Cryobiology 93 (2020) 33–36

Contents lists available at ScienceDirect

Cryobiology

journal homepage: http://www.elsevier.com/locate/cryo

Does human oocyte cryopreservation affect equally on embryo chromosome aneuploidy?

Nataliia Buderatska [a], Juliia Gontar [a], Ihor Ilyin [a], Serhii Lavrinenko [a], Maryna Petrushko [b], Taisiia Yurchuk [b,*]

[a] Medical Center "IGR", Pobedy Avenue, 121 B, Kiev, Ukraine[a]
[b] Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, 23 Pereyaslavskaya Str, Kharkov, 61015, Ukraine[b]

A B S T R A C T

Oocytes cryopreservation as an important part of assisted reproductive technologies, which should ensure after warming not only intact oocyte morphological characteristics, but also their genetic apparatus stability. However, the meiotic spindle is very sensitive to the temperature fluctuations that can lead to unequal chromosome segregation during meiosis and as a consequence can cause embryo aneuploidy after oocyte fertilization. The aim of the study was to estimate the oocytes cryopreservation impact on human embryo chromosome aneuploidy. It has been shown that fertilization rate of the cryopreserved oocytes did not differ from fresh ones (83.1% vs 84% respectively). The number of blastocysts obtained from cryopreserved oocytes was less than that obtained from fresh oocytes, however, their morphological characteristics were better if compared the fresh oocytes. Our results showed different cryopreservation impact on aneuploidy rates of certain chromosomes in embryos obtained from cryopreserved oocytes. They had an increased aneuploidy of chromosome 13 and a decreased nondisjunction of chromosome 18 and sex chromosomes.

Oocytes cryopreservation is an important part of assisted reproductive technologies (ART) [12]. According to a new trend of delayed maternity, the oocytes and embryos are cryopreserved at early women reproductive age. Such an approach of fertility preservation permits to plan the most convenient life period for maternity, to accumulate oocytes for women with low ovarian reserve, to treat infertility by ART with oocytes donation and to spare fertility in women undergoing gonadotoxic medical treatment [1,6,13]. In this regards, it is important to preserve not only morphological and functional characteristics of oocytes but either to keep intact their genetic apparatus. Oocytes' meiotic spindle is very sensitive to the environmental changes and their microtubules gets depolymerize by slight temperature fluctuation [9,15]. Meiotic apparatus disorders may cause an improper interaction between the oocyte chromosomes and as a result the number of aneuploid embryos could be increased. Using a preimplantation genetic testing for aneuploidy (PGT-A) permits to select the embryos with a diploid chromosome number [14]. It has been reported that physical stress during oocyte vitrification affect the chromosome configuration and DNA integrity and suggested to determine the optimal condition, in particular, changing equilibration (up to 10 min) [8]. However, other authors reported that oocyte cryopreservation did not increase an aneuploidy in embryos, the implantation and live birth rates compared with the embryos derived from fresh oocytes cycles [7].

The aim of this work was to assess the impact of human oocytes cryopreservation on chromosomal status of preimplantation embryos.

1. Materials and methods

The study was performed in the "IGR Medical Center" from April 2015 to April 2018. All the manipulations with gametes and embryos were approved by the Steering Committee in Bioethics "The protection of human embryo *in vitro*. Report by the working part in the protection of human embryo and fetus (CDBI-CO- GT3) (Strasbourg, 19 June 2003) and with the decision of the Bioethics Committee of "IGR Medical Center" and Biomedical Ethical Committee of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, in line with the Law of the Ukraine "On Personal Data Protection" (2010). Women involved as the oocyte donors corresponded the requirements of the Ministry of Health of Ukraine No. 787 dated of September 9th, 2013 "On approval of the procedure for using the assisted reproductive technologies in Ukraine". All the oocyte donors were anonymous. Patients who underwent an infertility treatment using

* Corresponding author.
E-mail address: taisiya.yur@gmail.com (T. Yurchuk).
[a] igrclinic@gmail.com
[b] cryo@online.kharkov.ua

https://doi.org/10.1016/j.cryobiol.2020.03.002
Received 3 December 2019; Received in revised form 30 January 2020; Accepted 3 March 2020
Available online 8 March 2020

donor oocytes and PGT-A have signed a written consent on these procedures, which fully comply with the legislation of Ukraine and the "Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine" (Oviedo, 1997).

