Original Article

The Influence Of Different Endometrium Preparation Regimen For Frozen Thawed Embryo Transfer On Perinatal Outcomes Of Singletons Pregnancy In Patients With Polycystic Ovary Syndrome: A Randomized Controlled Trial

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Abstract

Introduction: According to the lack of strong evidence, whether the different frozen thawed embryo transfer (FET) protocols in patients with polycystic ovarian syndrome (PCOS) have any effect on its success is still unclear. This study was performed to compare the effect of hormone replacement treatment (HRT) protocols and gonadotropin-releasing hormone GnRH agonist (GnRHa)-HRT protocols on perinatal outcomes of singletons pregnancy in patients with PCOS.

Material and Methods: The study design was a randomized controlled trial (RCT) conducted at a tertiary referral hospital. A total of 132 patients with PCOS undergoing FET cycles were included in this study. The patients were randomly divided into two groups according to a computer-generated method. The GnRHa-HRT group (n = 66) had pituitary suppression before steroid hormone administration whereas HRT group (n = 66) commenced steroid supplementation without prior pituitary desensitization. Primary outcome was considered birthweight of singleton newborns.

Results: There were no differences in birth weight of newborns between two groups (p=0.50). No significant differences were found between two groups for preeclampsia, gestational diabetes mellitus, IUGR, first trimester bleeding, and preterm labour. Moreover, neonatal anomalies were not significantly different between two groups, but no neonatal anomalies were reported from group HRT.

Conclusion: The findings indicated that neonatal birth weight and other prenatal outcome were not significantly different between two groups. It seems that the use of GnRH agonist creates additional burdens and adverse events for the patient, and the use of estradiol alone is an effective, less complicated and economically cost-effective protocol for patients with PCOS.

Keywords: Prenatal**,** Neonatal**,** FET cycles, GnRH Agonist, PCOS.

INTRODUCTION

Since Trouson performed the world's first frozen thawed embryo transfer (FET) in 1983 resulting in a successful clinical pregnancy, FET has played an important role in assisted reproduction technology [1]. FET provides the surplus embryos generated by IVF/ICSI treatment to be stored and transferred at a later date. FET increases the cumulative pregnancy rate in the single egg retrieval cycle, and reduces the occurrence of moderate and severe ovarian hyperstimulation syndrome (OHSS), and the risk of multiple pregnancy. This is also simpler and easier method than fresh cycles, causing less pain to patients and reducing

time and costs [2, 3]. Many studies have concluded that FET reduces the risk of preterm delivery and low birth weight [4, 5]. Furthermore, FET is a common procedure in women with polycystic ovarian syndrome (PCOS), with high responses to controlled ovarian hyperstimulation (COH), and a high risk of developing OHSS. To prevent the OHSS, the high cancellation rates occur in fresh cycles [3]. However, the best regimen for endometrium preparation in ovulatory women is still a matter of debate. To perform the FET, endometrial preparation should be accomplished. The most commonly used protocol is traditional hormone replacement treatment (HRT). In HRT cycle, estrogen is administered for two weeks for endometrial proliferation and dominant follicle growth suppression. However, HRT cannot definitely suppress pituitary, so that follicles growth and ovulation escape may happen occasionally [6-8].

An easy way to prepare the endometrium for FET is to use a gonadotropin-releasing hormone (GnRH) agonist, with better pituitary down-regulation and spontaneous ovulation prevention. As another FET protocol, GnRH agonist combined with hormone replacement treatment (GnRHa-HRT) has achieved good reproductive outcomes in patients with endometriosis and repeated implantation failure [9-11]. GnRHa- HRT is the application of a GnRH agonist (GnRHa) in the preparation of endometrium using inhabitation of the surge of luteinizing hormone (LH) before estrogen administration [12, 13]. Since the beginning of endometrial hyperplasia, continued application of estrogen alone has been shown to be sufficient to suppress ovulation through the negative feedback mechanism of the hypothalamic-pituitary ovarian axis [14]. In the initial stage of estrogen administration alone, the endometrium thickens is maintained, whereas follicular development is inhibited. Daily progesterone administration is started 4-6 days before the embryo transfer. Estrogen keeps the proliferative phase to maintains the endometrium in a pre-ovulatory state until the start of progesterone to induce the endometrium to transform into an embryo-accepting state [15].

