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PREGNANCY RATES IN IN VITRO FERTILIZATION CYCLES AFTER THE TRANSFER OF EMBRYOS WITH MOSAIC KARYOTYPE IN PATIENTS WITH ADVANCED MATERNAL AGE

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The technique of preimplantation genetic testing of embryos for aneuploidy makes it possible to select embryos with a balanced karyotype in case when assisted reproductive technologies are used. The literature provides data on the possibility of getting a normal pregnancy in patients when embryos with a low level of mosaicism of 20–40 % in the karyotype are transferred. The rates of blastocyst formation with balanced and mosaic karyotypes in women of different ages, and the clinical pregnancy rates after embryo transfer with mosaic karyotype in patients with advanced maternal age, were investigated in the present work. Increasing women's age was associated with decreased total blastocyst formation rate and the rate of blastocysts with high morphological quality (p=0.0044 and p=0.0190, respectively). Women aged 38 years or older are associated with an increased frequency of aneuploid embryo and blastocyst formation with a mosaic karyotype when using assisted reproductive technologies (p=0.00065 and p=0.0330, respectively). No statistically significant difference was found in clinical pregnancy rates between transferring embryos with a balanced karyotype and transferring blastocysts with a low level of mosaicism (p>0.05). A decrease in microscopic parameters of ejaculate leads to failures in the ability of sperm to fertilize a mature oocyte, even when the method of intracytoplasmic sperm injection was used when manipulating gametes in vitro (p=0.00504, p=0.000292, respectively).

Key words: preimplantation genetic testing for aneuploidy, mosaicism, clinical pregnancy, assisted reproductive technologies.

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НАСТАННЯ ВАГІТНОСТІ У РЕЗУЛЬТАТІ ПЕРЕНОСУ ЕМБРІОНІВ ІЗ МОЗАЇЧНИМ КАРІОТИПОМ ПРИ ПРОВЕДЕННІ ЕКСТРАКОРПОРАЛЬНОГО ЗАПЛІДНЕННЯ ЖІНКАМ СТАРШОЇ ВІКОВОЇ КАТЕГОРІЇ

Застосування передімплантаційного генетичного тестування ембріонів на анеуплоїдії дає можливість обрати ембріони зі збалансованим каріотипом при застосуванні методів допоміжних репродуктивних технологій. У літературі наведені дані про можливість отримати нормальну вагітність у пацієнток при переносі ембріонів з низьким рівнем мозаїцизму 20–40 % у каріотипі. У даній роботі досліджено частоту формування бластоцист зі збалансованим та мозаїчним каріотипом у жінок різного віку та перевірено частоту настання клінічної вагітності при переносі ембріонів з мозаїчним каріотипом у пацієнток старшої вікової групи. Показано, що збільшення віку жінки веде до зниження загальної частоти формування бластоцист та частоти формування бластоцист високої морфологічної якості (p=0,0044 та p=0,0190, відповідно). Вік пацієнтки старше 38 років асоціюється з ростом частоти формування анеуплоїдних ембріонів та бластоцист з мозаїчним каріотипом при застосуванні методів допоміжних репродуктивних технологій(p=0,00065 та p=0,0330, відповідно). Не виявлено статистично значущої різниці у частоті настання клінічної вагітності при переносі ембріонів із збалансованим каріотипом й при виконанні ембріотрансферу бластоцист з низьким рівнем мозаїцизму (p>0,05). Зниження мікроскопічних параметрів еякуляту призводить до порушення здатності сперматозоїдів запліднювати зрілі ооцити метафази II, навіть за умови використання методу інтрацитоплазматичної ін'єкції сперматозоїда при роботі з гаметами in vitro (p=0,00504, p=0,000292, відповідно).

Ключові слова: передімплантаційне генетичне тестування на анеуплоїдії, мозаїцизм, клінічна вагітність, допоміжні репродуктивні технології.

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The selection of the embryo with the highest implantation potential is the main purpose when the method of in vitro fertilization is used in infertility treatment. The main criterion for assessing the quality of an embryo is its morphological properties. The most perspective is embryo that has formed a blastocyst on the fifth or sixth day of development. A blastocysts' morphology is classified according to the Gardner system [6]. Additionally, in practice, morphokinetic observation is used for embryo selection. However, the morphological and morphokinetic features of the blastocyst do not provide information about the embryo karyotype [2]. On the other hand, the use of preimplantation genetic testing of embryos for aneuploidy makes it possible to select embryos with a balanced karyotype for transfer into the uterus cavity of patients undergoing infertility treatment using assisted reproductive technologies (ART) [7]. However, according to literature data, the chances to get an embryo with a balanced karyotype in women older than 39 years do not exceed 10 % [5]. On the other hand, recently, a number of studies have presented information on the possibility to achieve a normal pregnancy in patients when transferring embryos with a low level of mosaicism of 20–40 % in the karyotype [1].

