ROLE OF LINC COMPLEX PROTEINS IN SPERM FORMATION

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Spermatogenesis is characterized by the significant changes of three-dimensional organization of the nucleus in spermatocytes, spermatides and spermatozoa. The functional cooperation between the nuclear envelope proteins and the acroplaxome/manchette is essential for nuclear elongation, acrosome biogenesis, formation of the flagellum. Furthermore, the nuclear envelope ensures the non-random chromosome arrangement within the nucleus. The LINC (linker of nucleoskeleton and cytoskeleton) complex proteins are involved in interaction between the cytoskeleton and the nucleoskeleton, as well as in the control of mechanotransduction. The LINC complex contains proteins of the outer and inner nuclear membranes: KASH and SUN, respectively. The LINC complex proteins are involved in formation of the sperm head and flagellum, and are, therefore, essential for male fertility. This review will consider the issues of the LINC complex protein localization in cells during the successive stages of spermatogenesis, the role in regulation of sperm maturation, and mutations of the LINC complex proteins resulting in male infertility.

Keywords: LINC complex, nuclear lamina, nuclear pores, globozoospermia, male infertility

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РОЛЬ БЕЛКОВ LINC-КОМПЛЕКСА В ФОРМИРОВАНИИ СПЕРМАТОЗОИДОВ

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Процесс сперматогенеза характеризуется значительными изменениями в трехмерной организации ядер в сперматоцитах, сперматидах и сперматозоидах. Элонгация ядра, биогенез акросомы, формирование жгутика требуют функциональной кооперации между белками ядерной оболочки и акроплаксомы/манжеты. Помимо этого, ядерная оболочка обеспечивает неслучайное распределение хромосом в ядре. Белки комплекса, связывающего нуклеоскелет и цитоскелет (linker of nucleoskeleton and cytoskeleton, LINC), участвуют во взаимодействии цито- и нуклеоскелета, а также управляют механотрансдукцией. В состав LINC-комплекса входят белки внешней и внутренней мембраны KASH и SUN соответственно. Белки LINC-комплекса вовлечены в формирование головки и жгутика сперматозоида, таким образом, они необходимы для мужской фертильности. В обзоре представлены вопросы локализации белков LINC-комплекса в клетках на последовательных стадиях сперматогенеза, роль в регуляции созревания сперматозоидов и мутации белков LINC-комплекса, приводящие к мужскому бесплодию.

Ключевые слова: LINC-комплекс, ядерная ламина, ядерные поры, глобулозооспермия, бесплодие

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Spermatogenesis, which is responsible for sperm differentiation, is coordinated in space and time. During the process, male germ cells undergo changes when going through three fundamental phases: spermatogonial stem cell self-renewal, spermatogonial proliferation, meiotic division of spermatocytes to produce haploid round spermatids, and transformation of spermatids into spermatozoa. Spermatozoa acquire optimal architecture; the nuclear structures undergo changes that ensure the pairing of homologous chromosomes during meiosis, formation of haploid cells, extremely marked chromatin condensation to protect the parental genome from chemical and physical stress, and sperm head size reduction. Spermatogenesis involves formation of the acrosome and the flagellum in round spermatids, cytoplasm elimination, and nuclear condensation that requires histone replacement by protamines. The nuclear envelope alterations affect both inner and outer nuclear membranes. The outer membrane is involved in nuclear positioning and movement; the inner membrane is associated with the nuclear lamina, the 3-dimensional protein meshwork of lamins together with lamina-associated proteins and chromatin. This regulates a broad range of functions of the nucleus, such as chromatin organization, DNA transcription and replication. Chromosomes occupy specific regions of cell nucleus, called "chromosome territories". Chromosomes in sperm nuclei are characterized by radial arrangement that affects gene expression. The nuclear envelope and integral proteins of the inner nuclear membrane play a key role in chromosome positioning and sperm head formation [1]. Nuclear reorganization is typical for both spermatocytes and spermatids. During meiotic prophase, the nuclear envelope proteins together with cytoskeletal elements control chromosome positioning. The nuclear envelope provides a platform for assembly of multiprotein complexes involved in gene expression regulation that results in morphological alterations of cells, such as nuclear elongation, acrosome

