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## COMPARATIVE ANALYSIS OF PROGRAMS WITH THE USE OF NATIVE AND CRYOPRESERVED OOCYTES IN IN-VITRO FERTILIZATION PROCEDURES

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### ANNOTATION

This article release data about comparative survival of oocytes after freezing, fertilization, blastocyst formation and clinical results of IVF/ICSI programs in International Clinical Centre of Reproductology Persona during period from 2016 to 2019.

**Key words:** IVF, ICSI, vitrification, oocyte, donation, cryopreservation, blastocyst.

**Abbreviations:** TVP - transvaginal puncture, OP - cancellation of transfers, PE - transfer of embryos, CNB - frequency of pregnancy, hCG - chorionic gonadotropin, IVF - in vitro fertilization, ICSI - intracytoplasmic injection of one sperm.

### INTRODUCTION

Due to the vetrification method, embryo cryopreservation has been a routine and effective procedure in ART programs(1,2,16) over the past decades. Until recently, the oocyte cryopreservation procedure was considered experimental due to the fragility of the oocyte and its inability to tolerate the slow freezing process. Using the vetrification method has made significant changes to reproduction. New data demonstrate a high survival rate of oocytes after thawing. Since October 2012, the Practice Committee of the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technologies have determined that oocyte freezing and thawing procedures shall no longer be considered experimental( 3 ).

Oocyte cryopreservation technology has become an extremely important option for preserving the fertility of patients undergoing surgical and chemotherapeutic treatment for oncological diseases (3-5), as well as for women who plan to delay maternity. It has been proven that the age of the oocyte at the time of extraction has a stronger effect on pregnancy than the age of a woman who plans to have the pregnancy (6,7).

Oocyte freezing is a popular procedure in the ART program using donor oocytes. This greatly simplifies the organization of the use of donor material: it allows the formation of oocyte donor banks and eliminates the need for synchronization of the menstrual cycles of the donor and recipient (3).

The data on the effectiveness of IVF/ICSI programs with cryopreserved donor oocytes are varied. So, Kushnir et al. (8) found that the program using donor cryopreserved oocytes had a live birth rate of 9.0% (47.1%) less than fresh cycles

(56.1%); perhaps this was due to the fact that the study did not take into account factors that could affect birth rates (husband's sperm counts, couple's age, etc.). Several other studies have shown that programs using frozen oocytes had close efficiency factors with cycles where fresh oocytes were used. (9 -13). There are also studies where programs with fresh and cryopreserved oocytes of the same donor were compared, but different recipients. Their results showed identical birth rate in both groups (14, 15, 17).

### PURPOSE OF THE STUDY

Compare rates of fertilization, fragmentation, blastulation, pregnancy (PR) in IVF/ICSI programs using vitrified and fresh donor oocytes.

### MATERIALS AND METHODS

We conducted a continuous study of 307 IVF/ICSI programs in couples diagnosed with infertility using donor oocytes from 2016 to 2019 on the basis of the International Clinical Center for Reproduction PERSONA.

Indication for the use of donor oocytes were: premature ovarian exhaustion or a decrease in the ovarian reserve of the recipients, an age factor, several unsuccessful IVF programs with recipient's own cells, genetic or chromosome pathologies of the recipient, when the use of their own oocytes was impossible.

All patients were divided into 2 groups: the 1 group using fresh donor oocytes (129 programs) and the 2 group using vitrified donor oocytes (178 programs).

The average age of the donors who participated in this study was  $28 \pm 3.5$ . Various protocols were used to stimulate

superovulation: a short protocol, a protocol with GnRH antagonists, and a protocol with micronized progesterone.

The ovulation induction protocol was selected individually for each donor. Stimulation began from the 2nd-3rd day of the menstrual cycle. Follicular growth was monitored by transvaginal ultrasound and hormonal monitoring. Upon reaching a minimum of three follicles with a diameter of 19 - 20 mm, an ovulation trigger was introduced (hCG 10,000 IU or GnRH agonists 0.2 mg), transvaginal puncture of the follicles and oocyte recovery were performed after 34-36 hours.

A total of 124 transvaginal puncture (TVP) programs were completed in 67 oocyte donors. Some of the obtained oocytes were distributed between the recipients, some were cryopreserved for asynchronous donorship. Oocyte freezing was performed 2-3 hours after TVP.

