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Fresh Versus Frozen Embryo Transfer Among Women Undergoing Assisted Reproductive Technology: A Retrospective Cohort Study

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ABSTRACT

Background: Frozen embryo transfer has become an integral part of in vitro fertilization (IVF). But there are wide variations in the reported fertility outcomes in fresh and frozen embryo transfers.

Objective: The objective of the study was to compare the fertility outcomes between fresh versus frozen embryo transfer in a specialty reproductive center.

Methods: A retrospective cohort study was conducted at Dubai healthcare city, among 121 women who underwent IVF treatment and had either fresh or frozen embryo transfer. Variables like fertility patterns, hormonal levels, treatment parameters, and outcome parameters were compared between the two groups. The study was conducted from January 2020 until October 2020 during the peak of the SARS COVID pandemic, and as a result, a greater number of women underwent frozen cycles.

Results: Among 121 women, 37 had fresh embryo transfer, and 84 underwent frozen embryo transfer. The mean age of the fresh group was 37.11 ± 4.2 (years), and it was 35.74 ± 4.42 (years) in the frozen group. In the fresh group, 13 (35.14%) participants and in the frozen group, 60 (71.43%) participants had clinical pregnancy. The difference in the proportion of clinical pregnancy between the type of ET was statistically significant ($P<0.001$).

Conclusion: The pregnancy rate significantly differed between the fresh embryo and frozen embryo groups. Frozen embryo transfer had a lower risk of ovarian hyperstimulation syndrome and a higher chance of successful pregnancy outcomes.

Keywords: Infertility; Assisted Reproductive Techniques; In Vitro Fertilization; Embryo Transfer; Fresh Embryo; Frozen Embryo; Retrospective Cohort Study.

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انتقال جنین تازه در مقابل جنین منجمد در زنان تحت فناوری‌های کمک باروری: یک مطالعه کوهورت گذشته‌نگر

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چکیده

زمینه و اهداف: انتقال جنین منجمد به بخشی جدایی‌ناپذیر از لقاح آزمایشگاهی تبدیل شده است. در عین حال اختلافات گسترده‌ای در نتایج باروری گزارش شده، بین انتقال جنین تازه و جنین یخ زده وجود دارد. هدف از این مطالعه مقایسه نتایج باروری بین انتقال جنین تازه در مقابل جنین منجمد، در زنان تحت فناوری‌های کمک باروری در یک مرکز باروری تخصصی بود.

روش ها: این مطالعه کوهورت گذشته‌نگر در "شهر درمانی دبی"، در بین ۱۲۱ زن که تحت لقاح آزمایشگاهی قرار گرفته و جنین تازه یا منجمد شده دریافت کرده بودند، انجام پذیرفت. متغیرهایی مانند الگوهای باروری، سطوح هورمونی، پارامترهای درمانی و نتایج حاصله، بین دو گروه مقایسه شد. این مطالعه از ژانویه ۲۰۲۰ تا اکتبر ۲۰۲۰ در زمان اوج همه‌گیری ویروس COVID-19 انجام شد و در نتیجه، تعداد بالاتری از زنان تحت روند انتقال جنین منجمد قرار گرفتند.

یافته ها: در بین ۱۲۱ زن این مطالعه، برای ۳۷ نفر جنین تازه منتقل شد و ۸۴ نفر نیز جنین منجمد دریافت کردند. میانگین سن در گروه جنین تازه، ۴,۲±۳۷,۱۱ سال و در گروه جنین منجمد ۴,۴±۳۵,۷۴ سال بود. در گروه جنین تازه، ۱۳ شرکت کننده (۳۵,۱۴٪) و در گروه جنین منجمد، ۶۰ شرکت کننده (۷۱,۴۳٪) حاملگی بالینی داشتند. تفاوت نسبت بارداری بالینی بین نوع انتقال جنینی از نظر آماری معنی‌دار بود.($P<0.001$)

نتیجه‌گیری: این مطالعه نشان داد که میزان بارداری بین روش انتقال جنین تازه و جنین منجمد، تفاوت معنی‌داری دارد. انتقال جنین منجمد خطر کمتری برای رخداد سندرم تحریک بیش از حد تخمدان داشته و از طرفی شانس بالاتری برای نتایج موفقیت‌آمیز بارداری داشت.

