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Influence of radioactive background on an idiopathic form of infertility incidence in men from different regions of Ukraine

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There are 143 men (group I) with idiopathic infertility in the form of asthenozoospermia of the II degree living in the territories of the Kiev region (Poles'ky, Chernobylsky, Ivan'kovsky, Borodyansky, Vyshgorodsky districts) were investigated in the Department of Sexual Pathology and Andrology of the SI «Institute of Urology of the National Academy of Medical Sciences of Ukraine» and exposed to ionizing radiation due to the Chernobyl accident in 1986. All individuals in the I group were born from parents who were in the accident at the Chernobyl nuclear power station in the area of radioactive contamination and permanently resided in areas with a high level of radioactivity. The control group was made up of persons born and living in the Ivano-Frankivsk region with a similar condition (group II, n=57). The mean age of the studied group I was 28,3±2.4 years, and persons of the II group were 30,1±2,8 years. Diagnosis of II was performed ambulatory in accordance with WHO and Ministry of Health of Ukraine standards. The study was to study and compare spermogram parameters in patients from both groups.

It has been established that the reproductive problems of men with idiopathic infertility living in radiation-polluted areas of the Kiev region of Ukraine consist in a significant violation of the quality indicators of ejaculate (mobility by the pool A+B is less than 1,6 times, p<0.05) correlating with modifications of physicochemicals (the average time of liquefaction is 1,4 times greater, p<0,05) and biochemical (decrease of levels of fructose by 2,2 times, citrate - by 1,6 times, p<0,01-0,05) data characterizing the effect of ionizing radiation, as one one of the leading factors of male genital function violation. The study found that the level of general indicators of spermogramm is significantly worse in the regions of Ukraine who were exposed to radioactive contamination due to the Chernobyl accident, in contrast to the indicators of persons from conditionally pure regions.

Keywords: idiopathic infertility, radiation contamination, asthenozoospermia, fructose.

Вплив радіоактивного фону на рівні захворюваності ідіопатичною формою безплідності у чоловіків з різних регіонів України

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У відділі сексопатології та андрології ДУ «Інститут урології НАМН України» було проведено дослідження репродуктивної функції у 143 осіб (І група) з ідіопатичним безпліддям у вигляді астенозооспермії П ступеню, які мешкають на території Київської області (Поліського, Чорнобильського, Іванківського, Бородянського, Вишгородського районів) та зазнали впливу іонізуючої радіації внаслідок аварії на ЧАЕС у 1986 році. Усі особи І групи були народжені від батьків, що перебували під час аварії на ЧАЕС у зоні радіаційного забруднення та постійно проживали на території з підвищеним радіоактивним рівнем. До групи контролю увійшли особи, які народилися та мешкали на території Івано-Франківської області, з аналогічним станом (ІІ група, n=157). Середній вік пацієнтів І групи становив 28,3±2,4 року, осіб ІІ групи – 30,1±2,8 року. Діагностику ІБ проводили амбулаторно згідно зі стандартами ВООЗ та МОЗ України. Дослідження полягало у вивченні та порівнянні показників спермограми у пацієнтів з обох груп.

Репродуктивні проблеми у чоловіків з ідіопатичним безпліддям, що мешкають у радіаційно забруднених районах Київської області України, полягають у достовірному порушенні якісних показників еякуляту (рухливість за даними пулу А+В є меншою в 1,6 разу; p<0,05), що корелюють із модифікаціями фізико-хімічних (середній показник часу розрідження більшій в 1,4 разу; p<0,05) та біохімічних (зниження рівнів фруктози у 2,2 разу, цитрату – в 1,6 разу; р<0,001-0,05) даних, що характеризує вплив іонізуючої радіації як один із провідних факторів порушення чоловічої генеративної функції. У дослідженні було встановлено, що рівень загальних показників спермограми є достовірно гіршим у регіонах України, які зазнали радіоактивного забруднення під час аварії на ЧАЕС на відміну від показників осіб з умовно чистих регіонів.

Ключові слова: ідіопатичне безпліддя, радіаційне забруднення, астенозооспермія, фруктоза.