Ovarian stimulation was controlled using the follicle-stimulating hormone ("Fostimon", IBSA, Switzerland) started on the 2nd day of the menstrual cycle, with the initial doses of 150–225 IU/day. Gonadotropin releasing hormone (GnRH) ("Cetrotide", Serono, Switzerland) was used as an antagonist (0.25 mg/day) with the dominant follicle size of 14 mm. Ovulation was induced by 0.4 mg GnRH agonist ("Diphereline", Beaufour Ipsen Internation, France), which caused the dominant follicle to reach the diameter of 20 mm, and the serum estradiol level corresponded to the required concentration. Oocytes were retrieved by a transvaginal aspiration using sonography-guided technique in 36 h after ovulation induction.

383 donor oocytes from 29 ART cycles were used for quantitative and morphological assessments of derived embryos (262 oocytes obtained in 19 fresh oocyte cycles (group A) and 121 oocytes in 11 oocyte cryo cycles (group B)). The mean age of the donors in groups A and B were 27.2 ± 2.4 and 27.6 ± 4.8 years respectively.

The retrieved oocytes after hyaluronidase exposure (Sydney IVF Hyaluronidase, Cook Medical, Spencer, IA, USA) were denudated from the surrounding cumulus cells. All the mature oocytes (metaphase II stage) were selected for further intracytoplasmic sperm injection (ICSI) which was performed either 4–5 h after oocytes retrieval, or 1–2 h after oocytes warming.

The vitrification procedure was performed using Cryotech carrier for open system and Cryotec vitrification method (Cryotech, Japan). The survival rate of the cryopreserved oocytes based on the morphological integrity of the oocytes.

1.1. Preimplantation genetic testing

Day 3 embryos *Zona pellucida* was partially dissected using a laser device Saturn (Research Instruments, UK) for aneuploidy preimplantation genetic testing (PGT-A). If a blastocyst begun hatching on day 5 then 3–5 trophectoderm (TE) cells were biopsied using 25 μm polished biopsy pipette with assisted cutting by laser (Fig. 1).

PGT-A was performed using fluorescent DNA probes for chromosomes 13, 16, 18, 21, 22, X and Y (PB MultiVysion and CepX/CepY, Abbott, USA) and fluorescence microscope Olympus BX-51 (Olympus, Japan) equipped with an appropriate filter kit and the ISIS image processing program (Meta Systems, Germany).

1.2. Data analysis

The quantitative data distribution was verified by Shapiro-Uilka and Kolmogorov-Smirnov methods. Results are presented as the mean ± standard deviation. Statistical hypotheses were checked using criteria t, $\chi 2$ for significance levels $p < 0.05$, $p < 0.01$, $p < 0.001$.

2. Results and discussion

The mean number of mature oocytes obtained per one cycle in group A was 16.67 ± 6.25 and in group B was 15.00 ± 4.32. The oocyte viability after warming was 96.0%. Fertilization rate has no significant difference between the studied groups. The number of zygotes in groups A and B were 83.2% ($n = 262$) and 82.7% ($n = 121$) respectively. 87.6% of day 3 embryos continue their development in group A and 69.7% of them had a high morphological quality (Table 1). In group B, 88.2% of embryos reached the cleavage stage and 67.7% of them were characterized as morphologically normal. There was no significant difference between the groups by the number of day 3 embryos ($p > 0.05$).

In group A, 71.1% of blastocysts developed from the total number of zygotes and only 38.5% of them corresponded to AA grade quality.

