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ПРЕИМПЛАНТАЦИОННЫЙ РОЗВИТОК ХРОМОСОМНО НЕЗБАЛАНСОВАНИХ ЕМБРИОНІВ 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 exceeds 13,1%.

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 examination 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 changing 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 diagnosed with infertility and its consequent comparison with regard to patient'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. Then 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 1360 females).

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

Table 1. Quantitative characteristic of karyotype abnormalities in female patients' group

	2009	2010	2011	2012	Total
Total number of patients undergoing karyotype study	130	209	427	594	1360
Number of patients with karyotype features	14	22	76	96	208
%	10.8	10.5	17.8	16.2	15.3
Translocations	4	6	4	6	20
%	3.10	2.90	0.94	1.01	1.47
Inversions	-	5	10	14	29
%	0	2.3	2.34	2.36	2.13
Variations of autosome heterochromatic regions	2	3	30	45	80
%	1.5	1.4	7.02	7.58	5.88
Increasing of stalk length and satellite size of acrocentric chromosomes	5	6	20	21	52
%	3.9	2.9	4.68	3.54	3.82
Changes of X chromosome number	3	1	15	14	33
%	2.3	0.5	3.51	2.36	2.43
Unbalanced karyotype: deletions, duplications and markers	-	-	1	4	5
%	0	0	0.23	0.67	0.38

Cytogenetic studies revealed such patterns of chromosome structure and number variations: translocations (1,47%), inversions (2,13%), polymorphism of autosome heterochromatic regions (5,88%), increasing of stalk length

and satellite size of acrocentric chromosomes (3,82%), numerical changes of chromosome X copies (2,43%), unbalanced karyotype involving deletions, duplications and markers (0,38%).

Table 2. Quantitative characteristic of karyotype abnormalities detected among female patients

	2009	2010	2011	2012	всего
Infertility	39	68	203	309	619
Missed abortion	55	53	66	112	286
Multiple miscarriages	17	28	44	26	115
Children with multiple congenital abnormalities in anamnesis	12	8	12	3	35
Family planning	7	52	102	144	305
Total	1360				

Karyotype studies were performed on the basis of such indications: infertility (45,51%), missed abortion (21,03%), multiple miscarriages (8,46%), family planning (22,43%), children born with multiple congenital abnormalities (2,57%). (Table 2). CA were detected in every of the aforementioned groups. The highest rate of karyotype disorders was identified in the sample of female with multiple miscarriages in

anamnesis (15,3%), the lowest one – among women whose karyotype was examined because of fetus malformations detected in previous pregnancies (4,3%).

In the group of male patients various features of chromosomal set were detected in 202 specimen constituting almost 17,8% of all the cases studied. (Table 3)

Table 3. Quantitative characteristic of karyotype abnormalities in male patients' group

	2009	2010	2011	2012	всего
Total number of patients undergoing karyotype study	146	195	322	472	1135
Number of patients with karyotype features	20	29	64	89	202
%	13.7	14.9	19.9	18.9	17.8
Translocations	3	3	2	5	13
%	2.05	1.54	0.62	1.06	1.15%
Klinefelter syndrome	1	3	-	2	6
%	0.68	1.54	-	0.42	0.53%
Инверсии	4	6	9	14	32
%	2.74	3.08	2.79	2.97	2.82%
Variations of autosome heterochromatic regions	2	5	21	29	57
%	1.37	2.56	6.52	6.14	5.02%
Increased heterochromatin of Y chromosome	-	3	12	9	24
%	-	1.54	3.72	1.91	2.11%
Small heterochromatic region in the long arm of Y chromosome	5	6	9	14	34
%	3.42	3.08	2.79	2.97	2.99%
Increasing of stalk length and satellite size of acrocentric chromosomes	7	8	13	19	47
%	4.79	4.11	4.04	4.03	4.14%

Cytogenetic studies conducted on the peripheral blood of male patients' revealed such patterns of chromosome structure and number changes: translocations (1,15%), Klinefelter syndrome (0,53%), inversions (2,91%), varia-

tions of heterochromatic region in the long arm of Y chromosome (5,10%), increasing of stalk length and satellite size of acrocentric chromosomes (4,14%).