کلید واژه‌ها: ناباروری؛ تکنیک‌های کمک باروری؛ لقاح آزمایشگاهی؛ انتقال جنین؛ جنین تازه؛ جنین منجمد؛ مطالعه کوهورت گذشته‌نگر.

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Introduction

Since its inception 40 years ago, in vitro fertilization has grown and evolved into a wide field in assisted reproductive technologies. Controlled ovarian stimulation with gonadotropins has enabled an increased number of oocytes being harvested and subsequent embryo cryopreservation, thus enabling future use of frozen embryos. These advancements resulted in an increase in the cumulative live-birth rates following in vitro fertilization (IVF) [1].

Embryo transfer has advanced in recent years with different techniques like fresh embryo transfer and frozen embryo transfer. As a routine practice, fresh embryo transfer is done, and any excess embryos were cryopreserved. The stored frozen embryos were used for the second pregnancy or after the failure of fresh embryo transfer. The first-ever live birth reported after frozen embryo transfer was in the year 1984[2]. With advanced laboratory technology in recent years, the number of frozen embryo transfers has increased [3]. Studies have shown that frozen embryo transfer has higher pregnancy rates compared to fresh embryo transfer [1,4,5]. The hypothesized mechanism is that frozen embryo transfer may create a favorable intrauterine and endometrial environment for the process of implantation and placentation. Through this mechanism, the pregnancy rates seem to be higher in frozen embryo transfers without creating the supra-physiological condition that occurs after stimulation of the ovary [6].

Selection of women for frozen embryo transfer is essential, and those at risk of ovarian hyperstimulation syndrome are ideal candidates [7]. This technique can also be used in embryo banking programs in order to preserve them for future use [8,9] and in women planned for chemotherapy, radiation, and other cytotoxic treatments [10].

Various factors play a role in the selection as well as the outcome process. Patient characteristics such as age, type, and etiology of infertility, other associated comorbidities may play a role in determining the outcome [11]. Hormonal status of the patient, hormonal replacement is done during the treatment process [12], the method of freezing and thawing [13], selection criteria for freezing the embryo [14] determine the success rate. The objective of the current study is to compare the various factors associated with fresh and frozen embryo transfer techniques.

Methods

Patient selection:

A retrospective cohort study was conducted at Orchid Fertility Centre, Dubai Health Care City, from January 2020 until October 2020. Informed written consent was obtained from all participants, and confidentiality was maintained throughout the study.

A total of 121 embryo transfers were studied of which 37 were fresh transfers, and 84 were frozen transfers.

The frozen embryo transfer group was considered as the study group and the fresh transfer group as the comparison group. The baseline parameters of the women, such as age, type, and duration of infertility, other causes of infertility, polycystic ovarian syndrome (PCOS), and morbidity status, were compared. Laboratory parameters, hormonal levels, treatment-related parameters, and outcomes were also compared between the two study groups. Outcome parameters considered were positive pregnancy test, clinical pregnancy, biochemical pregnancy, and not pregnant.

All women with primary and secondary infertility or miscarriage and with any other cause of infertility undergoing IVF treatment were included in the study. Women who had embryo arrest and nothing to transfer were excluded from the study.

Ovarian stimulation and embryo transfer technique:

A pre-standardized ovarian stimulation strategy was followed for all the participants. Standard antagonist protocol was used for IVF stimulation. Controlled ovarian stimulation was done by human menopausal gonadotropins (HMG) and recombinant follicle-stimulating hormone (FSH). Doses were adjusted to the measurement of serum sex steroids and ovarian response. Transvaginal ultrasonography for follicular monitoring, as well as serial hormonal assessment, was done to confirm the ovarian response. When 3-4 follicles of 17 to 18 mm in the ultrasound were measured, either gonadotropin-releasing hormone (GnRH) agonist or recombinant human chorionic gonadotropin (HCG) was administered. This was done for final oocyte maturation based on ovarian response and estradiol levels. At the end of 35-36 hours, the retrieval of oocytes under anesthesia was done, followed by intracytoplasmic sperm injection. In the case of normal response with optimal estradiol levels, fresh embryo transfer was done on day three or day five. In patients with the hyper response with many follicles and high likelihood of ovarian hyperstimulation syndrome (OHSS) and/or patients selected for PGS, an agonist trigger was given. All embryos were cryopreserved, and frozen embryo transfer was done in a subsequent cycle. Standard protocols were followed at all the stages of the cycle uniformly for all the participants.

