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Artificial Cycle with or without a Depot Gonadotropin-releasing Hormone Agonist for Frozen-thawed Embryo Transfer: An Assessment of Infertility Type that Is Most Suitable*

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Summary: The clinical outcomes of five groups of infertility patients receiving frozen-thawed, cleavage-stage embryo transfers with exogenous hormone protocols with or without a depot gonadotropin-releasing hormone (GnRH) agonist were assessed. A retrospective cohort analysis was performed on 1003 cycles undergoing frozen-thawed, cleavage-stage embryo transfers from January 1, 2012 to June 31, 2015 in the Reproductive Medicine Center of Wuhan General Hospital of Guangzhou Military Region. Based on the infertility etiologies of the patients, the 1003 cycles were divided into five groups: tubal infertility, polycystic ovary syndrome (PCOS), endometriosis, male infertility, and unexplained infertility. The main outcome was the live birth rate. Two groups were set up based on the intervention: group A was given a GnRH agonist with exogenous estrogen and progesterone, and group B (control group) was given exogenous estrogen and progesterone only. The results showed that the baseline serum hormone levels and basic characteristics of the patients were not significantly different between groups A and B. The live birth rates in groups A and B were 41.67% and 29.29%, respectively (P<0.05). The live birth rates in patients with PCOS in groups A and B were 56.25% and 30.61%, respectively (P<0.05). The clinical pregnancy, implantation and on-going pregnancy rates showed the same trends as the live birth rates between groups A and B. The ectopic pregnancy rate was significantly lower in group A than in group B. We concluded that the live birth rate was higher and other clinical outcomes were more satisfactory with GnRH agonist co-treatment than without GnRH agonist co-treatment for frozen-thawed embryo transfer. The GnRH agonist combined with exogenous estrogen and progesterone worked for all types of infertility tested, especially for women with PCOS.
Key words: frozen-thawed embryo transfer; gonadotropin-releasing hormone agonist; polycystic ovary syndrome

With the development of cryopreservation, the survival of a greater number of optimal embryos after vitrification has significantly increased compared with slow freezing protocols, and frozen-thawed embryo transfer (FET) is associated with higher pregnancy rates than fresh embryo transfer[1]. A large body of evidence has demonstrated that controlled ovarian hyperstimulation (COH) is accompanied by poorer outcomes when fresh transfers are performed, due to changes in the endometrium during stimulation[2]. Additionally, to prevent the occurrence of secondary ovarian hyperstimulation syndrome (OHSS)[3], more centers choose ''freeze-all'' protocols[4, 5]. As a result, endometrial preparation for FET has received

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increasing attention.

The natural cycle FET (NC-FET) is the simplest method required for endometrium preparation, but the disadvantages include difficulties in transferring of the embryo at the correct time and the risk of unexpected ovulation. Ovulation induction is commonly used for patients with irregular menstruation, and the disadvantage of this approach is unexpected ovulation. In artificial cycle FET (AC-FET), estrogen and progesterone are used to imitate the endocrine surroundings of the endometrium; however, the administration of these hormones does not completely guarantee pituitary suppression, and unexpected ovulation may occur. For this reason, gonadotropin-releasing hormone (GnRH) agonist (GnRH-a) can be used[6, 7].

GnRH-a co-treatment can suppress the pituitary and subsequently prevent unexpected ovulation. Moreover, lower estrogen levels after down-regulation can avoid the shutdown of the "implantation window" in advance[8]. Furthermore, the GnRH gene is expressed in the human endometrium[9], and GnRH expression can directly inhibit inflammatory factors and increase endometrial adhesion molecules[10]. In 1991, one trial[11] showed that the clinical pregnancy rate was higher with FET in patients with irregular cycles using leuprolide acetate. Another retrospective cohort analysis[12] that included 1391 cycles suggested that the live birth rate was higher in GnRH-a synthetic cycles than in natural cycles for FET. However, three reports[13–15] showed similar outcomes between patients undergoing a GnRH-a synthetic protocol and those undergoing an exogenous hormone protocol.

The previous trials did not determine which type of infertility patients had better clinical outcomes after FET with down-regulation by a GnRH-a. Therefore, we performed this retrospective cohort study to compare multiple clinical outcomes, namely, the live birth, clinical pregnancy, implantation, and on-going pregnancy rates, in patients with different types of infertility with or without a depot GnRH-a for frozen-thawed cleavage-stage embryo transfer.