Some of the mentioned CA are presented on fig. 1, 2, 3.

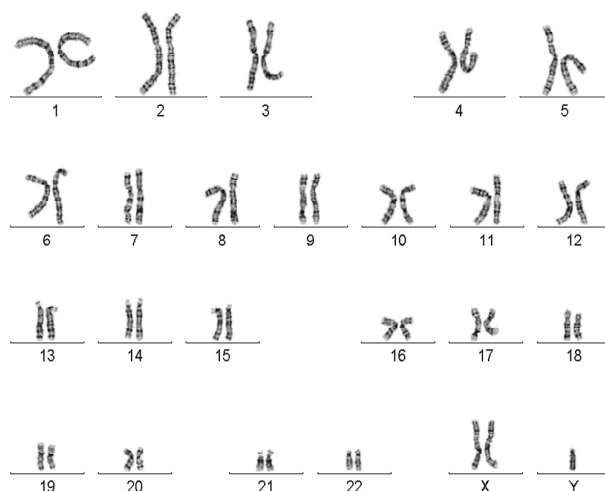


Fig.1. Karyotype with two chromosomes X and one chromosome Y

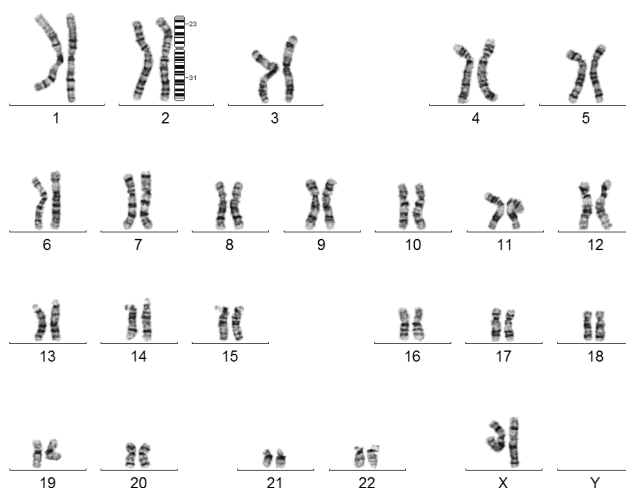


Fig.2. Female karyotype with chromosome 2 pericentric inversion

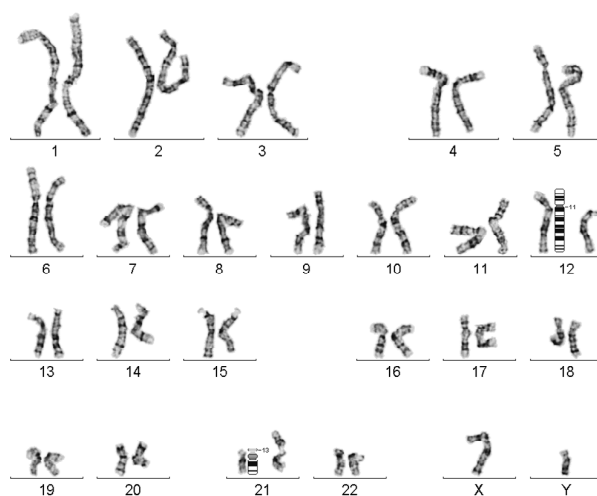


Fig.3. Male karyotype with reciprocal translocation involving chromosome 12 and chromosome 21

Statistical comparison of karyotype features rate between male and female group asserted the absence of statistical difference between values considered ($p \geq 0,001$).

This observation testifies that CA occurrence is not affected by the sex.