biogenesis, and flagellum formation [2]. A physical link is formed between the nucleoskeleton and cytoskeleton, which is essential for the nuclear positioning and motion. The LINC protein complex, the linker of nucleoskeleton and cytoskeleton, is formed by two transmembrane protein systems: the Nuclear Envelope Spectrin repeat proteins (NESPRIN), located in the outer nuclear membrane, and Sad1p-UNC84 (SUN), located in the inner nuclear membrane [3–5]. The central domains of NESPRIN proteins are of various lengths. C-terminal KASH (Klarsicht, ANC-1, Syne homology) transmembrane domain promotes the nuclear envelope localization and N-terminal domain is linked to the cytoskeleton [6–8]. On the contrary, SUN protein has a nucleoplasmic N-terminal domain linked to the nucleoskeleton, and a C-terminal domain located to the perinuclear space [2]. Nesprins include four proteins identified as KASH proteins, encoded by four genes, known as SYNE1, SYNE2, SYNE3, and SYNE4. The SUN family is comprised of five proteins (SUN1–5) [6, 9, 10]. The most common Nesprin isoforms, KASH1 and KASH2, are able to interact with F-actin. KASH3 is associated with intermediate filaments through the plectin-binding sequence [11]. KASH 4 interacts with kif5b, the microtubule-dependent motor kinesin-1 subunit, and, therefore, with microtubules [2]. In addition to these four members, KASH5 is the most divergent one; the KASH5 N-terminal domain interacts with dynein–dynactin. KASH5 mediates attachments between microtubules and chromosomes, and contributes to the rapid movement of chromosomes in the nuclei, thus facilitating chromosome pairing. Furthermore, this protein plays an important role in human fertility [12]. Among SUN proteins, SUN1 and SUN2 are expressed in somatic cells, while SUN3, SUN4 (the alternative name is SPAG4), and SUN5 (the alternative name is SPAG4L) are expressed exclusively in the testis [2, 9, 13]. The intracellular signaling pathways involve not only the LINC complex, but also the nuclear pore complex [14]. Firstly, SUN proteins located in the inner nuclear membrane require protein import into the nucleus, i.e. the properly functioning nuclear pores are necessary. Secondly, SUN1, colocalized with nucleoporin Nup153, affects the arrangement of the nuclear pore complexes [15]. The combination of SUN1 and SUN2 interacts with the mRNA particles, directly involved in the mRNA export through the nuclear pores in mammalian cells [16]. The regulatory function of the LINC complex during spermatogenesis was assessed in a number of studies. It has been shown that the LINC complex proteins are involved in proper chromosome pairing, meiotic chromosome recombination, telomere motion and attachment. SUN1 deficiency in mice prevents telomere attachment to the nuclear envelope, effective pairing of homologous chromosomes, and synapsis formation during prophase of meiosis. KASH5, being a specific partner of SUN1, is also involved in these events. SUN2 binds to KASH5 to form the SUN1-SUN2-KASH5 complex involved in meiotic telomere attachment to the nuclear envelope [2, 17]. SUN5 is involved in meiotic recombination as well. It is assumed that the SUN5-KASH2-LINC complex is involved in meiotic division of murine spermatocytes [18]. This function is associated with the presence in the cells of the properly functioning nuclear lamina, the fibrous layer underlying the inner nuclear membrane. In mouse cells, KASH5, colocalized with lamin B1, is involved in the nuclear motion during meiosis [19]. The LINC complex is also involved in sperm head formation and head-to-tail linkage. Thus, the LINC complex proteins play important role in male fertility [13, 20, 21]. Further study of characteristics of proteins of this complex will make it possible to create a relevant express test system, allowing us to assess the contribution of each protein to the development of spermatozoa, impaired spermatogenesis and infertility treatment adjustment.