Влияние радиоактивного фона на уровни заболеваемости идиопатической формой бесплодия у мужчин с разных регионов Украины

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В отделе сексопатологии и андрологии ГУ «Институт урологии НАМН Украины» было проведено исследование репродуктивной функции у 143 мужчин (І группа) с идиопатическим бесплодием в виде астенозооспермии ІІ степени, проживающих на территории Киевской области (Полесского, Чернобыльского, Иванковского, Бородянского, Вышгородского районов) и подвергшихся воздействию ионизирующей радиации вследствие аварии на ЧАЭС в 1986 году. Все лица І группы были рождены от родителей, которые находились во время аварии на ЧАЭС в зоне радиоактивного загрязнения и постоянно проживали на территории с повышенным радиоактивным уровнем. В группу контроля вошли лица, которые родились и жили на территории Ивано-Франковской

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области, с аналогичным состоянием (II группа, n=157). Средний возраст пациентов I группы составлял $28,3\pm2,4$ года, а лиц II группы – $30,1\pm2,8$ года. Диагностику ИБ проводили амбулаторно в соответствии со стандартами ВООЗ и Минздрава Украины. Исследование заключалось в изучении и сравнении показателей спермограммы у пациентов из обеих групп.

Было установлено, что репродуктивные проблемы мужчин с идиопатическим бесплодием, проживающих в радиационно загрязненных районах Киевской области Украины, заключаются в достоверном нарушении качественных показателей эякулята (подвижность по данным пула A+B меньше в 1,6 раза, p<0,05), коррелирующие с модификациями физико-химических (средний показатель времени разжижения больший в 1,4 раза; p<0,05) и биохимических (снижение уровней фруктозы в 2,2 раза, цитрата – в 1,6 раза; p<0,001–0,05) данных, что характеризует воздействие ионизирующей радиации как одного из ведущих факторов нарушения мужской генеративной функции. В исследовании было установлено, что уровень общих показателей спермограммы является достоверно худшим в регионах Украины, подвергшихся радиоактивному загрязнению вследствие аварии на ЧАЭС, в отличие от показателей лиц из условно чистых регионов.

Ключевые слова: идиопатическое бесплодие, радиационное загрязнение, астенозооспермия, фруктоза.

A ccording to the world statistics data about 10% of men on the Earth are infertile, and not less than 20% of men have quality of sperm below accepted by WHO of fertility standards. It is proved that the men's factor makes 20–50% of cases of infertility of married couple. In infertile couples that are exposed to auxiliary reproductive technologies, the idiopathic infertility presents about 30% of male infertility cases from all reasons.

According to WHO, the idiopathic infertility is an inability of couple to conceive the child when causes of fertility disturbance remain obscure. Now it is considered that the idiopathic infertility frequency tends to decrease that is connected to improvement of diagnostic methods which are used in modern reproductology. Many researchers consider that the male idiopathic infertility is much more frequent than a female one and its reason it is not possible to establish in every third case. The idiopathic infertility reasons in married couple often remain obscure and can arise thanks to a summation of negative factors often unknown to science and connected not only to fertility [1].

There are factors which increase the probability of the idiopathic infertility risk: male sex, age more than 30 years and a way of life (pernicious habits, household factors, noncompliance with occupational health, presence of frequent stresses, etc.).

Confirmation of idiopathic genesis of infertility at men who have no posterity during 1 year is carried out at an exception of a number of pathological states (lack of disturbances in endocrine and sexual systems; existence of insignificant disturbances like astenozoosperm or normosperm; absence of sexually transmitted diseases; lack of antisperm antibodies and similarity on HLA antigens; absence of genetic diseases, etc.).

The hidden factors in genesis of the idiopathic infertility can explain male infertility: enzymatic disturbances of spermatogenesis processes, pathology of calicrein kinin system [2, 3], damage of spermatozoa DNA, the increased levels of indicators of lipid peroxidation in biomembranes, genetic pathologies are not found (gametes with karyotype imbalance, chromosomal heteromorphism, etc.), genetically caused violation of any link of a spermatogenesis (ultrastructural defects of a nucleus, acrosome, centriole, flagellum, chromosomal – a deviation in a spermatozoon, etc.).

Negative impact of a radiation factor on a male reproductive system was open at the beginning of the XX century and now is one of the leading factors of the idiopathic infertility formation. The human reproductive system is considered as one of the most sensitive to radiative effects even of small doses of ionizing radiation, showing at the same time acute malfunction of a spermatogenesis system, with oppression of all its links and formation of total or partial sterilization.

