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Research Article

Evaluation of Efficiency of Cryopreservation of Human Gametes and Embryos in Assisted Reproductive Technology Program

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Abstract

Cryopreservation of gametes and embryos is one of the most essential problems of modern reproductive medicine. A comparative analysis of the effectiveness of the use of native and frozen material in the practice of an embryological laboratory is carried out in this article. We studied 120 semen samples of healthy men (sperm donors). One hundred cases (83%) demonstrate mobility attenuation after defrosting from 67% to 84% versus initial value. During the investigation of 35 native and 23 frozen oocytes, we taped an insignificant decreasing of cleavage rate (from 98,4% to 84,2%, p = 0,018). We performed 1289 defrosted gametes and embryos transfers and used closed (n = 611, CryoTip, USA) and open (n = 678, CryoTop, Japan) type carriers. Most of the indicators turned out to be better when using open carriers: the survival rate was 84.8% for the closed type and 95.1% for the open type (p < 0.0001), the pregnancy rate was 39.5% and 44.2% (p = 0.001), the birth rate of 72.7% and 67.3% (p = 0.003), the loss of 27.3% and 24.3% (p = 0.044). The data obtained allow us to conclude that vitrification of morphologically normal gametes does not impair the effectiveness of cryoprograms and maintains their reproductive status. The use of open-type carriers for vitrification and storage of embryos demonstrates higher embryo survival after cryopreservation, pregnancy rates and low loss rates. It is assumed that this occurs due to direct contact of the biological object (embryo) with liquid nitrogen and a high freezing rate, which may be a decisive factor in determining the success of vitrification on open carriers.

Keywords: *Assisted Reproductive Technology; Cryopreservation; Human Embryo*

Abbreviation

ART: Assisted Reproductive Technologies

Introduction

The development of assisted reproductive technologies (hereinafter - ART) requires a high level of cellular technology at the embryological stage. Cryopreservation of gametes and embryos is a clinically significant method for increasing the cumulative frequency of pregnancy [1]. During the standard procedure for ovulation stimulating and *in vitro* fertilization of oocytes in more than 60% of cases, embryos suitable for freezing remain after transfer. As a result, cryopreservation creates the possibility of treatment continuing in the absence of pregnancy in the stimulated cycle. Frozen embryos can be thawed and transferred to the patient's uterus in subsequent cycles that do not require the use of expensive hormonal stimulation schemes for superovulation [2]. Another obvious advantage of cryopreservation is undoubtedly the possibility of canceling embryo transfer in the stimulated cycle if the patient develops severe hyperstimulation syndrome and also if there are risk factors for implantation impairment (bleeding, insufficient secretory transformation and endometrial polyps, as well as extremely difficult embryo transfer) [3].

Study Objective

The study objective is to compare the effectiveness of native and frozen gametes use in cycles of assisted reproductive technologies and to evaluate the effect of the carrier type for freezing and storing embryos on the results of cryoprograms and cryoprotocols.

Materials and Research Methods

During the study we used oocytes, sperm and human embryos, the investigation of which was carried out in compliance with international ethical and legal standards for the treatment of human embryos [Art. 18 of the Council of Europe Convention on the Protection of Human Rights and Dignity of Human Being when Using the Advances in Biology and Medicine, 1997]. The study of gametes and embryos was carried out as part of *in vitro* fertilization cycles at the embryological laboratories of Closed Joined Stock Company "Medical IDK company", Samara, Russia. The use of germ cells and human embryos in scientific research was authorized by the ethics committee of Samara State Medical University of the Russian Ministry of Health. Gametes and embryos were identified under the control of a stereo microscope (Nikon, Japan). For incubation under conditions of 5% O_2, SOOC incubators (Australia) were used. Vitrolife media (Sweden) were used to cultivate embryos up to 5 - 6 days of embryonic development. Kitozato media and protocols (Japan) and open media types of CryoTop carriers (Japan) were used for vitrification of oocytes. FertiPro media (France) and closed carriers - 0.25 straws (France) were used for vitrification of germ cells. Irvine Scientific media (USA) and carriers of open CryoTop (Japan) and closed CryoTip (USA) types were used for vitrification of embryos. Blastocysts of 5 - 6 days cultivation were evaluated according to the international classification [4].

Statistical processing of the results was performed on a computer in the statistical calculations environment R (R v.3.5.3, RStudio v.1.1.463), the primary data input was performed using MS Excel spreadsheets. We used descriptive statistics methods, tests for comparing proportions, including the exact binomial for small samples and the one-sample proportional test with continuity correction for large samples.