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Women in the fresh embryo transfer group received luteal support with progesterone injections and suppositories. The progesterone support was started on the evening of oocyte retrieval, and the transfer was done either on day three or day five, blastocyst stage and continued for 12-14 days until pregnancy test. If the pregnancy test was positive, then all support medications were continued until 12 weeks of pregnancy.

In the frozen embryo transfer group, the embryo was cryopreserved for later use. The women in the study group underwent endometrial preparation either by natural cycle, modified natural cycle, or HRT cycle, and after five days

of progesterone, the frozen embryo was thawed and transferred. Luteal phase support was given similar to the fresh group.

Pregnancy was confirmed when the serum beta hCG levels were more than 10 mIU/ml, and serial β-HCG was done to see the doubling rate. Transvaginal ultrasonography was performed to confirm clinical pregnancy and heartbeat at six weeks and also to confirm singleton or multifetal pregnancy. The clinical outcome of all participants was followed up using medical records.

Statistical methods:

Type of embryo transfer (fresh vs. frozen) was considered as the primary explanatory variable. Biochemical pregnancy, clinical pregnancy, pregnancy status, and the number of sacs were considered as outcome variables. Various demographic and clinical parameters were considered as potential confounders.

Examination of the distribution of the variables showed that all departed considerably from the normal distribution. For normally distributed quantitative parameters, the mean values were compared between study groups using an independent sample t-test (2 groups). For non-normally-distributed quantitative parameters, Medians and interquartile range (IQR) were compared between study groups using Mann-Whitney U test (2 groups). Categorical outcomes were compared between study groups using the chi-square test/Fisher's Exact test. P-value<0.05 was considered statistically significant. The "coGuide" software version v.1.0 was used for statistical analysis [15].

Results

A total of 121 subjects were included in the final analysis. There was no statistically significant difference between the two groups in baseline parameters like age, marriage duration, regular mensural cycle, type of infertility, previous miscarriage, hypothyroid, and number of previous IVF cycles (*P>0.05*). (Table 1)

There was no statistically significant difference between the two groups in baseline parameters like follicle-stimulating hormone values (mIU/ml), luteinizing hormone (mIU/L), prolactin (ng/ml), and estradiol on day two (pg/ml) (*P>0.05*). There was a statistically significant difference between the two groups parameters, such as thyroid-stimulating hormone (μIU/L) and total no of antral follicles (*P<0.05*). (Table 2)

There was a statistically significant difference between the two groups in other baseline parameters like starting dose, final dose, total dose, days of stimulation, and day of trigger (*P<0.05*). (Table 3)

There was a statistically significant difference between the two groups in other baseline parameters like estradiol value on hCG day, progesterone value on the day of HCG, number of large follicles, number of small follicles, number of oocytes retrieved, number of MII oocytes, number of fertilized embryos, number of cleaved embryos, day of transfer, number of embryos transferred, the total number of blastocyst formation and number of good quality embryos available (*P<0.05*). In the fresh group, 13 (35.14%) participants and in the frozen group, 60 (71.43%) participants had clinical pregnancy. The difference in the proportion of clinical pregnancy between the type of ET was statistically significant (*P<0.001*). The difference in the proportion of the number of sacs between the type of Embryo Transfer (ET) was statistically significant (*P<0.001*). (Table 4)

Table 1: Comparison of baseline causes of infertility parameters between the type of ET

Parameter	Type of ET		P Value
	Fresh (N=37)	Frozen (N=84)	
Age (years)	37.11 ± 4.21	35.74 ± 4.42	0.113 *
Marriage Duration (years)	5.57 ± 1.79	5.57 ± 1.93	0.992 *
Regular Menstrual Cycle	31 (83.78%)	67 (79.76%)	0.603 †
Previous Miscarriage	2 (5.41%)	10 (11.9%)	0.341 ‡
Type of Infertility			
Primary	17 (45.95%)	55 (65.48%)	0.044 †
Secondary	20 (54.05%)	29 (34.52%)	
Hypothyroid	1 (2.7%)	6 (7.14%)	0.674 ‡
No. of Previous IVF Cycles			
Up to 1	35 (94.59%)	79 (94.05%)	1.000 §
2 or more	2 (5.41%)	5 (5.95%)	

