THE BIOLOGICAL ROLE OF APOPTOSIS IN SPERMATOGENESIS: A REVIEW

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Abstract. Apoptosis as a fundamental biological phenomenon is a special, genetically programmed form of physiological death of cell and is a prerequisite for the development and normal reproduction of living biodiversity, in particular the functioning of the reproductive system and the regulation of spermatogenesis. Apoptosis plays an important role in the regulation of spermatogenesis, a complex multi-stage process of sperm formation from primary germ cells, which begins during puberty and lasts throughout. The role of apoptosis in the regulation of spermatogenesis is well determined, since it controls the number of germ cells and eliminates defective gametes, but the role of apoptosis in ejaculate fertility impairment has not been elucidated yet. The biological role of apoptosis mechanisms in spermatogenesis is to maintain a constant number of cells in the spermatogenic system, to ensure the correct ratio of cells of various types and to remove genetically defective cells. Apoptotic changes in spermatozoa may result from the process initiated during spermatogenesis due to genetic disorders. So, a distortion of chromosome segregation, fixed at the verification points of the cell cycle, can lead to a switch to the apoptosis pathway. Thus, on the basis of the synthesis of information, it can be concluded that the biological role of apoptosis in the regulation of spermatogenesis is huge and inevitable for preserving biodiversity, however, to date, the patterns of sperm cell apoptosis realization and apoptosis markers initiation in the process have not been completely clarified. At the same time, it has been shown that the regulation of sperm cell apoptosis is directly dependent on the influence of various factors; in particular, it depends on the properties, concentration and time of exposure of the damaging agent to spermatogenesis.

Keywords: apoptosis, spermatogenesis, biodiversity, spermatogenic system.

Rezumat. Rolul biologic al apoptozei în spermatogeneză: un review. Apoptoza ca fenomen biologic fundamental este o formă specială, programată genetic de moarte fiziologică a celulelor şi constituie o condiţie prealabilă pentru biologia dezvoltării şi reproducerea normală a biodiversităţii viului, în special, pentru funcţionarea sistemului reproductiv şi reglarea spermatogenezei. Apoptoza joacă un rol important în reglarea spermatogenezei, care începe intensiv în perioada pubertăţii şi continuă pe tot parcursul vieţii organismului. Rolul apoptozei în reglarea spermatogenezei este bine definit, realizând controlul determinat al numărului de celule germinale şi eliminarea gameţilor patologici, iar rolul apoptozei în dereglarea fertilităţii ejaculatului încă nu este elucidat. Rolul biologic al mecanismelor de apoptoză în spermatogeneză este de a menţine un număr constant de celule reproductive în sistemul spermatogen, de a asigura raportul optim dintre celule nou formate de diverse tipuri şi de a elimina celulele spermatogene defecte genetic. Modificările procesului de apoptoză ale spermatozoizilor pot rezulta dintr-un proces iniţiat în timpul spermatogenezei şi din cauza tulburărilor genetice. Prin urmare, o dereglare a segregării cromozomilor, fixată la nivelul punctelor de reconciliere ale ciclului celular, poate provoca trecerea evoluţiei la fenomenele apoptozei. Astfel, în baza sintezei informaţiei existente, este posibil de concluzionat că rolul biologic al apoptozei în reglarea derulării spermatogenezei este imens şi inevitabil pentru păstrarea biodiversităţii, însă până în prezent, nu sunt elucidate complet schemele de realizare a apoptozei spermatozoizilor şi procesul de iniţiere a markerilor apoptozei. În acelaşi timp, s-a demonstrat că reglarea apoptozei celulelor reproductive este direct proporţională cu influenţa multiplilor factori, în special, depinde de proprietăţile, concentraţia şi durata de acţiune a agentului nociv asupra spermatogenezei.

Cuvinte cheie: apoptoză, spermatogeneză, biodiversitate, sistemul spermatogen.

INTRODUCTION

Despite the fact that many fundamental laws of the processes of formation, development and differentiation of spermatogenic cells are already well studied at all levels of the organization of living creatures, research activity in the field of male gametogenesis continues to be very intense. This is not only due to the cognitive interest in this key problem of biology, but is dictated by the need to solve an ever-expanding range of urgent practical problems of great biological and social importance.

