Nosenko O. M., Seylova A. I. Embryological results in married couples with combination of male and female factors. Journal of Education, Health and Sport. 2019;9(1):519-534. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.3612171
http://ojs.ukw.edu.pl/index.php/johs/article/view/7673

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 03.01.2019. Revised: 11.01.2019. Accepted: 31.01.2019.

EMBRYOLOGICAL RESULTS IN MARRIED COUPLES WITH COMBINATION OF MALE AND FEMALE FACTORS

O. M. Nosenko, A. I. Seylova

Odessa National Medical University

Abstract

The treatment of married couples with a combination of male and female infertility factors is quite complicated and often requires assisted reproductive technologies (ART), but according to ESHRE, after ICSI, the pregnancy rate is 28.4% for aspiration and 35.0% for transfer. One of the ways to improve the effectiveness of ART in this case is to use additional methods of selection of sperm and fertilized oocytes. **The purpose of the study** was to evaluate the embryological results of conducting cycles of ART in married couples with a combination of female and male infertility factors using modern methods of selection of sperm and embryos. **Material and methods.** There were 213 married couples of group I under prospective observation with a combination of male and female factors who had applied for ART. All men in group I suffered from pathozoospermia. Control group K consisted of 34 couples with secondary infertility of tube genesis, who consisted of conditionally somatically healthy women with a history of childbirth, without fallopian tubes, and their men with normozoospermia. The study group I was randomized into two groups: the main group A (n = 100) - the ART cycle was performed according to the proposed personalized method with additional methods for the selection of gametes and embryos; comparison group B (n = 113) - pre-conceptual training and the ART cycle were performed according to conventional methods. Groups A and B were stratified each into 3 subgroups. The A1 (n = 32) and B1 (n = 36) subgroups included married couples in whom women had

chronic anovulation; in subgroup A2 (n = 37) and B2 (n = 35) - married couples in whom women had tubal infertility; in subgroup A3 (n = 31) and B3 (n = 42) are married couples in which women had a combination of tubal and anovulatory factors of infertility. Group A used additional methods for the selection of sperm and embryos: fluorescence in situ hybridization, examination of morphology of organelles of moving sperm at magnification × 6 600, time lapse microscopy of embryos and comparative genome-hybridization, sequencing of the new generation. **Results.** The use of modern methods for the selection of gametes and embryos has resulted in a statistically significant increase in the percentage of mature oocytes from the total number of oocytes by 1.18 times, the number of received embryos from one woman - by 1.27, the number of received embryos of excellent and good quality per woman - by 1.85, the percentage of embryos of excellent and good quality from the total number of embryos - 1.45 times. **Conclusions.** In patients with a combination of male and female infertility factors, additional selection of gametes and embryos is a way to improve the effectiveness of infertility treatments in the ART cycles.

Key words: infertility; combination of female and male infertility factors; fluorescence in situ hybridization; motile sperm organelle morphology examination; time lapse of embryos; comparative genomic hybridization; next generation sequencing; embryological results.

The treatment of married couples with a combination of male and female infertility factors is quite complex and often requires the use of assisted reproductive technologies (ART). Given the low quality of sperm in these cases, such a method of ART is widely used as an intracytoplasmic sperm injection (ICSI) [19]. According to the latest data from the European Society for Human Reproduction and Embryology (ESHRE), the European IVF Monitoring Consortium (EIM) (2018), a total of 776,556 cycles were conducted in 2014 from 39 countries and 1,279 establishments offering ART services treatment, including 146 148 - by *in vitro* fertilization, 362 285 - by ICSI, ie the number of ICSI cycles exceeded the similar in vitro fertilization rate by 2.48 times. However, after ICSI, the pregnancy rate was 28.4% for aspiration and 35.0% for transfer [19].

These data need to further seek to improve the conduct of ART in married couples with a combination of male and female infertility factors. One way to address this is to use additional methods for the selection of sperm and fertilized oocytes.

Genetic FISH research on sperm is recommended for men with severe oligozoospermia [6], as well as with severe teratozoospermia regardless of its type and is a

necessary study for infertility [5, 14]. At the same time, the literature emphasizes the need to perform genetic FISH screening for all men who plan to perform ART / ICSI regardless of sperm count, which will allow them to make informed decisions about the feasibility of performing ART, taking into account the possible risks, as well as subsequent genetic transplantation. diagnostics [10]. The method of FISH analysis (Fluorescence in situ hybridization) allows you to objectively identify individual chromosomes and their individual parts on metaphase plates (chromosomes in a state of maximum condensation and visualization) or interphase nuclei (decondensed chromosomes, without a clear morphological structure) -genetic structure [13].