Research Results and Discussion

One of the main characteristics that determine the quality of spermatozoa and their cryotolerance is the kinetic characteristic. Sperm motility was evaluated before and after freezing and thawing procedures. 120 sperm samples of healthy men (sperm donors) were investigated. In 100 cases (83%), the decrease in mobility after thawing ranged from 67% to 84% of similar values of the indicator. The study showed that in 25% of cases there was a low cryotolerance of sperm. Subsequently, selected semen samples were used for fertilization in ART programs. The pregnancy rate in programs of *in vitro* fertilization and intracytoplasmic injection of sperm into the egg using cryopreserved donor sperm was 42%. To assess the effect of vitrification, we compared the efficiency of using native and frozen oocytes (Table 1).

Indicators	Native (fresh) oocytes	Vitrified (frozen) oocytes	P
Number of cases	35	23	-
Average age of patients, years	38,9	38,8	-
Average number of oocytes transferred to patient	9,6	9,3	-
% of thawing	Not rated	90	-
% of fertilization	85,6	73,1	0,061
% of fission	98,4	84,2	0,018*
% of growth to blastocyst	61,9	53,4	0,170
Average number of embryos per transfer	1,2	1,2	-
Positive test for chorionic gonadotropin, %	48,6	56,5	0,219
Pregnancy rate, %	45,7	47,8	0,470
Implantation frequency, %	44,1	39,3	0,394

Table 1: *Main indicators of ART programs quality using native (fresh) and vitrified (frozen) oocytes.*

From the data above it is clear that the indicators of the group of vitrified oocytes were slightly lower compared with the group of native oocytes. This may be due to a violation of the restoration of the cleavage spindle, incomplete recovery of oocyte organelles after thawing. However, the indicators of pregnancy rate when using fresh and frozen oocytes are not statistically different, which allows us to use oocyte cryopreservation technology at the embryological stage of ART programs without reducing chances of getting pregnant.

An important element of the freezing process is the carrier in which the sample is located during cryopreservation and subsequent storage [5]. Carriers for cryopreservation of oocytes and embryos should ensure reliable preservation of embryos during cooling, storage and heating. Carriers are divided into two groups - open and closed. In the case when the carrier is of an open type, a drop of a cryoprotective solution with the embryo is in direct contact with liquid nitrogen (for example, CryoTop, Japan). In a closed carrier (for example, CryoTip, USA), the cryoprotective solution does not contact with liquid nitrogen and cooling occurs through the protective cover. Both open and closed carriers have a protective cover that prevents mechanical damage to the frozen embryo.

To assess the effectiveness of different carriers, the following indicators were investigated: survival rate, average transfer embryo (s) score, average number of transfer embryos, pregnancy rate, birth and loss rates (Table 2).

Indicators	Closed carrier (CryoTip)	Open carrier (CryoTop)	P
Average age of patient, years	34,1	33,5	-
Number of thawed embryos	611	678	-
Survival rate, %	84,8	95,1	< 0,0001*
Average embryos score/transfer	3,9	4,1	-
Average number of embryos/transfer	1,2	1,3	-
Frequency of pregnancy, in % for transfer	39,5	44,2	0,001*
Birth rate, in % on clinically verified pregnancy	72,7	67,3	0,003*
Loss rate, in % on clinically verified pregnancy	27,3	24,3	0,044*

Table 2: *Main criteria for effectiveness of cryoprograms when using embryos vitrified in closed and open types of carriers.*

During 2015 - 2016, 1289 embryos were thawed on the types of carriers under consideration. Embryo survival rate on closed carriers was 84.8%, on open - 95.1% and this difference is statistically significant ($p < 0.0001$). The average transfer embryo score in group I was 3.9, in group II - 4.1. The average number of embryos per transfer is 1.2 and 1.3, respectively. The pregnancy rate was statistically significantly different ($p < 0.0001$) and amounted to 39.5% (group I) and 44.2% (group II). The birth rate for clinical pregnancy, taking into account the known outcomes, was 72.7% in group I, 67.3% in group II, and losses were 27.3% and 24.3%, respectively. The rates of birth and loss were statistically significantly different. In general, the results of the study allow us to conclude that using open carriers lead to a higher level of embryo survival during cryopreservation.

Conclusion

Thus, vitrification of gametes and embryos allows preserving the quality and normal morphological and physiological status of cells and makes it possible to use them in the future. This allows us to apply this technology on a par with native material without compromising the quality and effectiveness of assisted reproductive technology programs. At the same time, open carriers for freezing and storing embryos demonstrate higher cryoprogram performance compared to closed ones. Clinical indicators (indicators of clinical pregnancy, childbirth and pregnancy loss), which directly depend on the level of survival, also demonstrate a statistically significant difference in favor of using open carriers in embryological practice.

Disclosure

The authors report the absence of any conflicts of interest in the planning, implementation, financing, and use of the study results.

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