The birth weight of neonates has long been regarded as an indicator of the offspring's health. Previous evidence has indicated that FET is associated with a higher birthweight and an increased risk of delivering large for gestational age (LGA) newborns compared to fresh embryo transfer. These findings imply that the process of cryopreservation the endometrial milieu or other features of the IVF cycle can adversely affect the embryo quality and developmental potential [16, 17].

The potential effects of different endometrial preparation protocols on future children is of particular interest. Few studies have been conducted on the selection of the best FET protocol in patients with PCOS for perinatal outcomes. This study was performed to compare the effect of HRT protocols and GnRHa-HRT protocols in endometrial preparation on perinatal outcomes, especially on birthweight of singleton newborns in PCOS patients.

Materials and Methods

The study design was a randomized controlled trial (RCT) conducted at a tertiary referral hospital. A total of 132 patients with PCOS undergoing FET cycles from 20 Jun 2020 to 27 August 2021 were included in this study. It was conducted in accordance with the Declaration of Helsinki (1989) and institutional ethics committee approved it (code: IR.TUMS.MEDICINE.REC.1400.092). It was registered in IRCT.IR with reference code of IRCT20090526001952N15. Informed written consent was obtained from all included patients.

The following patients were included in to the study: The Rotterdam criteria define PCOS by the presence of at least two out of three criteria: oligo- and/or anovulation, clinical and/or biochemical hyperandrogenism and polycystic ovaries, maternal age <40 years, the first or the second FET cycle, previous conventional IVF/ICSI with two or more good embryo cryopreservation on day 3 or day 5 and normal intrauterine cavity after pretreatment assessment. Exclusion criteria consisted of use of testicular sperm for ICSI, early (day 3) follicular phase FSH levels 12 IU/l or above, egg donor, surrogate mothers, or patients with hydrosalpinx, uterine anomalies, and sub-mucosal myomas.

The patients were randomly divided into two groups according to a computer-generated method. Group GnRHa-HRT (n = 66) had pituitary suppression before steroid hormone administration whereas HRT group (n = 66) commenced steroid supplementation without prior pituitary desensitization.

Endometrial Preparation Before Embryo Transfer

After completing standard in vitro fertilization and intracytoplasmic sperm injection along with whole embryo freezing, the patient returned after her second menstrual period. On day 2 of spontaneous menses, the patients underwent a baseline transvaginal ultrasound and assessment of antral follicular count (AFC), endometrium thickness, and cysts.

In the HRT strategy, on day 2 cycle patients then began administration of oral estrogen (Estradiol Valerat 2 mg Aburiahan Pharmaceutical CO. Tehran-IRAN), 2 mg twice daily for 3 days, followed by 6 mg daily. After one week, patients underwent transvaginal ultrasound for endometrium thickness assessment. Oral estrogen was administered to induce endometrial proliferation while suppressing dominant follicle development. We performed transvaginal ultrasound every 3 days to assess the

recipients' endometrium, with the first ultrasound occurring within 7 days of initiating estrogen supplementation. When endometrial thickness was ≥8mm, progesterone supplementation was initiated as 50 mg intramuscular (Femogex-IH 50mg IRAN Hormone Pharmaceutical CO.) or 400 mg vaginally (Fertigest Suppository 400mg Aburiahan Pharmaceutical CO. Tehran-IRAN). When endometrial thickness was≤ 7 mm, estrogen supplementation was administered and performed transvaginal ultrasound every 3 days until 21 days. The cycle was cancelled when there was not appropriate endometrial thickness after 21 days.

