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The Effect of Immediate Transfer of Euploid Embryos on Outcome in Patients with Recurrent Implantation Failure

Tekrarlayan İmplantasyon Başarısızlığı Olan Hastalarda Euploid Embriyolarin Transfer Zamanlamasının Değerlendirilmesi

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ABSTRACT

Objective: We aimed to investigate the association of immediate transfer of embryos undergoing PGT-A with the clinical pregnancy rates of women with recurrent implantation failure. **Materials and Methods:** In total 502 patients with euploid blastocyst transfer were included in this retrospective study. For each woman, one vitrified-warmed embryo transfer cycle after PGT-A was analyzed. We assessed clinical pregnancy outcomes, categorizing them as either positive or negative. We grouped the patients according to cryo-storage duration: patients with cryo-storage duration of less than 90-days were grouped as Group A; and the ones with more than 90-days, were included in Group B. **Results:** The quantities of retrieved, metaphase II or fertilized oocytes, vitrified or transferred embryos, and blastocysts at the stage were comparable between the two groups. The overall clinical pregnancy rate was 77.1%, and this rate was statistically similar between the Grup A and Group B groups (p=0.747). For Group B, the subgroup with a positive pregnancy outcome had a greater number of transferred embryos but a shorter cryo-storage duration and this features did not change the general outcome between the two groups (p=0.049 and p=0.040, respectively). Within Group A, the subgroup with a positive pregnancy outcome had a higher proportion of Day 5 blastocysts (p=0.001). **Conclusion:** Immediate euploid embryo transfer of less than 90 days do not effect the pregnancy rate. A higher number of transferred embryos and a shorter storage duration may increase the possibility of pregnancy, if cryo-storage exceeds 90 days. Day 5 blastocyst transfer rather than Day 6 seems to be associated with a higher pregnancy rate when the transfer is done in less than 90 days period after the ovum pick up procedure.

Keywords: Blastocyst transfer; pregnancy; euploid embryos; preimplantation genetic diagnosis; cryo-storage duration

ÖZET

Amaç: Tekrarlayan implantasyon başarısızlığı olan kadınların PGT-A uygulanan embriyoların hemen transfer edilmesi ile klinik gebelik oranları arasındaki ilişkiyi araştırmayı amaçladık. **Gereç ve Yöntem:** Bu retrospektif çalışmaya öploid blastokist transferi yapılan toplam 502 hasta dahil edildi. Her kadın için PGT-A'dan sonra bir dondurulmuş-çözülmüş embriyo transfer siklüsü analiz edildi. Klinik gebelik sonuçlarını pozitif veya negatif olarak sınıflandırarak değerlendirildi. Hastaları kriyo-saklama süresine göre gruplandırdık: 90 günden daha az süre saklanan kriyo grubu Grup A, 90 gün ve daha fazla süre saklanan embriyolar Grup B olarak tanımlandı. **Bulgular:** İki grup arasında alınan toplam oosit sayısı, metafaz II oositlerin, döllenmiş oositlerin, vitrifiye edilmiş veya transfer edilmiş aşamadaki blastokistlerin sayısı iki grup arasında eşitti. Genel klinik gebelik oranı %77,1 idi ve bu oran her iki grupta da istatistik olarak benzerdi (p=0,747). Grup A içerisinde gebelik sonucu pozitif olan alt grupta 5. gün blastokist oranı daha yüksekti (p=0,001). Grup B için, gebelik sonucu pozitif olan alt grupta daha fazla transfer edilen embriyo sayısı vardı ancak bu grup içindeki hastalar karşılaştırıldığında kriyo-saklama süresi daha kısa olma eğlimindeydi. (sırasıyla p=0,049 ve p=0,040). **Sonuç:** 90 günden kısa süren öploid embriyo transferi daha geç yapılan öploid embryo transferlerine göre daha iyi bir klinik gebelik oranını vermemiştir. Yumurta toplama gününden transfere kadar geçen süre 90 günü aşan grupta (GrupB) klinik gebelik pozitif olanlarda transfer edilen embriyo sayısı daha fazla olduğu görülmüş ve grup içindeki saklama süresi daha kısa olma eğiliminde olduğu izlenmiştir. Bu bulgular Grup A ve Grup B arasındaki genel sonucu etkilememiştir. Transferin yumurta toplama işleminden sonraki 90 günden daha kısa sürede yapılması durumunda, 6. Gün yerine 5. Gün blastokist transferi daha yüksek gebelik oranı ile ilişkili görünmektedir (p=0,001).