The issues regarding the role of proteins in the development of mature spermatozoa and the role of the LINC complex protein mutations in male infertility are discussed in this review.

Role of LINC complex in mechanotransduction

Mechanobiology describes how mechanical forces influence cell morphology and physiology. Such exposure is involved in regulation of cell development and differentiation. The majority of mechanical effects result in nuclear motion, alterations in the nuclear shape, chromatin structure, and gene expression. The LINC complex plays a crucial role in these processes. Via mechanotransduction, cells convert mechanical stimuli to biochemical signals, thus providing the link between the cytoskeleton and the nucleoskeleton. Mechanical forces applied to cells are converted to biochemical signals transmitted from the cytoskeleton to the nucleus, providing modulation of the nuclear envelope composition, nuclear shape, and gene expression. The LINC complex is hub for signal transduction from the cytoskeleton to the cell nucleus. This idea emerged after the study of the isolated nuclei exposed to mechanical stimuli. This experiment showed that the LINC complex disruption or impaired communication between the LINC complex and the nuclear lamina caused cytoskeletal disorganization and affected both signal transduction through the cytoskeleton to the nucleus together with gene expression [22]. Actin plays a key role in mechanotransduction. Actin is involved in cellular motility, signal transduction and response to mechanical stress. Some other functions of actin have been reported recently: the network formed by actin filaments near the nuclear envelope may be involved in regulation of the nuclear motion and may affect gene expression [23]. The LINC complex provides mechanical coupling between the nucleus and the actin cytoskeleton that affects nuclear motion and positioning. Binding between actin and LINC complex is mediated by KASH proteins, which on the one hand interact with SUN proteins of the nuclear envelope, and on the other, interact with the cytoskeleton via the actin-binding domain [24]. Actin forms thread-like structures that cover the surface of the nucleus. In these structures, actin interacts with KASH2 or KASH3 by contributing to the nuclear orientation and shaping, and protects the nucleus from mechanical deformation. Mutations of KASH2/KASH3 are associated with inhibition of the perinuclear actin cap assembly and adversely affect mechanotransduction [26]. Hippo kinase signaling cascade is one of the major pathways for signal transduction from mechanoreceptors. This evolutionary conservative pathway is involved in regulation of cell proliferation and tissue differentiation, as well as in specifying the organ size. The cascade of reactions induces activation of actin. The signaling pathway involves Yes-associated protein (YAP) and the homologous TAZ protein (transcription co-activator) with PDZ binding motif. PDZ motif is a sequence of 80–90 amino acids, arranged in six β-sheets (βA–βF) and two α-helices (αA, αB). Moving between the nucleus and the cytoplasm, YAP and TAZ transmit signals from mechanoreceptors to the nucleus [26]. During mitosis, actin and the LINC complex proteins regulate centrosome and chromosome positioning; during meiosis, actin is also involved in nuclear motion [27]. Actin and the LINC complex proteins control gene expression. Thus, actin is involved in the determination of the nuclear volume, chromatin conformation, and chromatin accessibility for transcription factors responsible for gene expression. Alterations in gene