In recent years, the efforts of many researchers have been focused mainly on the study of the biological processes of the reproductive system, ensuring continuous production of sperm throughout the life of eukaryotes and restoring the integrity of the spermatogenic epithelium in case of damage. The value of these studies is determined not only by their significance for theoretical biology, but also by the possibility of obtaining knowledge of interest from the point of view of tissue system regeneration through apoptosis. The revealed patterns of apoptosis have opened up great prospects in researching the fundamental aspects of spermatogenesis, elucidating new aspects of gene and cellular male infertility, creating transgenic organisms, and preserving rare, endangered species of biodiversity (MEACHEM et al., 2001; APONTE et al., 2005; EHMCKE et al., 2006; OATLEY & BRINSTER, 2006; BRINSTER, 2007; BROMFIELD et al., 2017).

THE ROLE OF APOPTOSIS IN THE REPRODUCTION

Apoptosis as a fundamental biological phenomenon is a special, genetically programmed form of physiological death of cell and is a prerequisite for the development and normal reproduction of living biodiversity, in particular the functioning of the reproductive system (PLOSKONOS, 2014) and the regulation of spermatogenesis (SELI & SAKKAS, 2005). Normally, apoptosis, the process of elimination of cells that have completed their functions or cells with an impaired

genetic apparatus, starts in 75% of male germ cells at the stage of spermatogonia (MILLIGAN & SCHWARTZ, 1997; KIERSZENBAUM, 2001; OLDEREID et al., 2001; MOIBENKO et al., 2005; BROMFIELD et al., 2019).

The process of apoptosis is characterized by certain morphological features – the nucleus and cytoplasm decrease in size, condense, fragment, the cell breaks up into several parts (apoptotic bodies) containing elements of the nucleus and intact organelles (ROBINSON, 1991; GRIGORIEV, 2003; NAGORNEV, 2003). The nucleus undergoes destruction through the formation of large fragments with their subsequent internucleosomal degradation. The plasma membrane of the cell undergoes a number of changes that make it recognizable for phagocytes. As a result, apoptotic bodies are rapidly absorbed by macrophages, as well as often by surrounding cells that do not specialize in phagocytosis (GRIGORIEV, 2003; NAGORNEV, 2003). Thus, the structural integrity of biological membranes during apoptosis is not affected. That prevents the release of the contents of the cytoplasm (including lysosomal enzymes) into the extracellular environment and the development of inflammation (VLADIMIRSCAIA, 2002; GRIGORIEV, 2003). Therefore, the process of apoptosis, as a rule, occurs without macroscopic signs, structural and functional defects of the tissue, and without the development of inflammation (GRIGORIEV, 2003).

Apoptosis plays an important role in the regulation of spermatogenesis, a complex multi-stage process of sperm formation from primordial germ cells, which begins during puberty and lasts throughout life (CHIU et al., 2014; EISENBERG et al., 2015; KURILO & STAUT, 2015; LAROSE et al., 2019). Sexual cells are the only cells in the multicellular diversity of the body that can actively counteract aging. In this, the process of spermatogenesis is accompanied by a kind of "rejuvenation" of maturing cells through the rigorous selection of apoptosis. In addition, the maturation of male gametes differs from the process of differentiation of somatic cells, first of all, by high requirements for the presence of preserved DNA. The insufficiency of apoptosis of germ cells differentiation leads to the formation of defective gametes (*gametopathy*). Gross defects in mature germ cells, as a rule, are not transmitted to the offspring, since such gametes are either destroyed by apoptosis some time after maturation, or they are unable to form a zygote, or the zygote formed with their participation dies at one stage or another of its development (KAMEL, 2013; STONE et al., 2013; LAROSEH et al., 2019).

The role of apoptosis in the regulation of spermatogenesis is well determined, since it controls the number of germ cells and eliminates defective gametes, but the role of apoptosis in ejaculation fertility impairment has not yet been elucidated. To maintain long-term fertility and sustain sufficiently high levels of spermatogenesis, a delicate balance needs to prevail between the different niche factors that control cell fate decisions of spermatogonial stem cells by promoting self-renewal, differentiation priming or spermatogenic commitment of undifferentiated spermatogonia (MÄKELÄ & HOBBS, 2019). Despite the obvious need, the topic has not been studied enough. Normally, apoptosis is not to affect spermatozoa, for which long viability is important. However, markers of apoptosis are detected in mature gametes, but the nature of their occurrence and their role, as well as the initiation process and the realization pattern of apoptosis in sperm are still unknown (LEWIS et al., 2013). Spermatozoa marked for death by apoptosis may be mobile and may not visually differ from normal gametes, but the process of apoptosis causes irreversible changes in the cells; such a sperm will not ensure normal development if used for fertilization (AGARWAL et al., 2015).

