Ref: Ro J Med Pract. 2021;16(Suppl6)
DOI: 10.37897/RJMP.2021.S6.3

Pregnancy and live birth rate after fresh embryo transfer versus freeze-all strategy: A prospective observational study

Diana Mihai[1,2], Andreea Carp-Veliscu[1,2], Alina Elena Bordea[1,2], Mihai Dumitrascu[2], Florica Sandru[2], Antoine Edu[2,3], Marina Antonovici[3], Claudia Mehedintu[2,3], Elvira Bratila[1,2]

[1]"Prof. Dr. Panait Sirbu" Clinical Hospital Obstetrics and Gynecology Bucharest, Romania
[2]"Carol Davila" University of Medicine and Pharmacy,Bucharest, Romania
[3]"Nicolae Malaxa" Clinical Hospital, Obstetrics and Gynecology Department

ABSTRACT

Objectives. To evaluate which type of embryo transfer (ET) has a superior pregnancy rate.
Material and method. Prospective observational non-randomized study including 79 women that underwent 145 ET.
Outcomes. The results show that the highest biochemical pregnancy rate (BPR) was in the fresh day 3 ET group (47.62%), but in this group there were also the highest abortion rate (80%) and the highest premature birth rate (100%), giving that, after 21 fresh day 3 ET did not result any term live birth. The frozen day 3 embryo has the lowest BPR (30%), but also the lowest abortion rate (33.33%) and the lowest premature birth rate (0%), in the present study after 13 frozen day 3 ET resulting 2 full-term births (15.38%). The results for the fresh day 5 blastocyst show a good BPR (43.48%), a low abortion rate (33.33%) and the lowest premature birth rate (0%), giving that in the study performed after 23 day 5 ET resulted 4 newborns at term (15.38%). The frozen day 5 blastocyst has a very good BPR (46.07%), the highest birth rate (21.62%), a high abortion rate (37.14%) and a low premature birth rate (7.41%), taking into account that in the present study after 89 day 5 frozen ET resulted 24 newborns, of which 22 full-term births (24.71%).
Conclusions. The use of frozen day 5 blastocysts had the best results in terms of the ultimate goal - the birth of a live fetus at term.

Keywords: embryo transfer, pregnancy rate, fresh embryo, frozen embryo, live birth rate

INTRODUCTION

The birth rate (BR) has decreased in most countries, and moreover, the natural increase has been negative for several years in Romania, according to the National Institute of Statistics. In addition, the global fertility rate is 2.1 (number of children per woman, World Population Data Sheet 2021) and the number of women over the age of 35 who want to become mothers has increased in Europe alone from 8 to 24% between 1990 and 2020. This aging of the population has medical, psychological and financial effects on society [1-6].

Assisted human reproduction techniques (ART) are developing rapidly, bringing to light a new perspective on couple infertility and new opportunities for these families. Moreover, the development of embryonic culture technology and cryopreservation techniques determined a significant progress in the world of ART, from the transfer of fresh embryos to the transfer of frozen (or cryopreserved) blastocysts [7-14].

OBJECTIVES

We conducted this study to analyze in parallel the 4 types of embryo transfers (ET) - fresh day 3, frozen day 3, fresh day 5 and frozen day 5 – in order to determine the highest pregnancy rates (PR) and live birth rates (LBR).

Corresponding author:
Andreea Carp-Velişcu
E-mail: andreea_veliscu@yahoo.com

Article History:
Received: 9 November 2021
Accepted: 8 December 2021

MATERIALS AND METHODS

The present study is prospective, observational, non-randomized. We included patients diagnosed with infertility, who underwent specialized treatments to obtain a pregnancy in the ART Department of "Prof. Dr. Panait Sîrbu" Clinical Hospital of Obstetrics and Gynecology.

The research methodology, the patient questionnaire and the informed consent have been endorsed by the Ethics Committee and comply with the legislation in force regarding research studies. This sample is representative of a Romanian population undergoing infertility treatment in a specialized center. The study took place between March 2018 and May 2020.

We used the following inclusion criteria: patients undergoing ART who have at least one embryo for transfer, who will undergo the ET procedure (fresh or cryopreserved) in the Clinical Hospital of Obstetrics and Gynecology "Prof. Dr. Panait Sîrbu", who completed an informed consent form in which they agree to be included in this study and keep in touch with the investigator during pregnancy and to update the online questionnaire until the moment of birth.