Anahtar Kelimeler: Blastokist transferi; gebelik; öploid embriyolar; implantasyon öncesi genetik tanı; kriyo-depolama süresi

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Preimplantation genetic diagnosis (PGD) involves examining cells acquired through biopsies of fertilized oocytes, cleavage-stage embryos, or blastocysts to identify genetic abnormalities.[1] Preimplantation genetic testing for aneuploidies (PGT-A) facilitates the selection of euploid embryos for transfer, especially in cases of advanced maternal age, recurrent miscarriages, or repeated in vitro fertilization (IVF) failures.[2] Hence, the chance of obtaining high-quality blastocysts might be increased.

In general, it is known that endometrial receptivity is impaired due to supraphysiological hormonal metabolism in the patient group which PGT-A is performed by trying to obtain many embryos. Due to advancements in cryopreservation methods, the success rate of achieving pregnancy through frozen embryo transfer (FET) has reached parity with or even exceeded that of fresh embryo transfer.[3,4] A recent extensive study demonstrated that the duration of storage for vitrified embryos did not impact pregnancy rates, regardless of whether cleavage-stage embryos or blastocysts were transferred, nor did it affect neonatal outcomes.[5] Nevertheless, the study did find that storage exceeding 7 years was associated with a lack of pregnancy success.[5] Similarly, another study, which included slow-frozen early-cleavage human embryos revealed that long-term cryopreservation did not impact pregnancy rates or neonatal outcomes.[6] However, contrasting findings were also reported.[7]

Cimadomo et al. investigated the clinical, obstetric and perinatal outcomes after transfer of vitrified-warmed euploid blastocysts, which were selected by PGT-A.[8] They stratified storage duration into 7 groups, and showed no differences in obstetric and perinatal outcomes among the groups. However, they found that a storage duration of >90 days was associated with a lower rate of live births, and suggested that a decline in blastocyst quality as the reason. Shi et al. revealed that the pregnancy rates of embryos following cleavage-stage PGT were not adversely affected by cryopreservation when compared to those from fresh cycles.[9] Studies regarding the duration of cryopreservation after FET with PGT-A are scant. Our objective was to examine the link between the duration of cryo-storage for embryos, which were selected for high quality through PGT-A, and the clinical pregnancy rate among women who have experienced recurrent failures in IVF attempts.

MATERIALS AND METHODS

STUDY DESIGN

This retrospective study was conducted in Kolan International Hospital Şişli Private IVF Center, and approved by the local Ethics Committee of Altinabaş University Ethic Commettee with approval number: 04.09.2023-56442. The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all of the participants. The data of the patients can be found In IVF archive of the Kolan International Hospital IVF Center, Şişli/İstanbul. Derived data supporting the findings of this study are available from the corresponding author on request.

STUDY POPULATION

Adult (>18-year-old) women, who underwent euploid blastocyst transfer in the IVF center of the Kolan Internaional Hospital Şişli between 2018 and 2022 were included retrospectively. Patients undergoing vitrified-warmed blastocyst transfer, after PGT-A for aneuploidies was performed, were analyzed. We defined PGT-A indication as recurrent implantation failure(RIF) which was defined as three unsuccessful IVF cycles with good quality embryos.[10,11] If more than one oocyte retrieval occurred during the study, we included only the first cycle. Those for whom data were missing were not included in the study. We excluded contributory factors such as uterin abnormalities, endocrine factors , bad embryo quality, immunological factors and genetic abnormalities such as single gene mutations or translocations as well as the male infertility problems.

DATA COLLECTION

Demographic parameters (age), and clinical parameters associated with the procedures (number of retrieved oocytes, number of metaphase II [MII] oocytes, number of fertilized oocytes, number of vitrified embryos, number of transferred embryos, and pregnancy result after embryo transfer) were recorded and analyzed.

The primary outcome was the occurrence of a clinical pregnancy result after embryo transfer.

We grouped the patients according to cryo-storage duration, which was defined as the duration between vitrification and warming (just before embryo transfer): <90-day, Group A; and ≥90-day, Group B. We grouped them also according to the clinical pregnancy result after embryo transfer as either positive or negative. We defined the clinical pregnancy rate as visible gestational sac or sacs by ultrasonography done 2 weeks after the blood pregnancy test result. It takes around 4-5 weeks before we get the results of PGT-A and the earliest Frozen-Thaw embryo transfer can be done 3 months after the egg collection of the patients. This is why we defined the patient groups as either being transferred during the first 3 months or more then 3 months after the ovum pick-up procedure.

