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DEVELOPMENTAL CAPACITY OF HUMAN EMBRYOS POSSESSING NUMERICAL CHROMOSOMAL ABNORMALITIES

The rate of numerical chromosomal abnormalities in the sample of 720 preimplantation human embryos was estimated and subsequently the developmental patterns of pathologic specimen were studied on cleavage and blastulation stage. Low prognostic value of morphologic criteria usage for noninvasive selection of euploid embryos in vitro was testified.

Key words: preimplantation genetic screening, embryo, morphology.

Introduction. In vitro fertilization (IVF) technique gave us the option of preimplantation human development investigation including different aspects of genetics and embryo development interrelation. During its early growth embryo should pass the number of crucial key points successfully to survive to the stage relevant to implantation [4]. As far as every step of ontogenesis is predetermined by the genetic component, aneuploidy may negatively affect even the earliest stages of embryos' growth being the cause of delayed development, morphologic disturbances and embryo inability to implant [2].

The newly developed preimplantation genetic screening (PGS) enables us to check whether the embryos obtained during the in vitro fertilization treatment carry chromosomal disorders and after that transfer only euploid samples to the patient's uterus. PGS helps to avoid the miscarriage and pregnancy termination induced by fetus aneuploidy therefore and contributes to the highest pregnancy and delivery rates in poor prognosis patients [8]. Inasmuch as PGS is an invasive, time-consuming and expensive procedure [2] the search for other criteria for euploid embryos selection during the embryo culture in vitro is continued. The investigation of developmental characteristics inherent to embryos possessing chromosomal anomalies appears to be the basis of elaboration of morphological criteria to distinguish euploid samples for the transfer without PGS.

Thus, **the aim** of the study was to analyze the morphological characteristics of aneuploid embryos on cleavage and blastulation stage in order to determine their actual developmental capacity.

Materials and methods. Between September 2010–December 2012 in Reproductive Genetics Institute (Kyiv) 925 embryos obtained in 82 infertility treatment cycles were subjected to preimplantation genetic screening for the most frequent numerical chromosomal abnormalities. Poor prognosis patients [6] who entered such treatment cycles didn't possess genetic reproductive risks and contraindications to therapy.

Insemination of the oocytes obtained in the IVF+PGS cycles was performed with intracytoplasmic sperm injection. Oocytes were checked for the presence of two pronuclei and second polar body in 16-18 h after insemination procedure. Overall, during the study 1058 mature oocytes were retrieved (12,86 ± 6,1/treatment cycle) and the rate of fertilization success was 86,28 %. Obtained zygotes were cultured in droplets of commercial media (MediCult, Denmark) for 5 days till the specimen considered to be euploid according to PGS results were transferred to the recipient's uterus. During the cleavage stage the number of blastomeres and the presence of morphological defects were investigated whilst the blastula structure was evaluated according to Gardner's tripartite scoring system [1].

Blastomeres for preimplantation genetic screening were biopsied on the 3rd day of embryo culture as at the cleavage stage embryonic cells maintain totypotency and loss of one blastomere can be compensated by the adjacent blastomeres division [3]. Embryos possessing ≥ 6 blastomeres were used for investigation [5], but slow developing samples (≤ 5 cells at the moment of biopsy) were also examined as a control.

Retrieved cells were hypotonically treated and their nuclei were fixated with the ethanol/acetic acid mixture [7]. A two-step FISH protocol was used to examine the number of chromosomes 13, 16, 18, 21, 22 (PB Multivysion, Vysis, USA), X and Y (CEPX, CEPY, Vysis, USA). The signal analysis was performed with the help of computer program ISIS (MetaSystems,Germany).

Results and discussion. Preimplantation genetic screening was held successfully for 720 embryos which were assigned to one of the categories depending on the diagnostic results [9]:

1. Euploid samples carrying 2 copies of every autosome investigated and the set of sex chromosome specific to males (XY) or females (XX);

2. Embryos with numerical chromosomal abnormalities. These are: а) samples with lack of chromosome (monosomic and nullisomic embryos); b) samples with an additional chromosome (presence of extra chromosome of certain pair); в) samples with complex chromosomal abnormality (disorders involving ≥2 pairs of chromosome simultaneously).