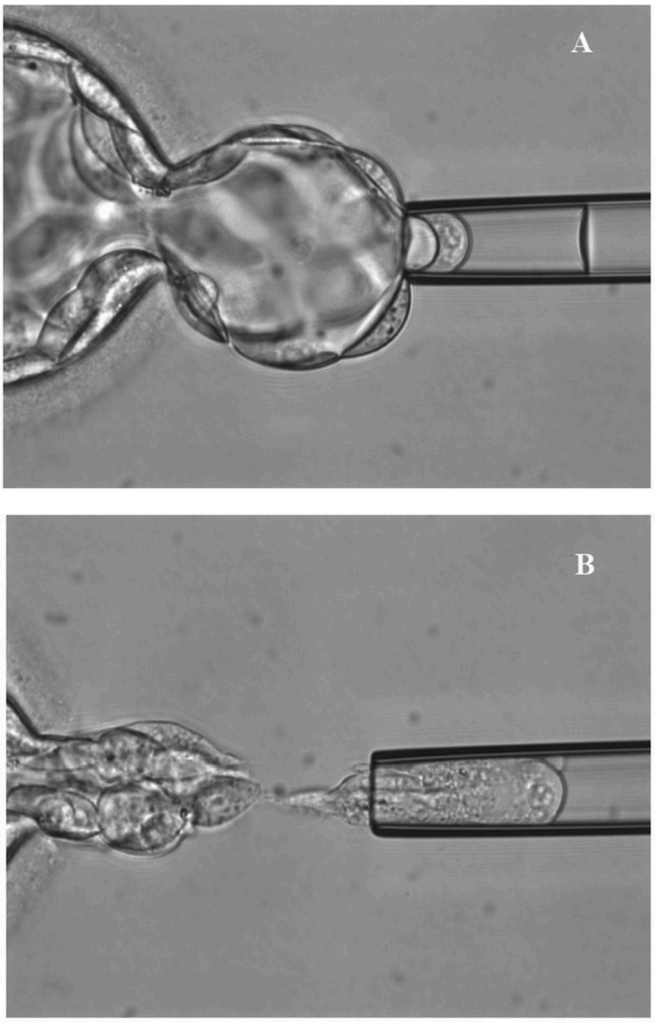

Fig. 1. Trophectoderm cell biopsy. A – trophectoderm cell aspiration, B – removal of biopsied trophectoderm cells, x400.

Table 1
Number of embryos resulting from *in vitro* fertilization of cryopreserved oocytes *vs* fresh oocytes.

Number of embryos	Group A, %	Group B, %
Zygotes	83.2 (218/262)	84.3 (102/121)
Day 3 embryos	87.6 (191/218)	88.2 (90/102)
High-quality day 3 embryos	69.7 (152/218)	67.6 (69/102)
Blastocysts	71.1 (155/218)	62.7 (64/102)*
High-quality blastocysts	38.5 (84/218)	55.8 (57/102)*

Note:* - difference is statistically significant in comparison with group A ($p < 0.05$).

Meanwhile, in group B 62.7% of embryos reached the blastocyst stage and 55.8% of them corresponded to AA grade.

Thus, despite the fact that the total number of blastocysts in group A was higher than in group B (71.1% *vs* 62.7% in groups A and B, respectively, $p < 0.005$), the number of good quality blastocysts was higher in group B compared with group A (55.8% *vs* 38.5% in groups B and A, respectively, $p < 0.05$). This morphological difference of embryos may result from the possible changes of oocyte chromosome set during cryopreservation [2,3]. Therefore, the next stage of the research was to study an influence of cryopreservation on the embryo chromosome status.

To determine an incidence of the chromosome nondisjunction 229

embryos in group A and 104 embryos in group B (Table 2) were analyzed.

There was no significant difference in the number of aneuploid embryos between the studied groups (54.1 *vs* 55.8% in groups A and B, respectively). At the same time the number of embryos with regular aneuploidies (71.2 *vs* 72.4% in groups A and B, respectively), embryos with ploidy disorders (20.2 *vs* 22.4% in groups A and B, respectively) and embryos with mosaic chromosome set (8.1 *vs* 5.2% in groups A and B, respectively) had no significant difference also.

The incidence of isolated and complex aneuploidies of certain chromosomes is represented in Table 3.

It was found that chromosomes 13 abnormality was increased in the embryos derived from cryopreserved oocytes compared with the fresh ones (36.2 *vs* 10.5%, group B and A, respectively). In addition, group B embryos showed a decreased aneuploidy rate of chromosome 18 and sex chromosomes (33.1 *vs* 17.2% and 39.5 *vs* 22.4% group A and B, respectively).

No significant differences in aneuploidy were found in other studied chromosomes (16, 21, 22). The frequency occurrence of isolated and complex abnormalities of all the studied chromosomes did not have significant differences between groups A and B (Table 3).