IVF Procedures

All patients were treated with recombinant follicle stimulating hormone (Gonal-F, Merck Serono, Italy) from cycle day 3 for ovarian stimulation until at least one dominant follicle reached a diameter of >18 mm, followed by injection of 250 μg of recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono, Italy) and 250 μg of GnRH antagonist (Cetrotide, Merck Serono) 36 hours before oocyte retrieval.

Embryo Culture, Te Biopsy And Next-Generation Sequencing

The retrieved oocytes were cultured before fertilization, in Quinn's Advantage Fertilization Medium (Sage Bio-Pharma, Inc., Trumbull, CT, USA) with a 15% serum protein substitute (SPS) (Sage Bio-Pharma, Inc.), in a triple gas phase of 5% CO_2, 5% O_2, and 90% N_2. The insemination or intracytoplasmic sperm injection (ICSI) was performed 38-41 hours after administration of the antagonist protocol. Following conventional insemination/ICSI, all embryos were further cultured in microdrops (Quinn Advantage Cleavage Medium (Sage BioPharma, Inc.), with 15% SPS under low oxygen (5% O_2) until Day 3. 70-72 hours after insemination/ICSI, all cleaved embryos were further cultured in microdrops of Quinn Advantage Blastocyst Medium (Sage Bio-Pharma, Inc.), with 15% SPS to blastocyst (Day5/6) for trophectoderm (TE) biopsy. The laser-assisted hatching was performed on day 4. The quality of blastocyst was defined according to the criteria presented by Gardner and Schoolcraft, only the expanded blastocysts considered to be of a desirable quality (4, 5, 6, AB, BA, and BB) with diameters more than 150 μm were defined as qualified blastocysts and suitable for subjecting to TE biopsy.[12] The Day 5 blastocyst stage without expansion (<150 μm) was further cultured until Day 6. TE biopsy was performed at Day 6 stage of blastocyst with an ICM and TE type A or B. The protocol of TE biopsy was performed as described by Chen et al.[13] The biopsied TE cells were immediately placed in an RNAse–DNAse-free polymerase chain reaction tube and amplified using the SurePlex DNA Amplification System (Illumina, Inc., San Diego, CA, USA). Extracted cells were placed in 2 μL of buffer and shipped frozen to Genesis Genetics for PGT-A using a high-resolution, next-generation sequencing (hr-NGS) platform.

Vitrification-Warming Protocol

The biopsied blastocysts were vitrified 2-4 hours after TE biopsy. The vitrification and warming protocols with Cryotech (Repro-Support Medical Research Centre, Co. Ltd., Tokyo, Japan) described by Gutnisky et al. were employed.[14] Single euploidy was selected for transfer, and warmed embryos were cultured in a blastocyst medium at 37˚C (5% CO_2 and 5% O_2) for 1-2 hours before transfer. The survival of warmed blastocysts was checked at the time of embryo transfer and defined as >80% of cells intact and full re-expansion. In this study, all of the warmed blastocysts survived with full re-expansion.

Endometrial Preparation And FET

All patientshad undergone an artificial cycle (AC) for endometrial preparation since day 3 of their natural menstrual cycle. Each woman was administered the same regimen during the menstrual cycle: oral estradiol valerate (Estrade, Synmosa, Taipei, Taiwan) at 4 mg daily on days 3 and 4, 8 mg daily from days 5-7, 24 mg daily from days 8-13, 12 mg on day 14, and 8 mg daily from day 15 to the day of the pregnancy test.

The transfer of single euploid blastocysts was performed on day 18 and required an endometrial thickness of at least 8mm. If the endometrial thickness was <8 mm, the transfer was canceled and shifted to the next cycle. On the 12th day of the menstrual cycle, a progesterone injection of 100 mg/day (Koçak Farma Pharmaceuticals and Chemicals Industry, Tekirdağ, Türkiye) for 17 days was started for luteal phase support. Estradiol and progesterone supplementation were continued until the day of the pregnancy test and, if the test was positive, estradiol and progesterone were continued until the 12th gestational week.