** Independent Sample T-Test, † Chi-square Test, ‡ Fisher's Exact Tet, § No statistical test was applied-due to 0 subjects in the cell*

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Table 2: Comparison of median lab parameters between the type of ET (N=121)

Laboratory Parameters	Type of ET		P Value*
	Fresh (N=37) Median (IQR)	Frozen (N=84) Median (IQR)	
Follicle Stimulating Hormone (mIU/ml)	7 (6,8)	6 (5,7.8)	0.077
Luteinizing Hormone (mIU/L)	7 (6,8)	7 (5.25,8)	0.945
Prolactin (ng/ml)	21 (11.5,22.5)	20.5 (12,22)	0.919
Thyroid Stimulating Hormone (µIU/L)	3 (2,3.5)	2 (2,3)	0.008
Anti-Mullerian Hormone (ng/ml)	0.8 (0.65,1.5)	2 (1.8,3)	<0.001
Estradiol on Day 2 (pg/ml)	33 (23,44.5)	34 (23.5,45)	0.514
Total Number of Antral Follicles	7 (6,8)	16 (12.25,19.75)	<0.001

***** *Mann-Whitney U Test*

Table 3: Comparison of treatment-related and process outcome parameters between the type of ET (N=121)

Parameters	Type of ET		P value
	Fresh (N=37)	Frozen (N=84)	
OCP Pretreat	0 (0%)	8 (9.52%)	*
Starting Dose (IU)	450 (450,450)	225 (200,300)	<0.001 †
Final Dose (IU)	600 (600,600)	300 (225,450)	<0.001†
HMG	36 (97.3%)	81 (96.43%)	1.000
rFSH	37 (100%)	84 (100%)	*
Antagonist	37 (100%)	84 (100%)	*
Total Dose (IU)	5600 (4500,6757.5)	4465 (3500,5400)	0.002 †
Days of Stimulation	12 (11,12)	11 (11,12)	0.004 †
Day of Trigger	12 (11,12)	11 (11,12)	0.006 †
Decapeptyl	0 (0%)	83 (98.81%)	*
HCG	0 (0%)	1 (1.19%)	*
rHCG	37 (100%)	2 (2.38%)	*
Process Outcome			
Estradiol, day of hCG (pg/ml)	2332 (1567,2456)	4719 (3506.5,5786.75)	<0.001 †
Progesterone, day of hCG (pg/ml)	0.8 (0.7,0.9)	0 (0,0.78)	<0.001 †
No. of Large Follicles	4 (2.5,4)	7 (5,8)	<0.001 †
No. of Small Follicles	3 (2,4)	7 (5,9)	<0.001 †
Endometrium Thickness (mm)	9 (8,9)	9 (8,9)	0.307 †
No. of Oocytes Retrieved	6 (4,9.5)	13.5 (9,17.75)	<0.001 †
Poor Oocyte Quality	2 (5.41%)	1 (1.19%)	0.221†
No. of MII Oocytes	4 (2.5,6)	11 (7,14)	<0.001 †
No. of Fertilized Embryos	2 (1,4)	9 (6,11)	<0.001 †
No. of Cleaved Embryos	2 (1,4)	8 (6,10.75)	<0.001 †
Day of Transfer	3 (3,5)	5 (5,5)	<0.001 †
No. of Embryos Transferred	1 (1,2)	1 (1,1)	<0.001 †
Total no of Blastocyst Formation	0 (0,2)	3 (1,5)	<0.001 †
No. of Good Quality Embryos	0 (0,2)	2 (0,4)	<0.001 †
Quality of transferred embryos			
Excellent	26 (70.27%)	76 (90.48%)	*
Average	10 (27.03%)	8 (9.52%)	
Poor	1 (2.7%)	0 (0%)	

** No statistical test was applied-due to 0 subjects in the cell., †-Mann Whitney U test*

Table 4: Comparison of outcome parameters between groups (N=121)