1 MATERIALS AND METHODS

1.1 Patients

Institutional review board approval was granted from the Reproduction Medicine Institutional Review Board of Wuhan General Hospital of Guangzhou Military Region for this study. Between January 1, 2012 and June 31, 2015, data from 1003 artificial endometrium preparation cycles for FET were analyzed. For the inclusion criteria, all the patients who underwent the transfer of their frozen-thawed embryos were enrolled. Embryos were cryopreserved at the cleavage stage by vitrification with cryoprotectants (KitazatoBiophama Co. Ltd., Japan). All COH cycles were included regardless of patients' age, diagnosis, stimulation protocol (GnRH-a protocol, GnRH antagonist protocol, natural cycle, and lutealphase ovarian stimulation), *in vitro* fertilization (IVF) or intracytoplasmic sperm injection. Exclusion criteria were as follows: blastocyst-stage embryos, donor oocytes, endometrial thickness that repeatedly did not reach 7 mm, and women with karyotype abnormalities.

The decision of whether to use a GnRH-a was made collaboratively by the physician and patients. Cycles were further separated into five subgroups: patients with tubal infertility, polycystic ovary syndrome (PCOS), endometriosis, male infertility, and unexplained infertility.

1.2 Preparation of Endometrium

In the GnRH-a group (group A), the protocol was initiated on day 3 of menstruation with a single intramuscular injection of a depot GnRH-a (Enantone 3.75 mg; Takeda, Japan). Four weeks later, the serum hormone levels were measured. If the estradiol level was <50 pg/mL and the progesterone level was <1.5 ng/mL, preparation of the endometrium was considered to have started. If these parameters were not met, 3.75 mg of the GnRH-a was injected again, and the serum hormone levels were measured again after 28 days.

In the control group (group B), a GnRH-a was not used. Preparation of the endometrium was started on day 3, as described below, and the serum hormone levels were measured. If the estradiol level was >80 pg/mL and the progesterone level was >1.0 ng/mL or if a follicle was over 10 mm in diameter per ultrasonography, the cycle was canceled.

In both groups, the preparation of the endometrium consisted of estradiol valerate (Progynova®; Bayer-Schering Pharma AG, Germany) at a dose of 2 mg twice daily for 4 days, followed by 3 mg for 4 days and then 4 mg for 4 days. On day 13, if ultrasonography confirmed an endometrial thickness of ≥7 mm and if the serum levels of progesterone were ≤1.5 ng/mL, progesterone in oil (Progesterone Injection; XianjuPharma, China) was administered intramuscularly at a dose of 60 mg. If the thickness of the endometrium was <7 mm, estradiol valerate was continued at 4 mg twice daily until the thickness reached ≥7 mm, and progesterone supplementation was started, as described previously. If the thickness was <7 mm despite a maximum of 7 days of additional supplementation, the cycle was canceled.

1.3 Assessment of Pregnancy Outcome

Cryopreserved embryos were transferred under ultrasound guidance 3 days after progesterone initiation. Luteal support was continued 2 weeks after transfer. Clinical pregnancy was defined as one or more gestational sacs present on ultrasonography 2–3 weeks later. Early pregnancy loss (spontaneous abortion and biochemical pregnancy) indicated the

loss of a pregnancy before 12 gestational weeks, and the remainder of pregnancies was defined as on-going pregnancies. Live birth was defined as the delivery of at least one live baby, regardless of the duration of pregnancy.

1.4 Statistical Analysis

Categorical variables were reported as percentages, and χ^2 analysis was performed. Continuous variables were reported appropriately as the mean or median with the associated SD and analyzed using the independent *t*-test. $P<0.05$ was considered statistically significant.

2 RESULTS

In total, 1003 cycles were included in the analysis. A total of 252 cycles were prepared with the GnRH-a co-treatment (group A), including 150 cycles with tubal infertility, 48 cycles with PCOS, 16 cycles with endometriosis, 36 cycles with male factor infertility, and 2 cycles with unexplained infertility. A total of 751 cycles were prepared without the GnRH-a co-treatment (group B), including 477 cycles with tubal infertility, 98 cycles with PCOS, 19 cycles with endometriosis, 139 cycles with male infertility, and 18 cycles with unexplained infertility.