In the GnRHa-HRT strategy, on days 18–21 of the previous cycle, the patient was administered injected 0.5 cc of Superfact (Buserlin acetat 1mg/cc Injection Sanofi Aventis). Vaginal ultrasound was performed 10-14 days later. If the endometrial thickness was less than 5 mm, and there were no ovarian cysts, estradiol was initiated like HRT Group treatment and with the start of estradiol, the dose of GnRH agonist is halved (0.25 cc superfact) and superfact was prescribed until progesterone was started. At the time of progesterone administration, the administration of superfact was discontinued. Administration of estradiol and progesterone would be the same as for the HRT group. After reaching the down-regulation standard, the administration of estradiol and progesterone as described for the HRT scheme**.**

At the timing of the FET, progesterone in the form of intramuscular or vaginal combined with administration of progesterone was performed daily. The route of progesterone supplementation was based on the patient's preference, as there is no medical indication for the use of one regimen over the other. Patients were administered intramuscular progesterone in oil or vaginally and a combination of oral estrogen, starting at 4 days before FET when transplanting the cleavage embryos, as well as 6 days before FET when transplanting the blastocysts.

After FET, daily estrogen and progesterone administration was continued until a negative pregnancy test was obtained. If pregnancy was achieved, hormone administration was continued until the expected luteoplacental shift in estrogen and progesterone production at approximately 8–9 weeks of gestation.

Embryo Vitrification, Thawing, and Transfer

Briefly, embryo vitrification was carried out using a Cyrotop carrier system with a solution of dimethyl sulfoxide, ethylene glycol, and sucrose used as a cryoprotectant. For thawing, embryos were transferred into dilution solution in a sequential manner (1–0.5–0 mol/L sucrose).

Cleavage-stage embryos (day 3) were graded according to the Cummins criteria. Grade A and B embryos were classified as high-quality and selected for vitrification. In all FET cycles, no more than three embryos were transferred. All embryos were thawed on the day of transfer, and post thaw embryos with R50% blastomeres intact were considered as having survived.

Outcome Parameters

The primary outcomes included birth weight (including absolute birth weight). The secondary outcome was medical complications during pregnancy (preeclampsia, preterm labour, and IUGR) and reproductive outcomes, including clinical pregnancy, ongoing pregnancy, abortion, and live birth rates. The clinical pregnancy rate per woman was defined as the presence of at least one gestational sac in the uterine cavity on ultrasound at 5 weeks after ET. The ongoing pregnancy rate per woman was defined as evidence of a gestational sac with fetal heart motion at 12 weeks as confirmed by ultrasound. The abortion rate was defined as a loss of clinical pregnancy before the 20 gestational week. The live birth rate per woman was defined as delivery of a live fetus after 24 completed weeks of gestation.

Statistical Analysis

Statistical analysis was conducted on an intention-to-treat basis that included all randomized patients who began this study. Descriptive statistics were carried out for each variable. Quantitative results are presented as mean (SD) and qualitative results are presented as distribution of frequencies. Means were compared by the two-sample t-test. Proportions for the two groups were compared using the Chi-squared test. $P < 0.05$ was considered statistically significant. The 95% confidence interval around the point estimates was calculated for baseline data and the treatment effect. For a sample of 66 women with PCOS in each group, a difference of 6.5% in the pregnancy rate per embryo transfer would be considered statistically significant at a power of 80%. SPSS statistical software package (SPSS, Inc., Chicago, IL, USA), version 22.0 and MedCalc software were used for statistical analyses.

Results

From 393 eligible PCOS patients, 132 women were randomly allocated to two groups. Finally, 131 women with PCOS

completed the trial, with 66 patients in group HRT and 65 patients in group HRT with GnRHa. The rate of loss to follow-up of all samples estimated in both groups was less than 10%. The patient recruitment and reasons for excluding participants before starting the trial and those who dropped out during the trial are presented in **Figure 1**.