We excluded from the study patients who refused to sign the consent or who during the study requested the waiver, patients with whom we lost contact during the procedures performed or due to their lack of response later, after obtaining the pregnancy.

Patients obtained the embryos following natural or controlled ovarian stimulation with different protocols, including the use of long, short, follicular or luteal phase protocols with FSH, LH, hMG, aromatase inhibitors, GnRH agonists or antagonists, in various combinations. The trigger constituted of r-hCG or triptorelin 0.1-0.3 mg/ml or a combination of these injected 35-36 hours prior to the ovarian puncture. Oocyte extraction was performed by ultrasound-guided transvaginal puncture and the oocytes were placed in Gamete Buffer culture media then moved to the fertilizing medium Fertilization. After being left in the incubator for one hour, they were fertilized by in vitro fertilization (IVF) or intracitoplasmatic sperm injection (ICSI) depending on the parameters of the semen analysis, the patient's history (miscarriages), the degree of sperm fragmentation.

After fertilization, the cells were left in the fertilization culture medium, in the incubator (Miri Esco) at 37 degrees Celsius, 6% CO2 and 5% oxygen. After 16-18 hours from fertilization, the oocytes were evaluated for the presence of pronuclei and moved to the One Step (Sage) culture medium. Then, the development of the embryos was evaluated at 3 or 5 days. The embryologist together with the attending physician determined, depending on the patient and the characteristics of the embryo, the transfer method: fresh, frozen, on day 3 or 5.

The embryos were frozen by vitrification in the cryopreservation medium Kitazato or Rapid VitOmni (Vitrolife) and stored in liquid nitrogen at -196 degrees Celsius, closed system type. For frozen embryos, thawing before the ET procedure was performed by removing the sequins from the device and deposit them in various thawing media (Kitazato, Thawing or Rapid Warm Omni - Vitrolife) on the day of the ET, 2-4 hours before the procedure. After thawing, the embryos were moved to the ET medium (EmbryoGlue).

The ET procedure was performed at the number of days corresponding to the implantation window depending on the age of the embryo (for example, ET on day 3 was performed 3 days after the puncture day for fresh embryos or 3 days after ovulation, in case of thawed transfer). Prior to ET, almost all patients received luteal phase supportive treatment with progesterone (mostly administered intravaginally) and treatment with Aspenter 75 mg in the evening. The ET was performed without anesthesia, with transabdominal ultrasound guidance, using a soft Wallace-type catheter. After every procedure, the embryologist checked the catheter to confirm the ET.

At least 10 days after the ET, patients performed a serum β-HCG assay, to establish the presence of biochemical pregnancy (BP). The diagnosis of clinical pregnancy was made at the time of visualization of the embryo with cardiac activity in the uterus. Pregnancy was monitored by quarterly tests: blood (HLG, coagulation tests, biochemistry, thyroid markers), urine, vaginal discharge, maternal serum screening, 1st, 2nd and 3rd trimester morphology, blood pressure monitoring plus other tests depending on patient characteristics.

The time of birth was determined by the attending physician according to the evolution of the pregnancy, without being influenced by the mode of ET performed. The method of birth (natural or cesarean section) was established together with the attending physician, in various maternity hospitals in the country or abroad.

If the same patient performed both ICSI and IVF procedures, only the data for the procedure that was successful and which resulted in at least one embryo were retained, the data related to the failed procedures being removed from the final analysis. For patients who underwent multiple ART procedures, but all were followed by failure, only data on a single randomly selected procedure were retained for analysis.

Statistical analysis

We performed various types of statistical analysis. A usual descriptive analysis with a simple uni-

variate binomial logistic regression was frequently used. The bidirectional χ^2 test was used to assess the difference between the success rate of one ET technique versus the other, and the Fisher exact test was used to assess whether the differences between the techniques are statistically significant.

For statistical analysis we used the R program, version 4.0.2 Copyright (C) 2020 "The R Foundation for Statistical Computing, R Core Team (2020). A: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria". URL https://www.R-project.org.

We considered the differences to be statistically significant at a $p < 0.05$.