The purpose of the study was to investigate the rates of formation of blastocysts with balanced and mosaic karyotype in women of different ages and to detect the clinical pregnancy rates after the transfer of embryos with mosaic karyotype in patients with advanced maternal age.

Materials and methods. Primary data collection and laboratory studies were carried out at the "Clinic of Professor Feskov O.M." (Kharkiv). The study was approved by the Ethics Committee of Sana-Med Ltd. (Human Reproduction Center " Clinic of Professor Feskov O.M.") in accordance with Protocol No. 2 dated December 5, 2022. The patients signed an Informed Consent for the Use and Processing of Data. During the period 2023–2024, totally 109 couples were considered. The study included only couples with male factor infertility; no gynecological pathologies were detected in the female partners. The results of preimplantation genetic testing for aneuploidy (PGT-A) of embryos in vitro were analyzed. Frozen blastocysts with a balanced or mosaic karyotype were transferred, in cases when the level of mosaicism was no more than 40 %. Embryos with mosaic aneuploidies involving certain chromosomes (13, 18, 21, X and Y), commonly referred to as ''viable aneuploidies'' in the nonmosaic state, were excluded from ETs. Taking into account the age of the women in the couple, the mentioned patients were divided into 2 groups.

Group 1 included 67 couples: the age of the women was in the range of 24–37 years; the age of the men was from 35 to 47 years. Group 2 was formed from 42 married couples: women aged from 38 to 43 years; the age of the men in this group was in the range of 35–49 years. Women aged 38 years and older were allocated to a separate group, since according to the presents in literature data, a part of embryos with a balanced karyotype is expected to decrease significantly right in female patients aged >38 years [1]. The Control Group was considered separately: embryos obtained using donor gametes. In total, preimplantation genetic testing was performed for 281 blastocysts: 144, 85 and 52 embryos in Groups 1, 2 and the Control Group, respectively. Microscopic analysis of ejaculate was performed according to WHO recommendations from 2021 [4]. A severe male factor of infertility (decreased motility, concentration, and pathological spermatozoa morphology) was detected in all men in couples in the considered Groups 1 and 2.

Controlled ovulation stimulation (COS) of patients in couples was performed using the ant-GnRH protocol. Fertilization of the MII oocytes was performed by the Intracytoplasmic Sperm Injection (ICSI) technique. Embryos were cultured to the blastocyst stage in GAIN medium Single-step (Austria) at a temperature of 36.9ºC-37.1ºC and a CO2 content of 5.5 %–5.7 %. The blastocysts were evaluated by morphological characteristics of the intracellular mass (ICM) and trophectoderm (TE), according to the criteria of D. Gardner [6]. The ICSI procedure, the evaluation of embryos' morphology and blastocysts' biopsy were carried out using the inverted microscope "Leica DMi 8" (serial number 418445). Freezing of embryos was performed by the method of vitrification according to M. Kuwayama, cryotop, modification [10]. Preimplantation genetic testing of blastocysts for aneuploidy was done with the technique of the next-generation sequencing [7].

The data were checked for compliance with the law of normal distribution. Statistical hypotheses were tested using the chi-square criterion at significance levels of 0.05, 0.025, 0.01 [3]. Calculations were performed using the Apache Open Office 4.0.0 software package (Sana-Light Ltd., Sana-Med Ltd.).

Results of the study and their discussion. Considering 281 blastocysts, biopsy was performed on the fifth day of culture for 143 embryos, on the sixth day for 131 blastocysts, and on the seventh day for 7 blastocysts, respectively. A photo of a blastocyst before the biopsy procedure is shown in Figure 1.