It is necessary to be careful in interpretation of direct link between influence of the environment factors and infertility during the analysis of factors of the idiopathic infertility genesis [4]. According to scientific data, within decades quality

of sperm worsened, and change of the general radioactive background is one of such reasons [5].

The scientific works devoted to comparison of radiation factor influence on mammals indicate changes in human testes, arising at lower doses at people, than at rodents and have more negative consequences. The processes arising at the same time are characterized by rapid morphological changes like aseptic inflammation with a tubular testicular necrosis, death of A-type spermatogonia with transition in hyalinosis and sclerosis and also decrease of mass of testicles.

The most sensitive to radiation are nucleus and mitochondrions of living cells where damage comes even of small doses of ionizing radiation and they are shown in the earliest terms in the form of change of physical and chemical properties of nucleoprotein complexes of cell. The effect of ionizing radiation on living cell is result of the complex interconnected and interdependent transformations in synthesis of DNA and RNA [6].

The growing percent unclear violations of a spermatogenesis is explained by influence of a set of undifferentiated factors of the environment on the state of health and a way of human life (intensive influence of heat, ionizing and not ionizing radiation, pesticides, etc.). It is established that radiofrequency electromagnetic fields (RF EMF) in vitro and in vivo cause harmful effects on sperm parameters (quantity of spermatozoa, morphology, mobility), have negative effect on function of a system of kinases in cellular metabolism, cause formations of genotoxicity and genomic instability. Researches of scientists proved that chronic influence of RF EMF causes an oxidizing stress with the increased level of active forms of oxygen, can lead to the idiopathic infertility formation [7].

Scientific research of ionizing radiation impact on process of a spermatogenesis that in vitro was carried out, established formations of radiation induced changes in the culture of testis cells of transgene rats of ACR GFP according to a fluorescent expression. There was an established fact of toxic influence of x-ray radiation (a monochromatic x-ray microbeam) on gametes of rat males in the form of development of temporary or irreversible infertility [8].

Pathological spermatogenesis in the form of apoptosis of spermatogenny cells with violation ectoplasmic specializations is the key biological mechanism that makes a basis of the idiopathic infertility in men. In physiological and pathophysiological conditions (hunger, intoxication, radiation) in Sertoli cells the autophagy mechanism is activated as compensatory process of adequate spermatogenesis support by oppression of apoptosis and stabilization of integrity of a spermatogenic epithelium by turn of cellular phenomena [9].

The studying of radiative effects in an experiment indicated radiation adverse effect on mobility of spermatozoa because of molecules chemical bonds destruction by radioactive shares at the expense of their huge speed and energy. On one hand it is caused by a direct harmful action on nucleinic acids and proteins, including water radiolysis with active

Quantitative and quality indicators of a spermograma at patients with idiopathic infertility

Index	Reference indicators	Group I, n=143	Group II, n=157
Volume	of 2,0 ml and more than	3,2±0,4	3,4±0,3
Fluidifying	no more than 60 min	55,5±3,8*	39,2±3,4
Acidity (pH)	from 7,2 to 7,8 7,9±0,2		7,5±0,3
Concentration of spermatozoa	here 15 million/1 ml	28,3±2,1	31,5±2,3
Mobility gradient	3–4 points	3,2±0,4*	3,7±0,4
Live spermatozoa, %	50% of the live spermatozoa (which are not painted with eosin)	65,2±3,4	69,7±3,7
Existence of mobile spermatozoa	<50% in categories (A+B)	31,2±2,8*	49,4±5,3
The category A is a fast progression	<25%	8,1±0,8*	19,2±1,3
The category B is a slow progression	<50%	23,1±2,0*	30,2±4,9
The category C is lack of a progression	≥25%	56,2±4,6*	43,2±2,6
The category D – motionless spermatozoa	≥10%	12,2±1,2*	7,2±0,6
Morphology by Kruger	more than 30% of spermatozoa with a normal form of a head	26,4±2,8*	38,2±3,0
Leukocytes	<1 million on 1 ml. 0,9±0		0,8±0,1
Round cages (youth spermatozoa)	no more than 5 million/1 ml	2,4±0,2	1,9±0,2

Note: * - r < 0.05 in comparison between groups.

radicals' formation, and on the other hand – activation of lipid peroxidation and influence secondary radiotoxins on integrity of biomembranes and intracellular organellas. The last processe leads to formation of mutations and spontaneous changes in cell biophysical structure. It demonstrates that radiation to which our organism adapted during many centuries, in the conditions of the modern world quickly leads to significant changes while processes of adaptation of an organism to these changes proceed slower.

