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PATHOPHYSIOLOGICAL AND AGE-SPECIFIC MECHANISMS OF MORPHO-FUNCTIONAL CHANGES IN SPERMATOZOA IN INFERTILITY

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The aim of the review is to analyze the causes and pathophysiological mechanisms of sperm alteration accompanying the development of male infertility, the sequence of the development of their dysfunctions in individuals of different ages. Male infertility is a worldwide problem; up to 20% of married couples are childless. In the Russian Federation, the problem is even more acute, which complicates the unfavorable demographic situation. Based on the generalization of the results of various screening studies, from 30 to 50% of men in the Russian Federation have impaired fertility. The review examines the main classifications and pathophysiological mechanisms of the development of male infertility. An extended assessment of changes in the morphology and functional properties of spermatozoa, relevant for the practice of *in vitro* fertilization, was carried out. The mechanisms of damage to spermatozoa and the sequence of development of degenerative changes in the cell are considered in detail. The mechanisms of sperm dysfunction development in men of different ages are compared. The conclusion is made about the need for further studies of the molecular mechanisms of fertility, deciphering the entire set of interactions between molecules and cells involved in the implementation of the reproduction function.

Keywords: male infertility; spermatozoa; pathophysiological mechanisms; reactive oxygen species; autoantibodies; *in vitro* fertilization; age-related changes.

ПАТОФИЗИОЛОГИЧЕСКИЕ И ВОЗРАСТНЫЕ МЕХАНИЗМЫ МОРФОФУНКЦИОНАЛЬНЫХ ИЗМЕНЕНИЙ СПЕРМАТОЗОИДОВ ПРИ БЕСПЛОДИИ

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Целью обзора был анализ причин и патофизиологических механизмов альтерации сперматозоидов, сопровождающих развитие мужского бесплодия, последовательности развития их функциональных дисфункций у лиц разных возрастов. Мужское бесплодие представляет всемирную проблему, до 20 % семейных пар являются бездетными. В Российской Федерации проблема носит еще более острый характер, что осложняет неблагоприятную демографическую ситуацию. Исходя из обобщенных результатов различных скрининговых исследований, от 30 до 50% мужчин в РФ имеют нарушенную фертильность. В обзоре рассмотрены основные классификации и патофизиологические механизмы развития мужского бесплодия. Проведена расширенная оценка изменений морфологии и функциональных свойств сперматозоидов, актуальных для практики применения экстракорпорального оплодотворения. Подробно разобраны механизмы повреждения сперматозоидов и последовательность развития дегенеративных изменений в клетке. Сопоставлены механизмы развития дисфункции сперматозоидов у мужчин разных возрастов. Сделан вывод о необходимости дальнейших исследований молекулярных механизмов фертильности, расшифровки всей совокупности взаимодействий между молекулами и клетками, вовлеченными в реализацию функции воспроизводства.

Ключевые слова: мужское бесплодие; сперматозоиды; патофизиологические механизмы; активные формы кислорода; аутоантитела; экстракорпоральное оплодотворение; возрастные изменения.

Problem of male infertility: General analysis

According to the WHO definition, infertility is the absence of pregnancy in a woman for 1 year or more in a sexually active couple which does not use contraception [1]. This problem has a worldwide character, up to 20% of married couples suffer from childlessness, while half of the cases of infertility are male infertility [1, 2]. In the Russian

Abbreviations

AMH — anti-Müllerian hormone; IL — interleukin; IVF — *in vitro* fertilization; ROS — reactive oxygen species; TGF — transforming growth factor; TNF — tumor necrosis factor; WHO — World Health Organization.

Federation, the problem is even more acute, which complicates the already unfavorable demographic situation. Based on the generalization of the results of screening studies, from 30 to 50% of men in the Russian Federation have impaired fertility, which reflects the increasing impact of psycho-emotional stress, adverse environmental, industrial and domestic factors [3, 4].