expression result from the LINC complex abnormalities and the LINC complex-dependent mechanisms. KASH protein downregulation leads to gene expression alterations in epithelial cells [28]. In other words, transcription is not only regulated via mechanosignaling, but also depends directly on the LINC complex protein integrity. Currently, it is still unclear whether the LINC complex proteins are directly bound with chromatin, or interaction is realized via the nuclear lamina. Furthermore, histones are replaced by protamines in the post-meiotic germ cells. In somatic cells, the Lamin B receptor (LBR), the integral protein of the inner nuclear membrane, interacts with lamin B1 and provides the link between the nuclear lamina and chromatin. The interaction involves heterochromatin protein 1 (HP1) and BAF protein (barrier to autointegration factor) [29, 30]. In germ cells, LBR can temporarily bind to protamine 1. During mammalian spermatogenesis, protamines are phosphorylated to ensure the binding of chromatin in spermatids and are completely dephosphorylated during sperm maturation [31]. In elongated spermatids, phosphorylation is required to ensure the temporary association of protamine P1 and LBR [32]. Localization of a number of the nuclear envelope proteins during spermatogenesis in humans was studied [30]. Polymerase chain reaction and immunfluorescence analysis revealed LEMD1 protein, together with ANKLE2, LAP2β and the short isoform of protein LEMD2 in spermatids. However, no emerin, LBR, full-length forms of LEMD2 and LEMD3 were found in the samples. Proteins LEMD1, ANKLE2, LAP2β, emerin, LBR, full-length forms of LEMD2 and LEMD3, lamins A, C and B2 were not found in ejaculated spermatozoa. Thus, the nuclear envelope proteins involved in interaction with chromatin in somatic cells change their localization in the nuclei of spermatids and spermatozoa in accordance with the chromosome positioning and stabilization in specific areas of the nucleus. In humans, such alterations in the composition of the nuclear envelope in spermatids may provide chromatin detachment from the nuclear envelope and lamina that increases the potential of the histone-to-protamine transition [30]. BAF and BAF-L (BAF-like) proteins were found in spermatozoa [33]. BAF homodimers are involved in chromatin condensation, BAF/BAF-like heterodimers are able to increase the massive histone-to-protamine transition due to more “open” chromatin conformation [30]. Actin, being the conventional cytoskeletal component that provides communication with the LINC complex, circulates between the nucleus and the cytoplasm. In the nucleus, actin regulates the activity of transcription factors, contributes to the assembly of some chromatin remodeling complexes, and appears to be associated with three different RNA polymerase complexes [34]. One of the mechanisms underlying mechanotransduction functions as follows: mechanical stimuli are able to induce physical alterations in the nuclear pore complexes regardless of the LINC complex [35]. Mechanical alterations can also modulate the phosphorylation status of the nuclear envelope proteins. Lamins A and C are examples of proteins, whose phosphorylation define nuclear stiffness in response to mechanical stimuli [36]. Chromatin organization can be changed under the influence of cell exposure to environmental factors and cytoskeletal reorganization; such alterations of the chromatin status affect gene expression. Perinuclear actin provides the LINC complex protein-mediated regulation of the lamins A and C hyperacetylation, chromatin decondensation, and gene expression activation. The nuclear import is an alternative mechanism designed for regulation of gene expression induced by mechanotransduction. Import of histone-lysine N-methyltransferase EZH2 causes gene silencing due to histone methylation, while import of histone deacetylase (HDAC) leads to gene repression due to hypoacetylation [37, 38].