The incidence of clinical pregnancy in group A was 64.7% and in group B made 62.5%, indicating high morphological and functional quality of cryopreserved oocytes ($p > 0.05$).

High survival rate of cryopreserved oocytes were reported in numerous studies [4]. Meiotic spindle and chromosomes of the young healthy donors oocytes are resistant to cryopreservation, since the level of aneuploidy in the embryos developed from frozen and warmed female gametes corresponds to the fresh oocytes characteristics. Munne and co-authors [10] also concluded that aneuploidy rate of embryos in young donors is low and cryopreservation does not increase its level.

According to our results, the number of blastocysts derived from cryopreserved oocytes was less than that, derived from fresh oocytes; however, their morphological characteristics exceeded the comparison group. This fact prompted the study of genetic status of embryos, since the literature widely discusses the relationship between morphological characteristics and chromosome euploidy [5]. An interesting fact of our research underlies in the fact that despite the absence of differences in aneuploidy rate of the embryos resulting from *in vitro* fertilization of either fresh or cryopreserved oocytes, there are differences in aneuploidy of certain chromosomes between these groups. The effect of cryopreservation on the completion of the meiotic second division increases aneuploidy in some chromosomes and a decrease in others. Thus, the embryos derived from cryopreserved oocytes had an increased rate of chromosome 13 aneuploidy and a decreased number of the embryos with nondisjunction of chromosome 18 and sex chromosomes. Since the chromosome 13 belongs to group D (large acrocentric chromosome) according to Patau classification, it is likely that this structural feature and the stability disturbances of the meiotic spindle tubulin filaments caused by cryopreservation leads to an unequal segregation of the chromosome 13 at the second stage of meiosis [11]. Taking into consideration the structural features of this chromosome we can assume that major difference between the arm lengths in this group can be the reason of oocyte aneuploidy after cryopreservation. The rest analyzed chromosomes are submetacentric or metacentric and less affected during segregation. Moreover embryos derived from cryopreserved oocyte had decrease level of chromosome 18 and sex chromosome aneuploidy compared with fresh oocyte group. Apparently, cryopreservation acts as a selection factor. In this case embryos with aneuploidy on these chromosomes do not develop up to the blastocyst stage, when genetic testing was carried out. These are novel findings in the understanding of selective impact of cryopreservation on certain oocyte chromosome ploidy and can be used in counselling of couples undergoing infertility treatment in donor programs.

Table 2
Chromosomal status of embryos obtained from fresh and cryopreserved oocytes.

Parameters	Group A, %	Group B, %
Number of euploid embryos including:	**45,9 (105/229)**	**44.2(46/104)**
Number of XX embryos	48.6 (51/105)	52.2 (24/46)*
Number of XY embryos	51.4 (54/105)	47.8 (22/46)
Number of aneuploid embryos including:	**54.1(124/229)**	**55.8(58/104)**
Embryos with regular aneuploidy	71.7 (89/124)	72.4 (42/58)
Embryos with ploidy disorders	20.2 (25/124)	22.4 (13/58)
Number of embryos with mosaicism	8.1 (10/124)	5.2 (3/58)

Table 3
Distribution of non-disjunction among 13, 16, 18, 21, 22 and sex chromosomes in embryos.

Parameters	Group A, % (n)	Group B, % (n)
non-disjunction of 13 chromosome including:	10.5 (13/124)	36.2 (21/58)*
isolated mono- or polysomy	23.1	28.6
complex chromosome abnormality	76.9	71.4
non-disjunction of 16 chromosome including:	20.2 (25/124)	22.4 (13/58)
isolated mono- or polysomy	20.0	23.1
complex chromosome abnormality	80.0	76.9
non-disjunction of 18 chromosome including:	33/1 (41/124)	17.2 (10/58)*
isolated mono- or polysomy	21.9	25.0
complex chromosome abnormality	78.1	75.0
non-disjunction of 21chromosome including:	23.4 (29/124)	21.1 (17/58)
isolated mono- or polysomy	27.6	17.7
complex chromosome abnormality	72.4	82.3
non-disjunction of 22 chromosome including:	37.9 (47/124)	31.0 (18/58)
isolated mono- or polysomy	21.3	16.7
complex chromosome abnormality	78.7	83.3
non-disjunction of sex chromosomes including:	39.4 (49/124)	22.4 (13/68)*
isolated mono- or polysomy	20.4	25.0
complex chromosome abnormality	79.6	75.0

Notes: isolated mono- or polysomia: one chromosome is involved.
*– differences are statistically significant in comparison with group A ($p < 0.05$).