RESULTS

The study groups

Of the 300 patients who initially agreed to be included in the study, only 79 kept in touch with the investigator and had the data included in the statistical analysis. On these patients were performed a total of 145 ET procedures.

The patients were between 27 and 47 years old and all stated that they did not consume alcohol, 19 patients stated that they smoked, and 4 patients reported using drugs.

The minimum BMI was 16.32 and the maximum – 38.56.

In regard to their family medical history, only 4 patients mentioned infertility, but we observed a high prevalence of diseases (62.02%).

The age at menarche was between 11 and 17 years, and 56 patients reported having dysmenorrhea, while 16 women reported having dyspareunia. Almost half of the patients in this group stated that they performed previous abortions (30 patients), half of them being on request (18) and 17 spontaneous.

The majority of women included in the study (86.08%) had no history of ectopic pregnancies. The diagnosis of endometriosis was relatively high, with 17 patients declaring that they were diagnosed with this pathology. Almost half of the included patients had impermeable fallopian tubes (30 patients) while their AMH ranged from 0.10 to 12.50, with a median of 2.00.

For 54 patients was performed IVF to obtain embryos, while for 24 was performed ICSI. Out of the 79 patients, 53 obtained biochemical pregnancies following the 145 ETs, 46 of these pregnancies were confirmed by ultrasound, and 27 of the pregnancies resulted in the birth of a live fetus.

Pregnancy rate

The analysis focused on the ability of ET techniques to obtain a BP. The deductible analysis must be carefully weighed as the study is not randomized. In this way we appreciate that there is a great possibility of bias that targets both the person who performed the procedure and the patient. The study was performed using 145 ETs performed in the study population comprising 79 patients (Table 1).

TABLE 1. Fresh vs Cryopreserved embryotransfer and biochemical pregnancy rate

Procedure	Failure - N (%)	Success - N (%)
Fresh day 3 embryo	11 (52.38)	10 (47.62)
Frozen day 3 embryo	7 (70.00)	3 (30.00)
Fresh day 5 blastocyst	13 (56.52)	10 (43.48)
Frozen day 5 blastocyst	48 (53.93)	41 (46.07)

We evaluated the existence of a statistical significance using the chi-square test of type χ^2 which is bidirectional for at least two samples that are different from each other. Table 2 shows the test result.

TABLE 2. χ^2 ET fresh versus Day 3 or Day 5 frozen test

Statistic χ^2	Degrees of freedom / Number	Value of p
1.02	4 / 145	0.7946

Since $p > 0.05$ there is no statistical significance. For this reason, we cannot say a difference between the success rate of one technique versus the other. We will analyze this result in more detail in the upcoming research.

The next aspect to be determined was which of the four different types of ET was the most successful in producing a BP, detected by high serum β-hCG levels.

We can see that the results with a success rate of 47.62% appeared in the case of using the fresh day 3 embryo, in this case half of the 21 ET having as finality the BP. This result is followed by a 46.07% success rate of frozen day 5 blastocyst, obtaining 41 pregnancies out of the 89 ET performed. In order of success rate follows the fresh day 5 blastocyst, with a percentage of 43.48%, respectively 10 biochemical pregnancies obtained out of a total of 23 transfers performed. According to the data of the study, the ET with frozen day 3 embryo had the most modest success rate obtaining 3 biochemical pregnancies from a total of 10 ETs.

Live birth rate

The best success rate for the birth of a live fetus, according to Table 3, was in the case of using the frozen day 5 blastocyst, with a percentage of approximately 21.62% and a number of 24 ETs that led to a live newborn. The ET using frozen day 3 embryo and fresh day 5 blastocyst had both success rates of 15.38% being at the same level. On the last position regarding the success rate was the ET made with fresh day 3 embryo which led to a single birth.

TABLE 3. Success rate on live birth for each type of ET

Procedure	Failure – N (%)	Success – N (%)
Fresh day 3 embryo	20 (95.24)	1 (4.76)
Frozen day 3 embryo	11 (84.62)	2 (15.38)
Fresh day 5 blastocyst	22 (84.62)	4 (15.38)
Frozen day 5 blastocyst	67 (78.38)	24 (21.62)

Premature birth rate

Table 4 shows the capacity of each type of ET to generate a premature birth rate.