There is no common classification of male infertility. More often than others, etiological factors, pathogenetic mechanisms of formation, topological level of damage, and methods of treatment are considered as the basis for classifying forms of male infertility [5]. Another approach involves the separation of male infertility, secretory infertility (associated with impaired spermatogenesis as a result of hormonal disorders of the central and peripheral levels) and excretory infertility (a consequence of diseases associated with impaired ejaculate excretion) [4].

The main etiological factors of male infertility are considered varicocele, urogenital infections, immunological factors, congenital malformations, external physical and chemical factors, as well as sexual dysfunctions, endocrine and idiopathic disorders. Idiopathic disorders are understood as deviations of unknown origin that do not fall under the other specified factors. Over the years, idiopathic disorders can manifest themselves in the pathophysiological mechanisms of male infertility based on one or more of these factors [6]. It is noteworthy that the proportion of etiological factors differs significantly in Russia and abroad. So, in Europe, idiopathic disorders are leading (more than 75% of cases of male infertility), followed by varicocele (12%), urogenital infections (up to 7%), and immunological and external unfavorable factors account for about 3% of cases. In Russia, idiopathic disorders are also confidently leading, although their level (38%) is 2 times less than in Europe, while urogenital infections hold second place, their contribution to the development of infertility is estimated at 32%. This is followed by varicocele (27.5%), immunological factors (17.5%), congenital malformations and unfavorable external factors (about 7% in both cases). The contribution of sexual dysfunctions and endocrine disorders to the development of male infertility in Russia is estimated at 0.5 and 2.5%, respectively [7, 8]. There is no consensus among russian experts regarding the significance of certain etiological factors of male infertility. According to V.A. Bozhedomov and co-authors the share of immunological factors is estimated to be 17.5% [7], comparing with only 2.5% in the works of V.N. Shirshov, repeating the data for Europe [8].

Idiopathic disorders leading to the development of male infertility are the most common cause of the disease both in Russia and in the world, however, it is this group of disorders that is very difficult to study due to the unclear genesis and complex nature of this pathology. The problem of urogenital infections as a cause of prostatitis deserves an additional study and is beyond the aim of this work. This problem is still far from being solved [9]. The problem of varicocele involving surgical treatment and the associated pathophysiological changes in the testis and maturing spermatozoa also deserves a separate review.

Among other etiological factors of male infertility, obesity is most often mentioned, the typical consequence of which is erectile dysfunction [10–12]. The key link in the pathogenesis of vasculogenic erectile dysfunction and obesity is dysfunction of the vascular endothelium. Other links between obesity and erectile dysfunction include high levels of free fatty acids and adipokines in the blood, severity of systemic inflammation, oxidative stress, and insulin resistance. In the case of endothelial dysfunction, a decrease in the production of nitric oxide NO causes a disturbance in the reactivity of vascular smooth muscles [13]. Obesity is often associated with changes in the level of sex hormones, while the exact mechanisms of the pathogenesis of hypogonadism in obesity are complex and are not fully understood [12].

Chronic obesity reduces testosterone production by Leydig cells and leads to their destruction, which is the result of an increase in TNFα production and macrophage activity. Obesity decreases intra-testicular testosterone levels due to leptin which suppress the expression of the cytochrome p450 gene involved in testosterone synthesis [14]. All this affects the spermogram parameters, morphology and functional state of sperm [15, 16].

The aim of the review is to analyze the causes and pathophysiological mechanisms of sperm alterations accompanying the development of male infertility, the sequence of development of functional sperm dysfunctions in persons of different ages.

Pathophysiological mechanisms of sperm alteration

To analyze the causes and pathophysiological mechanisms of sperm alteration during male infertility, it is reasonably to divide its causes into secretory and excretory [4, 9]. A separate group is made up of genetically determined malformations, associated with point mutations or chromosomal aberrations which lead to abnormalities in the structure of the reproductive organs and various severe deviations in the process of spermatogenesis in both secretory and excretory forms. Further analysis concerns the secretory form of male infertility.