MECHANISMS OF APOPTOSIS IN SPERMATOGENESIS

Current knowledge of the molecular mechanisms of apoptosis development leaves no doubt that the regulation of apoptosis is directly dependent on the influence of exo-and endogenous factors and can be either direct or indirect in nature (SELYASKIN, 2014). In response to regulatory signals, developing germ cells express biologically active substances (proteins of cell proliferation and apoptosis, growth factors, differentiation factors, etc.), which are the final link in the regulation of spermatogenesis, mediating the entire cascade of effects on spermatogenesis. However, there is very little information about the factors regulating sperm apoptosis and, especially, about the regulatory effects of biochemical components of seminal plasma. The presence of at least two mechanisms of apoptosis activation and a complex system of its regulation does not allow us to speak about the unambiguity of the available results, and a comparative assessment of the sensitivity of various methods of apoptosis documentation is significantly difficult due to the wide variety of assessed signs and markers of apoptosis. The processes that allow the cell to adapt to the variability of influencing factors and determine the possibility of its further coexistence with others should be sought in the peculiarities of apoptosis regulation mechanisms (DISK et al., 2013).

The biological role of apoptosis mechanisms in spermatogenesis is to maintain a constant number of cells in the spermatogenic system, to ensure the correct ratio of cells of various types and to remove genetically defective cells. Sex cells undergoing such programmed death actively use a genetically controlled program aimed at their own death, thereby committing a kind of suicide. The death of gametes occurs in favour of the cell community; therefore, we can talk about the social behaviour of cells (BUNGUM et al., 2013; KOMIJA et al., 2014). J.R. Kerr (1972) outlined the morphological picture of cell death and the dynamics of structural changes in tissues: changes in the structure of the cytoskeleton, collapse of cell organelles in the cytoplasm, condensation and wrinkling of granules, disintegration of the nucleus and the formation of apoptotic bodies, and at the next stage, phagocytosis of apoptotic bodies by macrophages or surrounding cells and their destruction under the action of lysosomal enzymes.

Later, when studying the biochemical and molecular mechanisms of apoptosis, it has been found that apoptosis as genetically programmed cell death is characterized by a number of morphological and biochemical changes, including a decrease in cell volume, bloating of the cytoplasmic membrane, condensation and primary localization of

chromatin on the inner surface of the nuclear membrane, the so-called marginalization, as well as the formation of apoptotic bodies. The inflammatory process with apoptosis does not develop, because of rapid cell phagocytosis (ZINI & AGARWAL, 2011; KOMIJA et al., 2014).

A characteristic feature of apoptosis is the externalization of phosphatidylserine from the inner to the outer sheet of the plasma membrane of the cell, resulting in its increased permeability. The transmembrane potential of mitochondria also changes. As a result of changes in the permeability of mitochondrial membranes, ion channels open; apoptosis induction factors such as AIF (apoptosis-inducing factor) enter the cell cytoplasm. A change in the membrane potential of mitochondria leads to the appearance of reactive oxygen forms in the cell. Spermatogonia, spermatocytes and spermatids can undergo apoptosis; however, germ cells at the stage of spermatogonia and round spermatids are more likely to undergo apoptosis (ZINI & AGARWAL, 2011).

Undoubtedly, spermatogenesis is a complex dynamic process of male germ cells development, culminating in the formation of a large number of mobile gametes capable of autonomous existence and transfer of the paternal genome to the ovum. Spermatogenesis is under strict genetic and hormonal control, is subject to spatial temporal regularities and includes processes such as apoptotic reactions of spermatogonial cell self-renewal (REDDIEN & HORVITZ, 2004). Spermatogenesis is also a highly ordered process in time and space, which is characterized by a certain dependence of differentiated to different degrees germ cells from each other. This makes it extremely sensitive to the action of damaging factors that do not affect the synchronism of development of spermatogenic epithelial cells, but cause their death and destruction of the seminiferous tubules (KURILO & STAUT, 2015). The cyclical progression of the seminiferous epithelium guarantees the appearance of specific germ cell subpopulations after fixed intervals providing a coordinated and efficient control mechanism for cell fate decisions within the seminiferous epithelium, such as onset of differentiation (MÄKELÄ & HOBBS, 2019).