For cryopreservation and thawing of oocytes, the Dr. Kuvayama's method of vitrification was used (16). Commercial kits (Vitrification and Warming KIT, Kitazato, Japan) were used throughout the study.

After thawing, the oocytes were transferred to Sage 1 Step culture medium with HAS (0.1 ml Origio, Denmark) coated with mineral oil (Liquid paraffin Origio, Denmark) under conditions of 6% CO<sub>2</sub>, 5% O<sub>2</sub>, 89% N<sub>2</sub> and a temperature of 37 °C in a SOOC Benchtop incubator for pre-incubation. After 1.5-2 hours, the oocytes were fertilized by ICSI method.

Fertilization of fresh oocytes was carried out either by the classical IVF method (kIVF), or using ICSI 2-4 hours after TVP. They were cultivated in universal IVF medium (0.1 ml; IVF Universal, Denmark) or Sage 1 Step culture medium with HAS (0.1 ml Origio, Denmark) coated with mineral oil (Liquid paraffin Origio, Denmark) under 6% CO<sub>2</sub>, 5 % O<sub>2</sub>, 89% N<sub>2</sub> and a temperature of 37 °C in a Benchtop SOOC incubator.

Fertilization was evaluated after 16-20 hours, and the presence of two pronuclei was considered a normal fertilized zygote. Embryos were cultured in microdrops with a volume of 50 µl (Sage 1STEP; Origio, Denmark) under mineral

oil ((Liquid paraffin; Origio, Denmark) in Oosafe plates (SparMedApS, Denmark).

Embryos were evaluated on the first, third, and fifth days of cultivation. Embryo transfer was carried out on the 3rd or 5th day. The remaining embryos, after receiving the informed consent of the recipients, were frozen by vitrification method.

Modern packages of statistical analysis MS Excel on PC were used to carry out the analysis of calculations. The data obtained during the study were subjected to statistical processing by the method of variation statistics using the online calculator [www.medstatistic.ru](http://www.medstatistic.ru). Arithmetic mean (M) and standard deviation ( $\pm$  SD) were calculated for quantitative indices, the data was presented as  $M \pm SD$ . Two-sided Student t-test was used to compare mean values. Absolute (n) and relative (%) values describe qualitative variables. The  $\chi^2$  criterion was used to compare frequencies and qualitative variables.

## RESULTS

From 2016 to December 2019, 309 programs with donor oocytes were carried out. In group I, 129 patients with native donor oocytes and in group II 178 patients with thawed donor oocytes.

Table 2 shows the characteristics of the recipients and the results of embryology.

The average count of oocytes in the first group was 35% higher ( $6.98 \pm 2.64$  oocytes) than the average count in the second group ( $4.56 \pm 2.1$ ), but the difference was not significant statistically. Oocyte survival after thawing procedure reached 87.8%.

Fertilization rates were similar in both groups (78% versus 76%, respectively,  $p > 0.05$ ). However, crushing rates and the growth rate of blastocysts in the groups had a significant statistical difference. The fragmentation in the first group was 98%, in the second group - 91.7% ( $\chi^2 = 30.792$ ,  $p < 0.001$ ). The blastulation frequency on the 5th day of cultivation in the first group was 59%, in the second group - 39%, which had a significant statistical difference ( $\chi^2 = 47.694$ ,  $P < 0.001$ ).

Table 1 - Characterization of embryological results of the programs with fresh and vitrified donor oocytes.

Characteristics	I group with native (fresh) oocytes	II group with vitrified (frozen) oocytes	P value
Number of programs	129	178	
The average age of the recipient, years	42,1 $\pm$ 6,16	41,8 $\pm$ 5,8	p=0,971
Oocytecount(MII)	901	924	
The average count of oocytes per patient	6,98 $\pm$ 2,64	4,56 $\pm$ 2,1	P=0,478
% thawingprocedure	-	87,8%	
% fertilization	78%	76%	P=0,340
% fragmentation	98%	91,7%	P<0,001
% reaching a blastocyst	59%	39%	p<0.001

Table 2 - Clinical results of the cycles performed with fresh and vitrified donor oocytes.

Characteristics	Native (fresh) oocytes	Vitrified (frozen) oocytes	P value
Totalembryo transfer	80	93	
% embryotransfer on day 3	12,5%	48%	P=20,008
% embryotransfer on day 5	87,5%	52%	
The average number of embryos per transfer	1,3±0,476	1,4±0,597	P=0,896
Embryocryopreservation%	76%	46%	P=12,994
HCG%	46%	36%	P=0,237
Pregnancy rate%	45%	35%	P=0,197

Embryo transfer in Group 1 was performed in 80 patients and in Group 2 - 93 patients. The average count of embryos for transfer in both groups was not statistically different ( $1.3 \pm 0.476$  versus  $1.4 \pm 0.597$ ).