The secretory form of infertility is associated with insufficient testis tissue and can be congeni-

tal or acquired caused by insufficiency of the gonads [17]. The general factor in the development of the secretory form of male infertility is a violation of the integrity of the blood-testicular barrier, which always leads to tissue alteration, damage to gametes at different stages of development, chronic inflammation and autoimmune damage to gametes and testes [18]. All of these causes are accompanied by violations of the integrity of the blood-testicular barrier due to chronic inflammation and accumulation of reactive oxygen species (ROS) in the testicular tissues [12, 19, 20]. Varicocele also leads to a violation of the blood-testicular barrier integrity, the trophism of cells of the seminiferous tubules and vascular endothelium [21]. Obesity also is accompanished to venous congestion and the accumulation of peroxide radicals (ROS and lipid peroxidation products) [22–24].

Pathological increased permeability of the blood-testicular barrier is a feature of infectious and non-infectious prostatitis [9]. In the presence of viral or bacterial (gonococcal and non-gonococcal) pathogens, the permeability of the blood-testicular barrier increases due to a weakening of cell contacts between vascular endothelial cells under the action of proinflammatory cytokines (IL-1, IL-6, IL-8, TNF) [25]. ROS production is an integral feature of acute inflammation in the prostate and gonads [26, 27]. The result of the chronization of the process is the replacement of the prostatic glandular tissue with fibrous connective tissue. It sharply impairs the sperm quality and complicates its evacuation. The same process takes place in sterile (stagnant) prostatitis, when massive death of spermatozoa leads to migration of neutrophils into the prostate and a further increase in local production of ROS [28]. The result again is an increase in the permeability of the blood-testicular barrier, damage to gametes and the infertility formation [29]. These studies also provide a basis for the search for new methods of pharmacological correction of ROS levels [30].

Alteration of tissues, acute and chronic inflammation, activation of phagocytes become stages of an autoimmune attack of sperm and testicles. This existence of autoimmune reactions was raised after attributing these structures as the barrier organs for the immune system and autoimmune orchitis was described [31]. This trend of research has become one of the most popular directions in the study of the mechanisms of male infertility in recent years. Now materials of high-level studies prove all stages of the development of autoimmune reactions directed to spermatozoa and gonads from processing and presentation of autoantigens to the formation of autoantibodies [32, 33].

According to the Gell and Coombs classification these developing autoimmune conditions can be attributed to type II and III allergic reactions [34]. In the course of type II reactions, formed antisperm antibodies belong to the IgG and IgA classes, but IgG autoantibodies have an important diagnostic value [33]. In this regard, the results of some authors, which emphasize the role of antisperm IgA antibodies in the development of pathology, require critical analysis [35]. Probably the ability to bind to spermatozoa is possessed by autoantibodies of both classes, which significantly affect the mobility of gametes and lead to their agglutination. However, IgA antibodies are unable to activate the classic complement cascade, so their damaging effect will not be critical. Moreover, in the acidic environment of the vagina and uterus during fertilization, a significant part of the immune complexes disintegrate. On the contrary, IgG-antibodies are able not only to bind to autoantigens, but also to activate the classical complement pathway as well as trigger neutrophil degranulation and type III allergic reactions after the large immune complexes formation.

The pathogenetic role of IgA localized on sperm membranes requires further research. The problem is the lack of data on the sites of the urogenital duct, where IgA binds to spermatozoa. The role of IgA in autoimmune reactions has not yet been described in principle [36]. On the contrary, in recent years the development of autoimmune pathologies has been associated with IgA deficiency [37]. The appearance of IgA on sperm membranes may be a consequence of its binding to herpetic determinants in the course of viral neutralization. Moreover, it is important to evaluate the molecular form in which IgA is found on the surface of the sperm. If this is serum monomeric IgA form, then its level can be considered as a criterion for the destruction of the blood-testicular barrier, and the binding itself can occur directly in the testis or in the epididymis. If bound IgA has dimeric or tetrameric form, then this will indirectly indicate its transport through the monolayer glandular epithelium of the prostate and other glands. Only glandular epithelium is capable of dimerizing IgA, supplying it with a secretory component and transporting it to the surface. In this case, the most likely that IgA bind to spermatozoa in the urethra during it passes through the prostate gland.