The studying of radiation influence on gonads in the context of dominant lethal mutations formation showed processes of the decrease of spermatozoa fertilizing ability that are not connected with genetic damages. 15 million spermatozoa in 1 ml were established as the lower level of norm, but only in case of their mobility and structure faultless [10].

Necessity of fertility maintaining in reproductive age men after chemotherapy concerning an oncopathology which deal with temporary, long-term or constant damage of gonads remains a serious problem too. There are experimental data about a possibility of reproductive function preservation in above-stated patients at prepubertal age and also artificial creation of the differentiated men's gametes from different types of stem cells, including the embryonic stem, induced pluripotent_stem and spermatogonia stem cells [11, 12]. These approaches give hope to patients at whom loss of gametes is connected with influence of various damages, including and radiation genesis (radiation professional, after chemotherapy), but still is experimental and preliminary. [13]

Two main strategies apply in therapy of the idiopathic infertility cases: expectations and empirical therapy or auxiliary reproductive technologies [14, 15].

MATERIALS AND METHODS

It was conducted the research of reproductive function at 143 men (group I) with an idiopathic infertility (astenozoosperm IInd degree) in department of sexual pathology and andrology of State establishment "Institute of Urology of Ukraine National Academy of medical sciences". The men of group I, living in territories of Kiev region (Polissya, Chornobyl, Ivankiv, Borodyanka, Vyshgorod the area) were affected by the ionizing radiation owing to accident on the Chornobyl atomic power station in 1986. Parents of all men from group I were in a zone of radiation pollution during accident on the Chornobyl atomic power station and constantly lived in the territory with the increased radioactive level. This group made 33% of all infertile men of this region that addressed to clinic. The group of control was made by men who were born and lived in the territory of the Ivano-Frankivsk region (the group II, n=157) that made 30% of all infertile men that asked for the specialized help in the region.

Middle age in group I was $28,3\pm2,4$ years, and in group II $-30,1\pm2,8$ years. Diagnostics of the idiopathic infertility at men was carried out according to standards of WHO and Ministry of Health of Ukraine. The research consisted in studying and comparison of spermogram indicators at patients from both groups.

Criteria of inclusion: Cases of idiopathic infertility in specified persons, was proved in specialized clinics according to the protocol of the Ministry of Health of Ukraine. Existence of the informed consent of the patient to carrying out a research.

Criteria of an exception: infertility cases that classified by other etiological factors, or cases where patients received treatment about it. Presence of a serious somatic illness, hormonal or genetic disorders. Simultaneous participation in other researches.

Different indicators of a spermogram of patients were criteria of comparison.

Processing of researches results carried out with use of the standard statistical methods on a computer Asus X51Lseries with a package QPRO, a statistical package "stargraf", determination of coefficient of probability (p).

Biochemical indicators of the ejaculate in patients with inflammatory diseases of genitals

Index	Reference values	Group I, n=39	Group II, n=47
Fructose (mmol/l)	11,9–28,8	8,7±0,4*	17,3±1,3
Citric acid (mmol/l)	23,4–31,2	17,5±1,8	27,3±2,2
Ceruloplasmin (g/l)	0,0442	0,0253±0,0031*	0,0382±0,0037
General proteolytic activity (c.u.)	12-15	7,6±0,7*	13,1±1,1

Note: * - p < 0.05 in comparison between groups.

RESULTS AND TO THEIR DISCUSSION

Quantitative and qualitative results of a research are given in table 1 and 2. The provided data are presented classical to spermogram indicators in the form of statistically worked average values which show quantitative and qualitative characteristics of men that addressed with fertility troubles to medical clinics of conditionally clean zone (The Ivano-Frankivsk region) and radiation polluted areas of Kiev region.