Funding

This study was supported by the National Academy of Science of Ukraine for Scientific Research, state registration No. 0116U003498.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cryobiol.2020.03.002.

References

[1] K. Baldwin, L. Culley, N. Hudson, H. Mitchell, S. Lavery, Oocyte cryopreservation for social reasons: demographic profile and disposal intentions of UK users, Reprod. Biomed. Online 31 (2015) 239–245.
[2] M.G.R.S. Camargos, J.K. Rodrigues, V.N. Lobach, T. El Cury-Silva, M.E.G. Nunes, A. F. Camargos, F.M. Reis, Human oocyte morphometry before and after cryopreservation: a prospective cohort study, Cryobiology 88 (2019) 81–86.
[3] J. Cheng, B. Jia, T. Wu, G. Zhou, Y. Hou, X. Fu, S. Zhu, Effects of vitrification for germinal vesicle and metaphase II oocytes on subsequent centromere cohesion and chromosome aneuploidy in mice, Theriogenology 82 (2014) 495–500.
[4] A. Cobo, J.A. García-Velasco, A. Coello, J. Domingo, A. Pellicer, J. Remohí, Oocyte vitrification as an efficient option for elective fertility preservation, Fertil. Steril. 105 (2016), 755-64.e8.

[5] A. Deng, W.H. Wang, Assessment of aneuploidy formation in human blastocysts resulting from cryopreserved donor eggs, Mol. Cytogenet. 8 (2015) 12.
[6] K. Devine, S.L. Mumford, K.N. Goldman, B. Hodes-Wertz, S. Druckenmiller, A. M. Propst, N. Noyes, Baby budgeting: oocyte cryopreservation in women delaying reproduction can reduce cost per live birth, Fertil. Steril. 103 (2015) 1446–1453, e1-2.
[7] K.N. Goldman, Y. Kramer, B. Hodes-Wertz, N. Noyes, C. McCaffrey, J.A. Grifo, Long-term cryopreservation of human oocytes does not increase embryonic aneuploidy, Fertil. Steril. 103 (2015) 662–668.
[8] Y.J. Jung, Y.P. Cheon, Improvement of the vitrification method suppressing the disturbance of meiotic spindle and chromosome systems in mature oocytes, Dev. Reprod. 18 (2014) 117–125.
[9] T. Lei, N. Guo, J.Q. Liu, M.H. Tan, Y.F. Li, Vitrification of in vitro matured oocytes: effects on meiotic spindle configuration and mitochondrial function, Int. J. Clin. Exp. Pathol. 7 (2014) 1159–1165.
[10] S. Munné, M. Alikani, L. Ribustello, P. Colls, P.A. Martínez-Ortiz, D.H. McCulloh, R. P. Group, Euploidy rates in donor egg cycles significantly differ between fertility centers, Hum. Reprod. 32 (2017) 743–749.
[11] K. Patau, The identification of individual chromosomes, especially in man, Am. J. Hum. Genet. 12 (1960) 250–276.
[12] M.P. Petrushko, Current state of cryopreservation of reproductive cells and embryos, Visnik NatsIonalnoi akademii nauk Ukraini 7 (2017) 44–53.
[13] A.M. Quaas, A. Melamed, K. Chung, K.A. Bendikson, R.J. Paulson, Egg banking in the United States: current status of commercially available cryopreserved oocytes, Fertil. Steril. 99 (2013) 827–831.
[14] L. Rienzi, C. Gracia, R. Maggiulli, A.R. LaBarbera, D.J. Kaser, F.M. Ubaldi, S. Vanderpoel, C. Racowsky, Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance, Hum. Reprod. Update 23 (2017) 139–155.
[15] T. Yurchuk, M. Petrushko, B. Fuller, Science of cryopreservation in reproductive medicine - embryos and oocytes as exemplars, Early Hum. Dev. 126 (2018) 6–9.