The main feature of ICSI is the direct introduction of sperm into the oocyte, which facilitates the development of fertilized embryos regardless of semen characteristics such as the concentration and motility of male gametes [25]. However, the outcome of ICSI depends on several factors, including oocyte quality, patient age, and the quality of single sperm selected for oocyte introduction. This choice is usually performed under an inverted microscope with magnification 200 × - 400 ×, which allows to detect both motility and normal morphology of sperm, based on the evaluation of their head, neck and tail. However, this selection revealed many limitations due to the use of a slight increase, which allows us to observe only the main morphological defects of sperm. To overcome the limits of conventional microscopy, a real-time, large-scale (greater than × 6,000) sperm examination technique known as motile sperm organelle morphology examination (MSOME), which deals exclusively with the presence of size, has been developed , number and location of vacuoles [4, 23, 27]. According to MSOME criteria, nuclear chromatin content is abnormal if the sperm head contains one or more vacuoles (0.78 ± 0.18 IM in diameter) occupying more than 4% of the normal nuclear region. The introduction of sperm selected by the above MSOME criteria is used in a modified ICSI technique, the so-called IMSI (intracytoplasmic morphologically selected sperm injection) [2, 12]. Accurate morphological assessment of the integrity of the sperm nucleus of humans is an important parameter associated, according to the authors of the method, with the frequency of pregnancy - at IMSI 66% compared with 30% with conventional ICSI.

IMSI is well described by the work of A.S. Setti et al. (2013), who reported that when sperm with large vacuoles were selected for injection, the possibility of such embryos to develop into blastocytes was lower [22]. This study is supported by the data of K. Knez et al. (2011) [11]. The data of A.S. Setti et al. (2013) also confirmed that IMSI increases the incidence of implantation in couples with male infertility and should be the method of choice

for these cases [21, 22]. This is consistent with data from other researchers suggesting that IMSI should have a clear role as a routine procedure in every IVF laboratory [3, 7, 9].

It is necessary to further study the features of the structure of sperm by MSOME at IMSI and the impact of this technique on the result of ARTin married couples with a combination of female and male infertility.

It is now known that the factor of infertility affects the quality of oocyte superovulation induced by induction, their ability to fertilize, the morphological characteristics of embryos, the nature of their crushing and development rate, the onset of pregnancy, the nature of its course and the frequency of complications. One of the most important criteria for the selection of embryos for further transfer into the uterine cavity is their ability to form a blastocyst. Embryo transfer in the blastocyst stage allows to increase the incidence of pregnancy up to 50–60% of cases for embryo transfer [24]. In the presence of marked changes in semen, despite the conduct of ICSI or IMSI, the quality of the embryos suffers [26].

To date, the only method of assessing embryo quality has been expensive and time-consuming pre-implantation diagnostics, requiring many conditions for its implementation, such as: presence of at least 5 embryos, development of embryos up to 6 days. However, in 2009 a new method appeared - embryoscopy using time lapse microscopy.

Embryoscopy - a technique based on time-lapse video technology, in which the embryo is scanned at 11 sections at certain intervals, and then the pictures are compiled into a video link [17]. It allows to increase the informativeness of estimation of growth and development of embryos, which facilitates and improves their selection, and, accordingly, helps to increase the frequency of onset of pregnancy [15].

Information on the morphokinetic features of embryos when combining male and female infertility factors is limited. The question of the effect of pathozoospermia on the quality and development of embryos remains open.

The prospect of embryo shooting is a promising technique with several potential benefits; however, data for available randomized controlled trials are still of very poor quality, and further studies are valid [1, 20]. There is insufficient evidence to support the use of infinite time compared to the traditional assessment of embryo selection.

In some cases, ART is perhaps the only way to achieve childbirth through couples through pre-implantation genetic research, a set of activities aimed at investigating the genetic structures of germ cells or embryonic cells at the stage prior to implantation of the latter into the uterine cavity [16].