LINC proteins and sperm head

Acrosomal and postacrosomal segments are distinguished in the sperm head. The nucleus containing highly compacted chromatin occupies almost the entire sperm head. It is covered partially by the acrosome, a cap-shaped organelle derived from the Golgi apparatus that contains enzymes and receptors for oocyte binding. The acroplaxome is a structure located between the acrosomal membrane and the nuclear membrane that surrounds the developing acrosome and attaches it to the nuclear envelope. The acroplaxome contains keratin 5, F-actin and profilin IV. The manchette is a transient structure containing microtubules of centrosome [39]. Sperm capacitation occurs in the female genital tract. Capacitation involves biochemical modifications essential for oocyte fertilization. The testis-specific form of the SUN protein (SUN1η is the most interesting component of this cascade [20]. SUN1η is located at the anterior pole of the nucleus and points to the acrosomal membrane instead of the inner nuclear membrane. This protein participates in assembly of the extranuclear LINC-KASH3 complex involved in the plectin-mediated interaction with the acroplaxome. The same complex is assembled on the posterior pole of the nucleus (except for the area of the implantation fossa) [40]. Recent studies have shown that SUN3 and SUN4 are essential for sperm head formation. During spermatogenesis, the expression of SUN3 increases when the round spermatids are formed, and further increases during formation of elongated spermatids. SUN3 is localized in the lateral and posterior parts of the spermatid nucleus, however, no SUN3 was found in the implantation fossa and the rearmost part of the nucleus. KASH1 is the partner of SUN3 in the sperm head. KASH1, colocalized with SUN3, is involved in the LINC complex assembly. The LINC complex is capable of interacting with actin by the actin-binding domain or with microtubules via the dynein–dynactin complex. It can improve the interaction between the manchette and the outer nuclear membrane [20]. SUN4 is expressed only during spermatogenesis. It is localized to the posterior poles of the round and elongated spermatids. The protein complex SUN3-SUN4-KASH1 provides the link between the manchette and the nuclear envelope. Abnormal numbers of round spermatids, impaired sperm head elongation, and nuclear envelope disintegration with subsequent production of the deformed spermatozoa could be observed in the Sun4 knockout mice. The decrease in the level of SUN4 induces the SUN3 protein migration to the cytoplasm and manchette disorganization. This indicates that SUN4 is essential for normal SUN3 and KASH1 localization in the cell [13]. In humans, SUN4 forms the complex with the cytoskeletal protein septin (SEPT12) expressed specifically in testes and lamin B1 involved in the sperm head formation in post-meiotic germ cells, as well as in the sperm flagellum arrangement [41]. Along with actin filaments, microtubules, and intermediate filaments, the cytoskeleton also contains septins engaged in various physiological functions. In particular, SEPT12 is involved in mammalian spermatogenesis: it is expressed around the manchette, in the necks of elongated spermatids and annuli of mature spermatozoa [42]. In the study of the role of the LINC complex in acrosome biogenesis, the new testis-specific protein SPAG4L-2 of the family SPAG4L was characterized. High level of protein expression was observed during spermatogenesis; the protein was localized in the apical region of the round spermatid nucleus that points at the acrosome [43]. During nuclear elongation, SUN5 progressively migrates to the posterior pole of the nucleus in elongated spermatids to finally reach the implantation fossa. Such redistribution of protein excludes the protein from

