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Comparison of Pregnancy Outcomes in Delayed Frozen Embryo Transfer and Immediate Transfer: Statistical Study

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ABSTRACT

Background & Objective: Ovarian Invitro Fertilization (IVF) is the best treatment for couples with multifactorial infertility. Controlled Ovarian Stimulation (COS) is the main factor for determining the success of Invitro Fertilization (IVF). Frozen-Thawed Embryo Transfer (FET) is performed to overcome supra-physiological hormone concentration of this process. Embryo transfer can be done immediately in the first menstrual cycle or delayed two or three cycles. The present study was performed with the aim of comparing pregnancy outcomes in immediate and delayed FET after COS.

Materials & Methods: This quasi-experimental interventional epidemiological study was performed on 202 infertile women aged 20-40 years who underwent puncture at the fertility center from 2021 to 2022. The subjects were randomly divided into two groups: immediate transfer group (n=100) and delayed transfer group (n=102). Then, the rate of chemical pregnancies, clinical pregnancies, live births, implantations, and abortions were compared Between the two groups by chi-square and Mann-Whitney tests. P<0.05 was considered statistically significant.

Results: Successful chemical pregnancy rate was 45% in the immediate transfer group and 40.2% in the delayed group (P=0.490). The live birth percentage was 33% in the immediate transfer group and 30.3% in the delayed group (P=0.482). The number of days of estradiol prescribed for the endometrial thickness to reach the threshold of 7 mm for embryo transfer was more in the immediate transfer group (P=0.032).

Conclusion: There was no significant difference Between the immediate and delayed frozen embryo transfer groups regarding fertility success percentage, but the only difference was in the number of days of estradiol administration.

Keywords: In Vitro Fertilization, Pregnancy, Fertility, Embryo transfer, Ovarian stimulation

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1. Introduction

Infertility is defined as the absence of pregnancy after one year of unprotected intercourse. About 10 to 15 percent of couples of reproductive age are infertile (1). National census studies indicate that in the United States, about 11% of women and 9% of men have fertility problems (2). In Iran, about a quarter of couples experience primary infertility during their life and at any point in time 4.3% of couples have primary infertility problems (3). In another study, the overall prevalence of primary infertility in Iran was reported as 20.2% (4). Various studies indicated that infertility has many psychological, social, and economic consequences (5,6). In recent years, with increasing demand for infertile couples to get pregnant, extensive studies have been conducted, and various assisted reproductive technologies have been developed (7). Invitro Fertilization (IVF) is a sequence of a series of procedures that starts with the induction and stimulation of the ovaries with exogenous gonadotropins and is followed by the extraction of oocytes from the ovaries under the guidance of transvaginal ultrasound, fertilization, and embryo culture in the laboratory and its transfer (1,8). Invitro Fertilization (IVF) is the best treatment for couples with multifactorial infertility (9). Controlled Ovarian Stimulation (COS) is the main factor for determining the success of Invitro Fertilization (IVF) in which several follicles are created simultaneously, and numerous oocytes are recovered in one cycle (10,11). Unfortunately, the supra-physiological hormonal

concentration of this process, especially in the aggressive stimulation regime and the use of GnRH analogs, can lead to a decrease in endometrial blood flow, endometrial maturation, and immunological change in the endometrial environment **(12-15)**. In the last decades, to overcome this problem, freezing and preservation of embryos after Controlled Ovarian Stimulation (COS) have been increasingly used for subsequent thawing and transfer, that is, the freeze-all policy. Embryos that are frozen and then thawed can be transferred immediately in the first menstrual cycle after Controlled Ovarian Stimulation (COS) (immediate transfer) or this transfer can be delayed by two or three cycles (delayed transfer) **(12-16)**. Today, the number of Frozen-Thawed Embryo Transfer (FET) cycles is steadily increasing worldwide. Postponing frozen-Thawed Embryo Transfer (FET) for at least one menstrual cycle after a failed new transfer or after a freeze cycle is standard clinical practice. The purpose of this approach is to minimize the possible negative effect of ovarian stimulation on the resumption of the normal cycle of ovulation and acceptance of the endometrium **(17,18)**. Although postponing frozen embryo transfer after Controlled Ovarian Stimulation (delayed transfer) is preferred in many infertility centers, this approach is experimental and has limited scientific evidence. On the other hand, the delay of frozen embryo transfer can cause unnecessary distress and anxiety in infertile couples, which can reduce the probability of pregnancy and increase the rate of treatment failure **(14-20)**.