The appearance of anti-sperm IgG-antibodies is a univocal consequence of chronic inflammation, since no epithelium has a special system for IgG transporting to the surface. Therefore, the release of IgG on the surface of the epithelium into the lumen of the canal is the result of weakening of the isolating contacts between epithelial cells under the action of inflammatory cytokines and ROS. The binding of IgG to sperm can occur in the seminiferous tubules in autoimmune orchitis, in the

epididymis under epididymitis, or in the urethra in the case of prostatitis. In any case the question of the pathogenetic role of IgA and IgG in male infertility requires further research.

The appearance of IgG on the spermatozoa membrane and the immune complex formation leads to the neutrophil activation and degranulation and a new round of ROS production, which is accompanied by further tissue damage. This has been shown in the framework of numerous clinical and experimental studies [38, 39].

The binding of autoantibodies to the spermatozoa can inhibit the acrosome reaction completely block its fertilizing ability [40]. Therefore, the assessment of the A23187-induced acrosome reaction was one of the first points of using flow cytometry in the study of sperm alteration in infertility [41, 42].

Changes in the morphology and functional characteristics of spermatozoa in infertility

A decrease in the concentration of sperm and their total number in the ejaculate is considered an important sign of male infertility. The Russian norm is based on the WHO indicators [1], amounting to at least 15×10^6 spermatozoa in 1 ml of semen (with lower values — oligozoospermia) with a total number of cells in the ejaculate equal to 39×10^6. Meanwhile, the British and American criteria for the norm are much stricter: a concentration equal to 13.5×10^6 spermatozoa in 1 ml is already interpreted as infertility and a firm conclusion about male fertility is made when the cell concentration is over 48×10^6 spermatozoa per 1 ml. This is more than 3 times higher than the WHO standards [43]. Other British experts urge to completely abandon the numerical norms of concentration and total sperm count, completely moving on to assessing the functional properties of the material [44].

The pathogenetic factors have a significant impact on the morphology of male gametes, which is revealed by the analysis of traditional spermogram. Normally, at least 4% of cells in the ejaculate have the classical morphology (a smaller proportion is called teratozoospermia). As a rule, the patient with infertility has two or all three morphological signs of it (oligozoospermia, asthenozoospermia and teratozoospermia) [8].

The influence of pathogenetic factors extends not only to the shape of the spermatozoa, but also to the structure of its nucleus, the density of chromatin packing, the state of the acrosome, the composition of membrane lipids and the state of the cell's recognition apparatus. The most detailed descriptions of the consequences of exposure to ROS, various viruses and autoantibodies.

The influence of oxidative stress is associated with a decrease in the reserve of catalase, glutathione peroxidase, glutathione-S-transferase, NO-synthase and superoxide dismutase under the action of ROS. These enzymes play an important role in spermatogenesis, and their deficiency critically affects the density of chromatin packing and protection of DNA from damage [45]. In the epididymis spermatozoa contain a non-compact DNA, which makes them inert [46]. Under the influence of hydrogen peroxide the oxidation of nuclear proteins occurs, which ensures the densification of DNA [47, 48]. Low concentrations of hydrogen peroxide also cause tyrosine phosphorylation, which increases the area on the sperm membrane where the sperm binds to the oocyte [49, 50]. There is also evidence that the presence of hydrogen peroxide is necessary for the acrosomal reaction to proceed, although the mechanism of the influence of H_2O_2, HO_2^-, and O_2^- is not completely clear [51]. Simultaneously the results confirm the danger of ROS high concentrations exposure to maturing and mature spermatozoa. ROS disrupt the work of mitochondria and reduce the energy reserve of the cell, which leads to a decrease in motility, as well as disrupt the acrosome function and reduce the ability of sperm to penetrate. ROS can also cause oxidative damage to DNA [19].