Outcome parameters	Type of ET		P value†
	Fresh (N=37)	Frozen (N=84)	
Not Pregnant	10 (27.03%)	21 (25%)	0.814
Biochemical Pregnancy	0 (0%)	5 (5.95%)	*
Clinical Pregnancy	13 (35.14%)	60 (71.43%)	<0.001
Pregnant	13 (35.14%)	60 (71.43%)	<0.001
Number of Sacs			
0	29 (78.38%)	26 (30.95%)	<0.001
1	8 (21.62%)	58 (69.05%)	<0.001
Miscarriage	0 (0%)	7 (8.33%)	*

*† Chi-square Test, * No statistical test was applied-due to 0 subjects in the cell.*

Discussion

With the advancement of assisted reproductive technology procedures, embryo cryopreservation has become an important part of in vitro fertilization treatment. In this study, 71.43% of the frozen embryo transfers had a positive outcome, that is, pregnancy. Among the frozen embryo group, 8.33% had a miscarriage that is a negative outcome. Controlled ovarian stimulation leads to a higher level of serum estradiol which may affect the outcome [16–18]. A previously published study has shown that the outcomes of IVF have been significantly improved by the following freeze-all strategy group when

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compared to the fresh embryo group [19], which is similar to the findings of this current study. Previously published authors recommend that patients with previous fresh IVF failures due to impaired endometrial receptivity should choose frozen transfer embryo transfer cycles with artificial endometrial preparation [20]. With the advancement in embryo freezing technology, vitrification techniques have given wider application and success rates [21]. Those who undergo IVF treatment are at an increased risk of exposure to prolonged levels of estrogen, thus increasing their risks of OHSS, ovarian hyperstimulation syndrome. Incidence of OHSS is drastically nil nowadays due to stratified IVF treatment with embryo cryopreservation. One such research was done by Shapiro et al. [20], in which they concluded that the clinical pregnancy rate was higher in the cryopreserved embryo transfer group compared to the fresh group. This was similar to the current study findings where there was 71.43% clinical pregnancy among the frozen embryo group compared to 35.14% in the fresh group. The difference observed was statistically significant. This is in contrast to the study by Xue Wang et al. [22], which showed the pregnancy rate was similar between frozen and fresh transfer. Follow up of the study participants were done by the researchers to compare the pregnancy outcome. The live birth rate in this study was significantly higher in the frozen embryo group compared to the fresh embryo. This finding was statistically significant. Such follow-up was not done in this current study due to resource-constraints, and it is considered a limitation. A recent meta-analysis was done by including four randomized clinical trials with 1892 compared the outcome of the transfers and concluded that freeze all policy had higher pregnancy outcomes [23]. The findings of the current study were similar to the previously published studies [19,24]. In the present study, the number of oocytes retrieved was higher in the frozen transfer group. This did not have any effect on the outcome as the patient had two to three natural cycles before the embryos were transferred back, thus reducing the negative effects of high estrogen levels on the endometrium. Researchers in the past had recommended embryo cryopreservation techniques in IVF. In 2015, Roque et al. [19] found that there was reduced obstetric complications, perinatal complication and good clinical outcome among the transfers done through freeze all technique. Recently in 2016, a Strength, Weakness, opportunity and threats (SWOT) analysis was done by Blockeel et al. [25] enlightened the areas on various aspects of freeze all technique. With the available literature, lacunae still transfer. To fill these lacunae, controlled clinical trials and follow-up studies are required.

Conclusion

The findings from this study showed that the clinical pregnancy outcome was higher in the frozen embryo group. Frozen embryo transfers can likely improve the overall outcome of assisted reproductive technology, and there was no disadvantage in following frozen embryo transfer. A well-defined policy for frozen embryo transfer in assisted reproduction cycle can act as an effective and safe strategy, specifically in hyper responders and in women opting for pre-implantation genetic screening.

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Ethics approval and consent to participate

Informed written consent was obtained from all participants, and confidentiality was maintained throughout the study. Ethical approval was not obtained due to lack of institutional ethics committee.

Conflict of interests

The authors declare no conflicts of interest.

Source of funding

The project was self-funded. No external agency had funded the project.

Author Contributions

PsD is responsible for the conceptualization and methodology. PsD and MB collected, analyzed, and interpreted the patients' data. PsD drafted the manuscript. PsD and MB revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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