The study revealed that 474 embryos examined (65,83 ± 1,77 %) possessed chromosomal disorders. The major part among the diagnosed embryos (Figure 1) comprised specimen with complex aberrations of chromosomal set (212 cases, 29,44 ± 1,7 % of the group). The quantity of mono- and polysomic embryos was almost equal and constituted 11,25 ± 1,18 % and 11,53 ± 1,19 % of the research group. Moreover, 13,61 ± 1,28 % of examined specimen were considered to be haploid (bearing one copy of each chromosome investigated) or polyploid (with a multiple increase in chromosomal sets). Notably, that only 34,17 ± 1,77 % of studied sample possessed two copies of target chromosomes, that gives us 3,0 ± 1,06 euploid embryos/diagnostic cycle. Established rate of normal embryos is slightly lower than the corresponding results obtained by other authors (44 % according to Munne [7], 39 % in Magli's research [6]) that maybe the consequence of sample formation bias or the natural attribute of our nation [3].

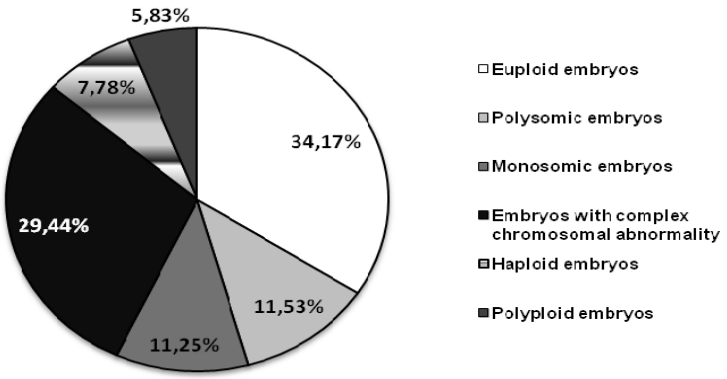

Figure 1. Assignment of embryos examined according to the results obtained during the chromosomal screening

Analysis of pathologic embryos' morphology on the 2nd day of their development revealed that major part of the sample comprised specimen with 4 cells, that constituted 54,33 ± 2,29 % of the group (Figure 2). Furthermore, at rate of 18,6 ± 1,79 and 14,8 ± 1,63 % the embryos consisting of 2 or 3 blastomeres were detected. As a result after 40–42 h of culture the optimal tempo of cleavage [1] was inhered by 87,55 ± 1,52 % of pathologic embryos, while 59 samples on the 2nd day of development had ≥ 6 cells and so transcend the expected speed of division. The average number of blastomeres was equal to 3,66 ± 1,02 while the structural aberrations such as cell fragmentation or uneven blastomeres were encountered at a rate of 6,96 ± 1,17 %.

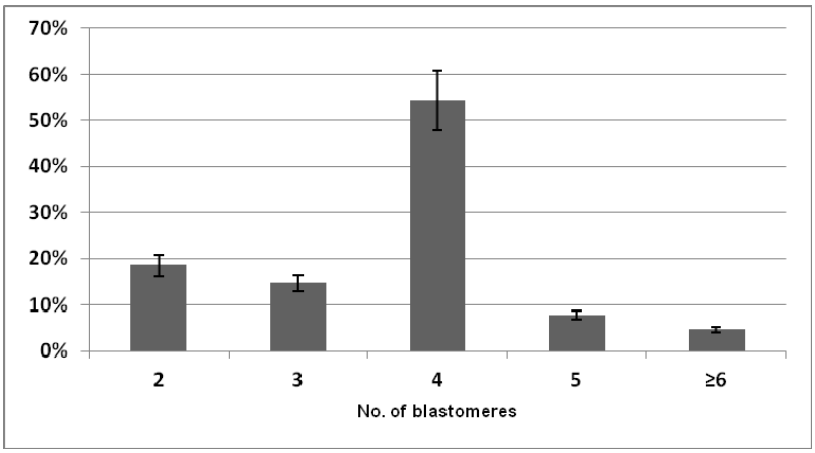

Figure 2. Distribution of aneuploid embryos in relation to the cellular state on the 2nd day of culture