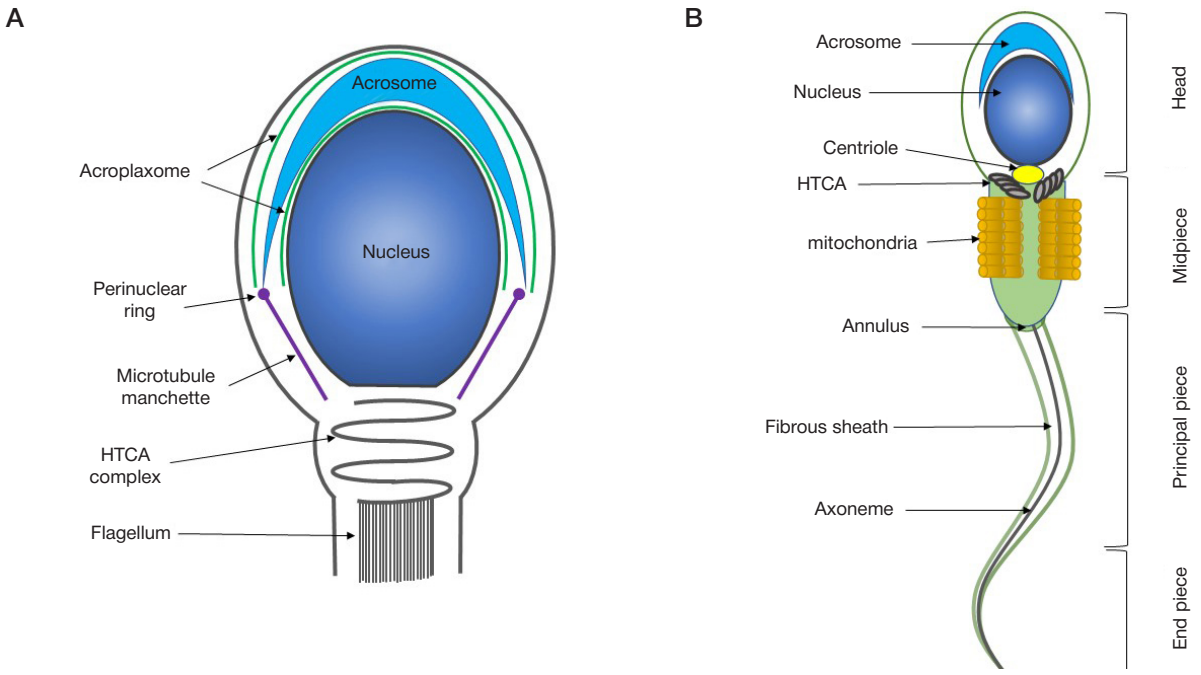

Fig. A. Spermatid structure. **B**. Structure of spermatozoon

the sperm head formation process. Sun5 knockout mice showed no disturbances of acrosomal development, however, abnormalities of the sperm flagella were found [44, 45]. Lamins are also involved in acrosome biogenesis. Lamins A and C were characterized as the components of the acroplaxome that is essential for acrosome biogenesis and spermatid head formation. Phosphorylation is an important mechanism responsible for the localization of lamins A/C in the cytoplasm, nucleoplasm or nuclear lamina [46]. Lamin B1 interacts with the testis-specific protein DRY19L2 synthesized mainly in spermatids. Disruption of DRY19L2 results in the altered localization of lamin B1 during spermatogenesis, as well as in the disturbed communication between the nuclear envelope and the acroplaxome during acrosome biogenesis. The sperm nucleus seems to be poorly compacted, and histones are not replaced by protamines. This results in globozoospermia, the condition characterized by abnormal sperm with rounded heads [47]. No KASH2 and KASH4 were found at any stage of the post-meiotic sperm development. The germ cell-specific protein KASH5 is localized to the cytoplasm. KASH5, forming complexes with SUN1 and SUN2, mediates telomere attachment to the cytoskeleton and chromosome movement in spermatocytes [2, 20, 48].