Presumably, apoptosis performs two functions during normal spermatogenesis. The first function is to numerically limit spermatogonial cells with Sertoli cells, which control gamete maturation (SELI et al., 2005). The microscopic examination of testis tissue easily reveals apoptotic patterns, as spermatozoa mature in large quantities. With age, the resources of precursors of germ cells with a preserved genotype are depleted, and gametogenesis ceases. In the male organism, the extinction of the reproductive function normally occurs over several decades. The spermatogenic epithelium continues to function in the old age, but more and more differentiating germ cells are destroyed by apoptosis, since among them the number of cells with a preserved genotype decreases; an increase in double-stranded DNA breaks occurs in them (SINGH et al., 2003). Therefore, in the seminiferous tubules of elderly and old males, in the absence of mature germ cells, many figures of apoptosis are detected, that indicates deterioration in the normal process of gamete selection with age (BUNGUM et al., 2013). The second function is the selective elimination of pathological gametes with abnormal morphology and impaired biochemical functions or DNA damage (SAKKAS, 2003; KAMEL, 2013). The role of apoptosis in regulating the number of germ cells is obvious, which cannot be said about the complete elimination of abnormal spermatozoa (SAKKAS, 2003).

Cell apoptosis involves the complex interaction of a large group of substances, among which a central place is occupied by special proteases – caspases (Cytosolic Aspartate-Specific cysteine Proteases). Caspases are aspartate-specific proteases that break down proteins at the locations of aspartic bases (VLADIMIRSCAIA, 2002; NAGORNEV, 2003). These compounds are inactive in the cytoplasm to exclude the possibility of accidental cell death (GRIGORIEV, 2003; NAGORNEV, 2003; ADAMS, 2003). Caspases consist of three domains: the N-terminal domain, the large subunit, and the small subunit. Caspase activation occurs by proteolytic cleavage of the N-terminal domain with the association of the subunits into a heterodimer and the formation of an active center (VLADIMIRSCAIA, 2002). According to their functional responsibilities and structural homology, caspases are divided into three groups: cytokine activators, inducers of effector caspase activation, effector caspases - performers of apoptosis (VLADIMIRSCAIA, 2002; NAGORNEV, 2003; ADAMS, 2003).

The main directions of the destructive effect of caspases on the male reproductive cell: inactivation of inhibitors of apoptotic proteins; direct cleavage of structural proteins of the cell; impairment of the regulation of protein synthesis; inactivation of proteins involved in DNA repair, mRNA formation, DNA replication (VLADIMIRSCAIA, 2002). It is customary to distinguish two fundamentally different mechanisms of caspase activation: the pathway of "death receptors" located on the cell surface is characteristic of intact cells; the mitochondrial pathway mediated by the Bcl-2 protein family is characteristic of pathologically altered cells (ADAMS, 2003; ITOH, 2004; BHANG et al., 2018).

It has been revealed that the spontaneous apoptosis of spermatogonia, spermatocytes and spermatids occurs in the male gonads (PRINT & LOVELAND, 2000). Mature spermatozoa can also have signs characteristic of a cell that has entered apoptosis (ZINI & AGARWAL, 2011). Moreover, spermatozoa show signs of apoptosis too (PLOSCONOS, 2013), a number of authors (CAYLI et al., 2004; GRUNEWALD et al., 2006; JI et al., 2009) describe the presence of apoptosis markers, such as externalization of phosphatidylserine, Fas-expression, increased caspase activity, DNA fragmentation, in normal spermatozoa and in cases of spermatogenesis disorders.

According to the literature (OOSTERHUIS et al., 2000; WANG et al., 2003) with various parameters of spermograms, on average, 20% of sperm cells show signs of apoptosis. Cells killed as a result of apoptosis are either phagocytosed by Sertoli cells or enter the lumen of the seminiferous tubules. In cases of spermatogenesis disorders, the number of germ cells entering apoptosis increases (GANDINI et al., 2000). At the same time, it is still not clear whether apoptotic markers of spermatozoa are the result of an apoptotic process that began before ejaculation during

spermatogenesis (SAKKAS, 2003), or whether they are a sign of apoptosis initiated in the post-ejaculatory period (TAROZZI et al., 2007). The apoptotic process may be associated with the premature departure of spermatids in the early stages of apoptosis from Sertoli cells, which, as a rule, are normally to actively participate in the elimination of such cell (MÄKELÄ & HOBBS, 2019).