2.1 Baseline Serum Hormone Levels

The baseline serum hormone levels of the patients are presented in table 1. The baseline luteinizing hormone (LH) serum levels in the patients with PCOS (6.58±3.92 IU/L in group A *vs*. 8.44±5.10 IU/L in group B) and with male factor infertility (9.40±5.32 IU/L in group A *vs*. 7.36±4.82 IU/L in group B) were significantly different between the two groups, but the levels of the other hormones showed no significant difference.

Table 1 Baseline serum hormone levels of the patients

Classification	Groups	*n*	FSH (IU/L)	LH (IU/L)	E_2 (pg/mL)	PRL (ng/mL)	T (ng/mL)
Tubal infertility	A	150	6.36±2.23	7.71±4.61	36.25±16.09	12.71±6.58	0.35±0.19
	B	477	6.63±228	7.87±5.04	34.51±15.70	12.90±6.40	0.33±0.19
PCOS	A	48	6.24±2.20	6.58±3.92*	38.37±21.45	13.08±6.69	0.31±2.98
	B	98	6.71±2.20	8.44±5.10*	35.70±19.78	12.18±6.89	0.32±0.19
Endometriosis	A	16	6.23±2.83	6.79±4.08	40.18±21.32	13.43±6.36	0.31±0.23
	B	19	6.96±2.21	8.51±4.40	34.62±17.68	14.18±4.46	0.31±0.21
Male infertility	A	36	7.10±1.84	9.40±5.32*	33.13±12.00	11.53±6.74	0.34±.018
	B	139	6.37±2.33	7.36±4.82*	34.27±18.03	12.95±6.95	0.31±0.20
Unexplained infertility	A	2	5.16±0.25	7.39±0.93	42.01±7.82	12.57±2.70	0.58±0.28
	B	18	6.68±1.98	7.77±2.87	36.51±10.85	13.64±5.33	0.42±0.22
Total	A	252	6.58±1.99	7.86±4.49	37.39±16.26	14.37±4.91	0.37±0.17
	B	751	6.82±1.96	8.12±4.81	35.82±15.66	14.68±4.63	0.35±0.18

FSH: follicle stimulating hormone; LH: luteinizing hormone; E_2: estradiol; PRL: prolactin; T: testosterone.
*$P<0.05$

2.2 Basic Characteristics

The basic characteristics of patients are presented in table 2. Age, male age, infertility duration, body mass index (BMI), the thickness of endometrium, the number of embryos transferred and the rate of high-quality embryos showed no significant difference between two groups.

Table 2 Basic characteristics of the patients

Classification	Groups	*n*	Age (years)	Male age (years)	Infertility duration (years)	BMI (kg/m^2)	Thickness of endometrium (mm)	Number of embryos transferred (n)	Rate of high-quality embryos (%)
Tubal infertility	A	150	31.05±5.15	34.27±5.79	3.14±2.20	22.38±3.59	9.11±1.44	2.31±0.50	70.7%
	B	477	31.20±4.72	34.20±5.83	3.10±2.16	22.21±3.34	9.04±1.43	2.20±0.48	67.9%
PCOS	A	48	28.31±3.43	31.23±2.98	2.71±1.58	22.55±3.53	8.96±1.17	2.13±0.39	68.8%
	B	98	29.41±4.62	32.05±4.63	2.98±2.04	22.70±3.55	9.16±1.37	2.26±0.46	74.5%
Endometriosis	A	16	32.69±4.29	34.31±5.12	2.38±1.15	22.66±3.11	8.63±1.50	2.38±0.20	75.0%
	B	19	32.84±4.02	32.84±3.27	3.21±2.35	23.59±3.57	9.00±1.29	2.16±0.50	78.9%
Male infertility	A	36	29.50±4.37	31.47±5.26	3.78±2.10	22.79±3.50	8.69±1.19	2.36±0.54	61.1%
	B	139	29.54±5.31	32.63±5.91	3.02±2.16	22.30±3.58	9.09±1.40	2.16±0.47	71.2%
Unexplained infertility	A	2	39.50±6.36	39.00±7.07	4.00±2.83	19.45±2.76	8.00±1.41	2.00±1.41	50.0%
	B	18	36.89±5.70	38.67±5.24	3.72±2.80	22.72±3.27	8.56±1.25	2.22±0.73	66.7%
Total	A	252	30.48±4.90	33.33±5.42	3.11±2.04	14.37±4.91	8.98±1.36	2.28±0.50	69.0%
	B	751	30.84±4.98	33.70±5.74	3.06±2.16	14.68±4.63	9.05±1.41	2.20±0.48	69.6%

