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Effects of artificial cycles with and without gonadotropin-releasing hormone agonist pretreatment on frozen embryo transfer outcomes

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Abstract

Objective: This study aimed to compare the pregnancy outcomes between women receiving frozen embryo transfer (FET) with hormone replacement treatment (HRT) with and without gonadotropin-releasing hormone agonist (GnRHa) pretreatment.

Methods: All consecutive women undergoing HRT cycles (2936 cycles) or HRT with GnRHa pretreatment (HRT + GnRHa, 303 cycles) at our reproductive center between January 2015 and December 2017 were analyzed retrospectively.

Results: The average age was higher in the HRT + GnRHa compared with the HRT group (34.0 ± 4.8 vs. 31.3 ± 4.4). However, the pregnancy outcomes were comparable between the two groups. The clinical pregnancy rate was significantly increased in younger women ($\leq$35 years) in the HRT + GnRHa group compared with the HRT group (56.8% vs. 48.7%), but the live birth rates were similar in the two groups (44.2% vs. 38.4%). The HRT + GnRHa protocol significantly increased the clinical pregnancy rate (55.6% vs. 43.2%) and live birth rate (43.5% vs. 33.5%) compared with the HRT group among women with endometriosis, and significantly decreased the abortion rate in women with polycystic ovarian syndrome (3.1% vs. 16.4%).

Conclusions: GnRHa pretreatment may improve pregnancy outcomes in women with endometriosis and polycystic ovarian syndrome.

Keywords

Frozen–thawed embryo transfer, gonadotropin-releasing hormone agonist, endometriosis, pregnancy outcome, hormone replacement treatment, *in vitro* fertilization

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Introduction

The use of fresh or frozen embryo transfer for *in vitro* fertilization (IVF) has been a hot topic for debate in recent decades. However, there has been no definitive answer to date, given that the use of fresh or frozen cycles may depend on the cause of the infertility.[1,2] Frozen–thawed embryo transfer (FET) is no longer considered as merely a supplement to fresh embryo transfer, and a "freeze-all" protocol has become routine procedure in IVF treatment, owing to the application of progestin-primed ovarian stimulation protocols and preimplantation genetic diagnosis/screening, and the higher risk of ovarian hyperstimulation syndrome, higher levels of progesterone or estrogen, and abnormal endometrial status in fresh cycles.[3,4] The current priority is thus to identify factors that could improve pregnancy outcomes in patients undergoing FET cycles.

Endometrial preparation protocols for FET cycles can be natural or artificial. Natural cycles are suitable for younger women with regular ovulation, and involve several courses of hormone testing and ultrasound monitoring without medical intervention; however, this protocol is less easy to control and less flexible, and carries a risk of asynchronization between the embryo and endometrium due to advanced ovulation or anovulation.[5] Artificial or hormone replacement treatment (HRT) cycles are usually recommended in older women and women with ovarian function disorders, irregular menstruation cycles, or ovulation disorders. HRT cycles are more flexible than natural cycles and are generally suitable for women with or without regular ovulation. Although this protocol is controlled by estrogen supplementation, it has been proved to be as successful as natural cycles.[5]

Programmed cycles using a gonadotropin-releasing hormone agonist (GnRHa) before HRT aim to achieve pituitary down-regulation and avoid spontaneous ovulation and cycle cancellation.[6] However, GnRHa administration increases the cost and number of treatment cycles, and its effect on pregnancy outcomes remains controversial. Several studies claimed no difference in pregnancy outcomes between HRT and HRT with GnRHa pretreatment,[7–9] and other prospective studies showed that pregnancy outcomes were significantly improved by GnRHa pretreatment in HRT cycles.[10,11] We therefore retrospectively analyzed the outcomes of patients treated with FET cycles in our reproductive center and compared outcomes between HRT and HRT + GnRHa cycles.

Materials and methods

Study design

This was a retrospective cohort analysis of women undergoing FET with HRT cycles at the Centre for Reproductive Medicine, Renmin Hospital of Wuhan University, between 1 January 2015 and 31 December 2017. All FET cycles in women receiving HRT or HRT + GnRHa cycles were included, regardless of age, diagnosis, stimulation protocol, embryo stage, or embryo transfer number.