To date, numerous cohort studies and three systematic review and meta-analysis studies have been published to compare pregnancy outcomes Between immediate and delayed frozen embryo transfer groups **(15-31)**. In a retrospective cohort study by He et al. in 2020, which was based on 4404 embryo transfer cycles in an infertility center in China, it was shown that there was no significant difference between the immediate and delayed frozen embryo transfer groups in terms of live birth rate, implantation, clinical pregnancy, multiple pregnancies, abortion in early pregnancy, premature birth and stillbirth **(26)**. In the meta-analysis study by Huang et al., twelve retrospective cohort studies including 18,230 cycles were examined, and no significant difference was found Between the two groups of immediate and delayed frozen embryo transfer in the rate of live birth **(16)**. In general, these studies showed that delaying embryo transfer to subsequent cycles has no benefit in Invitro Fertilization (IVF) results **(15-31)**. However, all studies are retrospective in design and publication, selection, and practice biases are possible. Therefore, there is a need for an interventional study to obtain better evidence for deciding whether to perform immediate transfer instead of delayed transfer **(16-20)**. Therefore, the present research was conducted with aim to compare pregnancy outcomes in delayed frozen embryo transfer and immediate transfer group.

2. Materials and Methods

This quasi-experimental interventional epidemiological study was performed in 2021-2022. The inclusion criteria were all infertile women aged 20-40 years who underwent puncture at Moulood Infertility Center (Zahedan, Iran) and had at least 2 grade A and grade B frozen embryos. Exclusion criteria were abnormal parental karyotype results, history of recurrent spontaneous abortion (defined as three or more spontaneous pregnancy losses), previous diagnosis of congenital uterine abnormalities (e.g., septate uterus and duplex) or acquired (for example, submucous myoma, and adenomyosis), use of donated embryos, moderate and severe ovarian hyperstimulation syndrome (OHSS) during Controlled Ovarian Stimulation (COS), severe azoospermia or oligospermia in the man and any underlying maternal disease (diabetes, hypertension, lupus, etc.).

According to the aim of the research, which compares the rate of fertility success in two groups of immediate and delayed transfer of frozen embryos after Controlled Ovarian Stimulation (COS), the formula for comparison of two independent proportions was used to determine the sample size **(32)**. Considering a confidence level of 95%, success proportion in immediate transfer group 35%, success proportion in delayed transfer group 21% **(33)**, and precision 20% after performing the calculations, 104 patients were required in each group to achieve a power of 80%.

Patients were randomly assigned to two groups by permuted block stratified randomization method. In this way, at first, eligible referring patients were classified according to age and BMI in the order of arrival. Then, they were assigned to the desired group based on quadruple blocks (consisting of two groups A and B, and two repetitions for each) that were randomly selected from among all possible states of permutations **(34)**. Two groups were matched by frequency matching in terms of age and BMI based on the stratified permuted block randomization procedure. In this research, single blinding method was implemented, so that the technician responsible for recording the results was not aware of the intended intervention **(35)**.

After collecting the data, the raw data were entered into the statistical software SPSS (version 22). In the first step, the quality of the data was evaluated using graphical methods and calculating descriptive indices. Frequency distribution tables and common central indices were used to describe qualitative and quantitative data, respectively. A point estimate and a 95% interval have been used to estimate the rate of fertility success. To compare the mean of quantitative variables Between the two groups, the independent t-test has been performed if the assumptions of parametric tests are established, and alternative non-parametric tests, such as the Mann-Whitney test, have been accomplished if the assumptions are not established. The chi-square test was used to compare the ratio Between the two groups (checking the

assumption of independence). P<0.05 was considered the significance level.

3. Results

Initially, 208 people participated in this research, including 104 people in the immediate transfer group and 104 in the delayed transfer group. In the endometrial preparation stage despite the administration of the maximum dose of estradiol, the thickness of the endometrium did not reach 7 mm in four cases from the immediate transfer group and two cases from the delayed transfer group. Therefore, these six people were excluded from the research statistics. Accordingly, a total of 202 people, including 100 people in the immediate transfer group and 102 in the delayed transfer group participated in this study.

The demographic information of the subjects studied was summarized in (Table 1).

Table 1. Demographic information of participants in this study

	Immediate transfer n=100	Delayed transfer n=102	Overall n=202	P-value
Age (years)	32.26	32.22	32.24	0.951
BMI (kg/m2)	23.85	23.49	23.67	0.589
Type of infertility				0.727
Primary infertility (%)	68	65.7	66.8	
Secondary infertility (%)	32	34.3	33.2	
Reasons of infertility				0.997
Female (Tubal factor, %)	17	15.7	16.3	
Female (Ovary factor, %)	21	22.5	21.8	
Female (Other factors, %)	6	4.9	5.4	
Male (%)	33	32.4	32.7	
Unexplained infertility (%)	9	8.8	8.9	
Female & Male (%)	14	15.7	14.9	