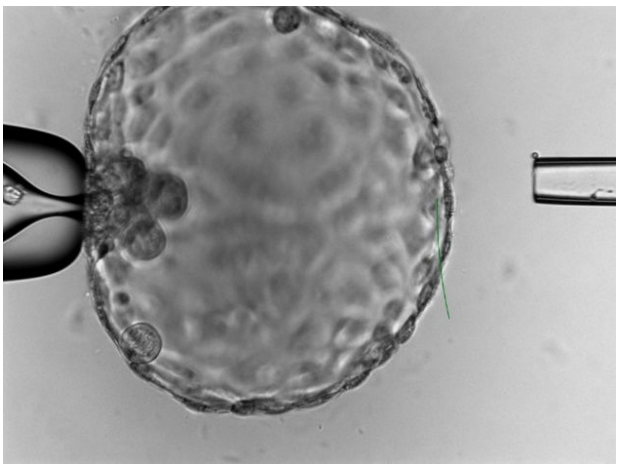

Fig. 1. Blastocyst 6AA according to the D. K. Gardner classification in vitro, before the procedure of biopsy. Magnification: x250.

As a result of COS, a total of 603 oocytes at the MII maturity stage were obtained from patients in Group 1. The fertilization rate in this group, when using the ICSI method, was 61.5 % (371 normal zygotes with two pronuclei). The total blastocyst formation rate (BFR) among patients in Group 1 was 43.6 % (162 blastocysts). Blastocysts of high morphological quality, suitable for biopsy and vitrification, developed from 38.8 % of fertilized oocytes (144 blastocysts).

Totally 512 mature MII oocytes were retrieved from women in Group 2. The fertilization rate in these patients was 60.4 % (309 zygotes with two pronuclei). The total BFR in Group 2 was 32.7 % (101 blastocysts). Blastocysts suitable for biopsy and vitrification developed from 27.5 % of fertilized oocytes (85 blastocysts).

In the control Group 3, 93 mature MII oocytes were fertilized by ICSI. The fertilization rate in this group was 80.6 % (75 zygotes with two pronuclei). The total BFR and the rate of formation of blastocysts of high morphological quality in Group 3 were 76. 0 % (57 blastocysts) and 69.3 % (52 blastocysts), respectively.

The rate of fertilization of MII-oocytes did not differ statistically between patients in Groups 1 and 2. However, the rate of fertilization of mature MII-oocytes in the Control Group was statistically higher, compared with the fertilization rates both among women in Group 1 (df = 1, $\chi2_{crit.}$=12.1007, $\chi2_{fact.}$=12.9214, p=0.00504) and patients with advanced maternal age in Group 2 (df = 1, $\chi2_{crit.}$=13.1183, $\chi2_{fact.}$=13.9799, p=0.000292). Data about the blastocyst formation rates in experimental Groups 1, 2 and the Control group are presented in Table 1.

Table 1

Blastocyst formation rates in patients with different maternal age

Group of patients	Total BFR		BFR with high-quality morphology	
	N	%	N	%
Group 1	162*,**	43.6	144***	38.8
Group 2	101*	32.7	85***	27.5
Control Group	57**	76.0	52	69.3
Statistics	*df = 1, $\chi2_{crit.}$=8.1123, $\chi2_{fact.}$=8.569, p=0.0044 **df = 1, $\chi2_{crit.}$=9.2236, $\chi2_{fact.}$=10.0329, p=0.0024 ***df = 1, $\chi2_{crit.}$=9.1485, $\chi2_{fact.}$=9.6481, p=0.0190			

Notes: p – significance level, N – the number of blastocysts.

The total blastocyst formation rate and the rate of formation of blastocyst with high morphological quality were significantly higher in patients in Group 1, compared with these indicators among patients with advanced maternal age in Group 2 (p=0.0044 and p=0.0190, respectively). Mentioned results demonstrated the negative effect of advanced maternal age on the process of blastocyst formation in in vitro fertilization cycles.

The results of preimplantation genetic screening for aneuploidy of blastocysts obtained from patients in Groups 1, 2 and the Control Group are presented in Table 2.

Table 2

Results of preimplantation genetic testing for aneuploidy of blastocysts obtained from women of different age categories

Group of Patients	Total number of blastocysts, N	Part of euploid blastocysts, N (%)	Part of aneuploid blastocysts, N (%)	Part of blastocysts with mosaic karyotype, N (%)
Group 1	144	81(56.3 %)[1]	51(35.4 %)	12 (8.3 %)[3]
Group 2	85	28 (32.9 %)[1,2]	41(48.2 %)	16 (18.8 %)[3]
Control Group	52	31 (59.6 %)[2]	16 (30.8 %)	5 (9.6 %)
Statistics	[1]df = 1, $\chi2_{crit.}$=10.7268, $\chi2_{fact.}$=11.6426, p=0.00065 [2]df = 1, $\chi2_{crit}$=8.3059, $\chi2_{fact.}$=9.3622, p=0.00395 [3]df = 1, $\chi2_{crit.}$=4.4567, $\chi2_{fact.}$=5.4806, p=0.0330			

Notes: p – significance level, N – the number of blastocysts.