Embryo cryopreservation and thawing

Embryos were cryopreserved on day 3, 5, or 6 of embryo culture. The embryos were placed into equilibrium solution (Kitazato Corporation, Tokyo, Japan) for 6 minutes in room temperature, transferred to vitrification solution (Kitazato Corporation) for 30 s, and then loaded on a Cryotop (Kitazato Corporation) and plunged into liquid nitrogen within 60 s, for no longer than 90 s after initial exposure to vitrification solution. For thawing, the Cryotop

was removed from liquid nitrogen and placed immediately into thawing solution (Kitazato Corporation) at 37°C for 1 minute, followed by a three-step rehydration protocol: dilution solution for 3 minutes, followed by two steps of washing solution for 5 minutes, respectively. The embryos were then transferred into a droplet of blastocyst medium in a pre-balanced culture dish in 37°C and 6.0% CO_2.

Endometrial preparation protocol

Artificial preparation of the endometrium consisted of treatment with estradiol valerate (Progynova®; Bayer-Schering Pharma AG, Berlin, Germany) 2 mg twice daily for 7 days, followed by two mg three times daily for 6 days. Progesterone supplementation was started on day 13 if the endometrium was at least 7 mm thick, a triple-line endometrium was present, and serum progesterone levels were <1.5 ng/mL. Day 3 embryos were transferred on the fourth day of progesterone exposure, and the blastocysts were transferred on the sixth day of progesterone exposure.

For HRT + GnRHa cycles, 3.75 mg leuprorelin acetate (Diphereline®, Ipsen, France) or 3.75 mg triptorelin acetate (Decapeptyl®, Ferring, Switzerland) was administered during the early follicular phase of the previous menstrual cycle (day one or two), and the HRT protocol was started 28 days later.

Assessment of pregnancy outcomes

Serum β-human chorionic gonadotropin levels were measured 12 days after embryo transfer. If the test was positive, daily estradiol valerate and progesterone supplementation was continued until the 12th week of pregnancy. An ultrasound scan was performed to determine fetal viability 30 days after embryo transfer. Clinical pregnancy was defined as the presence of at least one fetus with a heart beat on ultrasound 45 days after embryo transfer. Pregnancy outcomes, including information on abortion, ectopic pregnancy, delivery conditions, and neonatal status, were collected at clinic visits and by telephone follow-up.

Ethical approval

This was a retrospective study that analyzed the electronic and paper databases in our hospital. All the participating partners signed informed consent for controlled ovarian hyperstimulation, oocyte and sperm collection, IVF or intracytoplasmic sperm injection treatment, embryo cryopreservation, embryo transfer, and follow-up visits. All the procedures complied with the Regulation of Human Assisted Reproductive Technology in China. The data collection and analysis were exempt from the need for ethical approval because the Ethical Review Board confirmed that it was a retrospective study with no extra interventions or bias in treatment. Patient consent for data collection and analysis was not required because the personal information was de-identified for tracking and searching.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA). Continuous variables were analyzed using independent *t*-tests or Mann–Whitney U tests, depending on the normality of the distribution. Categorical variables were analyzed by Pearson's χ^2 or Fisher's exact tests. $P<0.05$ indicated statistical significance.

Results

Baseline characteristics

A total of 3239 FET cycles were evaluated and included in this study, including 2936 HRT cycles and 303 HRT + GnRHa cycles. The baseline characteristics of both groups are presented in Table 1. The average age was significantly higher in the HRT + GnRHa compared with the HRT group ($P<0.0001$) and the proportion of older women (>35 years) was also significantly higher in the HRT + GnRHa group ($P<0.0001$). The proportion of women with endometriosis was significantly higher and the endometrial thickness on the progesterone-administration day was significantly lower in the HRT + GnRHa compared with the HRT group (both $P < 0.0001$). The type of infertility, number of FET cycles, number of transferred embryos and type of embryos, and serum estradiol level on the progesterone-administration day were all similar in both groups.

Pregnancy outcomes in the HRT and HRT + GnRHa groups

The pregnancy outcomes are presented in Table 2. The overall clinical pregnancy rate (CPR) and live birth rate (LBR) were similar in the two groups. However, among younger women (≤35 years), the CPR was significantly higher in the HRT + GnRHa group ($P = 0.04$), but the LBR remained similar in both groups. Among older women, the LBR was slightly lower in the HRT + GnRHa compared with the HRT group, but the difference was not significant. The abortion rate and sex ratio at birth (female versus male) were similar in both groups.