In 64–66 h after fertilization embryos possessing 8 blastomeres prevailed among the pathologic group (35,65 ± 2,2 %). Overall, optimal morphological characteristics [1] were attributable to 68,99 ± 2,12 % of examined embryos (Figure 3), moreover 3,59 ± 0,85 % of sample had more than 10 cells or even formed morula. At the same time, 29 abnormal embryos comprised from ≤ 4blastomeres, while the development of 0,84 ± 0,42 % of specimen was arrested. On the average, there were 7,09 ± 1,66 blastomeres in every embryo on the 3rd day of embryo culture. Noteworthy, structure abnormalities were detected in 53,38 ± 2,29 % of examines specimen. Among them cell size inequality predominated (31,22 ± 2,13 % of group) and the estimated rates of other morphology defects were almost similar (7,17 ± 1,19 for fragmentation and 7,38 ± 1,22 for cytoplasmic bulbs).

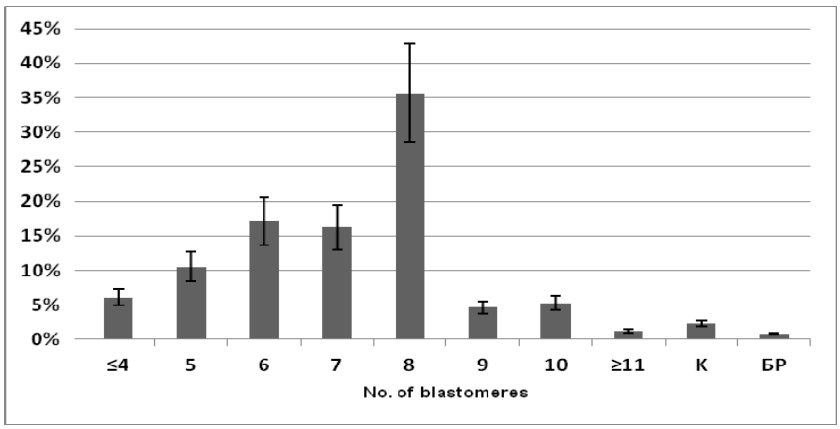

Figure 3. Percentage of aneuploid embryos in relation to the cellular state (64–66 h after fertilization) with M for morula and DA for developmental arrest

On the 4th day of development aneuploid embryos were graded according to their developmental stage (table 1). More specifically, the lowest grade "0" was conferred to embryos that didn't exhibit signs of progressive development during last 24 h of observation including samples with distinct marks of degradation. On the contrary, fully developed blastocyst that successfully completed the hatching process were rated as "9" grade.

***Table* 1. Embryo grading depending on its morphological characteristics in 88-114 h after fertilization**

Grade	Developmental characteristics of embryo
0	No evidence of progressive development for last 24 h of culture
1	Embryo possesses 8-16cells, it's development is impaired, but no signs of degeneration are observed
2	Morula – embryo contains 12-16 tightly packed cells, thus resembling a mulberry
3	Compactisation – the gap junctions are formed, cells are bound tightly, no distinct outlines of particular cells can be seen
4	Cavitation is the initial step of blastocoels formation. The cavity size is less than half of the embryo. Corresponds to grade 1 of Gardner embryo classification [1]
5	Blastocoel enlarges and occupies more than a half of the blastocyst flattening the surrounding cells. Gardner's grade 2
6	Blastocoel is formed completely. It's of round shape. Embryoblast and trophectoderm are clearly seen. The latter is comprised of numerous tightly packed cells. Zona pellucida is stretched. Grade 4 in Gardner embryo classification
7	Expanded blastocyst with very thin zona pellucida. Gardner's grade 4.
8	Hatching blasctocyst. Gardner's grade 5.
9	Embryo managed to leave the zona and is ready for implantation. Corresponds to grade 6 of Gardners embryo classification.