No statistically significant differences were found in baseline characteristics, of demographic and clinical data, i.e. age, Body mass index (BMI), duration of infertility, type of infertility, previous fresh and frozen embryo transfer, menstruation, gestational age, gravid, parity, abortion history (**Table 1**). Hormonal profiles of patients in both groups were compared. Total consumed estrogen concentration was significantly higher in group HRT with GnRH agonist (15.13±2.01versus 73.33±1.73, P = 0.008). The concentration of AMH was also significantly higher in group HRT with GnRH agonist (10.20±0.77 versus 8.26±0.63 0.63, P =0.050). The mean number of consuming estrogen days was significantly higher in group HRT with GnRH agonist (15.38±2.52 versus 14.17±2.29, p=0.006). Quality assessment of embryo transferred revealed that the number of high -quality embryo transferred (grade: A, AB) was significantly more in group HRT with GnRH agonist (p<0.000). However, the number of embryos transferred with grades B, BC was significantly remarkable in group *HRT* (p<0.001) (**Table 2**).

There were no differences in birth weight of newborns between two groups (p=0.50). No significant differences were found between two groups for preeclampsia, gestational diabetes mellitus, IUGR, first trimester bleeding, and preterm labour. Moreover, neonatal anomalies were not significantly different between two groups, but no neonatal anomalies were reported from group HRT. There were no differences in β-HCG positive rate, multiple pregnancy, ongoing pregnancy rate, and clinical pregnancy rate between the two study groups. However, the number of early miscarriages, clinical pregnancy, live birth was clinically higher in HRT with GnRH agonist group compared to the HRT group. (**Table 3).**

Discussion

To the best of our knowledge, the present study was first prospective investigation to provides information on the neonatal outcomes of the two most commonly used protocols for preparation of the endometrium in FET cycles. The present findings revealed that no significant difference in the rate birth weight of newborns preeclampsia, gestational diabetes mellitus, IUGR, and preterm labour comparisons between HRT and HRT with GnRH agonist. These results were not similar to findings by Wang et al. in 2020. They retrospectively found that among newborns from natural cycle FET, artificial cycle FET, and stimulated cycle FET, a higher mean birth weight was observed in the artificial cycle FET group than in the stimulated cycle FET group[18]. Another retrospective cohort study also showed that after controlling for a variety of covariates, singletons from the artificial cycle FET group had a higher mean birth weight and Z-score than those from the natural cycle FET group and stimulated cycle FET group [19]. The results indicated a link between the absence of the corpus luteum and adverse perinatal outcomes, However, further studies are needed to detect the underlying mechanism.

Birth weight is determined by both the duration of gestation and the rate of fetal growth [18]. In the present study, singletons conceived with two preparation methods had a same duration of gestation. The present findings did not show significantly different difference of preterm birth between groups.

Finding the appropriate protocol for endometrial preparation in patients with PCOS is more complicated than regularly ovulating patients. In this study, GnRH agonist combined with HRT did not improve the clinical outcomes of frozen embryo cycles in PCOS patients. Preparation of the endometrium prior to embryo transfer is an important consideration in implantation and development of pregnancy of FET cycles [20, 21]. Despite the existing many studies in this issue, the beneficial effect of GnRHa in PCOS patients before FET for endometrial preparation is not known. There were several advantages for the use of GnRH agonist in hormonal replacement treatment protocol has. First, it enables reduction in the cancellation rate and the flexibility in time of embryo transfer. Second, GnRHa pretreatment for PCOS can suppress LH level, E2 level, hyper-androgenic level, and GnRH-HCG axis function through with inhibition of endometrial inflammation and enhanced expression of endometrial adhesion molecules [22]. However, this protocol has disadvantages. First, the preparation is prolonged; second; the cost increases; and third the GnRHa can have side effects and may delay the resumption of spontaneous ovulation if FET fails [23]. HRT without the prior administration of GnRH agonist may result in a rise in LH, ultimately negatively altering the receptive window of implantation. However, it simultaneously simplifies and reduces the time and money consumption and may thus be more convenient and acceptable for patients if it achieves a similar clinical outcome [24, 25].