Modern molecular diagnostics have led to the use of highly sensitive methods of pre-implantation genetic diagnostics, which are based on the phenomenon of nucleic acid hybridization. They can be divided according to the depth of the study into methods of restricted screening (FISH, polymerase chain reaction (PCR)) and methods of full-genome screening (chip diagnostics, sequencing of "new" generation, karyomaping); diagnostic based on amplification phenomenon (PCR) methods, FISH, and chip diagnostics, including “next generation” sequencing.

A new step in the development of pre-implantation PCR research has been a modification that provides the possibility of conditionally full-genome screening, based on the phenomenon of quantitative PCR [8]. Working with the troptoderm, it was possible to eliminate the phenomenon of "allele fallout" and to provide diagnostic accuracy at the level of 98.6%. However, the reaction requires quantitative real-time PCR at 96 loci in 4 repetitions, requiring one 3845-well plate per embryo and significantly limiting laboratory throughput without addressing the problem of exclusion of microdeletion syndromes.

The purpose of the study was to evaluate the embryological results of conducting cycles of ART in married couples with a combination of female and male infertility factors using modern methods of selection of sperm and embryos.

Material and methods

There were 213 married couples with a combination of male and female infertility factors of group I under prospective observation, who applied for artificial insemination in the reproductive medicine cycles of the Reproductive Medicine Center of the Center for Rehabilitation and Reconstructive Medicine (University Clinic) of the Odessa National Medical University. All men in group I suffered from pathozoospermia. Control group K consisted of 34 couples with secondary infertility of tube genesis, who consisted of conditionally somatically healthy women with a history of childbirth, without fallopian tubes, and their men with normozoospermia.

The study group I was randomized into two groups: the main group A (n = 100) - the ART cycle was performed according to the proposed personalized method with additional methods for the selection of gametes and embryos; Comparison group B (n = 113) - the ART cycle were performed according to the conventional method, following the requirements of the protocol on infertility treatment approved by the order of the Ministry of Health of Ukraine.

Groups A and B were stratified each into 3 subgroups. The A1 (n = 32) and B1 (n = 36) subgroups included married couples in whom women had chronic anovulation; in

subgroup A2 (n = 37) and B2 (n = 35) - married couples in whom women had tubal infertility; in subgroup A3 (n = 31) and B3 (n = 42) are married couples in which women had a combination of tubal and anovulatory factors of infertility. That is, the distribution of the main forms of infertility of groups A and B were homogeneous.

The inclusion criteria for Group I were: 22 to 38 years of age; the presence of a combination of male and female infertility factors; pathoospermia; the need for treatment of infertility by the methods of ART; women are good defendants.

Exclusion criteria from group I: genital defects, genital endometriosis, diabetes mellitus, HIV infection, thyrotoxicosis, malignant tumors, acute pelvic infectious process, azoospermia.

Spectral analysis on the AFS-500-2 sperm analyzer (BIOLA, CIS) was used to evaluate the semen fertilization ability. The norm was taken into account by semen suggested by WHO (2010).

Additional selection of sperm was performed by FISH and due to examination of morphology of organelles of mobile sperm at magnification × 6 600.

Anomalies in the sex chromosomes (X, Y) and autosomes (13, 18, 21) were determined using FISH. FISHs were examined using an Olympus BX60 fluorescence microscope at × 1000 magnification. Used VysisTM Aqua / Green / Orange Triple Band Filter Set and VysisTM DAPI / Green / Orange Triple Band Filter Kit.

The morphology examination of motile sperm organelle morphology examination (MSOME) was performed using inverted microscopy (ECLIPSE TE 2000 U, Nikon, Japan). Images were captured using a DC2 IMSI camera (Nikon, Japan) with a DICOM-compatible medical image monitor (EIZO, Ishikawa, Japan). The introduction of MSOME-selected sperm into the eggs of patients in group A was applied by a modified ICSI technique, the so-called intracytoplasmic morphologically selected sperm injection (IMSI).

Time-lapse microscopy was used for morphokinetic analysis of embryos from 100 married couples of group A. Fertilized oocytes were cultured using an integrated time-lapse monitoring system, which allows to perform morphokinetic analysis of embryo development over time with the help of measuring at specific time points (PrimoVision, Cryo-Innovation Ltd., Hungary, periodic green illumination) under standard cultivation conditions in an incubator for i IVF (37.0 °C, 6% CO_2 in humid air). Images were taken every 15 min in seven different focal points for at least 72 hours for each embryo. The video changes created from these images were used to control the cell cleavage anomalies and to determine the timing of

certain events in the cell cycle. The analysis was performed separately for each embryo. The splitting time and the time between splitting were evaluated.