STATISTICAL ANALYSIS

Data obtained in the study were analyzed statistically using SPSS 25.0 software (IBM Corporation, Armonk, New York, United States). The conformity of the univariable data to normal distribution was evaluated using the Shapiro-Wilk Francia test. When comparing two independent groups according to qualitative variables, the Mann Whitney U test was used with Monte Carlo results. When comparing categorical variables to each other, the Pearson Chi-square test was used with the Monte Carlo simulation technique. Quantitative variables were stated as median (1st Quartile-3rd Quartile) (q1-q3) values, and categorical variables as number (n) and percentage (%) in the table. Variables were evaluated at a 95% confidence level, and a value of $p<0.05$ was accepted as statistically significant.

ETHICAL APPROVAL

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Altınbaş University (Date: 04.09.2023 Number:56442)

RESULTS

A total of 502 women was analyzed, and the median age was 33 (29-37). Group A consisted of 211 women and Group B, 291 women. The number of collected, MII or fertilized oocytes, the number of vitrified or transferred embryos, or blastocyst stage were similar in both groups. The positive pregnancy rate was 76.3% in Group A and 77.7% in Group B (p=0.747) and no significant difference was found (Table 1). Within Group A, the proportion of positive clinical pregnancies correlated with a higher ratio of Day 5 blastocyst transfers (p=0.001). In Group B, while the subgroup with positive clinical pregnancies had a greater number of transferred embryos, their cryo-storage duration was notably shorter (p=0.049

TABLE 1: Comparison of clinical features and pregnancy results in all patients.

	Clinical pregnancy		
	Negative (n=115)	Positive (n=387)	
Total (n=502)	**Median (q1-q3)**	**Median (q1-q3)**	**p**
Maternal age (year)	33 (29-37)	33 (29-37)	0.419
Cryo-storage duration (day)	120 (61-227)	102 (65-194)	0.579
Number of retrieved oocytes	11 (5-19)	11 (7-22)	0.519
Number of MII oocytes	9 (5-18)	9 (6-18)	0.523
Number of fertilized oocytes	8 (4-13)	8 (5-13)	0.416
Number of vitrified embryos	4 (3-8)	4 (3-8)	0.273
Number of transferred embryos	1 (1-1)	1 (1-2)	0.057
	n (%)	**n (%)**	
Cryo-storage			
<90-day (Group A)	50 (43.5)	156 (40.3)	0.590[c]
≥90-day (Group B)	65 (56.5)	231 (59.7)	
Blastocyst stage			
Day 5	97 (21.0)	366 (79.0)	**0.001**[c]
Day 6	18 (46.2)	21 (53.8)	

[u] Mann-Whitney U Test(Monte Carlo), [c] Chi-Square Test (Monte Carlo), q1: 1st quartile, q3:3rd quartile MII: metaphase II

and p=0.040), respectively. It seems logical that as the number of transferred embryos increases (Median 1.5 embryos per transfer) the pregnancy rate will be better. This cannot make any difference in interpretation of results because in overall patients the number of transferred embryos between negative pregnancy results patients and positive pregnancy results patients is statistically same (Table 2). The shorter cryo-storage duration of Group B in positive pregnancy subgroup reminds as that prolonged duration decreases the pregnancy rates. Maybe in different studies which have a third group with more than 168 days duration; which is the median cryo-storage duration of the positive pregnancy subgroup in Group B , the negative effect of prolonged cryo-duration of euploid blastocysts can be shown. Additionally clinically pregnancy-positive cases were compared according to the 90-day cut-off point and no significant difference was found (p = 0.59).

DISCUSSION

Our findings indicated that the embryo transfer of euploid embryos , whether less or more than 90 days after the egg collection , did not correlate with the rate of positive pregnancy outcomes. When the cryo-storage period was ≥90 days, a greater number of transferred embryos and shorter storage duration were linked to a higher likelihood of clinical pregnancy. On the other hand, if the cryo-storage duration was <90 days, an earlier blastocyst stage was associated with clinical pregnancy. We included euploid blastocyst transfer and found a clinical pregnancy rate of about 77%, which was about 3-times that found in a previous study in which embryo transfer was performed without PGT-A.[15] We showed that a longer storage duration was negatively associated with the pregnancy rate in the ≥90-day cryo-storage group, and that pregnancy was independent of the number of transferred embryos in the <90-day cryo-storage group. The findings suggest that extending the duration of storage (in the >90-day cryo-storage group) could potentially have a negative impact on the viability of euploid embryos and could be linked to a reduction in embryo quality. Therefore, transfer of more than one embryo might increase the possibility of pregnancy in this group. In a retrospective cohort study, pregnancy and birth rates were observed not to be affected by storage duration within a range of 1-84 months, but clinical pregnancy could not be achieved with vitrified embryos with a cryo-storage duration of >84 months.[5] Shi et al. revealed no effect of long-term cryopreservation on clinical pregnancy and birth rates in a cohort of slow-frozen early-cleavage human embryos.[6] The study's discov-

TABLE 2: Comparison of groups features between Group A and B.