There are a few numbers of studies comparing HRT and GnRHa + HRT cycles, however, the results have been conflicting. In the present study, it was tried to evaluate the information of the cycles in details, for example preeclampsia, Gestational diabetes, preterm labour, etc. The present findings indicated that the hormonal profile of patients did not significantly affect complications during pregnancy between both groups. However, no neonatal anomalies were reported from group HRT. So, the use of GnRH agonist creates additional burdens and adverse events for the patient, the use of estradiol alone is an effective, less complicated and economically cost-effective protocol for patients with PCOS [26].

The clinical pregnancy rate is a common outcome measured in comparative effectiveness research. Findings of the present study

are in line with a previously published prospective randomized trial [26], which shows no significant differences were observed in the pregnancy rate per transfer, miscarriage rate, and live birth rate between the studied groups. Another randomized controlled trial showed no difference between HRT, and GnRHa with HRT even in patients with repeated implantation failure and in patients with normal ovarian function [27, 28]. A review conducted on 7 RCT concluded that no evidence of a difference between the two groups in the clinical pregnancy rate [8]. A meta-analysis also showed that neither of the two regiments had a significant advantage in terms of the clinical pregnancy rate [29]. However, this present study results obviously contrast with another randomized trial and a retrospective study, which concluded that the supplement of GnRH agonist associated with a higher pregnancy rate and live birth rate [30, 31]. Although the number of high-quality frozen embryo was slightly higher in HRT with GnRH agonist, physicians usually chose the best quality embryo for transfer in the FET cycle.

In this clinical trial, it was not possible to complete randomized double-blind comparison. Considering the possible effect of the previous physiological dose in the previous cycle, it was not possible to investigate the estradiol level for the previous physiological dose in the previous cycle that could affects.

Conclusion

The findings indicated that neonatal birth weight and other prenatal outcome were not significantly different between two groups. It seems that the use of GnRH agonist creates additional burdens and adverse events for the patient, and the use of estradiol alone is an effective, less complicated and economically cost-effective protocol for patients with PCOS. Further large prospective studies should be carried out to confirm these results and the underlying mechanisms should also be investigated.

Conflict of Interest Statement

There is no conflict of interest between authors of this article.

