Original Article

MEDICINE PALLIATIVE CARE

DOI: 10.47582/jompac.1219205

J Med Palliat Care 2023; 4(2): 89-93

The pregnancy results were not affected from the administration day of Depot GnRH agonists in artificial cycle frozen-thawed embryo transfers

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Cite this article as: Ağar M, Ayar Madenli A, Dokuzeylül Güngör N, Alanya Tosun Ş. The pregnancy results were not affected from the administration day of Depot GnRH agonists in artificial cycle frozen-thawed embryo transfers. J Med Palliat Care 2023; 4(2): 89-93.

ABSTRACT

Aim: In frozen-thawed embryo transfers (FET), Gonadotropin-Releasing Hormone (GnRH) agonists have recently been used to improve implantation results. It is preferred to administer it in the luteal phase of the previous cycle. The objective was to compare the effects of different administration days of depot GnRH agonists on implantation and pregnancy rates in the artificial cycle of FET.

Material and Method: A retrospective case-control study was conducted in an in vitro fertilization (IVF) center in a university hospital, including all women starting an artificial cycle of FET. One thousand two hundred and twenty-seven (n:1227) FET cycles were scanned from the files from October 2014 to December 2021. Depot agonists (Lucrin depot 3.75 mg sc Abbott USA.-leuprolide acetate) were used in 219 patients with endometriosis. In 58 patients, it was administered on day 21 of the previous cycle (Group 1), and in 161 patients, it was administered on day 2 of the same cycle (Group 2).

Results: This study showed no statistically significant difference between the two groups in laboratory parameters and endometrial thickness ($p>0.05$). There was no statistically significant association between the abort rate and transfer day ($p>0.05$). There was no statistically significant association between the pregnancy results and transfer day ($p>0.05$). The ongoing pregnancy rate (OPR) rate was relatively high in the second group compared to the twenty-first day of the previous cycle (87/161(54%) vs. 30/58 (51.7%)). The biochemical pregnancy was relatively high in the second-day group compared to the twenty-first day of the previous cycle (62/161(38.5%) vs. 21/58 (36.2%)). The abort rate was relatively high in the twenty-first-day group compared to the second day of the cycle (25/87(28.75%) vs. 9/30(30%)).

Conclusion: In conclusion, the impacts of various administration days of depot Gonadotropin-releasing hormone (GnRH) agonists on implantation and pregnancy rates were not statistically significant.

Keywords: Assisted reproduction technology, FET cycle, GnRH agonist, implantation

INTRODUCTION

Infertility and the inability to reproduce have always been problems humans face, leading to several psychological and social outcomes for the individuals and families involved (1,2). With the progress of science and technology, doctors and researchers have recently investigated the efficiency of in vitro fertilization methods, which are generally known as assisted reproduction technology (ART) today, giving desirable and promising results (3). Since the publication of the first successful reports regarding frozen-thawed embryo transfer cycle (FET) in the 80s, cryopreservation has become a very important procedure for the treatment of infertile couples (4). Human implantation is a complicated and multifactorial procedure (5). Implantation needs a receptive endometrium, a healthy embryo, and a synchronized molecular dialogue (6). The factors that affect the success of implantation and FET methods have been reviewed in many articles. Many researchers seek to investigate the effect or the extent of the effect of different factors on the success of ART methods. In recent years, the role of timing in FET has received the attention of researchers, and the effect of progesterone administration on pregnancy results in HRT cycles is being studied (7).

Cryopreservation of human embryos significantly improved in the last decade with the introduction of

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Received: 14.12.2022 **Accepted:** 24.02.2023

vitrification protocols (8-10). Embryo vitrification has been routinely applied for the "freeze-all" (FA) strategy, based on ovarian stimulation segmentation, the ovulation triggering, vitrified-warmed embryo transfer in subsequent natural or artificial cycles, and all viable embryos' elective cryopreservation (11). There have been popular FA policies in recent years.

Compared to other protocols related to the growth stimulation of different follicles, FET protocols are simpler. Their primary and foremost purpose is limited to preparing the endometrium for embryo reception(12). Some authors believe that FA strategy increased pregnancy outcomes and decreased the risks of ovarian hyperstimulation syndrome (OHSS) (13).