The lowest rate of premature birth was recorded in the case of fresh day 5 blastocyst and in the case of frozen day 3 embryo, both procedures obtaining full-term infants. In the case of the frozen day 5 blastocyst, 21 full-term fetuses were born out of a total of 23. The most modest results appeared when using the fresh day 3 embryo, the only newborn obtained being premature.

TABLE 4. Fetal premature birth rate by each type of ET

Procedure	Failure – N (%)	Success – N (%)
Fresh day 3 embryo	1 (100.00)	0 (0.00)
Frozen day 3 embryo	0 (0.00)	2 (100.00)
Fresh day 5 blastocyst	0 (0.00)	4 (100.00)
Frozen day 5 blastocyst	2 (7.41)	21 (92.59)

Abortion rate

According to Table 5, the highest abortion rate (80%) occurred with fresh day 3 ET. The next procedure as abortion rate (approximately 37.14%) was that with frozen day 5 blastocyst. The ET with fresh day 5 blastocyst and the one with frozen day 3 embryo had very low and approximately equal rates of abortions.

TABLE 5. Rate of abortion by ET

Procedure	Yes - N (%)	No - N (%)
Fresh day 3 embryo	4 (80.00)	1 (20.00)
Frozen day 3 embryo	1 (33.33)	2 (66.67)
Fresh day 5 blastocyst	2 (33.33)	4 (66.67)
Frozen day 5 blastocyst	13 (37.14)	22 (62.86)

DISCUSSIONS

The highest success rate of BP was 47.62% and occurred in the use of fresh day 3 embryo, the next being 46.07% of frozen day 5 blastocyst, then fresh day 5 blastocyst - 43.48% and ET with frozen day 3 embryo - 30%.

The best success rate for the birth of a live fetus was when we used frozen day 5 blastocyst (21.62%), while when using ET with frozen day 3 embryo and fresh day 5 blastocyst the live birth rate was 15.38% and ET with fresh day 3 embryo led to a single birth (4.76%) (although in the previous study this type of embryo had the highest BPR).

The lowest rate of premature birth (0%) was recorded in the case of fresh day 5 blastocyst and in the case of frozen day 3 embryo, both procedures obtaining only full-term newborns, then 7.41% using frozen day 5 blastocyst, and an interesting result (100% rate) was in the case of fresh day 3 embryo, the only newborn being premature (although in our previous study this type of embryo had the highest rate of biochemical pregnancies).

The highest abortion rate (approximately 80%) occurred in fresh day 3 ET, then 37.14% in frozen day 5 blastocyst, while ET with fresh day 5 blastocyst and frozen day 3 ET have the lowest abortion rates 33.33%.

Due to these results, we highlight the importance of reporting LBR after ART procedures and not to focus on the BPR. It is obvious that one can have very good PR after a certain type of ET, but after this, because of high abortion rate and high premature births, to result a very low number of children. And we should not forget that the success of the procedures is represented by the number of healthy children the couples will take home.

International specialty literature reports show similar results, a favorable prognosis for the transfer of frozen blastocysts in terms of implantation rate and live birth rate, if after fertilization all embryos are frozen and transferred on subsequent cycles (RR 1.30) BPR (45.8% vs 64.3% cryopreserved) LBR (45% vs. 60.55%) [15]. Nevertheless, it was observed a favorable prognosis of thawed ET in terms of spontaneous abortion rate, perinatal mortality rate, preeclampsia rate, low birth weight rate, premature birth rate and antepartum hemorrhage rate [16]. This is due to the fact that during the cycle of ovarian hyperstimulation that results in obtaining oocytes, drugs negatively influence the quality of the endometrium. Thus, by impairing the implantation, the number of pregnancies obtained will be affected consecutively. Uterine contractility can also be increased in the controlled hyperstimulation cycle, and the implantation window is sometimes already exceeded at the time of transfer [17,18]. At the same time, were observed reduced associated malformations in the case of thawed ET [19] and the low incidence of hyperstimulation syndrome [20-24].