Studies have confirmed the significant effect of herpes and papilloma viruses on sperm motility. Based on ejaculate analysis of 71 infertile patients aged 22 to 44 years with the papilloma virus, it was shown that among the variants of pathozoospermia, asthenozoospermia was most often detected (56% of cases). Asthenoteratozoospermia (21%) and oligoasthenoteratozoospermia (16%) were less common. Oligoasthenozoospermia had the lowest frequency (6%). In most cases pathozoospermia was combined with papillomavirus types 16, 18, 33 [25]. Similar results were obtained in infertile patients infected with herpes viruses types 1-7 [52, 53].

Finally, ROS have a significant effect on the density of chromatin packing and the level of apoptosis of spermatozoa. Based on the examination of 433 infertile and 35 fertile men, it was shown that the activation of free radical processes and the imbalance of pro- and antioxidant systems play a leading role in the pathogenesis of male immune infertility. There is a direct relationship between ROS synthesis, the percentage of IgG^+-spermatozoa and apoptotic gametes with DNA fragmentation [39]. Moreover, infertility is caused not only by the initiation of DNA fragmentation, but also by ROS-induced changes in the histone/protamine ratio in the chromatin structure which leads to an increased risk of developing fetal abnormalities and miscarriage [54, 55]. There are also results of clinical observations indicating the

modification of the sperm membrane and its recognition apparatus under the action of ROS [56].

The literature contains data on the sequence of sperm alteration leading to the male infertility. The first event indicating a decrease in sperm fertility is a change in glycocalyx structure associated with decreasing of membrane charge. The second event is the destruction of the chromatin as well as decreasing in the mitochondrial potential. Finally, the destruction of the chromatin and a decrease in ATP production are accompanied by a decrease in intracellular transport and inhibition of acrosome function, sharply weakening the sperm ability to penetrate. Of these factors, the mitochondrial potential is the most reversible and widely variable at the stage of early apoptosis. The rest of the indicators vary within very narrow limits, which indicates the irreversibility of the changes [57].

Age-related changes in the structure and properties of spermatozoa

Literature data unanimously confirm the obvious fact of age-related decline in male fertility [58]. However, there are two aspects of age-related decrease in male fertility: the first is associated with the analysis of the age-related dynamics of spermogram indices, and the other — with the physiological and pathophysiological interpretation of these data.

Analysis of age-related changes in spermogram. Very important results for the analysis of the age-related aspect of male fertility are given in Ibishev et al. [25]. Based on the analysis of a large cohort of patients the authors indicate that the proportion of patients under 24 years old is only 1.4%, 24-26 years old — 9.9%, 26-28 years old — 26.8%, 28-30 years old — 9.9%, 30-32 years old — 12.7%, 32-34 years old — 11.3%, 34-36 years old — 5.6%, 36-40 years old — 7.0%, 40-42 years old — 11.3%, over 42 years — 4.2%. Apparently, this age distribution is associated with a whole complex of socio-psychological reasons, the analysis of which is not the purpose of this review. However, it should be borne in mind that after 24 years, patients begin to realize the need to seek medical help in connection with problems in the reproductive sphere. Of no less interest are the data on the number of spermatozoa and their motility in patients: in 56% of cases, asthenozoospermia was revealed, in 21% of cases — asthenoteratozoospermia, in 17% — oligoasthenoteratozoospermia, and in 6% of cases — oligoasthenozoospermia. The effect of various papilloma viruses on the number of sperm in ejaculate, the percentage of mobile forms and the percentage of morphologically normal cells was also assessed. There was a significant correlation between the sperm motility and the number of types of papilloma viruses detected in the patient, although with a low degree of conjugation ($r = -0{,}267$ at $p < 0{,}05$) [25].

Age-related changes in standard spermogram indices were first published back in 1950-1953 by J. McLeod and R. Gold in a series of 7 papers devoted to different indicators of semen analysis in men with infertility [59]. They proved a twofold decrease in the intensity of sexual activity in fertile men after 30 years, a decrease in ejaculate volume, absolute and sperm count, as well as their mobility. In men with infertility, these changes were more pronounced.