Comparative genomic hybridization, next-generation sequencing was performed for the purpose of pre-implantation diagnostics. The cells of the troptodermal blastocyst membrane were examined, using a sequencing machine (Life-Thermofisher, USA). Embryos have been diagnosed as euploid, aneuploid or chaotically abnormal.

Statistical processing of the obtained data was performed using the program Excel. In the statistical processing of the study materials, the parameters of the general population were estimated according to the sample data; it was determined the mean M and the standard deviation error SE; the eligibility of the hypotheses was determined by statistical criteria: the t-test was used to compare the mean of the independent samples and the related (dependent) samples; χ^2-criterion - for analysis of conjugation of signs, comparison of frequencies of events.

Results of the study and their discussion

The mean age of the patients in group I was 30.67 ± 0.30 years, in the control - 31.12±0.58.

All men of women of the studied groups with a combination of female and male infertility were observed pathozoospermia.

In group I, sperm thinning time was 54.48 ± 0.03 min. and was longer than the same in group K (40.09 ± 1.55 min) by 1.36 times ($p < 0.01$), with the number of patients with prolonged sperm thinning time being 39.44%, whereas in all patients in group K had sperm thinning time within the WHO reference range. The mean volume of ejaculate was equal in men with pathozoospermia 3.23 ± 0.01 ml versus 4.01 ± 0.15 ml in patients with normozoospermia, ie it was less than 1.24 times ($p < 0.01$) due to the presence in group I in 50.70% of cases of high viscosity of semen.

No statistical difference was observed between groups I and K in levels of pH (7.46 ± 1.13 vs. 7.43 ± 0.03).

24.21% of men with pathozoospermia had oligozoospermia. Accordingly, the average number of sperm per 1 ml in patients in group I was less than the control by 1.95 times (48.44 ± 0.42 vs. 94.32 ± 2.79 million, $p < 0.01$).

A characteristic feature of spermatological study in group I was the presence of asthenosoospermia: in 66.20% of patients ($p<0.01$) the number of actively motile sperm was less than 25% and in 69.01% of men the total number of actively and slowly motile sperm was recorded at less than 50% ($p<0.01$), whereas no control was observed in such cases.

Accordingly, the percentage of actively motile sperm in men with pathozoospermia was lower 1.56 times (20.70 ± 0.03 vs. 32.32 ± 0.60%, p <0.01), slow-moving in 1.34 (24 , 66 ± 0,03 against 32,94 ± 1,37%, p <0,01), the total percentage of actively and slowly mobile - in 1,44 (45,36 ± 0,41 against 65,26 ± 0,93 %, p <0.01).

79.62% of patients in group I and 61.76% of group K had sperm without progressive movement, which had no statistically significant difference, but the average percentage of sedentary sperm in men with pathoospermia was 1.68 times higher (10.18 ± 0, 03 vs. 6.06 ± 1.14%, p <0.01).

The average percentage of fixed sperm in Group I exceeded the same in control by 1.55 times (44.46 ± 0.03 vs. 28.68 ± 0.34%, p <0.01). In 35.68% of persons with pathozoospermia registered specific gravity of the presence of fixed sperm below 50%, in the control of such men were not (p <0.01).

In men with pathoospermia, semen agglutination was observed in 45.07% of cases, in control less than 3.83 times (11.76%, p <0.01).

23.47% of group I patients suffered from piospermia, which was not observed in group K (p <0.01). The average number of leukocytes in the field of view was accordingly 2.57 times higher - 6.89 ± 0.03 versus 2.68 ± 0.29, p <0.01.

Normal sperm morphology in group I was recorded 1.53 times less frequently (67.74 ± 0.86 vs. 44.17 ± 1.23%, p <0.01), whereas degenerative forms were more frequently 1.73 times (55.83 ± 0.94 vs. 32.26 ± 0.86%, p <0.01). The average percentage of pathology of the head of sperm in group I was more 1.92 times (33.29 ± 0.40 vs. 17.38 ± 0.70%, p <0.01), necks - 1.51 (19.66 ± 0.15 vs. 13.00 ± 0.42%, p <0.01), tail - in 1.44 (2.88 ± 0.80 vs. 2.00 ± 0.01%, p <0.01).