C. Lachaud et al. (LACHAUD et al., 2004) assessed the death of spermatozoa – apoptosis and necrosis among freshly isolated sperm cells and after 24 hours of *in vitro* incubation, and found that spermatozoa do not start the apoptosis process *in vitro*, and their death is most often realized by necrosis rather than apoptosis. Thus, the presence of apoptotic markers in spermatozoa is a consequence of the processes initiated before ejaculation, which confirms the theory of apoptosis.

As you know, the site of spermatogenesis is testicular tissue, but after maturation, already at the post-testicular level, normal sperm can undergo physiological or non-physiological damage. So, if sperm is located in the epididymis for a long time, which serves as storage for spermatozoa for 12-14 days, sperm cells age or may be exposed to increased levels of reactive oxygen species (ROS) for a long period before ejaculation (GABER et al., 1983; LAMIRANDE et al., 1995). ROS is a known inducer of apoptosis in somatic cells (RATAN et al., 1994) and in mature spermatozoa at the testicular level (GORCZYCA et al., 1993; AITKEN, 2020). Damage caused by sperm storage is called the effect of aging, or overripeness, and is reflected in the degree of chromatin condensation and the ability of the genome to produce normal embryonic development. It is known that if fertilized with stale sperm, the animals' embryonic mortality increases, and aborted embryos show chromosomal abnormalities. In extracts from animals' old sperm cells, an increase in the content of antigens was found (GABER et al., 1983).

The destruction and elimination of aging or pathological forms of spermatozoa occurs by apoptosis, due to using the ability of the epithelium of the appendage, and spermiophages play an important role in this process. However, when ejaculating from the tail of the epididymis, the spermatozoa do not emerge in a homogeneous population, but vary in maturity (GABER et al., 1983), therefore, there is a certain percentage of not only atypical, but also normal spermatozoa in the ejaculated sperm, which are at the beginning or at different stages of aging and apoptosis. The aging of sperm cells and the apoptosis process launched in them are especially important to be considered in the practice of auxiliary reproductive technologies during artificial insemination procedures.

In addition, spermatozoa can undergo apoptosis under the action of agents of various natures, which are a part of the secretion of the accessory sex glands when they merge with it at the time of ejaculation. Thus, the percentage of truly apoptotic spermatozoa (PLOSCONOS, 2014) among the total number of spermatozoa with signs of apoptosis in fertile ejaculates is not clear.

Some studies have shown the important role of various pro- and anti-apoptotic factors in spermatogenesis (PRINT et al., 2000; TAROZZI et al., 2007) mechanisms are known by which the Sertoli cells trigger and regulate male gamete apoptosis (RICHBURG, 2000). Proteins of the Bcl-2 family, both pro-apoptotic (Bax, Bak, Bcl-xs, Bad) and anti-apoptotic (Bcl-2, Bcl-xL), participate in the regulation of apoptosis. The ratio of pro- and anti-apoptotic proteins determines the fate of the cell. So, in case of overexpression of the Bax and Bak protein genes, an inactivation of the Bcl-2 protein is observed, and the cells enter apoptosis (SINHA HIKIM et al., 1998). It was proved that the transgenic overexpression of the Bcl-2 protein led to the blockade of apoptosis and, as a result, to a disorder of normal spermatogenesis and male infertility (FURUCHI et al., 1996).

Some authors described morphological changes in the nucleus and cytoplasm of spermatozoa that are characteristic of apoptosis and similar to those in somatic cells, but they had their own features (GANDINI et al., 2000; MURATORI et al., 2000; MARTIN et al., 2004).

Other authors described the presence of apoptosis molecular markers in spermatozoa: Fas-expression, decreased transmembrane potential of mitochondria, externalization of phosphatidylserine, increased caspase activity, change in the expression level of apoptosis regulators (proteins of the Bcl family, etc.), DNA fragmentation (CAYLI et al., 2004; JI et al., 2009; ROBINSON et al., 2012; LEWIS et al., 2013).

However, it is not clear whether the apoptosis markers are the result of a failed apoptotic process that began before ejaculation during spermatogenesis (SAKKAS, 2003; TESARIK & MARTINEZ, 2002), or whether they are signs of apoptosis initiated in the post-ejaculatory period (SELI & SAKKAS, 2005; TAROZZI et al., 2007). The apoptotic process is probably associated with the premature departure of spermatids at the very early stages of apoptosis from Sertoli cells, which, normally, are actively involved in the elimination of such cells.