Pregnancy results of FETs in the immediate and delayed groups were presented in Table 2. The rate of chemical pregnancy was 45% in the immediate transfer group and 40.2% in the delayed transfer group. In other words, the immediate transfer method has been 4.8% more successful; the results of the chi-square test showed no statistically significant difference Between the two groups in this regard (P=0.490). Moreover, the clinical pregnancy rate was 41% in the immediate transfer group and 36.3% in the delayed transfer group. In other words, the immediate transfer method was 4.7% more successful; the difference Between the two groups was not statistically significant (P=0.490). In addition, the live birth rate was 33% and 30.3% in the immediate and delayed transfer group, respectively. It means that the immediate transfer method had 2.7% more live births; no statistically significant difference was found Between the two groups (P=0.482). Also, the abortion rate in the immediate and delayed transfer groups was 0.6% and 5.9%, respectively; the two groups did not significantly differ in terms of abortion rate (P=0.972). Moreover, the rate of implantation in the immediate transfer group was 55/220 (25%) and in the delayed transfer group it was 208/48 (23%). By examining the results of the Shapiro-Wilk test, the skewness, and the quantile-quantile diagrams, it was observed that the distribution of the implantation rate in the two groups was not normal. Therefore, the results of the Mann-Whitney test showed no statistically significant difference Between the two groups in terms of implantation rates in women (P=0.734) (Table 2).

As shown in Table 3, the mean number of days of estradiol prescribed to reach the thickness of the endometrium to 7 mm was 14.27 days in the immediate transfer group and 13.48 days in the group of delayed transfer of frozen embryos after controlled ovarian stimulation (COS) and. Examining the Shapiro-Wilk test results, skewness and quantile-quantile diagrams showed that the distribution of the number of days of estradiol prescribed in the two groups was not normal. Therefore, the non-parametric Mann-Whitney test was used to compare the mean number of days of estradiol prescribed. According to the findings, the average number of days of estradiol prescribed in the immediate and delayed transfer groups was 15 and 14 days, respectively. The results of the statistical test showed that the difference Between the mean number of days of estradiol prescribed to reach the thickness of the endometrium to 7 mm was statistically significant in the two groups (P=0.032). Also, the mean number of days to increase the estradiol dose to reach the

endometrial thickness to 7 mm was 4.23 days in the immediate transfer group and 3.48 days in the delayed transfer group. Based on the results of the Shapiro-Wilk test, the degree of skewness, and the quantile-quantile diagrams, it was revealed that the distribution of the number of days of increasing the dose of estradiol prescribed in the two groups was not normal. Therefore, the results of the Mann-Whitney test demonstrated that the difference Between the mean number of days of increasing the dose of estradiol prescribed was statistically significant in women of the two groups (P=0.040).

Table 2. Pregnancy results of FETs in the immediate and delayed groups

	Immediate transfer n=100	Delayed transfer n=102	Overall n=202	P-value
Chemical pregnancy rate	45	40.2	42.6	0.490
Clinical pregnancy rate	41	36.3	38.6	0.490
Live birth rate	33	30.3	31.6	0.482
Implantation rate (sac/fetus)	25(55/220)	23(48/208)	24(103/429)	0.734
Abortion rate	6	5.9	5.9	0.972

Table 3. Comparison of endometrial characteristics Between immediate and delayed groups

	Immediate transfer n=100	Delayed transfer n=102	Overall n=202	P-value
Endometrial thickness during FET transfer (mm)	8.18	8.20	8.19	0.822
The average number of days to increase the estradiol dose to reach the endometrial thickness to 7 mm	14.27	13.48	13.87	0.032
The average number of days to increase the estradiol dose to reach the endometrial thickness to 7 mm	4.23	3.48	3.85	0.040

The mean size of the endometrium during the transfer of frozen embryos in the immediate and delayed transfer groups was 8.18 mm and 8.20 mm, respectively. By examining the results of the Shapiro-Wilk test, the degree of skewness, and the quantile-quantile diagrams, it was observed that the endometrial size distribution during the transfer of frozen embryos in the two groups was not normal. Therefore, the results of the Mann-Whitney test showed that the difference Between the mean size of the endometrium was not statistically significant in women of the two groups (P=0.822).

4. Discussion

To the best of our knowledge, this study was the first interventional study which compared the pregnancy outcomes Between two groups of immediate and delayed frozen embryo transfer. So far, all studies in this field have been retrospective cohorts (14-31) and only one large clinical trial has been conducted in Denmark **(20)**.