The simplest method is the natural cycle FET (NC-FET) needed for preparation of endometrium, but the disadvantages are the risk of unexpected ovulation and difficulties of transferring the embryo at the proper time (14). Induction of ovulation is usually applied for patients with irregular menstruation, and unexpected ovulation is the disadvantage of this approach (15). In artificial cycle FET (AC-FET), progesterone and estrogen are applied to imitate the endometrium's endocrine surroundings. Still, these hormones' administration does not fully guarantee pituitary suppression, leading to unexpected ovulation. For this reason, one can use the gonadotropin-releasing hormone (GnRH) agonist (GnRH-a) (16). GnRH agonists in FET have recently been used to improve implantation results. It should be administered in the previous cycle's luteal phase (17).

In the present study, the effects of the administration day of Depot GnRH-a in artificial cycle of FET on ongoing pregnancy rate (OPR), biochemical pregnancy results, and abortion rate in the women included in the research were investigated. The results of this study are valuable for patients and health centers for more accurate planning and reducing patient waiting time. Based on our knowledge, no study in the literature shows that Depot GnRH agonists are made on different days in FET cycles. Since this study is the first in the literature, it is important.

MATERIAL AND METHOD

This retrospective, cross-sectional study conducted in Bahcesehir University Göztepe MedicalPark Hospital IVF Clinic. The study was carried out with the permission of Ordu University Clinical Researches Ethics Committee (Date 22.07.2022, Decision No:170). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A retrospective case-control study was performed in an IVF center in a university hospital, including all women starting an artificial cycle of FET. One thousand two hundred and twenty-seven (n:1227) FET cycles were observed from the files from October 2014 to December 2021. Depot agonists (Lucrin depot 3.75 mg sc Abbott USA.-leuprolide acetate) were used in 219 patients with endometriosis. In 58 patients, it was administered on day 21 in the previous cycle (Group:1), and in 161 patients, it was administered on day 2 of the same cycle (Group:2).

Women between the ages of 25 and 39 were included in this study. The exclusion criteria were as follows: 1) known chronic disease, 2) over 39 years of age, 3) history of recurrent miscarriage, 4) known chromosomal disorder, and 5) history of fetus with anomaly.

The detection made the diagnosis of endometriosis-adenomyosis of endometrioma or adenomyotic focus by ultrasonography. The analog application was made on the 2nd or 21st day, according to the patient's admission day. Blood values are checked on the 2nd or 3rd day of menstruation when the fresh cycle starts. All cases were transferred within 3 months after the fresh cycle. A single dose of analog was used in all patients. 2 mg estradiol (E2) hemihydrate (17beta-estradiol) treatment was started orally three times a day and was continued at the same dose for at least seven days. The dose was increased (2 x 2 per oral per day) in cases of a thin endometrium (< 7mm) or serum E2 did not reach 300 pg/ml. Intramuscular progesterone injection of 1x100mg per day was started when the endometrial thickness was more than 7mm. These medications were used until a βhCG test. Controlled ovarian stimulation was done by recombinant follicle-stimulating hormone (r-FSH; Gonal-F®, Serono, Geneva, Switzerland), and suppression for LH surge was done by a gonadotropin-releasing hormone (GnRH) antagonist, cetrorelix acetate (Cetrotide®), Merck KGaA, Serono, Geneva, Switzerland). Final follicular maturation has been completed by the analog trigger, Leuprolide acetate (Lupron; TAP Pharmaceuticals, North Chicago, IL, USA). Finally, ovum pick-up is performed after 35-36 hours with transvaginal ultrasound. The fertilization process is done with Intracytoplasmic sperm injection (ICSI) for all patients.

The estradiol levels and endometrial thickness measured on a triggering day in the case group of the study were 1578.25±989.7 and 9.93±1.05, respectively. The estradiol levels and endometrial thickness measured on a triggering day in the case group of the study were 1611.09±973.71 and 9.98±1.73, respectively.

Statistical Analysis

The Kolmogorov-Smirnov test was performed to check the normality, and the nonparametric tests were performed given the non-normality of the groups before the statistical analyses. Mean and standard deviations (SD) were measured to check each continuous variable, including age, BMI, Total oocytes, MII oocytes, PN, AMH, Prolactin, FT4, TSH, FSH, LH, E2, and Endometrial thickness. The Mann-Whitney U test was performed to study the difference between the two groups. SPSS v22 was used for statistical analyses. A value of $p< 0.05$ was accepted as statistically significant. We employed the GPower 3.1 program to estimate the sample size. The two groups' total mean was measured based on the Mann-Whitney test with a power of 90%, effect size of 40%, and 0.05 type 1 error for at least 226 patients (18).