The average number of live sperm in men with pathoospermia was less than that in the control by 1.25 times (71.45 ± 0.80 versus 89.38 ± 0.26%, p <0.01), while the dead was greater in 2, 95 (31.38 ± 0.80 vs. 10.62 ± 0.26%, p <0.01).

Groups A and B were homogeneous in terms of spermatological examination.

According to FISH results, the level of aneuploidy in the sperm of men in group A ranged from 0.11 to 8.55%, while in the control from 0.07 to 0.19% (reference limit - up to 0.25%). The number of patients with elevated sperm aneuploidy in group A was 81/100 (81.00%). The mean level of aneuploidy in group A was 1.94 ± 0.38% and exceeded that in the control (0.12 ± 0.02%) 16.17 times (p <0.01).

A direct correlation was found between the level of aneuploidy and the presence of: asthenozoospermia (r = 0.39, p <0.01) and teratozoospermia (r = 0.43, p <0.01). No

significant correlation between the level of aneuploidy and the presence of oligozoospermia has been reported. Among the aneuploidy, the most common were: the presence of two 18 chromosomes in 62/81 (76.54%) cases, two X chromosomes in 14/81 (17.28%) cases, the absence of one of the sex chromosomes - 6/81 (7, 41%).

The morphokinetic analysis of the embryos selected for the first transfer (Table 1, Table 2) was performed.

Table 1

The time division of blastomeres according to time-lapse microscopy, M ± SE, in hours

Group	t1	t2	t3	t4	t5	t6	t7	t8
A1, n=32	28,38± 0,77 a3,k	28,89± 0,91 a3,k	38,07± 0,75 a2,k	48,03± 0,95 a2,a3,k	55,38±0,79 a2,a3,k	56,92± 0,99 a2,a3,k	58,18±1,58 a2,a3,k	63,85± 1,72 a2,a3,k
A2, n=37	24,41± 0,62 a1,a3	26,80± 0,61 a1,a3	37,45± 0,63 a3	42,70± 0,93 a1,a3	49,10± 0,81 a1,a3	51,62± 0,92 a1,a3,k	54,29± 1,16 a1,a3,k	57,82±1,41 a1,a3,k
A3, n=31	28,40± 0,89 a2,k	29,28± 0,81 a1,a2,k	40,39±0,78 a1,a2,k	54,47± 1,68 a1,a2,k	55,14± 0,93 a1,a2,k	56,13± 1,08 a1,a2,k	59,71±1,95 a2,k	68,53± 1,86 a2,k
K, n=34	24,15± 0,79	26,62± 0,37	36,32±0,87	39,09± 0,63	51,01± 1,29	53,95± 1,06	55,82± 1,10	59,31± 1,54

Note. a1, a2, a3, k – statistically significant difference with indicators, subgroups A1, A2, A3, group K ($p<0,05$)

Table 2

The distribution of time intervals between the separation of embryos into blastomeres according to tetrafer microscopy, M ± SE, in hours

Group	cc1	cc 2	cc 3	cc 4	cc 5	cc 6	cc 7
A1, n=32	0,51± 0,04 a2,k	4,58± 0,78 a2,a3,k	5,55± 0,57 a2,a3,k	16,36± 0,91 a2,a3,k	1,44± 0,32 a3,k	1,32± 0,33 a2,a3,k	5,64± 0,15 a2,a3,k
A2, n=37	1,92± 0,21 a1,a3	9,52± 1,28 a1,a3	2,51± 0,34 a1,a3	12,02± 0,54 a1,a3,k	1,79± 0,16 a1,a3,k	1,58± 0,15 a1,a3,k	3,47± 0,21 a1,a3
A3, n=31	0,69± 0,10 a2,k	8,31± 0,89 a1,k	6,44± 0,65 a1,a2,k	15,05± 0,68 a1,k	1,52± 0,12 a1,a2,k	1,66± 0,37 a2,k	8,69± 0,77 a1,a2,k
K, n=34	2,07± 0,22	9,74± 0,66	2,70± 0,13	11,01± 0,34	1,94± 0,65	1,83± 0,67	3,67± 0,59

Note. a1, a2, a3, k – statistically significant difference with indicators, subgroups A1, A2, A3, group K ($p<0,05$)