The number of cases with a positive level of  $\beta$ -hCG in the blood 14 days after ET and the pregnancy rate in the groups with fresh and vitrified oocytes did not significantly differ in the groups and amounted to 46% against 36%, and 45% against 35%, respectively.

Table 3 - Reasons for canceling embryo transfers in the programs with fresh and vitrified oocytes.

Group	Cancellation of embryo transfers				
	No fertilization	No fragmentation	Stagnation	Cryopreservation of embryos	Total
1 group	-	-	2 (4.3%)	45 (95.7%)	47 (36.4%)
2 group	1	1	20 (25%)	58 (72.5%)	80 (44.9%)

The number of transfer cancellations in group 1 was 47 (36.4%); of which 2 cases (4.3%) were associated with embryostagnation (on day 5 of cultivation), and 45 patients underwent cryopreservation of embryos in connection with segmentation of the cycle. In group 2, Transfer cancellation was 80 (44.9%); of which 20 cases (25%) were associated with stagnation (in two cases there was no fertilization, in one case there was no fragmentation, in the remaining cases there was no reaching the blastocyst), 58 cases (72.5%) were associated with cryopreservation of embryos.

### INFERENCE

In the course of the study, we established the absence of statistically significant differences in fertilization rates in groups using native and cryopreserved donor oocytes.

There was a significant difference in the percentage of fragmentation and blastulation in favor of programs with fresh donor oocytes, which may be the result of incomplete restoration of oocyte organelles after thawing procedure. But the survival rate was 87.8%, which corresponds to the data of

world clinics. It was established that the average number of thawed oocytes transferred to recipients for fertilization was lower by 35% compared to fresh oocytes.

The difference in the frequency of pregnancy was 10% more in group 1 with fresh oocytes than in group 2 using vitrified oocytes, but it was statistically insignificant, which allows the use of oocyte cryopreservation technology at the embryological stage in ART programs without significantly reducing the chances of getting pregnancy.

### CONCLUSION

To increase the effectiveness of IVF programs, we recommend using from 8-9 vitrified donor oocytes.

Strictly assess the morphological characteristics of donor oocytes before the vitrification procedure.

Make a careful selection of donors for further stimulation and freezing of oocytes, taking into account the criteria of cryotolerance, fertilization, crushing, growth to blastocysts and BNB.

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## РЕЗЮМЕ

**СРАВНИТЕЛЬНЫЙ АНАЛИЗ ПРОГРАММ С ИСПОЛЬЗОВАНИЕМ НАТИВНЫХ И ВИТРИФИЦИРОВАННЫХ ООЦИТОВ В ПРОЦЕДУРАХ ЭКСТРАКОРПОРАЛЬНОГО ОПЛОДОТВОРЕНИЯ**

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В статье приведены данные сравнения выживаемости ооцитов после разморозки, оплодотворения, частоты дорастания до бластоцисты и клинические результаты программ ЭКО/ИКСИ с использованием свежих и витрифицированных донорских ооцитов на базе МКЦР PERSONA в период с 2016 по 2019 г.

*Ключевые слова:* ЭКО, ИКСИ, витрификация, ооцит, донорство, криоконсервация, бластоциста.

## ТҮЙІНДЕМЕ

**НАТИВТЫ ЖӘНЕ КАТЫРЫЛҒАН ООЦИТТЕРДІ ҚОЛДАНУ АРҚЫЛЫ ДЕНЕДЕН ТЫС ҰРЫҚТАНДЫРУ БАҒДАРЛАМАЛАРЫН САЛЫСТЫРУ**

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Мақалада 2016 жылдан бастап 2019 жылға дейін PERSONA ICRC негізінде жаңа және витрификацияланған донорлық ооциттерді қолданумен IVF / ICSI бағдарламаларының клиникалық нәтижелері және ерітілгеннен, ұрықтанудан кейін ооциттердің өмір сүруін салыстыратын мәліметтер келтірілген.

*Түйін сөздер:* ЭКҰ, ИКСИ, витрификация, ооцит, донор болу, криоконсервация, бластоциста.