RESULTS

This study included two hundred nineteen (n:219) age-matched (30.75±3.39) and body mass index (BMI)-matched (23.78±2.28) women. The majority of study participants try IVF for the first time(71.7%). **Table 1** shows descriptive statistics of study parameters.

Table 2 compares case and control groups on the laboratory values. As stated in **Table 2**, a Mann-Whitney test did not find a statistically significant association between the case and control in regard to total oocytes ($p>0.05$). There was no statistically significant difference between groups in terms of MII oocytes and PN ($p>0.05$).

AMH of the second-day group (Mean=1.68) was comparable to the 21st-day group (Mean=1.67). A Mann-Whitney test indicated that this difference was not statistically significant ($p>0.05$).

Table 1. Descriptive statistics of study parameters in women (n=242).

Study parameters	median (range) mean±SD
Maternal characteristics	
Age	32(20-35)30.75±3.39
BMI	23.8(19-29.8)23.78±2.28
Laboratory values	
Total oocytes	9(7-14)8.87±1.23
MII oocytes	8(6-11)7.84±0.98
PN	7(6-10)7.48±0.85
AMH	1.8(1-3.64)1.67±0.44
Prolactin	17.6(8.2-25)17.54±4.97
FT4	1.02(0.31-1.62)1.02±0.28
TSH	1.62(0.63-2.46)1.58±0.53
FSH	7(2.3-9.86)6.88±1.47
LH	7.23(3.52-15.2)7.52±1.71
E2	42(29-51.2)39.89±6.57
Endometrial thickness	9(9-12)9.82±1.04

SD, standard deviation.

There was no statistically significant difference between groups in terms of Prolactin (p=0. 981), FT4 (p=0.955), TSH (p=0.440), FSH (p=0.534), LH (p=0.704) and E2 (p=0.853).

The endometrial thickness of the second-day group (Mean=9.82) was comparable to the 21st-day group (Mean=9.83). A Mann-Whitney test indicated that this difference was not statistically significant ($p>0.05$).

As stated in **Table 3**, a chi-square test found no statistically significant association between the pregnancy results and transfer day ($p>0.05$).

As stated in **Table 4**, a chi-square test found no statistically significant association between the abort rate and transfer day ($p>0.05$).

Table 2. Comparison of case and control groups

Study parameters	Second day Case (n=161) median (range) mean±SD	21st day Control(n=58) median (range) mean±SD	p
Laboratory values			
Total oocytes	9(7-14)8.88±1.27	9(7-12)8.84±1.14	0.968
MII oocytes	8(6-11)7.84±1	8(6-10)7.83±0.94	0.887
PN	7(6-10)7.51±0.85	7(6-10)7.41±0.84	0.422
AMH	1.92(1-3)1.68±0.41	1.51(1-3.64)1.67±0.52	0.951
Prolactin	17.78(8.2-25)17.56±4.81	17.2(8.48-25)17.48±5.45	0.981
FT4	1.02(0.31-1.62)1.02±0.28	1.02(0.31-1.62)1.04±0.29	0.955
TSH	1.54(0.63-2.46)1.57±0.52	1.76(0.63-2.46)1.6±0.56	0.440
FSH	7(3.96-9.86)6.87±1.51	7(2.3-9.86)6.92±1.36	0.534
LH	7.2(4.72-12.6)7.51±1.17	7.27(3.52-15.2)7.54±2.7	0.704
E2	42(29-50)39.86±6.64	40.5(30-51.2)39.97±6.41	0.853
Endometrial thickness	9(9-12)9.82±1.04	9(9-12)9.83±1.05	0.923

M, Mean; N, number of subjects; AMH, Anti-Mullerian hormone; PN, multi-pronuclei; FT4, Free T4; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, ; All variables tested by a Mann-Whitney U test.

Table 3. The relationship between pregnancy results and transfer day

Variables	Second day Case (n=161) n (%)	21st day Control (n=58) n (%)	p
Pregnancy results Bhcg(+)(%)			0.762*
Yes	87 (54)	30 (51.7)	
No	74 (46)	28 (48.3)	
Ongoing pregnancy rate(%)			0.582*
Yes	62 (38.5)	21 (36.2)	
No	99(61.5)	37(63.8)	

*A Chi-square test.