The pregnancy outcomes in women with endometriosis and polycystic ovarian syndrome (PCOS) are shown in Table 3. Among women with endometriosis, the CPR and LBR were both significantly higher in the HRT + GnRHa group compared with the HRT group (P=0.04 and $P = 0.02$, respectively). Among women with PCOS, the CPR and LBR were comparable in the two groups, but the abortion rate was significantly lower in the HRT + GnRHa group ($P = 0.04$).

Discussion

In this study, we analyzed 3239 FET cycles to compare the pregnancy outcomes between women receiving HRT cycles and HRT + GnRHa cycles. CPR and LBR were similar in both HRT protocols, with or without GnRHa pretreatment. However, given that this was a retrospective study with significant differences in participants' ages and infertility diagnoses, the results must be interpreted with caution.

GnRHa may be given in addition to HRT to suppress hormone production by the ovaries and inhibit spontaneous ovulation in artificial cycles. In this study, the average age was significantly higher in the HRT + GnRHa group compared with the HRT group. According to feedback from clinic physicians, this was partly because GnRHa pretreatment could prolong the menstruation cycle and decrease the cycle cancellation rate in older women. A previous retrospective study also found that the average age of women undergoing FET cycles with GnRHa pretreatment was higher than that for women undergoing cycles without GnRHa, because physicians preferred to use GnRHa to prevent cancellation in women of advanced age.[8]

Natural and artificial FET cycles can achieve equivalent pregnancy outcomes in women with regular ovulation and well-preserved ovarian function.[12,13] A prospective randomized clinical trial found no difference in pregnancy outcomes between HRT cycles with and without GnRHa

Table 1. Baseline characteristics of women receiving HRT or HRT + GnRHa.

Characteristic	Study group		*P* value
	HRT	HRT + GnRHa	
Transfer cycles (n)	2936	303	
Age at transfer (years)	31.3 ± 4.4	34.0 ± 4.8	<0.0001[a]
≤35	2455 (83.6)	190 (62.7)	<0.0001[b]
>35	481 (16.4)	113 (37.3)	
Type of infertility			0.28[b]
Primary infertility, n (%)	1291 (44.0)	143 (47.2)	
Secondary infertility, n (%)	1645 (56.0)	160 (52.8)	
Indication for fertility treatment			<0.0001[b]
Tubal factor, n (%)	1892 (64.4)	121 (39.9)	<0.0001[b]
Male factor, n (%)	352 (12.0)	23 (7.6)	0.03[b]
PCOS, n (%)	354 (12.1)	32 (10.6)	0.05[b]
Endometriosis, n (%)	206 (7.0)	108 (35.6)	<0.0001[b]
POF, n (%)	27 (0.9)	4 (1.3)	0.71
Other, n (%)	105 (3.6)	25 (5.0)	0.0001
Number of FET cycles	1.3 ± 0.6	1.3 ± 0.7	0.19[a]
Endometrial thickness (cm)	0.94 ± 0.19	0.85 ± 0.27	<0.0001[a]
Embryo transfer	2.0 ± 0.4	2.0 ± 0.4	0.94[a]
Embryo transfer type			0.14[b]
Day 3, n (%)	2332 (79.4)	229 (75.6)	
Blastocyst, n (%)	604 (20.6)	74 (24.4)	
E_2 level (pg/mL)	339.5 ± 14.0	355.0 ± 443.0	0.13[a]

Data presented as mean ± standard error or n (%).[a]Student's *t*-test;[b]Pearson's χ^2 test. HRT, hormone replacement treatment; GnRHa, gonadotropin-releasing hormone agonist; PCOS, polycystic ovarian syndrome; POF, premature ovarian failure; FET, frozen-thawed embryo transfer; E_2, estradiol.

Table 2. Pregnancy outcomes in women receiving HRT or HRT + GnRHa in relation to age.