Comparing the physical and chemical indicators of the ejaculate showed in tab. 1 it was established that ejaculate volume in groups I and II authentically did not differ $(3,2\pm0,4\,\text{ ml})$ and $(3,4\pm0,3\,\text{ ml})$ respectively), the indicator pH in sperm at patients of group I differed in pathological increase $(7,9\pm0,2)$ in contrast to persons of group II $(7,5\pm0,3)$. Violations of spermoplazma physical and chemical properties consisted in reliable increase in indicators of the ejaculate fluidifying time at the group II $(55,5\pm3,8\,\text{min.})$, in comparison with patients of group II (fluidifying time $(39,2\pm3,4\,\text{min.})$; p<0,05).

According to these tab. 1 it was revealed statistically reliable violations of the ejaculate quality indicators in the form of spermatozoa mobility reduction at doubtful decrease in quantitative indices at patients of group I. So, spermatozoa with a fast progression on average values in groups I and II differed among themselves by 2,4 times $(8,1\pm0,8\%)$ and $19,2\pm1,3\%$ respectively; p<0,001), indicators with a slow progression of mobility $(23.1\pm2.0\% \text{ and } 30.2\pm4.9\% \text{ respectively})$ in group I were 1,3 times lower (p<0.05), indicators with mobility progression lack (56,2±4,6% and 43,2±2,6% respectively) were 1,3 times lower (p<0,05), and quantity of motionless spermatozoa was 1.7 times more at patients of group I, than patients in group II (12,2±1,2% and 7,2±0,6% respectively; p<0,05). Cases of idiopathic infertility at persons from group I are characterized by reliable reduction of quality indicators of the ejaculate (the mobility according to A+B pool is 1,6 times smaller; <0,05) that correlate with violation physical and chemical (an average time fluidifying value more than by 1,4 times; p<0,05) and biochemical (decrease in fructose levels by 2,2 times, citrate - by 1,6 times; p<0,001-0,05) data that characterizes influence of the ionizing radiation as one of the leading factors of men's reproductive function troubles.

The assessment of ejaculate morphological properties was carried out in two parameters: Percent of live spermatozoa in an ejaculate and Kruger's indicator. The percent of live spermatozoa in groups among themselves authentically did not differ, $(65,2\pm3,4\%)$ and $69,7\pm3,7\%$ respectively; p>0.05). Kruger's indicator was 1,4 times higher in an ejaculate of persons who lived in conditionally clean zone (respectively in groups I and II $-26,4\pm2,8\%$ of spermatozoa with a normal form of a head and $38,2\pm3,0\%$ of spermatozoa with a normal form of a head; p<0,05).

On an indicator of quantity of round cells in ejaculate samples groups authentically did not differ (in groups I and II $2,4\pm0,2$ million/ml and $1,9\pm0,2$ million/ml respectively; p>0,05).

It indicates that the leading pathological process in patients with idiopathic infertility have ejaculate kinesis troubles in the absence of probable changes in the majority of quantitative indices. In contrast to patients of group I, at patients group II ejaculate kinezograms were characterized considerably by the best indicators with respectively the highest (though it is not probable) quantitative indices. In general, spermogram of the patients living in conditionally clean regions of Ukraine showed authentically better data, than at inhabitants of the corresponding zones whose parents were in territories with the raised radiation background after accident on the Chornobyl atomic power station.

It was analyzed the ejaculate kinezograms in the context of possible violations of a spermoplazma. For this purpose, it was applied selective biochemical researches of ejaculate markers (fructose, citrate, ceruloplasmin and the spermoplazma general proteolytic activity) that can be the reason of the idiopathic infertility (table 2). Such changes at idiopathic infertility are presented in the form of classical violation of the ejaculate enzyme and isoenzyme range and decrease in concentration of citric acid and fructose [16, 17].

Fructose is the indicator of secretory function of seminal vesicles and also a power source for ejaculate spermatozoa. Its synthesis completely happens in seminal vesicles under the influence of exogenous androgens. Fructose splitting process speed (fructolysis) is connected to spermatozoa mobility and viability.

Decrease in concentration of fructose in ejaculate testifies the hypoandrogenic state, existence of inflammatory changes, atresia or obstructive process in seminal vesicles. Reduction of fructose concentration in ejaculate can be permanent or constant that leads to decrease of spermatozoa mobility and viability.