Table 4. The relationship between abort rate and transfer day

Variable	Second day Case (n=87) n (%)	21st day Control (n=30) n (%)	p-value
Abort rate (%)			0.159*
Yes	25 (28.75)	9 (30)	
No	62 (71.25)	21 (70)	

*A Chi-square test.

DISCUSSION

In the present study, different administration days (The 21st day of the previous cycle and the second day) were compared in regard to laboratory parameters, endometrial thickness, and pregnancy results.

The effect of endometrial thickness on the reproductive outcome is apparent (19). The interaction between the receptive endometrium and the embryo is a complicated molecular process that results in effective implantation (20). When the level of P4 reaches a critical threshold, it drives an orderly and timely secretory transformation of the endometrium, leading to receptivity (21). The present study reported the same endometrial thickness transition in two groups. No significant effect was observed between days 2 and 21.

This study showed no statistically significant difference between the two groups in laboratory parameters. In some parameters such as Total oocytes, MII oocytes, PN, AMH, and Prolactin, the second-day values were relatively higher than 21st day. In other parameters such as FT4, TSH, FSH, LH, and E2, that was vice versa.

In the current study, the OPR rate was relatively high in the second group compared to the twenty-first day of the previous cycle (87/161(54%) vs. 30/58 (51.7%)). The biochemical pregnancy was relatively high in the second group compared to the twenty-first day of the previous cycle (62/161(38.5%) vs. 21/58 (36.2%)). The abort rate was relatively high in the twenty-first group compared to the second day of the previous cycle (25/87(28.75%) vs. 9/30(30%)). However, the differences did not achieve statistical significance in terms of OPR, biochemical pregnancy, and abort rate ($p>0.05$).

The effects of the endometrial preparation protocol artificial cycle (with/without GnRH-a suppression) vs. natural cycle (true/modified) vs. stimulated cycle on the risk of live birth rate, OPR, and early pregnancy loss after FET was the topic of many reports (22-25). Different results about the benefits and disadvantages of protocols were reported (26,27). Conversely, it needs to be more studies regarding the effects of timing on the success of protocols. To the best of our knowledge, this study is the first research that addresses the administration day of depot GnRH-a in artificial cycle FET outcomes. GnRH-a suppresses ovarian steroidogenesis by a downregulation of pituitary GnRH receptors that affects gonadotropin secretion. Pituitary GnRH receptors are downregulated to suppress ovarian steroidogenesis, with GnRH-a affecting gonadotropin secretion. One can administer GnRH -a through different routes, but since the sc way can be easily used, it is preferred. Successful medical management of endometriosis has been done using GnRH-a for many years. Endometrial implants are found to be affected by lower estrogen production or counteract E action at a peripheral level. Deep suppression of luteinizing hormone is done while FSH levels significantly decrease only in the 1st month of therapy, before a constant increase. Suppression of the pituitary gonadotropin secretion in COH caused to prevent premature luteinization of LH and surge, reducing the cancellation rate and improving the assisted reproduction's routine organization (28,29). GnRH-a formulations were used to increase the patients' and clinicians' convenience (3). The difference in the administration of depot GnRH-a and the possible effects of various versions on the pregnancy results was the motivation for this research. Because of the lack of similar study in the literature, the issue was raised as a fundamental question for the authors of this article. The pregnancy results administered on day 2 of the previous cycle were more successful than those in another group. However, there was no statistically significant. It is recommended to conduct the protocol on the second day.

The imbalance between the two groups is one of the most critical limitations of this study (Group 1:58 vs. Group 2:161). However, a sufficient number of patients have compensated for this weakness. Another limitation of this study is its single center. Using data from several centers in future studies is recommended to create a sample.

CONCLUSION

As a result, the effects of different administration days of depot GnRH-a on implantation and pregnancy rates were not statistically significant. Medical centers and hospitals can determine the day of transfer based on the patient's condition and the center's facilities. What

is clear is that the day does not significantly affect the reproductive system's success. The second day for transfer is recommended because the patient will wait less in this condition, and the treatment period will be shorter.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ordu University Clinical Researches Ethics Committee (Date 22.07.2022, Decision No:170)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients**.**

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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