2.3 Clinical Outcomes

The clinical outcomes are presented in table 3. The live birth rate was significantly higher for all cycles in group A (41.67%) than that in group B (29.29%) and significantly higher for the patients with PCOS in group A (56.25%) than for those with PCOS in group B (30.61%). The other outcomes, including the clinical pregnancy, implantation and on-going pregnancy rates, were equivalent to the live birth rates. The ectopic pregnancy rate was significantly lower for all patients in group A (0%) than in group B (4.98%) and significantly lower for the patients with tubal infertility in group A (0%) than for those with tubal infertility in group B (6.78%).

Table 3 Clinical outcome

Classification	Groups	*n*	Clinical pregnancy rate (%)	Multiple pregnancy rate (%)	Ectopic pregnancy rate (%)	Implantation rate (%)	Early pregnancy loss rate (%)	On-going pregnancy rate (%)	Live birth rate (%)
Tubal infertility	A	150	43.33%	20.00%	0.00%*	22.25%	15.38%	36.00%	35.33%
	B	477	37.11%	15.25%	6.78%*	19.08%	11.86%	30.19%	29.56%
PCOS	A	48	68.75%*	24.24%	0.00%	40.20%*	15.15%	58.33%*	56.25%*
	B	98	39.80%*	25.64%	0.00%	22.62%*	23.08%	30.61%*	30.61%*
Endometriosis	A	16	62.50%	30.00%	0.00%	36.84%	20.00%	50.00%	50.00%
	B	19	36.84%	14.29%	14.29%	21.95%	28.57%	21.05%	21.05%
Male infertility	A	36	47.22%	29.41%	0.00%	25.88%	5.88%	44.44%	44.44%
	B	139	37.41%	23.08%	1.92%	21.33%	11.54%	32.37%	30.22%
Unexplained infertility	A	2	50.00%	0.00%	0.00%	25.00%	0.00%	50.00%	50.00%
	B	18	33.33%	50.00%	0.00%	22.50%	16.67%	27.78%	16.67%
Total	A	252	50.00%*	23.02%	0*	26.96%*	14.92%	42.46%*	41.67%*
	B	751	37.42%*	18.86%	4.98%*	20.12%*	13.88%	30.36%*	29.29%*

*$P<0.05$

3 DISCUSSION

A recent Cochrane Review[6] and a meta-analysis[7] found no evidence to support the GnRH-a co-treatment protocol over other protocols. However, no study has discussed which type of infertility patient would have a better clinical outcome with GnRH-a co-treatment. Our data were classified into five subgroups of infertility: tubal infertility, PCOS, endometriosis, male factor infertility and unexplained infertility.

Our data demonstrated that the live birth, clinical pregnancy, implantation, and on-going pregnancy rates were significantly higher and that the ectopic pregnancy rate was significantly lower in the cycles with GnRH-a co-treatment than in those without the co-treatment. These findings were consistent with those reported by Muasher *et al*[11] and Hill *et al*[12]. However, Dal Prato *et al*[13], van de Vijver *et al*[14], and AzimiNekoo *et al*[15] determined that the treatment with GnRH-a was unnecessary, given similar outcomes between groups and the added expense of the treatment.

In our study, when a single depot GnRH-a (3.75 mg) was injected intramuscularly on days 2–3 of the menstrual cycle, the preparation of the endometrium began after 28 days, even though conventionally, the injection was given during the mid-luteal phase and endometrium preparation was started at subsequent menses. A seminal study[16] showed that the responses were similar when a single depot GnRH-a (3.75 mg) was injected during the early follicular or mid-luteal phase. After 3 or 4 weeks, the FSH levels rose to normal, but the LH and estrogen levels increased until the 7th and 8th week. Menses was postponed until the 11th and 13th week after the injection. The results of the experiments suggested that GnRH mRNA and protein were expressed in peri-implantation human embryos and stimulated human chorionic gonadotropin (hCG) secretion and that the GnRH-hCG system played an important role in successful embryonic implantation and development[17, 18]. In our study, at the 4th week after GnRH-a (3.75 mg) was injected, endometrial preparation was started and lasted for 12 days, while the implantation was performed almost 7 weeks after GnRH-a injections. This indicated that the pituitary suppression by the GnRH-a depot was relieved and that the GnRH-hCG system could perform its function. However, implantations were performed at 4th week in other studies, in which the injections were performed during the mid-luteal phase and in which endometrial preparation was started at subsequent menses. At this time, the pituitary is still desensitized, and the GnRH-hCG system does not function.