Methods of documentation of sperm cell apoptosis can be divided into three groups: 1) identification of morphological changes characteristic of the apoptotic cell; 2) identification of DNA changes in spermatozoa that entered apoptosis; 3) identification of structural biochemical changes occurring outside the nuclear apparatus of the cell.

The detection of the expression of the apoptosis marker Fas on the cell is not an evidence of apoptosis, but only an indication of the potential readiness of the cell for Fas-dependent apoptosis (DONNELLI et al., 2001; CHEN et al., 2004), although the externalization of phosphatidylserine is one of the earliest biochemical changes that occur with apoptosis (ANZAR et al., 2002; VRIES et al., 2003).

Among the markers of apoptosis, the most studied are the cellular receptor Fas (CD95), which indicates the readiness of cells for Fas-induced apoptosis, and its ligand FasL (CD154), the main inducer of the signal for triggering apoptosis (NAGATA, 1999; FRANCAVILLA et al., 2000; PLOSCONOS, 2012). Fas and FasL are surface proteins of the cell membrane, the key molecules that trigger the apoptosis process. However, it should be emphasized that the

expression of Fas on cells only indicates the potential readiness of the cells for Fas-dependent apoptosis, but in no way is an evidence of apoptosis (SION et al., 2004).

The functions of the Fas-receptor-ligand system are to regulate the number of germ cells at the level of spermatogenesis, as well as to track various injuries and malfunctions during spermatogenesis and ensure the removal of damaged cells (PRINT & LOVELAND, 2000; PLOSCONOS, 2012).

The Fas-receptor-ligand system is involved in protection against immunological reactions in immunologically privileged tissues, including the testes (PLOSCONOS, 2012). One of the possible functions of FasL, recently discussed, is the function of self-defense of the male gamete from antisperm-activated lymphocytes in the male germ tract. Thus, the expression of FasL on spermatozoa may be one of the factors that increase their survival (ANZAR et al., 2002; PLOSCONOS, 2013, 2014).

The literature includes rather conflicting data on the presence of the apoptosis markers Fas and FasL on the surface of spermatozoa. Thus, some researchers find expressions of Fas and FasL on spermatozoa of fertile and subfertile ejaculates, while others do not. This may be due to different methods for determining the markers, as well as to the quality of reagents (for example, the use of different antibodies to detect Fas and FasL). Some researchers believe that with various disorders of spermatogenesis, there is a change in the expression of the apoptosis markers Fas and FasL on spermatozoa, reflecting the activity of pathological processes in the male reproductive system (SAKKAS, 2003; MCVICAR et al., 2008; PLOSCONOS, 2012, 2013). Thus, to date, there is no consensus among researchers in the world literature regarding the expression of Fas and FasL on germ cells and in the male reproductive system as a whole, which necessitates the expansion of work in this direction. In addition, few researchers have attempted to identify a correlation between the expression of Fas and FasL on sperm cells and some functional and morphological parameters (morphology of spermatozoa, their concentration, motility) of ejaculates of men recommended by the WHO for research (SAKKAS, 2003; MCVICAR et al., 2008).

Among the markers of apoptosis, the cell death initiation receptor Fas is more studied, and its detection on the cell indicates its readiness for Fas-induced apoptosis, and not apoptosis itself. Also, the FasL ligand, the initiator of the molecular component of apoptosis, the main inducer of the signal for triggering apoptosis, plays a decisive role in the process of apoptosis (DONNELLI et al., 2001; CHEN et al., 2004).

Fas – a glycolized surface protein of the cell membrane - is found on the surface of fertile and infertile sperm cells, a marker of cell readiness for apoptosis (SAKKAS, 2003; MCVICAR et al., 2008).

The specific transmembrane protein FasL from the cytokine family is a ligand of the Fas-receptor. In the reproductive system, FasL, unlike Fas, is mainly expressed on activated follicular cells of the testes – Sertoli cells, supporting epitheliocytes and sustenocytes. It is bound to Fas in cells sensitive to apoptosis.