In subsequent years, the level and accuracy of research increased significantly, although the methodological differences require certain clarifications when comparing the results of the 1970s-1980s with modern data [60, 61]. The results of studies of fertile men 21-50 years old indicate that the minimum ejaculate volume was observed before 25 years (about 3.4 ml) and after 46 years (about 3.2 ml). The sperm concentration was minimal up to 25 years (about 97×10^6 in 1 ml), increased to $100\text{-}106 \times 10^6$ after 26 years of life and remained at this level up to 46-50 years. Moreover, in men after 40 years, there was a slight decrease in sperm motility and the proportion of cells with normal morphology [61]. In the same years, a morphological analysis of material after testicular biopsy in men 21-50 and 51-80 years old was carried out [62], which showed age-related extinction of the functions of the testes, which was expressed in a decrease in the mass of the organ, the volume and area of the spermatic cord, and a reduction in the area of interstitial tissue while replacing glandular tissue with fibrous connective tissue.

Recent studies have expanded the age interval of the surveyed population and gave an opportunity to compile a more accurate picture of age-related changes. On the basis of 25-year studies of a cohort of men (1023 people) with 5-year intervals, the age-related dynamics of indicators in the same individuals was traced [63]. It was found that the volume of ejaculate began to decrease after 46 years (which is confirmed by the data by Schwartz et al. [61]), but only after 55 years it decreased by 2 times from the indicators of the same men before 30 years. The concentration of spermatozoa in the ejaculate remains stable in the age range of 30-50 years, but decreases by 20-25% (to 42×10^6) after 55 years with a large scatter of data. It is important to pay attention to a significant decrease in the concentration of spermatozoa in the ejaculate by 2008 compared to the late 70s — early 80s, as indicated by many authors [5, 8]. Sperm motility begins to decline after the age of 46, and this process continues smoothly until the age of 55 or more. Finally, the proportion of morphologically normal forms turned out to be a very stable

individual indicator, which slightly decreased (from 6.0 to 5.0% of morphologically normal forms) over 25 years of observation [63].

Mechanisms of age-related changes in spermatozoa. The list of reasons of age-related changes in sperm is quite similar with features of infertility indicated above. It includes long-term exposure to ROS, leading to DNA damage and the formation of numerous mutations in germ and somatic cells, aging of chromosomes associated with shortening of telomere regions [64, 65], chronic (mainly viral) infections [25], age-related decrease testosterone production [66, 67], metabolic disorders associated with diabetes and obesity [12, 68], as well as numerous concomitant pathologies, including diseases of the cardiovascular system [69, 70].

However, some of the possible causes of age-related changes in the morphology and functional properties of sperm cells mentioned in the literature require additional analysis. Cellular aging can manifest itself at several levels. By-products gradually accumulate in senescent cells, as a result of which the level of activity of all physiological systems gradually decreases. This is what V.M. Dilman, putting forward the accumulation component of the aging process [68]. Mitochondrial changes are some of the most notable features of senescent cells, and several theories place mitochondria at the center of aging as an example of long-term ROS cell and tissue damage, although alternative theories exist [71]. However, oxidative stress is not associated with mitochondrial aging only. The level of energy metabolism, changes in mitochondrial DNA, and mitochondrial testosterone production are no less significant. The age-related decrease in mitochondrial functions is sometimes associated with the accumulation of many toxic factors in the mitochondria of all cells, including spermatozoa. With age, this process can lead to a significant decrease in their energy function. Particular emphasis is placed on the decisive importance of sperm mitochondria for male reproductive function, so mitochondria can be a link between aging and loss of fertility [72].