Nowadays, the embryo freezing policy is used for the preservation and transfer of embryos in the Invitro Fertilization (IVF) cycle. Frozen thawed embryos can be transferred immediately in the first menstrual cycle after Controlled Ovarian Stimulation (COS) (immediate transfer) or this transfer can be carried out in two or three cycles (delayed transmission) **(12-14)**. Although selective postponement of embryo transfer may delay pregnancy, it is thought that immediate embryo transfer may be ineffective in a clinical setting and increase the risk of cycle cancellation **(14-18)**. Lattes et al. reported that there is no difference Between the immediate and delayed groups in the rate of chemical pregnancy (positive pregnancy test) **(24)**. In the present study, successful chemical pregnancy (positive pregnancy test) was 45% in the immediate transfer group and 40.2% in the delayed transfer group. In other words, the immediate transfer method was 4.8% more successful. Also, the clinical pregnancy rate (seeing a fetus with a heart in ultrasound) was 41% in the immediate transfer group and 36.3% in the delayed transfer group. It means that the immediate transfer method was 4.7% more successful. However, the findings of statistical tests showed that these chemical and clinical differences were not statistically significant Between the immediate and delayed embryo transfer groups. Our study rejects the idea that the supraphysiological effects of hormones used in Controlled Ovarian Stimulation (COS) can increase the probability of failure of transfer cycles. Moreover, as evidenced by the findings of the present study, there is no statistical difference Between the two groups of

immediate and delayed transfer of frozen-thawed embryos in terms of pregnancy results.

Although the adverse effect of hormones from Controlled Ovarian Stimulation (COS) on implantation and live birth rates in the fresh transfer has led to the use of the frozen embryo transfer policy, there is no study on the time needed for the endometrium to regain its receptive potential. However, delayed transfer can increase the couple's anxiety without affecting the rate of live birth **(24)**. In the meta-analysis study by Burningham et al., which evaluated 15 retrospective cohort studies including 6304 immediate transfer cycles and 13851 delayed transfer cycles, the results showed that the rate of live birth is slightly higher in the immediate transfer group than that of delayed transfer **(18)**. In the current study, the live birth rate was 33% in the immediate transfer group and 30.3% in the delayed transfer group. In other words, the immediate transfer method had 2.7% more live births, but the difference Between the two groups was not statistically significant, therefore, there is no need to delay the menstrual cycle to reduce the probability of endometrial acceptance. Contrary to what was previously thought that the rate of implantation is more influenced by the hormonal effects of ovarian stimulation, our study exhibited that there is no difference Between the two groups in the rate of implantation. A similar finding was also reported by Matorras et al. in a meta-analysis study **(19)**. Moreover, in the present study, no difference was observed Between the immediate and delayed embryo transfer groups in terms of the rate of abortion in early pregnancy. He et al. also reported similar results **(26)**.

According to the obtained findings in the present study, the mean number of days prescribed estradiol in the immediate and delayed transfer groups was 15 and 14 days, respectively. So that in the immediate transfer group, it was one day more than the delayed group. Also, the mean number of days of estradiol dose increase in the immediate and delayed transfer groups was 4.23 and 3.48, respectively. Statistical tests revealed that this difference Between the two groups is statistically significant. Actually, in the immediate transfer group, we needed a higher dose of estradiol and more days of administration for the endometrium to reach the threshold of 7 mm for embryo transfer which was not mentioned in similar works. This case can indicate the remaining negative effects of the hormones used in the Controlled Ovarian Stimulation (COS) in the first cycle compared to the next cycle. Our findings demonstrated that there is no need to postpone the menstrual cycle to overcome these negative effects of the remaining hormones. On the other hand, only more time and more estradiol are needed to prepare the endometrium.

5. Conclusion

As evidenced by the results of the present study, there was no significant difference Between the two groups of immediate and delayed transfer of frozen embryos regarding the rate of chemical pregnancy, clinical pregnancy, live birth, implantation, abortion, and generally the rate of reproductive success. The only difference Between the two groups is the higher dose of estradiol and more days of its administration for the endometrium to reach the threshold of 7 mm in the immediate transfer group. Since immediate transfer reduces the stress and anxiety of infertile couples and also reduces costs, immediate transfer (transfer in the first cycle) of frozen embryos is suggested. However, further study and investigation are needed in this field.

6. Declarations

Acknowledgments

The present study was financially supported by Zahedan University of Medical Sciences, Zahedan, Iran. The authors declare that they have no conflict of interest.

Ethical Considerations

This study was approved by the local Ethical Committee of Zahedan University of Medical Sciences with ethical code: (IR.ZAUMS.REC.1400.176). All the patients signed the informed consent form before being enrolled into the study groups.

Authors' Contributions

Elahe Bijari contributed to study design and preparation and data collection. Marzieh Ghasemi performed conceptualization and designing the article. Abolfazl Payandeh performed data analysis and interpretation. Narjes Noori contributed to data collection. All authors contributed to the writing of the manuscript and discussed the manuscript.

Conflict of Interest

The authors declared no conflict of interest.

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