Our data showed that the patients with PCOS received the best clinical outcomes with GnRH-a co-treatment among the five subgroups. PCOS is a complex endocrine disorder that involves multiple factors, such as the overproduction of androgen; the reduced expression of αvβ3 integrin and glycodelin; hyperinsulinemia that locally down-regulates insulin-like growth factor binding protein-1; high levels of plasminogen activator inhibitor; and the increased resistance of uterine arterial blood flow, which leads

to reduced endometrial receptivity[19]. One study[20] showed similar outcomes in patients with PCOS using ovulation induction and AC-FET; however pretreatment to promote pituitary suppression was not performed. Conveniently, the GnRH-a co-treatment reduces the serum levels of LH, which promotes the embryo-endometrium interaction; directly inhibits inflammatory factors and increases endometrial adhesion molecules; and improves endometrial receptivity and clinical outcomes[21].

No ectopic pregnancies occurred in the cycles with the GnRH-a co-treatment, and this result was especially notable for the patients with tubal infertility. One study[22] demonstrated that tubal infertility, pelvic inflammatory disease, endometriosis, a history of tubal surgery, cigarette smoking and a previous ectopic pregnancy were the most significant risk factors for ectopic pregnancy secondary to IVF-embryo transfer. In our study, the ectopic pregnancy rates of the patients with tubal infertility (6.78%) and endometriosis (14.29%) were obviously higher, which was consistent with the above study. Two studies[23, 24] suggested that high levels of estrogen could increase uterine peristaltic activity, leading to ectopic pregnancy, especially during the COH. The GnRH-a co-treatment could reduce the estrogen levels that could promote embryo implantation and maintenance.

Multiple reports[25, 26] have shown that patients with endometriosis have improved clinical outcomes after GnRH-a co-treatment. The clinical pregnancy rates and implantation rates of the patients with endometriosis were obviously higher after the GnRH-a co-treatment in our study, but the results were not significant. The lengthy protocol improved endometrial receptivity, so the protocol resulted in a good clinical outcome with fresh embryo transfer in patients with endometriosis due to the small sample size.

A recent study[27] showed that the decreased expression of endometrial adhesion molecules (such as leukemia inhibitory factor and αvβ3 integrin) was evident in unexplained infertility patients and that GnRH-a co-treatment could eliminate this effect. A similar report[28] showed that GnRH-a co-treatment could be used for patients who had idiopathic repeated implantation failures of IVF, and the treatment may help increase the receptivity of the endometrium. The live birth and clinical pregnancy rates of patients with male infertility and unexplained infertility obviously increased after the GnRH-a co-treatment. Due to the small sample size, no significant differences were evident.

The hypothesis of our study is that pituitary down-regulation (including the suppression of LH levels, E_2 levels and the GnRH-hCG system) after GnRH-a lasts for 7 or 8 weeks, while the inhibition of inflammatory factors and increase in endometrial adhesion molecules lasts longer. This dynamic process indicates that the lower levels of LH and E_2 are better for endometrial preparation during 7 weeks after the GnRH-a injection and that subsequently, the recovery of the GnRH-hCG system and the continued inhibition of inflammatory factors and increase in endometrial adhesion molecules are better for implantation. The weaknesses of our paper are the lack of basic research that shows the durations of the inflammatory factor inhibition and adhesion molecule increase. Moreover, to our knowledge, no related studies exist.

In conclusion, the live birth rate and other clinical outcomes, including the clinical pregnancy, implantation, and on-going pregnancy rates, were superior for the exogenous hormone stimulation protocol with a depot GnRH-a in FET. This protocol worked for all types of infertility, especially for women with PCOS.

Conflict of Interest Statement

The authors declare no conflict of interest.

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