In a Cochrane meta-analysis that included 27 randomized clinical trials involving 4031 women we observed the following results: in the “fresh group”, the rate of live infants after fresh blastocyst was higher than that after fresh embryo transfer (13 randomized clinical trials including 1630 patients, low quality evidence, OR 1.48, 95%CI 1.20 to 1.82, I^2 = 45%). No differences in cumulative pregnancy rates were observed after a single ovarian puncture between the fresh and cryopreserved

group (5 randomized clinical trials, including 632 patients) [25-28]. Obstetric and perinatal patient outcomes were better also in the frozen groups OR 0.89, CI = 95%; I2 = 71% (with very low-quality results) [11]. Comparing the groups with blastocysts, a higher rate of clinical pregnancies was observed for the use of fresh blastocysts (27 randomized clinical trials, 4031 patients, OR 1.30, 95%CI 1.14 to 1.47, I2 = 56%, evidence of moderate quality) [29-32].

We can observe that the majority of studies have results with not enough statistical significance, and we found the same aspect in our study. Analyzing this aspect, we concluded that there are two neuralgic points to mention in this study: the first is that we have very few cases of transfer with frozen day 3 embryos and a biochemical success rate of only 30% and there is practically the possibility that the tests performed, related to test strength and sample size to be wrong - for this reason we did not go further with the analysis for patients with day 3 embryos and we focused more on day 5 blastocysts.

The second problem is that we studied patients with various procedures (some patients underwent several IVF or ICSI procedures and transferred several embryos, some fresh, some cryopreserved, some – day 3 and some – day 5). The multiple statistical analysis performed tried to cover and compensate this aspect, in order to obtain correct results. The results are accurate, at least for patients who received day 5 blastocysts (fresh or frozen).

Another aspect worth mentioning is the number of patients included. As the pregnancies obtained in the ART clinic then went to the attending physician for monitoring, perhaps in another city or even another country, in many cases the contact with the patient was lost. We included in the final study only the patients who signed the consent for inclusion in the study, then filled in the online questionnaire, updated the data in the questionnaire during pregnancy and then at birth and kept in touch with the investigators. Because we wanted to investigate several variables (pregnancy rate, pregnancy progression and birth outcome), many patients were excluded because they did not respond later on (out of 500 patients invited, only 300 patients signed consent, and of these only 79 kept in touch with investigators throughout the study).

The patients' explanations for this phenomenon were represented by the fact that contacting investigators to update the data along the way creates a negative psychological impact, as many patients did not get pregnant after the first or second ET and the investigators reminded them of this failure by asking the updating questions.

We are talking about emotionally sensitive patients who are under intense psychological stress. A retrospective and not a prospective study could have been a solution in this respect but given that patients after ART procedures return to the attending physician in the city of residence, it would have been much more difficult to establish contact with them later, as they would not have signed the informed consent giving us the right to contact them during pregnancy and after birth. If we would have analyzed only the rates of biochemical and clinical pregnancies, a retrospective study would have been easier to achieve than a prospective study. But because we wanted to be able to analyze the evolution throughout pregnancy and birth, we would not have had access to these data of patients who gave birth in another maternity than "Prof. Dr. Panait Sîrbu" Clinical Hospital of Obstetrics and Gynecology, Bucharest.

This project was developed in order to follow the evolution of these embryos from the cell stage to the first years of life, to observe how we can improve in the future the chances of couples with infertility to get pregnant quicker on one hand and to achieve healthier babies on the other hand, by taking into account what happens after birth and the evolution of children. The patients signed in the consent that they agree to be contacted annually even after the birth of the children, in order to obtain comparative data on the evolution of the children obtained after fresh ET versus cryopreserved ET. This long-term study can come as a complement to the present trial.

CONCLUSIONS

The fresh day 3 embryo has the highest BPRs, but also the highest abortion rate and the highest premature birth rate, in the study performed not resulting any term birth.

Frozen day 3 embryo has the lowest BPRs, but also the lowest abortion rate and the lowest premature birth rate.

Fresh day 5 blastocyst has a good BPR, low abortion rate and the lowest premature birth rate.

Frozen day 5 blastocyst ET has a very good BPR, the highest birth rate, high abortion rate and low premature birth rate.

After this study we can conclude that the use of frozen day 5 blastocyst had the best results in terms of the ultimate goal: the birth of a live fetus at term.

Conflict of interest: none declared
Financial support: none declared

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