LINC complex and sperm flagellum

The sperm flagellum is divided into the connecting piece, the midpiece, the principal piece, and the end piece (see Figure). The axoneme that comprises two central and nine pairs of peripheral microtubules, is the core structure of the flagellum. The axoneme is surrounded by periaxonemal structures: nine longitudinally oriented outer dense fibers in the midpiece and principal piece, the fibrous sheat in the principal piece, and mitochondria that form a mitochondrial sheat surrounding the outer dense fibers in the midpiece of the flagellum. The flagellum is responsible for sperm motility that is essential for fertilization. Flagellar motility is ensured by the normally functioning cytoskeleton, ATP production by mitochondria, and correct arrangement of the axonemal components. ATP synthesis is ensured by both glycolysis and oxidative phosphorylation. Implantation fossa, i.e. the segment providing the head-to-tail connection, plays a vital part in sperm function. The coupling apparatus is an asymmetric structure formed during spermatogenesis that contains centrioles (proximal and distal), dense fibrous stucture (capitulum), and segmented columns [2]. The LINC complex proteins are involved in interaction between the coupling apparatus and the sperm head: particularly, SUN4 and SUN5 are involved in the head-to-tail anchorage [21]. SUN4 protein contributes to the outer dense fiber positioning, providing a link between axonemal microtubules and outer dense fibers. The researchers studied the mechanisms responsible for such interactions [9]. Sun4 knockout mice have been reported [13]: the flagellum is wrapped around the murine sperm head, suggesting incorrect connection of these structures. Coupling apparatus abnormalities associated with the SUN4 deficiency were not found, however, the lower head-to-tail linkage efficiency was observed [48]. The coupling apparatus is to a lesser extent attached to the lateral areas of the nucleus, suggesting that SUN4 is involved in the interaction. Such proteins as ODF1, SEPT12 and lamin B1 are the partners of SUN4. SEPT12 is involved in formation of the flagellum; together with SUN4 and lamin B1, SEPT12 is associated with the sperm neck [49]. According to available data, SUN5 is essential for the head-tail anchoring. SUN5 is localized in the nuclear envelope during spermatogenesis, however, in mature sperm, SUN5 is found exclusively in the head-tail coupling apparatus (HTCA) in the implantation fossa essential for the head-tail connection. Destroyed connection of the coupling apparatus to the sperm head with subsequent releasing of the flagellum into the lumen of the seminiferous tubule and retention of sperm head within the tubular epithelium was found in elongated spermatids of the Sun5 knockout mice [45]. A similar disorder was discovered in men with homozygous deletions of Sun5 associated with the emergence of decapitated spermatozoa [50]. To ensure interaction, SUN5 can co-operate with the heat shock protein DNAJB13, the structural component of axoneme found in spermatids and spermatozoa. It is believed that SUN5 prevents separation of the sperm head from the flagellum during sperm migration into the lumen of the seminiferous tubule [45].

Table. Nuclear envelope proteins involved in human spermatogenesis

Protein	Cells	Localization	Functions	Reference
Lamin B1	Spermatocytes	Nuclear envelope (NE)	The decreasing levels of lamin B1 are essential to reduce mechanical forces of NE, which is necessary to ensure chromosome movement during meiotic prophase. Ensures the specific non-random arrangement of chromosomes in the nuclei during cell maturation. Progressive decrease in lamin B1 is essential for the sperm head formation	[51]
	Round spermatids	Nuclear envelope, posterior pole of the nucleus Nucleoplasm		
	Elongating spermatids	Posterior pole of the nucleus		
	Elongated spermatids	Posterior pole of the nucleus		
	Mature sperm	Posterior pole of the nucleus		
LAP1	Spermatogonia	Nuclear envelope	Ensures the specific non-random arrangement of chromosomes in the nuclei during cell maturation. Ensures manchette formation	[52]
	Spermatocytes	Nuclear envelope, cytoplasm		
	Round spermatids	Posterior pole of the nucleus		
	Elongating spermatids	Posterior pole of the nucleus		
	Elongating spermatids	Posterior pole of the nucleus		
LAP2	Round spermatids	Nuclear envelope, nucleoplasm (for LAP2beta)	Ensures the specific non-random arrangement of chromosomes in the nuclei during cell maturation. Ensures chromatin compaction (together with BAF, BAF-L)	[30]
LEMD1	Round spermatids	Nucleoplasm, posterior pole of the nucleus	Ensures chromatin compaction (together with BAF, BAF-L)	[30]
	Elongating spermatids	Posterior pole of the nucleus		
	Elongated spermatids	Posterior pole of the nucleus		
SUN4	Round spermatids	Nuclear envelope	Ensures manchette microtubule attachment to the nucleus together with SUN3 and nesprin-1. Lateral binding of the coupling complex in the implantation fossa essential for the tight head-to-tail anchorage. Ensures sperm flagellum arrangement during spermiogenesis together with ODF1	[13]
	Elongating spermatids	Neck		
	Elongated spermatids	Neck		
	Mature sperm	Axoneme		

LINC complex and infertility

The LINC complex proteins are crucial for sperm differentiation. Impaired SUN and KASH protein interactions result in significantly lower sperm quality.