A new approach links age-related decline in sperm quality with depletion of spermatogenic epithelial cells, which is also affected by all of the above damaging factors [73]. This hypothesis leads to a completely new treatment for male infertility associated with the transdifferentiation of the patient's somatic cells into induced pluripotent stem cells. Initially, this method was developed to obtain mesenchymal stem cells capable of providing the renewal of fibroblastic cells. However, it must be taken into account that the rudiments of future germ cells are very early isolated from other main directions of embryonic development. The solution of the proposed problem is tantamount to dedifferentiation of somatic cells to the stages of primary ecto- and endoderm. Therefore, despite the large amount of research in this area, the assigned tasks are still very far from being solved.

The study of anti-Müllerian hormone (AMH), a glycoprotein belonging to the family of transforming growth factor (TGF), showed some possibilities of its application for treatment of age-related disorders of the male reproductive system. AMH plays a fundamental role in the regression of the Müllerian ducts in the male embryo. In boys, it is largely produced in the Sertoli cells until puberty, and then slowly decreases to residual values throughout the rest of the life of men. Sertoli cells produce AMH throughout their life not only into the blood, but also into the seminal plasma [74]. It is assumed that AMH can lengthen the activity of the germinal epithelium, which leads to a possible cooperation of this method and the method of renewal of the germinal epithelium due to inducible human stem cells [75].

Age and epigenetic changes. Epigenetic changes in spermatozoa can be an important bridge between many mechanisms of the male infertility formation, including age-related changes [76]. Available data suggest that there are very clear patterns of aging in the sperm epigenome that can be directly detected in DNA methylation patterns. Importantly, these alterations are so consistent that a predictive model has been successfully generated to predict an individual's age based only on sperm DNA methylation signatures. DNA methylation plays an important role in the development of negative changes in the genome, since it is associated with variations in the promoter regions [77]. Methylation marks at cytosine residues, typically found at cytosine phosphate guanine dinucleotides (CpGs), and have been shown to be capable of transcription regulation based on the presence or absence of this mark [78]. The link between hypermethylation of the *MTHFR, PAX8, NTF3, SFN, HRAS, JHM2DA, IGF2, H19, RASGRF1, GTL2, PLAG1, D1RAS3, MEST, KCNQ1, LIT1* and *SNRPN* genes with low sperm fertility and male infertility has been proven [79]. In addition to DNA methylation, sperm fertility can also be influenced by the modification of histones [80], changes in the chromatin structure [81], and the expression of noncoding RNA [82]. It is significant that the negative consequences of epigenomic changes for male health may be the result of exposure throughout life from the embryonic period to old age [83]. The same epigenomic changes are associated with exposure to adverse environmental factors at any period of life [84]. However, all these changes are reversible; therefore, one of the main research goals in this area is to assess the age of a man and/or a combination of factors, after which these changes become irreversible and lead to male infertility [76].

All the age-related changes in spermatozoa inevitably affect their fertilizing ability [85]. However, changes in the modern lifestyle, an increase in the proportion of the urban population, a lengthening of the period of professional growth and ever later periods of family construction lead to the need to create, use and improve methods for maintaining and prolonging male fertility.

Conclusion

In a sense, the situation in modern spermatology can be characterized as a methodological dead end, when the classical strictly regulated approaches based on microscopy and routine biochemical analyzes have practically exhausted themselves and are subjected to justified criticism, and innovative developments based on genomics and proteomics are still very far from perfect [86, 87]. The trivial sperm analysis, which has been the cornerstone of fertility verification for more than 50 years, is losing its former importance because it does not provide comprehensive information for many forms of male infertility.

One of the reasons for the slowdown in the rate of progress in the field of infertility diagnostics is that understanding of the basic mechanisms of life of mature spermatozoa is still limited. This mainly concerns the molecular basis of fertility, deciphering the entire set of interactions between chemical compounds that are involved in the implementation of the reproductive function. The practice of IVF, which in fact is the locomotive of research on the physiology and pathophysiology of spermatozoa, adds new problems associated with the search for new methods for assessing the viability and fertility of sperm in individuals of different ages.

Conflict of interests. The authors declare no conflicts of interest.

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