In the A1 subgroup with the combination of anovulatory and male infertility factors and in the A3 subgroup with the combination of anovulatory, tubal and male infertility factors, a reduced time of the former was observed (0.51 ± 0.04 and 0.69 ± 0.10 vs. 2.07 ± 0,22 h, p<0.01), second (4.58 ± 0.78 and 8.31 ± 0.89 vs. 9.74 ± 0.66 h, p <0.01), sixth (1.44 ± 0.32 and 1.52 ± 0.12 vs. 1.94 ± 0.65 h, p <0.01) and the seventh (1.32 ± 0.33 and 1.66 ± 0.37 vs. 1.83 ± 0.67 h, p<0.01) separation, while the fourth (5.55 ± 0.57 and 6.44 ± 0.65 vs. 2.70 ± 0.13 h, p<0.01), n (16.36 ± 0.91 and 15.05 ± 0.68 vs. 11.01 ± 0.34 h, p<0.01) and eighth (5.64 ± 0.15 and 8.69 ± 0,77 vs. 3.67 ± 0.59 h, p <0.01) separation was longer with and sometimes compared to similar indicators of group K.

t1-t4, t8, and cc1-cc3, cc7 in the A2 subgroup with the combination of tubal and male infertility factors were not significantly different from those in control, whereas the fifth (10.02 ± 0.54 vs. 11.01 ± 0, 34 h, p <0.01), sixth (1.79 ± 0.16 vs. 1.94 ± 0.65 h, p <0.01) and seventh (1.58 ± 0.15 vs. 1.83 ± 0.67 h, p <0.01), the separation was shorter.

Subgroup A3 was characterized by the longest time between division into 2 blastomeres and division into 3 cells (cc2 -12,80 ± 0,94 h), and the incidence of biochemical pregnancies was 55,00%.

Based on the analysis of the time of division into blastomeres, depending on the subsequent implantation (Table 3), it was found that those embryos that did not implant were divided more slowly than those that were implanted.

Table *3*

Time division of blastomeres depending on subsequent implantation, M ± SE, in hours

Time of division into blastomeres	The embryos that were implanted	Embryos that were not implanted
t1	24,12±0,86*	26,27±0,92
t2	26,75±0,92*	28,64±1,02
t3	36,48±1,12*	40,12±1,26
t4	39,41±1,14*	42,68±1,27
t5	51,05±1,27*	54,13±1,36
t6	53,02±1,35*	56,97±1,48
t7	55,61±1,45*	59,85±1,55
t8	59,63±1,56*	64,02±1,73

Note. * – statistically significant difference between the compared groups (p<0,05).

11 (11.00%) patients with a previous unsuccessful attempt at ART were pre-implanted genetic diagnostics by comparative genomic hybridization, sequencing of a new generation of troptoderm biopsies (Table 4). Of the 72 embryos, 4 were uninformative due to amplification failure.

Table 4

Results of pre-implantation genetic diagnosis of embryos by next-generation sequencing

Indicator	n (%)
Total embryos were analyzed	72
The number of informative embryos	68(94,44)
The number of euploid embryos	36/68 (52,94)
Number of aneuploid embryos	32/68(47,06)

Among 68 informative sequencing embryos, 36 (52.94%) were euploid with normal / balanced karyotype and 32 (47.06%) were aneuploid with abnormal / unbalanced karyotype.

The use of additional gamete and embryo selection techniques, as well as IMCI in Group A against traditional preconception training, traditional gamete and embryo selection techniques, and ICSI artificial fertilization in Group B led to improved embryological results (Table 5).

Table 5

Embryological results in the study groups

Indicator	Group A (IMCI) (n=100)	Group B (ICSI) (n=113)	Group K (ICSI) (n=34)
The average number of aspirated follicles per woman, M±SE	20,11±0,85 [k]	19,65±0,77 [k]	12,46±0,38
The average number of oocytes received per woman, M±SE	15,11±0,77 [k]	14,55±0,84 [k]	11,12±0,36
% of oocytes from aspirated follicles	75,14	74,05	89,25
The average number of mature oocytes per woman, M±SE	12,31±0,58 [k,b]	10,05±0,59 [k,a]	9,78±0,43
% of mature oocytes from the total number of oocytes	81,47 [b]	69,07 [k,a]	87,95
The number of embryos received per woman, M±SE	8,08±0,51 [k,b]	6,34±0,47 [k,a]	6,04±0,48
% of embryos from the total number of oocytes	53,28	43,57 [k]	54,32
The number of received embryos of excellent and good quality per woman, M±SE	6,55±0,49 [k,b]	3,54±0,38 [k,a]	5,13±0,39
% of embryos of excellent and good quality from the total number of embryos	81,06 [b]	55,84 [k,a]	89,93

Note. [k, a, b] – статистично значуща різниця з показниками груп К, А, В ($p<0,05$).