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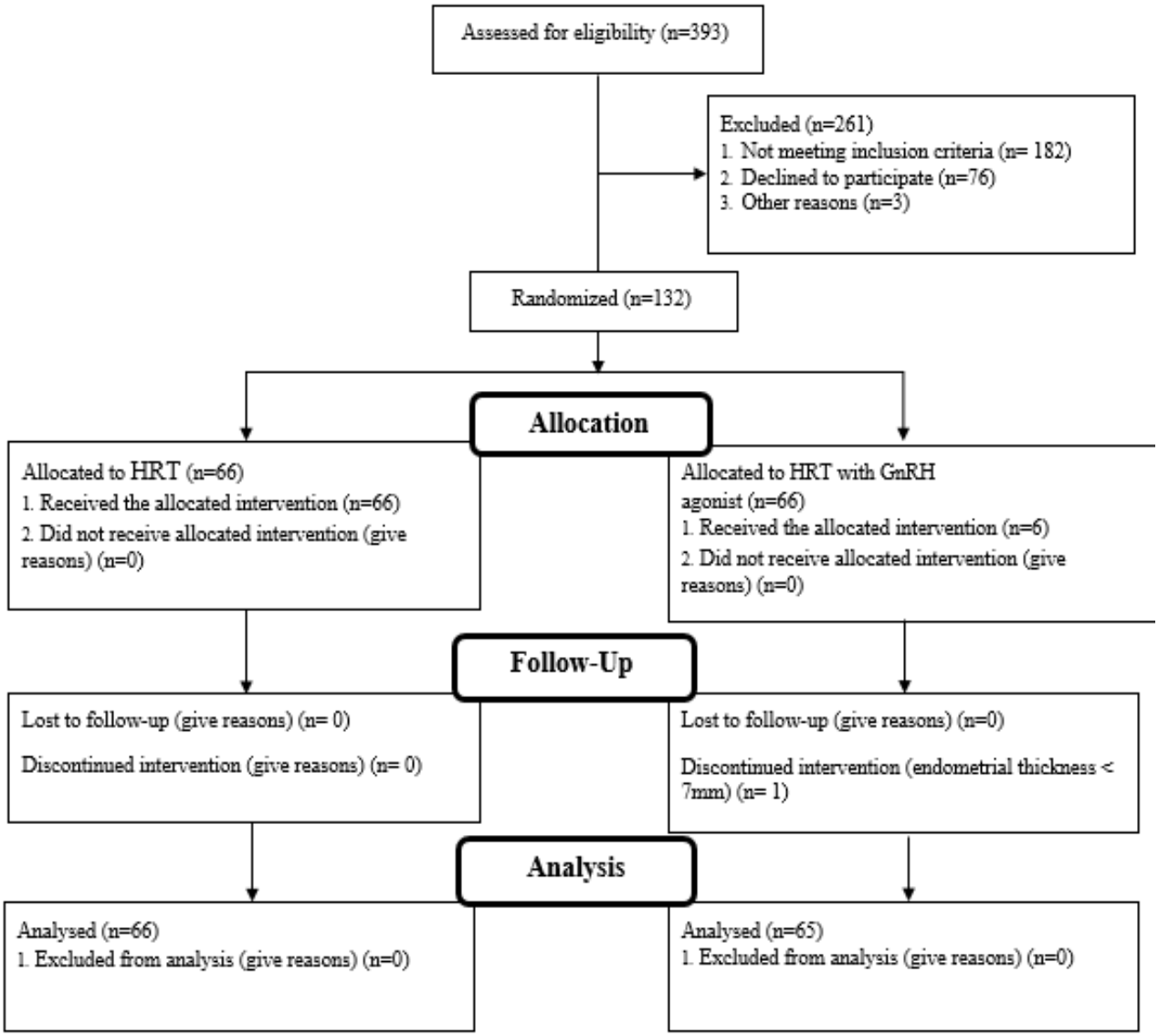

Table 1: The baseline characteristics of two study groups

Characteristics		**HRT**	**HRT with GnRH agonist**	**P-value**	**Mean difference /OR**	**95% CI**	
						Lower	**Upper**
Age, years		31.44±5.02	30.61±4.76	0.336	0.83	-0.87	2.53
BMI, kg/m2		25.85± 3.43	26.52± 4.30	0.059	-0.68	-2.18	-0.18
Duration of infertility, years		6.06± 3.81	7.64± 4.33	0.058	-1.58	-2.99	-0.17
Type of infertility	Primary	55 (89.5%)	59 (90.8%)	0.321	-	-	-
	Secondary	9 (14.1%)	5 (8.7%)				
Previous ART cycle (fresh and frozen embryo transfer)		0.97± 0.70	1.14± 0.69	0.161	-0.17	-0.42	0.07
Menstruation	Oligomenorrhea	48 (72.7%)	48 (76.2%)	0.665	-	-	-
	Amenorrhea	0 (0.0%)	1 (1.6%)				
	Regular	16 (24.2%)	13 (20.6%)				
	Irregular	2 (3%)	1 (1.6%)				
Gestational age, Weeks		36.8± 4.86	37.00± 3.31	0.890	-0.20	-3.39	2.99

Gravid		0.39± 0.58	0.28± 0.49	0.058	0.11	0.013	0.39
Parity		0.11± 0.31	0.09± 0.34	0.81	0.014	-0.09	0.13
Abortion history	Yes	14 (21.5%)	6 (9.4%)	0.56	2.65	0.95	7.42
	No	51 (78.5%)	58 (90.6%)				

Values are reported as means ± SD.

Independent sample t-test was used for all statistical analysis.

P < 0.05 is statistically significant.