	Group A Cryo-storage <90-day (n=211) Clinical pregnancy			Group B Cryo-storage ≥90-day (n=291) Clinical pregnancy		
	Negative (n=50)	Positive (n=161)		Negative (n=65)	Positive (n=226)	
	Median (q1-q3)	Median (q1-q3)	p	Median (q1-q3)	Median (q1-q3)	p
Maternal age (year)	34 (29-39)	32 (29-36)	0.194	33 (30-37)	33 (29-37)	0.892
Cryo-storage duration (day)	57.5 (51-75	59 (52-75)	0.842	193 (136-321)	168 (113-276)	**0.040**
Number of retrieved oocytes	15 (6-25)	11 (7-19)	0.446	9 (4-15)	12 (7-25)	0.169
Number of MII oocytes	12 (5-19)	9 (6-18)	0.505	9 (3-14)	9.5 (6-19)	0.174
Number of fertilized oocytes	13 (5-18)	8 (5-13)	0.587	7 (3-13)	8 (5-13)	0.148
Number of vitrified embryos	4 (3-9)	4 (3-8)	0.716	4 (3-7)	4 (3-8)	0.243
Number of transferred embryos	1 (1-1)	1 (1-2)	0.591	1 (1-1)	1.5 (1-2)	**0.049**
	n (%)	n (%)		n (%)	n (%)	
Blastocyst stage						
Day 5	41 (20.8)	156 (79.2)	**0.001**[c]	56 (21.1)	210 (78.9)	0.128 [c]
Day 6	9 (64.3)	5 (35.7)		9 (36.0)	16 (64.0)	

[u] Mann-Whitney U Test(Monte Carlo), [c] Chi-Square Test (Monte Carlo), q1: 1st quartile, q3:3'd quartile MII: metaphase II

ery of diminished embryo quality among individuals with cryopreservation periods exceeding 60 months, in comparison to those under 60 months, did not yield statistically significant results.

While we categorized cryo-storage durations into two groups using a threshold of 90 days, it's worth noting that the maximum duration observed in our study was 1,442 days. This extended timeframe could potentially involve the transfer of embryos of relatively lower quality. Various studies have reported conflicting results regarding the effect of prolonged cryo-storage on clinical pregnancy rates.[16-21]

Studies investigating an association of cryo-storage duration with clinical outcomes after euploid blastocyst transfer are scant. Cimadomo et al. conducted a retrospective analysis of clinical outcomes after 2,688 vitrified-warmed euploid blastocyst transfers.[8] They found that live birth rates were lower for transfers with a cryo-storage duration of between 90-720 days compared to those with a duration of ≤60 days. As a possible explanation, they proposed that the highest quality embryos were prioritized for initial transfer, while embryos of lower quality were reserved for subsequent procedures. However, this was not seen to be statistically significant in the multivariate analysis. Ultimately, the live birth rate was similar in those groups where cryo-storage duration was >720 days and <60 days, and no difference was found between consecutive euploid single embryo transfers, in terms of the live birth rate. For such a study design, in order to obtain a clear clinical interpretation, possible confounders should be adjusted during analysis. In a small study analyzing fresh and frozen-thawed embryo transfer cycles after cleavage-stage PGD, pregnancy, implantation, abortion, and live birth rates were found to be similar in both the fresh cycle and frozen-thawed embryo transfer cycle.[9] Cryo-storage duration was not analyzed in that study. Other studies showed that incubator type or post-thawing culture duration might affect the blastocyst formation rate.[22,23] In our study, we could not analyze embryo quality, birth rate or neonatal outcomes. We used the same incubator, culture media and genetic technique for all transfer procedures. We excluded factors such as sperm quality or male fertility.