Characteristic	Study group		*P* value[a]
	HRT	HRT + GnRHa	
Transfer cycles (n)	2936	303	
Clinical pregnancy rate (per transfer cycle)	46.1% (1353/2936)	48.5% (147/303)	0.45
≤35 years old	48.7% (1196/2455)	56.8% (108/190)	0.04
>35 years old	32.6% (157/481)	34.5% (39/113)	0.74
Abortion rate (per transfer cycle)	8.5% (249/2936)	11.2% (34/303)	0.13
≤35 years old	8.5% (209/2455)	10.0% (19/190)	0.60
>35 years old	8.3% (40/481)	13.3% (15/113)	0.11
Live birth rate (per transfer cycle)	35.8 (1051/2936)	35.0% (106/303)	0.83
≤35 years old	38.4% (942/2455)	44.2% (84/190)	0.13
>35 years old	22.6% (109/481)	19.5% (22/113)	0.53
Sex ratio at birth(female: male)	1: 1.20 (598: 718)	1: 1.22 (59: 72)	>0.95

Data presented as mean ± standard error. [a]Pearson's χ^2 test. HRT, hormone replacement treatment; GnRHa, gonadotropin-releasing hormone agonist.

Table 3. Pregnancy outcomes in women with endometriosis or PCOS receiving HRT or HRT + GnRHa.

Characteristic	Study group		*P* value
	HRT	HRT + GnRHa	
Endometriosis	206	108	
Age (years)	30.8 ± 4.2	31.4 ± 3.66	0.21[a]
Clinical pregnancy rate	43.2% (89/206)	55.6% (60/108)	0.04[b]
Abortion rate	7.8% (16/206)	9.3% (10/108)	0.67[b]
Live birth rate	33.5% (69/206)	43.5% (47/108)	0.02[b]
PCOS	354	32	
Age (years)	30.1 ± 3.9	31.3 ± 2.8	0.11[a]
Clinical pregnancy rate	51.4% (182/354)	50.0% (16/32)	0.85[b]
Abortion rate	16.4% (58/354)	3.1% (1/32)	0.04[b]
Live birth rate	34.5% (122/354)	46.9% (14/32)	0.18[b]

Data presented as mean ± standard error. [a]Student's *t*-test; [b]Pearson's χ^2 test. HRT, hormone replacement treatment; GnRHa, gonadotropin-releasing hormone agonist; PCOS, polycystic ovarian syndrome.

pretreatment in women with regular menstrual cycles.[7] HRT cycles can therefore be applied in younger women with normal ovulation and ovarian reserve function, to minimize clinic visiting times and costs. However, the LBR was slightly lower and the abortion rate was higher among older women (>35 years) receiving HRT + GnRHa compared with HRT. Likewise, in controlled hyperstimulation ovulation, a long pituitary-suppression protocol with GnRHa did not produce favorable results in older women or women with poor ovarian reserve, possibly because GnRHa may cause oversuppression of hypothalamic-pituitary-ovarian function and negatively affect uterine receptivity.[14–16] The effect of GnRHa pretreatment on pregnancy outcomes in women older than 35 years needs to be further validated.

Endometriosis is a main reason for subfertility and failure of embryo implantation.[13,17] GnRHa has been used to treat endometriosis by long-term pituitary suppression to improve uterine receptivity.[18–20] In this study, HRT cycles with GnRHa pretreatment significantly increased the CPR and LBR in women with endometriosis. Previous results also suggested that FET following GnRHa treatment tended to increase the pregnancy rate in women with endometriosis or adenomyosis.[21,22]

Infertile women with PCOS have an increased risk of early pregnancy loss, possibly as a result of hyperandrogenism, aberrant uterine receptivity, insulin resistance, and high body mass index.[23–25] In terms of IVF treatment, women with PCOS are usually transferred to FET cycles because of a high risk of ovarian hyperstimulation syndrome, and it is important to decrease the rate of pregnancy loss in women with PCOS.[26] In this study, although the abortion rate among women with PCOS was significantly higher in the HRT group compared with the HRT + GnRHa group, the LBR was similar in both groups, possibly because of the small sample size in the HRT + GnRHa group. A previous retrospective study showed that GnRHa pretreatment during FET significantly increased the ongoing pregnancy rate in women with hyperandrogenic PCOS.[27] However, testosterone levels were not monitored in women with PCOS to determine the mechanism of GnRHa in the prevention of pregnancy loss.

Conclusion

We recommend that women undergoing FET be treated individually in terms of endometrial preparation, based on their diagnosis and age. GnRHa pretreatment could significantly increase CPR and LBR in women with endometriosis and decrease the abortion rate in women with PCOS. However, this was a retrospective clinical study with a limited sample size in the HRT + GnRHa group, and further prospective, randomized clinical studies are needed to validate the optimal protocol for FET cycles.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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