Table 2: Hormone characteristics and embryo assessment in two study groups

Characteristics		**HRT**	**HRT with GnRH agonist**	**P-value**	**Mean difference /OR**	**95% CI**	
						Lower	**Upper**
Total consumed Estrogen, pg/ml		73.33±1.73	15.13±2.01	0.008	-7.12	-12.34	-1.91
AMH, ng/ml		8.26±0.63	10.20±0.77	0.050	-1.94	-3.89	0.004
TSH, IU/l		2.03±0.18	2.29±0.38	0.519	-0.27	-1.10	0.56
LH, IU/l		7.73±0.97	9.07±1.85	0.56	-1.34	-5.91	3.24
Total estrogen days		14.17±2.29	15.38±2.52	0.006	-1.20	-2.05	-0.35
Number of embryo transfer		2.31±0.063	2.15±0.070	0.094	0.16	-0.03	0.34
Embryo grade A	No	43 (66.2%)	56 (96.6%)	0.000	0.070	0.016	0.313
	Yes	22 (33.8%)	2 (3.4%)				
Embryo grade B	No	47 (72.3%)	16 (27.6%)	0.000	6.854	3.106	15.127
	Yes	18 (27.7%)	42 (72.4%)				
Embryo grade C	No	64 (98.5%)	54 (93.1%)	0.133	4.74	0.514	43.695
	Yes	1 (1.5%)	4 (6.9%)				
Embryo grade AB	No	17 (26.2%)	48 (73.8%)	0.000	0.173	0.079	0.376
	Yes	39 (67.2%)	19 (32.8%)				
Embryo grade BC	No	66 (100.00%)	49 (84.5%)	0.001	1.184	1.060	1.322
	Yes	0 (0.0%)	9 (15.5%)				

Values are reported as means ± SD. Independent sample t-test was used for all statistical analysis. P < 0.05 is statistically significant.

Table 3: Cycle outcome and pregnancy complications in two study groups

Characteristics		HRT	HRT with GnRH agonist	P-value	Mean difference /OR	95% CI	
						Lower	Upper
Endometrial thickness, mm		9.11± 1.45	9.3±1.4	0.44	-0.19	-0.69	0.30
β-HCG positive		19 (28.7%)	25 (38.5%)	0.24	0.6	0.31	1.34
Clinical pregnancy		17 (25.7%)	24 (36.9%)	0.17	0.59	0.28	1.25
Multiple pregnancy		4 (6.06%)	2 (3.07%)	0.42	2.03	0.35	11.5
Miscarriage		3 (4.5%)	6 (9.2%)	0.29	0.46	0.11	1.95
EP		1(1.5%)	1 (1.5%)	0.99	0.98	0.06	16.08
Ongoing pregnancy		15 (22.7%)	18 (27.6%)	0.51	0.76	0.34	1.69
Live birth		14 (21.2%)	16 (24.6%)	0.64	0.82	0.36	1.86
Complications during pregnancy	Preeclampsia	2 (14.2%)	3 (18.7%)	0.78	0.76	0.11	5.23

	GDM	4 (28.5%)	2 (12.5%)	0.28	2.8	0.42	18.37
	Placental abruption	2 (14.2%)	2 (12.5%)	0.88	1.66	0.14	9.58
	IUGR	1 (7.1%)	0 (0.0%)	0.43	3.66	0.13	97.49
	First trimester bleeding	0 (0.0%)	2 (12.5%)	0.31	0.2	0.008	4.54
	Preterm labour	1(7.1%)	2(12.5%)	0.62	0.53	0.04	6.66
Neonatal anomaly	Cleft palate	0 (0.0%)	1(6.2%)	0.49	0.32	0.01	8.08
	Hydrocephalus	0 (0.0%)	1(6.2%)	0.49	0.32	0.01	8.08
Sex	Male	8 (57.1%)	7 (43.7%)	0.62	1.39	0.36	5.35
	Female	9 (64.2%)	11 (68.7%)				
Weight (g)		3215.78±400.79	3155.00±654.27	0.50	60.78	-384.06	505.60

Values are reported as means ± SD. Independent sample t-test was used for all statistical analysis. $P < 0.05$ is statistically significant.