In a previous report, an endometrial preparation protocol employing an artificial cycle was identified as a significant factor negatively impacting the birth rate, although it did not exhibit the same effect on the clinical pregnancy rate.[15] We used an artificial cycle protocol in all patients. The study also demonstrated that transferring blastocysts, in comparison to cleavage-stage embryo transfer, led to higher clinical pregnancy and birth rates. We performed the transfer of blastocysts on Day 5 or Day 6. In many previous studies of artificial reproductive technology in which PGT-A was not performed, a Day 5 blastocyst transfer was shown to yield a higher implantation and clinical pregnancy rate compared to a Day 6 blastocyst transfer.[24-26] In one study, it was suggested that embryo quality or aneuploidy rate might be important with respect to the differences seen between Day 5 and Day 6 blastocyst transfer.[27] In one study, which included 527 frozen-thawed blastocyst transfers cycles in which PGT-A was performed, clinical outcomes were compared between Day 5 and Day 6 blastocyst transfer.[28] Implantation rate, clinical pregnancy or live birth rates were shown to be similar, but birth weight was found to be higher in the Day 5 blastocyst transfer. They did not take into account cryo-storage duration. We revealed a high pregnancy rate in euploid embryo transfers, and found that the earlier blastocyst stage (Day 5) was associated with a higher pregnancy rate only in the cryo-storage group of <90-day. One could argue that extending cryopreservation beyond 90 days might mitigate potential distinctions between Day 5 and Day 6 blastocysts. To our knowledge, the impact of cryo-storage duration on the correlation between blastocyst stage and pregnancy rate has not been investigated previously. In one study, which included 431 frozen-thawed embryo transfers, in which PGT-A was not performed, the clinical pregnancy rate was 25.5%, and the age and number of transferred embryos were found to influence the birth rate, but cryo-storage duration was not analyzed.[15]

We observed a relation between a higher number of transferred embryos and the pregnancy rate, specifically within the cryo-storage group of ≥90

days. However, no significant association was noted between age and the pregnancy rate . We propose that the factors influencing a higher pregnancy rate appear to change as the duration of cryo-storage becomes longer. Further research is necessary, encompassing embryo transfer cycles that involve PGT-A, and examining a broad range of factors across various durations of cryo-storage In a large study, cryopreservation time was found not to affect clinical outcomes of vitrified-warmed blastocysts within the same grade.[17] Zhang et al. showed that extended cryopreservation was inversely associated with pregnancy rates, particularly evident in subgroups involving high-quality blastocyst transfer,, a maternal age of <40-years-old, and a higher (>3) number of oocyte retrievals.[16] It's important to note that our study also included high-quality blastocysts.

STRENGTH AND LIMITATIONS

Our study aimed to explore the correlation between cryo-storage durations and clinical pregnancy rates within a specific subset of vitrified-warmed blastocyst transfers, where PGT-A was employed. We analyzed only one cycle for each woman included. We used the same incubator and culture media, biopsy and genetic techniques, vitrification-warming, endometrial preparation and transfer protocols for all transfer procedures. We excluded factors such as sperm quality and male infertility as well as the causes of recurrent implantation failure caused by female factors. We could not analyze, live birth rates or neonatal outcomes. As we all know that the most important prognostic marker in IVF treatments is the take on hand baby rates. Unfortunately we were not able to follow these patients till the delivery and this is why we emphasized that this is the most strong limitation of our study.

CONCLUSIONS

Our findings suggest that cryo-storage of less or more than 90 days seems not to change clinical pregnancy rates after euploid blastocyst transfer. It could be suggested that, in cases of cryo-storage exceeding 90 days, opting for double embryo transfer and maintaining shorter storage times might enhance the chances of pregnancy. Conversely, for cryo-storage durations less than 90 days, the transfer of Day 5 blastocysts might be more favorable for increasing pregnancy likelihood. To minimize possible confounders, we used the same techniques and protocols in all transfers, but could not analyze live birth rates or neonatal outcomes. Further studies including euploid embryo transfer cycles should analyze possible factors across a wide range of cryo-storage durations.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Alper Şişmanoğlu, Ulun Uluğ;* ***Design:*** *Alper Şişmanoğlu;* ***Control/Supervision:*** *Alper Şişmanoğlu;* ***Data Collection and/or Processing:*** *Alper Şişmanoğlu;* ***Analysis and/or Interpretation:*** *Ulun Uluğ, Alper Şişmanoğlu;* ***Literature Review:*** *Alper Şişmanoğlu;* ***Writing the Article:*** *Alper Şişmanoğlu;* ***Critical Review:*** *Ulun Uluğ.*

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