1. Knockout of Sun1 in mice results in infertility due to impaired interactions between SUN1 and telomeres during recombination in primary spermatocytes. Since the SUN1–KASH5 complex regulates telomere motion, KASH5 mutations result in inefficient telomere attachment to the nuclear envelope, and compromises homologous chromosome pairing [17]. It was shown that Sun1 knockout mice were infertile. Furthermore, cells of testes of such mice contained no coding or non-coding RNAs essential for spermatogenesis. This fact shows the new role played by the SUN1 protein in gene regulation [2].

2. Sun3 knockout mice are infertile; they display disruption of both flagellum and head. The main defects emerge during formation of elongated spermatids, since spermatid nuclei are unable to elongate due to impaired signal transduction between the cytoskeleton and the nuclear envelope. Animals producing a low concentration of spermatozoa are characterized by acrosome defects, low motility, and globozoospermic phenotype. It has been shown that SUN3 is engaged in the manchette attachment to the nuclear envelope via SUN3-SUN4-KASH1 [13]. Sun3 knockout mice demonstrate lower expression of SUN4 protein in association with abnormal manchette formation. Spermatogenesis of SUN4-deficient mice progresses normally before meiosis. However, impaired post-meiotic differentiation results in infertility. Spermatid nuclei are round-shaped or altered, impaired chromatin remodeling is observed. The disorganized manchette is unable to ensure the nuclear envelope attachment necessary for the sperm head formation; acrosomal alterations occur and formed spermatozoa show signs of globozoospermia. Sun4 knockout mice show the reduced amount of SUN3 in spermatids along with abnormal sperm head elongation. Instead of the typical distribution of SUN3 polarized to the posterior pole of the nuclear envelope, this protein moves to the cytoplasm. The altered amounts of SUN4 correlate with inefficient sperm head-to-tail anchorage. In particular, spermatids of the Sun4 knockout mice show detachment of the coupling apparatus from the nuclear membrane that adversely affects the head-to-tail anchorage [48]. Taking into account the SUN4 capability of binding to SEPT12, the need to study the role of SEPT12 in infertility becomes clear. Indeed, Sept12 knockout mice produce sperm with multiple defects of flagella and heads, disrupted nuclei, premature chromosome condensation. Spermatozoa obtained from such mice cause preimplantation embryo developmental failure [2].

3. Sun5 knockout mice produce acephalic spermatozoa. SUN5 protein is essential for the sperm head-to-tail anchorage; knockout leads to their separation in elongated spermatids, thus causing infertility [51].

CONCLUSION

Male infertility remains a pressing issue of human reproduction. Infertility affects 15% of couples of reproductive age in the world. Quantitative, kinematic and morphological characteristics of sperm are assessed in order to estimate the fertilization potential of spermatozoa. According to the current WHO Manual for human semen analysis (WHO, 2021), in 95% of fertile men, ejaculate contains at least 4% of spermatozoa with the morphology typical for the potentially fertile subpopulation, that penetrated through cervical mucus *in vivo* after the coitus. The rest of the sperm subpopulation is characterized by morphological heterogeneity: spermatozoa have various structural defects of the head, acrosome, neck, and flagella. In particular, globozoospermia is diagnosed when the sperm morphology assessment reveals head defects, such as round head (100% of spermatozoa for type 1 globozoospermia, and 40–80% for type 2 globozoospermia), along with no acrosomes or pronounced acrosomal defects [53].

Studying the disorders associated with male infertility is a challenge. Testicular integrity and sperm quality depend on both genetic and epigenetic factors. The effects of harmful chemicals, stress, and diets can be critical for spermatogenesis and embryo formation. Various protein complexes and hormones control the process of sperm differentiation. In particular, the LINC complex proteins ensure the functions of spermatozoa and their precursor cells throughout the entire differentiation process (see Table). The use of knockout animal models is required for exact determination of the function of each protein. It is also necessary to analyse the composition of the nuclear envelope during the sperm differentiation. This will make possible to identify new therapeutic targets in animals and later in humans to overcome the problem of infertility.

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