It was found out that the part of euploid embryos was statistically higher in patients in Group 1 and in the Control Group compared to the data obtained for patients with advanced maternal age in Group 2 (p=0.00065 and p=0.00395, respectively). In addition, it was found that the part of blastocysts with a mosaic karyotype was higher in women in Group 2 than among patients under 38 years of age in Group 1 (p=0.0330, respectively). In six patients from Group 2 there were no embryos with a balanced karyotype or blastocysts with a low level of mosaicism in the karyotype. The mentioned in this study results confirm that the part of aneuploid embryos increases with the age of the woman [9, 13].

Totally 83 frozen embryo transfers (ETs) were performed within the cryoprotocols. Of these, 47 transfers of embryos with balanced karyotypes were performed for Group 1 patients; 22 transfers of blastocyst with normal karyotypes and 14 ETs of blastocyst with low level of mosaicism in karyotype were performed for women in Group 2. The clinical pregnancy rate following balanced karyotype embryo transfer was 48.9 % (23 pregnancies) for Group 1. Successful implantation among Group 2-women was 40.9 % (9 pregnancies) and 35.7 % (5 pregnancies) after ETs of euploid blastocysts and ETs embryos with mosaicism levels not higher than 40.0 %, respectively. The clinical pregnancy rate was not statistically different in the considered groups, both when transferring embryos with a balanced karyotype and when performing ET with blastocysts with a low level of mosaicism (p>0.05).

Thus, in the present work it was shown that poor microscopic sperm parameters lead to a decrease in fertilization rates even when the method of ISCI is used. Currently, the data presented in the literature according to the influence of spermogram parameters on the fertilization rates in vitro are ambiguous. For example, Toan D. Pham (2025) and co-authors showed a negative effect of a decrease in the part of sperm

with normal morphology on the fertilization rates and the blastocyst formation rates when using ART methods [11]. On the other hand, Francesco Del Giudice and colleagues (2022) claim in their study that spermogram parameters do not affect the fertilization process and the onset of clinical pregnancy in ART [8]. This work did not reveal the effect of the paternal or maternal age on the frequency of fertilization of mature MII oocytes in in vitro fertilization cycles.

In our study a negative effect of advanced maternal age on the process of formation of blastocysts with normal morphology has been demonstrated. The literature data on this is contradictory. For example, V. S. Vanni et al. (2017) showed in their study that increasing of maternal age negatively affects the process of blastocyst formation [12]. On the other hand, in the work of Korkmaz C. et al. (2015), it was not proven that the women's age over 35 years reduces the rates of blastocyst formation [9].

It was shown that the clinical pregnancy rates after the transfer of blastocyst with balanced karyotype and transferring low mosaic karyotype-embryo are comparable. Currently, the results of clinical pregnancy when using embryos with a mosaic karyotype, obtained by a number of authors in different studies, are ambiguous. For example, Lei Zhang (2019) claims that the implantation rate of embryos with a normal karyotype does not differ from the clinical pregnancy rate when transferring blastocysts with a low level of mosaicism [14]. At the same time, Ying Xin Zhang and colleagues (2020) showed that the implantation potential of a mosaic embryo is significantly lower than in the case of transferring a blastocyst with a balanced karyotype [15].

Conclusions

1. The negative impact of a decrease in male fertility indices on the ability of sperm to fertilize a mature oocyte has been demonstrated, even when using the ICSI technique when manipulating gametes in vitro (df = 1, $\chi 2_{crit.}$=12.1007, $\chi 2_{fact.}$=12.9214, p=0.00504; df = 1, $\chi 2_{crit.}$=13.1183, $\chi 2_{fact.}$=13.9799, p=0.000292).

2. It has been shown that increasing of maternal age leads to a decrease in the total blastocyst formation rates and the rates of formation of the blastocyst with high morphological quality (p=0.0044 and p=0.0190, respectively).

3. It was proved that the women's age over 38 years is associated with an increase in the frequency of formation of aneuploid embryos and blastocysts with a mosaic karyotype when using ART methods (p=0.00065 and p=0.0330, respectively).

4. No statistically significant difference was found in the clinical pregnancy rates when transferring embryos with a balanced karyotype and when performing embryo transfer of blastocysts with a low level of mosaicism (p>0.05).