According to proposed arrangement in 88-90 h after insemination average developmental grade of aneuploid embryos was 1,87 ± 1,07 (Figure 4). Among the studied samples 36,21 ± 2,21 % formed morulae, 28,63 ± 2,07 % demonstrated the initial steps of compactisation process whereas 68 specimen failed to develop steadily (14,32 ± 1,61 % of the group). On the 5th day of culture the development of almost 38,48 ± of pathologic embryos was ceased, thus lowering the average developmental grade index to 3,07 ± 2,1 (corresponds to compaction stage). But still 48,99 ± 2,36 % of sample reached the blastocyst stage. Among them 70 embryos proceeded the hatching and 44 were of highest quality according to Gardner's classification. This implies that taking into consideration the morphologic characteristics solely almost 15,6 % of abnormal embryos would be the samples of choice for the transfer in corresponding IVF treatment cycles [4].

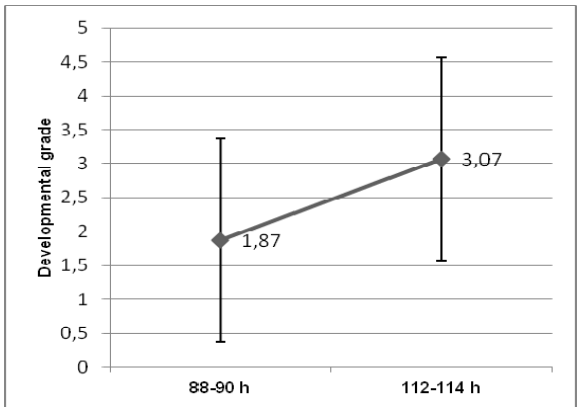

Figure 4. Aneuploid embryos' development on the blastulation stage

Conclusions. The preimplantation genetic screening revealed that 65,83 % of studied human preimplantation embryos possessed numerical chromosomal abnormalities the majority of which constituted combined aneuploidy. The detrimental effect of detected chromosomal abnormalities wasn't visible on the cleavage stage as majority of pathologic samples corresponded to high quality developmental criteria. Till the 5th day of culture 38,5 % of aneuploid embryos degraded but still 15,0 % chromosomally unbalanced sample were able to form morphologically optimal blastocysts. The obtained results prove that morphological evaluation of early embryos is ineffective for euploid embryos' selection, therefore the preimplantation chromosomal investigation is recommended for poor prognosis patients' treatment.

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ПРЕІМПЛАНТАЦІЙНИЙ РОЗВИТОК ХРОМОСОМНО НЕЗБАЛАНСОВАНИХ ЕМБРІОНІВ ЛЮДИНИ

Встановлено частоту виникнення кількісних хромосомних аномалій різних типів серед преімплантаційних ембріонів людини, вказані особливості розвитку патологічних зразків на стадії дроблення та бластуляції. Доведена низька прогностична цінність дослідження морфологічних характеристик культивованих in vitro зразків для неінвазивної селекції еуплоїдних ембріонів перед ембріотрансфером.

Ключові слова: преімплантаційний генетичний скринінг, ембріон, морфологія.

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ПРЕИМПЛАНТАЦИОННОЕ РАЗВИТИЕ ХРОМОСОМНО НЕСБАЛАНСИРОВАННЫХ ЭМБРИОНОВ ЧЕЛОВЕКА

Установлена частота возникновения количественных хромосомных аномалий разного типа среди преимплантационных эмбрионов человека, проведена оценка особенностей их развития на стадии дробления и бластуляции. Показана низкая прогностическая ценность исследования морфологических характеристик культивируемых in vitro эмбрионов с целью неинвазивной селекции хромосомно сбалансированных образцов перед эмбриотрансфером.