The Fas-receptor-ligand system is involved in protecting against immune responses in immunologically privileged tissues, including the testes. So, sperm cells in young organisms (young males) contain antigens that have not previously been in contact with immune cells, so an immune response may develop on them (ANZAR et al., 2002; MCVICAR et al., 2008). To avoid this, spermatozoa develop in spermatogenic tubules, whose walls allow the passage of nutrients, oxygen, hormones, but do not allow contact with immune cells circulating in the blood. All the structures of the seminiferous tubule, and first of all Sertoli cells, form a hemato-testicular barrier (BOZHEDOMOV et al., 2011). Normal spermatogenesis depends on Sertoli cells, mainly due to their influence on nutrient supply, maintenance of cell junctions, and support for germ cells' mitosis and meiosis (Ni, Hao, Yang, 2019). Sertoli cells constantly produce high levels of FasL (FRANCAVILLA et al., 2000), which is bound on the surface of lymphocytes entering the testis parenchyma.

Riccioli et al. suggested revising the role of FasL in the testes, which consists in maintaining their immune privilege and regulating physiological apoptosis of germ cells, since the hypotheses are based on erroneous cell localization of FasL in the vas deferens epithelium (RICCIOLI et al., 2003). Riccioli et al. showed that FasL is present on the surface of epididymal sperm cells, and not on Sertoli cells, and is based on spermatozoa, which allowed the authors to propose a new function for FasL - the function of self-protection of the male gamete from lymphocytes in the female genital tract (RICCIOLI et al., 2003). However, the involvement of the Fas-system in the apoptosis of epididymal spermatozoa in the absence of androgens is controversial (SUGIHARA et al., 2001).

The association of apoptosis markers with sperm cell death, considered by various authors (BLANC-LAYRAC et al., 2000; RAMOS et al., 2001; SAKKAS, 2003), can be explained by increased sensitivity to the effects of external damaging factors of those spermatozoa whose apoptosis was started in the last stage of spermatogenesis, but interrupted after spermation. About 20% of sperm cells are detected as apoptotic (BACCETTI et al., 1996). Perhaps this is due to membrane or mitochondrial damage caused by the onset of apoptosis.

The process of sperm apoptosis has its own features associated with the unique organization of DNA and the cell as a whole. It is suggested that in germ cells closely associated with Sertoli cells, DNA fragmentation occurs without activation of caspases, and the process is not accompanied by externalization of phosphatidylserine. After release from Sertoli cells, spermatids and spermatozoa use their independent cell death signalling pathway (GRECO et al., 2004; TESARIK & MARTINEZ, 2004; BHANG et al., 2018).

Apoptotic changes in spermatozoa may result from the process initiated during spermatogenesis due to genetic disorders. So, a distortion of chromosome segregation, fixed at the verification points of the cell cycle, can lead to a switch to the apoptosis pathway (BRUGNON et al., 2006).

In a few studies, an attempt was made to identify a correlation between sperm cell apoptosis and traditional sperm parameters (sperm cell morphology, concentration and motility) (RICCI et al., 2002; CHEN et al., 2004; MURATORI et al., 2008). However, the effect of apoptosis on sperm functions and quality is largely elusive.

The assumption about the correlation of sperm cell morphological parameters with DNA fragmentation was put forward on the basis that the role of somatic cell apoptosis is to eliminate pathological cells, and DNA in abnormal spermatozoa is destroyed by analogy with somatic cells (OEHNINGER, 2003; WANG et al., 2003). Apoptotic changes of chromatin in spermatozoa are often associated with poor sperm counts (SIVANARAYANA et al., 2012). At the same time, some authors state that there is no clear correlation between DNA fragmentation and ejaculate parameters (concentration, motility and morphology) (MURATORI et al., 2000; RUBES et al., 2005). Other authors show that the percentage of apoptotic sperm cells in the ejaculate correlates with sperm cell motility and concentration (GLANDER & SCHALLER, 1999; OOSTERHUIS et al., 2000), and by inducing apoptosis in spermatozoa, it is assumed that motility may serve as a marker of intact DNA (RAMOS & WETZELS, 2001).

CONCLUSIONS

Thus, on the basis of the synthesis of information, it can be concluded that the biological role of apoptosis in the regulation of spermatogenesis is huge and inevitable for preserving biodiversity; however, to date, the patterns of sperm cell apoptosis realization and apoptosis markers initiation in the process have not been completely clarified. At the same time, it has been shown that the regulation of sperm cell apoptosis is directly dependent on the influence of various factors; in particular, it depends on the properties, concentration and time of exposure of the damaging agent to spermatogenesis.

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