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ELECTROCARDIOGRAPHIC DYNAMICS OF THE ST SEGMENT AND T WAVE IN NON–ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION

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To assess the characteristics and dynamics of ST-segment and T-wave changes in non–ST-segment elevation myocardial infarction, 200 patients were examined, including 164 men and 36 women; the mean age was 62.21±9.38 years. Based on coronary angiography findings, patients were divided into two groups: Group I included 94 patients with non–ST-segment elevation myocardial infarction and non-obstructive coronary artery lesions (stenosis <50 %); Group II included 106 patients with non–ST-segment elevation myocardial infarction and obstructive coronary artery lesions (stenosis≥50 %). In Group I, predominantly transient electrocardiographic changes were observed. During the acute phase, ST-segment depression in leads V4–V6 was most common (62.8 % of patients), and less frequent in leads II, III, and aVF (21.3 %). In Group II, ST-segment depression was deeper and more persistent; ST depression≥1 mm was recorded in 79.2 % of patients, predominantly in leads V4–V6 and II, III, aVF, which was significantly more frequent than in Group I (p=0.016). No significant intergroup differences were found for isolated T-wave inversion or for the combined ST↓+T pattern. ST-segment normalization on days 5–7 occurred significantly more often in Group I (p<0.001). Progression to transmural myocardial infarction (ST-segment elevation myocardial infarction) was observed only in Group II (p=0.019).

Key words: non-ST-segment elevation myocardial infarction; electrocardiogram; ischemic heart disease; obstructive and non-obstructive coronary artery lesions.

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ЕЛЕКТРОКАРДІОГРАФІЧНА ДИНАМІКА СЕГМЕНТА ST І ЗУБЦЯ Т ПРИ ІНФАРКТІ МІОКАРДА БЕЗ ПІДЙОМУ СЕГМЕНТА ST

З метою оцінки особливостей динаміки змін сегмента ST і зубця Т у пацієнтів з інфарктом міокарда без підйому ST обстежено 200 пацієнтів, з них 164 чоловіки і 36 жінок, середній вік 62,21±9,38 років. Пацієнти були розділені на 2 групи: І група включала 94 пацієнтів з ІХС та інфарктом міокарда без підйому сегмента ST з необструктивним ураженням коронарних артерій; ІІ група – 106 пацієнтів з ІХС та інфарктом міокарда без підйому сегмента ST з обструктивним ураженням коронарних артерій. У пацієнтів І групи відмічені переважно транзиторні зміни ЕКГ. У гострому періоді на ЕКГ переважала горизонтальна або низхідна депресія сегмента ST у відведеннях V4–V6 (у 62,8 % пацієнтів), рідше — у відведеннях II, III і aVF (у 21,3 %). У ІІ групі депресія сегмента ST була вираженою і більш стійкою. У 79,2 % пацієнтів реєструвалася депресія ST≥1 мм, переважно у відведеннях V4–V6 і II, III, aVF, що в порівнянні з І групою мало значущий характер (p=0,016). Щодо інверсії зубця Т і поєднання ST↓+T між групами істотних відмінностей не відзначалося. Нормалізація ST на 5–7 добу значно частіше відзначалася у пацієнтів в І групі. Перехід в трансмуральний інфаркт міокарда спостерігався лише у пацієнтів ІІ групи. Найбільш виражена відмінність між групами виявлена за ознакою переходу в трансмуральний інфаркт міокарда (p<0,001).

Ключові слова: інфаркт міокарда без підйому сегмента ST, електрокардіограма, ішемічна хвороба серця, обструктивне та необструктивне ураження артерій.

Non–ST-segment elevation myocardial infarction is a type of acute coronary syndrome characterized by partial coronary artery occlusion, leading to reduced oxygenated blood flow to the myocardium and myocardial necrosis. This condition requires urgent medical care because it may result in fatal outcomes [3, 10, 12, 15].

According to published data, approximately 800,000 cases of acute coronary syndrome are registered annually, and about 60 % of these cases correspond to non–ST-segment elevation myocardial infarction. The electrocardiogram is most often the first diagnostic test performed in suspected acute coronary syndrome; ST-segment depression and/or T-wave inversion may be detected. Knowledge of electrocardiographic patterns that help localize the culprit coronary artery in non–ST-segment elevation myocardial infarction is clinically important; however, it remains insufficiently studied and contemporary evidence is limited [2, 6, 14].