Ключевые слова: преимплантационный генетический скрининг, эмбрион, морфология.

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KARYOTYPE DISORDERS AMONG MEN AND WOMEN DIAGNOSED WITH INFERTILITY

This article shows the frequency of chromosomal abnormalities in women and men with reproductive disorders and infertility.

Key words; karyotype, infertility, cytogenetic studies, translocation, inversion, mosaicism.

Introduction. Infertility problem has become increasingly relevant lately: according to WHO data every fifth couple in the world is infertile. At the same time a lot of breakthroughs referring to infertility correction have been developed recently. For example, intracytoplasmic sperm injection (ICSI) elaboration significantly contributed to infertility treatment practice. Genetic disorders those are responsible for natural conception failure can be inherited by progeny conceived with the help of assisted reproductive technologies (ART). Therefore attention should be paid to the genetic factor of patients undergoing infertility treatment as the central task of ART is healthy child birth [10].

For the selection of appropriate treatment approach complete diagnostic examination is necessary. One of the most important genetic tests is karyotyping that studies the numerical and structural characteristics of chromosomal set. Numerous researches report that the most frequent chromosomal disorders found in the group of infertile patients are: numerical abnormalities of sex chromosomes; 2. balanced reciprocal translocations; 3. Robertsonian translocations; 4. inversions; 5. marker chromosomes; 6. mosaic forms of pathologies mentioned above [5].

Generally the rate of chromosomal abnormalities (CA) in population is low and accounts for 0,5-3,0%, while corresponding CA frequency among infertile patients is about 4,3 – 9,6% though its phenotypical manifestation is rare. [4]. According to some authors [6,2] CA rate among patients undergoing ICSI procedure exceeds13,1%.

Risk of conceiving a child with physical and mental disorders (including severe mental retardation) is significantly higher for carriers of balanced structural aberrations in comparison with population [7]. Recurrent pregnancy loss is also common in patients carrying chromosomal aberrations. Consequently the karyotype exmination is extremely important for counseling of infertile couples or patients with spontaneous abortions. That is why methods of prenatal diagnostics of chromosomal disorders are evolving rapidly nowadays and its practical application in public health service helps to prevent the birth of severely affected children [3].

As chancing the parents' karyotype is impossible the awareness of such problem gives patients the opportunity to do the genetic examination of fetus in time thus revealing and solving the problem after careful consideration. Being well-informed about all the risks considering clinical prognosis couple can plan the pregnancy. Some of them apply for donor programs. In case of IVF treatment the option of preimplantation genetic testing can be the subject for a debate [1].

So the aim of this research is determination of CA rate among female and male patients diagnoswd with infertility and its consequent comparison with regard to patent's sex.

Materials and methods. The primary information search was conducted between 2009–2012 years in the cytogenetic laboratory of Reproductive Genetics Institute clinic headed by Illin I.E., MD. The results of cytogenetic tests of 2495 patients (1135 males and 1360 females) were processed.

Standard procedure of peripheral blood lymphocytes culture and chromosome smears preparation was used. The blood for cytogenetic testing were obtained from cubital vein and added to the culture media. The specimen were incubated at +37°C for 72 hours. For metaphase harvesting colchicine was added into the culture tubes. Than the specimen were centrifuged and treated with hypotonic solution of 0,075M potassium chloride for no longer than 20 min at +37°C. The cells were fixated by means of three replacements of iced to +4°C fixative made ex tempore of ethanol and acetic acid in the ratio of 3:1. After the last centrifugation and supernatant aspiration, cell suspension was dropped on wet cold slides which then were air-dried. The chromosome preparations were GTG-banded [9]. 15-30 metaphase spreads at 550 bands resolution analyzed for every patient [11].

In order to detect differences of CA distribution between males and females the sampling ratios of groups were compared [8].

Results and discussion

The study included the results of karyotyping conducted for 2495 patients (1135 males and и 1360 females).

Among the studied females 208 patients possessed chromosome abnormalities that accounted for 15.3% out of all the specimen studied (table 1).