As can be seen from the table 5, groups A and B differed in all embryological parameters from group K except the percentage of oocytes from aspirated follicles (75.14%, 74.05%, and 89.25%). The average number of aspirated follicles per woman in groups A (20.11 ± 0.85) and B (19.65 ± 0.77) was statistically significantly higher than in group K (12.46 ± 0.38), respectively, in 1,61 and 1.58 times; the average number of oocytes received per woman (15.11 ± 0.77 and 14.55 ± 0.84 vs. 11.12 ± 0.36) was 1.36 and 1.31 times, respectively; the average number of mature oocytes per woman (12.31 ± 0.58 and 10.05 ± 0.59 vs. 9.78 ± 0.43) was 1.26 and 1.03 times, respectively; the number of embryos obtained from one woman (8.08 ± 0.51 and 6.34 ± 0.47 vs. 6.04 ± 0.48) was 1.34 and 1.05 times, respectively. But the proportion of mature oocytes from the total number of oocytes in group A (81.47%) and group B (69.07%) was lower than in group K (87.95%) in 1.08 and 1, 27 times; the percentage of embryos from the total number of oocytes (53.28 and 43.57% versus 82.03%) at 1.54 and 1.88; the number of obtained embryos of excellent and good quality per woman in group A (6.55 ± 0.49) was higher and in group B smaller (3.54 ± 0.38) compared to group K (5.13 ± 0, 39) - respectively 1.28 and 1.45 times; the percentage of embryos of excellent and good quality from the total number of embryos was lower in both groups (81.06, 55.84 versus 89.93%) - by 1.11 and 1.61 times, respectively.

The development of personalized pre-conceptual training and techniques of ART compared to traditional ones resulted in a statistically significant increase in the percentage of mature oocytes from the total number of oocytes by 1.38 times, the number of received embryos from one woman - by 1.17, the number of received embryonic embryos per woman - 2.13%, the percentage of embryos of excellent and good quality of the total number of embryos - 1.83 times.

Conclusions

Patients with pathoospermia requiring treatment with DRI require higher genetic alertness, as their average aneuploidy is 16.17 times higher ($p < 0.01$) than in men with normozoospermia. Among the aneuploidy the most common in patients with pathozoospermia are: the presence of two 18 chromosomes (76.54%), doubling of the X chromosome (17.28%), the absence of one of the sex chromosomes (7.41%).

The morphokinetics of transferred embryos in pairs with different forms of combination of female and male infertility is different. Embryos from couples with chronic anovulation and the male factor divide the slowest, leading to a low number of pregnancies. Embryos from pairs with a combination of tubular factor and pathoospermia split compared to controls up to the 5th blastomere slower and 6 to 8 faster, whereas the pregnancy rate was not

significantly different from the control level. The morphokinetics of embryos from couples with a combination of chronic anovulation, tubal factor, and pathoospermia differ asynchronously and with the longest division up to 8 blastomeres, the absence of separation of several embryos, which is also accompanied by a low pregnancy rate.

The use of morphokinetic analysis of embryos allows them to be observed throughout the in vitro cultivation process. Estimation of the morphokinetic parameters of the embryo, namely the dynamics of its development in the early stages of embryogenesis, facilitates the selection of the most promising embryos for selective transfer into the uterine cavity.

Preimplantation genetic diagnosis by sequencing of a new generation of blotocyst troptoderm biopsies is an effective method of breeding embryos with a balanced karyotype.

The use of modern methods of selection of gametes and embryos leads to a statistically significant increase in the percentage of mature oocytes from the total number of oocytes by 1.18 times, the number of received embryos from one woman - by 1.27, the number of received embryos of excellent and good quality per woman. 1.85, the percentage of embryos of excellent and good quality from the total number of embryos - 1.45 times, as well as a decrease in the amount of spent RSFS 1.23 times.

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