The Citric Acid is synthesized in structures of a prostate vesicular complex. It acts as a factor of ejaculate fluidifying, of hyaluronidase activation and other factors which promote processes of spermatozoon penetration to an ovum. Definitions of citric acid in sperm gives information about secretory function of a prostate gland. Reduction of concentration of citric acid is sign of chronic inflammatory process of bacterial and abacterial genesis, including its subclinical forms and also a hypogonadic state of various genesis.

Level of ceruloplasmin is an indicator of local reaction of free radical oxidation in structures of a prostate vesicular complex. Decrease of ceruloplasmin level in ejaculate of patients with inflammatory diseases of genitals is connected to excessive activity of lipid peroxidation and the increased use of bioantioxidants in reproductive organs, being a separate etiological factor of fertility troubles of the ejaculate of men.

The biochemical research of the ejaculate showed existence of violations of fructose secretion in seminal vesicles

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and of citric acid in prostate gland at the patients with the idiopathic infertility living in territories of Kiev region and were affected by the ionizing radiation owing to accident on the Chornobyl atomic power station in 1986. Decrease in the fructose level by 2,2 times in spermogram of patients from group I, in comparison with data of patients from group II (8,7±0,4 mmol/l and 17,3±1,3 mmol/l respectively; p<0,05) violation of spermatozoa mobility and viability correlated with the expressed processes. It was confirmed by the data obtained in the research, in the form of the established statistically reliable difference of an average value of quantitative maintenance of fructose in the studied groups of patients.

The average indicator of concentration of citric acid which reflects a prostate gland functional condition and endocrine function of testicles also was much lowered in the patients with the idiopathic infertility living in territories of Kiev region, making 17,5±1,8 mmol/l. In the group II this indicator was 27,3±2,2 mmol/l respectively, that is approached indicators of healthy men. The difference in groups I and II made 1,6 times (p<0.05).

Proceeding from ideas of locality of inflammatory process in a prostate gland, comparative studying of antioxidant enzyme contents – ceruloplasmin (iron II – oxygen oxyreductase), so-called universal "cleaner" of products free radical oxidation in blood and in ejaculate of men was carried out. Contents of the ceruloplasmin in blood and in ejaculate were defined behind reaction with n-phenylenediamine dichloride. The control group was made of 12 almost healthy men of the same age. Patients with generative function violation on the background of ionizing radiation small doses had decrease in level of ceruloplasmin in ejaculate (patients of group I – 0,0253 \pm 0,0031 g/l). In the group II this indicator equaled 0,0382 \pm 0,0037 g/l that is 1.3 times higher and approaches an indicator of control group of men – 0,0442 g/l. At the same time the level of ceruloplasmin in blood of group II of

patients, in our opinion, shows local, but not system activation of reactions free radical oxidation with strengthening of digestion of antioxidants, in this case of ceruloplasmin.

Thus, decrease in levels of ceculoplasmin in ejaculate of the patients with idiopathic infertility living in territories of Kiev region is probable and were affected by the ionizing radiation owing to accident on the Chornobyl atomic power station in 1986, connected with excessive activity of lipid peroxidation processes with increase in utilization bioantioxidants in organs of a reproductive system. At patients with generative function violation on the background of ionizing radiation small doses decrease in levels of proteolytic activity to 7.6 ± 0.7 c.u. is established. In the group II average values of this indicator equaled 13.1 ± 1.1 c.u. that is 1.7 times higher (p<0.01) and comes nearer to normal values (12-15 c.u.).

CONCLUSIONS

- 1. It is established that the level of the general indicators of a spermogram is authentically worse in regions of Ukraine which underwent radioactive pollution during accident on the Chornobyl atomic power station in contrast to indicators of persons from conditionally clean regions;
- 2. Reproductive problems at the men with idiopathic infertility living in radiation polluted areas of Kiev region of Ukraine consist in reliable violation of quality indicators of the ejaculate (the mobility according to A+B pool is 1,6 times smaller; p<0,05) that correlate with physical and chemical modifications (an average value of fluidifying time 1,4 times longer; p<0,05) and biochemical (decrease in levels of fructose by 2,2 times, citrate by 1,6 times; p<0,001–0,05) data that characterizes influence of the ionizing radiation as one of the leading factors of violation of men's generative function.
- 3. Perhaps pathospermia among infertile men, with socalled idiopathic infertility can be carried to a secretory form of male infertility that needs further researches.

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