Conclusions. The high frequency of chromosomal abnormalities among patients with fertility requires more attention from doctors of reproductive medicine. Peripheral blood cells' karyotyping appears to be one of the most important steps in couple's examination held before their entrance the in vitro fertilization program. This test is crucial for the calculation of risk of chromosomally abnormal child conceiving and subsequent choice of the most appropriate treatment approach involving additional diagnostic methods, e.g. preimplantation genetic diagnosis. This procedure allows to avoid the embryotransfer of aneuploid or chromosomally unbalanced embryos into the uterus what is extremely helpful in case of one of the parents is a carrier of balanced chromosomal aberrations or other abnormality.

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ПОРУШЕННЯ КАРІОТИПУ СЕРЕД ЧОЛОВІКІВ І ЖІНОК З ДІАГНОЗОМ БЕЗПЛІДДЯ

Дана стаття висвітлює частоту хромосомних аномалій серед жінок і чоловіків з порушенням репродуктивної функції та безпліддям.

Ключові слова: каріотип, безпліддя, цитогенетичні дослідження, транслокації, інверсії, мозаїцизм.

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НАРУШЕНИЕ КАРИОТИПА СРЕДИ МУЖЧИН И ЖЕНЩИН С ДИАГНОЗОМ БЕСПЛОДИЕ

Данная статья освещает частоту хромосомных аномалий у женщин и мужчин с нарушениями репродуктивной функции и бесплодием.

Ключевые слова: каріотип, бесплодие, цитогенетические исследования, транслокации, инверсии, мозаицизм.

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FEATURES CONDITIONS SELECTION AND CULTIVATION MOUSE BONE MARROW ADHESIVE FRACTION MONONUKLEAR CELLS

Was established that the parameters centrifugation of mouse bone marrow cells in density gradient fikol-verohrafin 1,074 by centrifugal force of 300 g is provide getting an adhesive cells fraction, the most enriched on mesenchymal stem cells. The conditions for the allocation of mouse bone marrow cells, in which a mixture contributes most proliferative activity of mesenchymal stem cells (MSC).

Key words: mesenchymal stem cells, fikol-verohrafin gradient, bone marrow, mouse.

Introduction. For the formation and growth of the colonies of mononuclear bone marrow cells requires some concentration and ratio of substances and factors that stand in the environment in the life of most cells. For a variety of techniques and correlation of these substances varies, which in turn affects the formation and growth of colonies of cells.

It is known that the cells of organism need to exchange information with each other to regulate its development of the tissue, to control growth process and division, to coordinate functions. The structure of the bone marrow are fibroblasts, macrophages, adipocytes, osteoblasts, osteoclasts, endothelial cells, mesenchymal and hematopoietic stem cells and their descendants [3,4].

Vital cells in culture greatly simplified compared to the functioning of the body, and is usually limited to division. Interactions of bone marrow cells supports stay mesenchymal stem cells in the inactive phase of mitosis (G0). The output of this phase of the transition in the mitotic cycle is regulated by changes in intracellular signals [5,1]. Inter-cellular interaction occurs through hormones, neurotransmitters, and histohormoniv. Obviously, the latter type of intercellular signaling is present between the cells of the

bone marrow, and the signals received by the bone marrow cells, and report violations of homeostasis in the body, coming through hormonal and neurotransmitter substances. These signals and give the command to exit stimulates a number of stem cells from dormant state, which then become the path of differentiation and migrate to the area of the pathological process under the action of the same intercellular signals [2,3]. The same signaling molecules can cause target cells unequal response. In some cases this is due to the fact that the signaling molecule binds to various proteins, receptors, in other cases, they bind to the same protein receptors, but activated in different cells different mechanisms of response. Some reaction cells are gradual and increase in direct proportion to the increase ligand. However, there is another group of cells that responds to an increase in the concentration of signaling substances on the principle "all or nothing", in this case, the target cells are not observed any changes until the ligand concentration reaches a threshold level, and the achievement of it – immediately starts maximal response [6].

As already noted, one of the hallmarks of MSCs is their high adhesive ability to culture dishes by culturing. It is