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Septins as key players in spermatogenesis, fertilisation and pre-implantation embryogenic cytoplasmic dynamics

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Abstract

Septins are a family of cytokinesis-related proteins involved in regulating cytoskeletal design, cell morphology, and tissue morphogenesis. Apart from cytokinesis, as a fourth component of cytoskeleton, septins aid in forming scaffolds, vesicle sorting and membrane stability. They are also known to be involved in the regulation of intracellular calcium (Ca^{2+}) via the STIM/Orai complex. Infertility affects ~ 15% of couples globally, while male infertility affects ~ 7% of men. Global pregnancy and live birth rates following fertility treatment remain relatively low, while there has been an observable decline in male fertility parameters over the past 60 years. Low fertility treatment success can be attributed to poor embryonic development, poor sperm parameters and fertilisation defects. While studies from the past few years have provided evidence for the role of septins in fertility related processes, the functional role of septins and its related complexes in cellular processes such as oocyte activation, fertilization, and sperm maturation are not completely understood. This review summarizes the available knowledge on the role of septins in spermatogenesis and oocyte activation via Ca^{2+} regulation, and cytoskeletal dynamics throughout pre-implantation embryonic development. We aim to identify the currently less known mechanisms by which septins regulate these immensely important mechanisms with a view of identifying areas of investigation that would benefit our understanding of cell and reproductive biology, but also provide potential avenues to improve current methods of fertility treatment.

Keywords Septins, Fertilisation, Oocyte activation, Reproduction, Infertility, Embryogenesis

Introduction

Infertility affects ~ 15% of couples globally, while male infertility affects ~ 7% of men [42]. While known genetic causes can be attributed to ~ 30% of infertility cases, ~ 50% of male factor infertility cases remain unexplained [31]. However, while assisted reproductive technology (ART; a suite of laboratory techniques used to combat cases of infertility) can remedy infertility, this seems only possible only following multiple cycles of fertility treatment. Global pregnancy and live birth rates following ART remain relatively low, rarely exceeding 22% per cycle [89]. Furthermore, there seems to be a decline in male fertility parameters by as much as 50–60% in western countries over the past 60 years

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[59]. Another contributing factor to such low success rates is also attributed to poor embryonic development or competency following fertility treatment [8, 40]. While poor embryogenesis can be attributed to poor sperm parameters [54, 68], another leading cause seems to be attributed to the early dynamics of pre-implantation embryogenesis, with fertilisation defects seemingly underlying poor quality of embryogenesis in the fertility clinic.

Wong et al. [110] suggested that the dynamics of cell division in zygotic/preimplantation embryo development was predictive of embryo quality in humans. Subsequently, examination of embryonic morphokinetic parameters have become a well-accepted measure of embryo quality and ultimately successful pregnancy and birth [16, 37]. Indeed, embryos exhibiting cellular dynamics beyond recommended ranges are thought to represent suboptimal cytoplasm/nuclear machinery, with Yang et al. [111] suggesting that earlier occurring irregularities in processes influencing fertilisation and pre-implantation embryogenesis exerting greater impact upon embryogenic efficacy, with ideal morphokinetic parameters indicating embryos that are able to arise from an undisturbed/ideal cell cycle progression, dependent upon embryonic cytoskeletal parameters [72].

Actin filaments, microtubules, and intermediate filaments are considered the main components of the cell cytoskeleton. However, 50 years ago, a fourth vital component of the eukaryotic cytoskeleton was discovered for the first time in yeast – subsequently named Septins [36]. This group of proteins plays an essential role in cellular structure and assembly, with currently 13 identified Septin genes in humans that engage with the actin and microtubule cytoskeleton along with membranes [74]. These proteins have been exhaustively studied, and they have been observed in different organisms apart from humans, including Schistosoma, Chlamydomonas, and S. cerevisiae. They are mostly referred to as the fourth component of the cytoskeleton due to their significant role in maintaining the cell's structural integrity. Septins are generally classified as small GTP-binding proteins with a molecular weight of 30–65 kDa, belonging to the phosphate-binding loop (P-loop) NTPases. Different domains have been recognized to play a critical role in facilitating the organization and function of Septins and the resulting complex structures [74]. These proteins consist of four structural components: a variable N-terminal region, a polybasic region for phospholipid binding, which allows the septin to interact directly with the phosphoinositides found on the plasma membrane, a conserved GTP-binding domain, and a predicted coiled-coil domain in the C-termini [10, 29, 82].

The septin family of proteins

Understanding the physiological role of septins in cellular processes necessitates understanding the septins' structure and their organization into high-order structures. In human cells, septins assemble and form a diverse set of complex and higher-order structures. Evidence suggests that contrary to RAS-like GTP-binding proteins, septins exist as either hetero-hexamers or hetero-octamers facilitated by the interaction between their GTP-binding domains and the N-terminal and C-terminal regions [74]. Mainly, it is the nucleotide binding and the GTP hydrolysis that govern and modulate the septin-septin interaction, except in the case of SEPT6 group septins, which are persistently bound to GTP and lack the hydrolysis capacity due to the absence of an essential Threonine residue – Thr78 [93].

The 13 septins found in humans can be categorized into four groups according to their phylogenetic analysis, named after their most extensively studied members: *SEPT2* (comprising *SEPT1*, *SEP*T4, and *SEPT5*), *SEPT3* (including *SEPT9* and *SEPT12*), *SEPT6* (including *SEPT8*, *SEPT10*, *SEPT11*, and *SEPT14*), and *SEPT7*, which stands alone in its group (Fig. 1). The number of mammalian septins is further expanded by the expression of isoforms, and these increase the diversity of septin filaments and may alter their functions in different tissues and cell types [20]. A striking property of septin complexes is their capacity to form non-polar filaments, making them highly stable cytoskeletal elements when compared to the dynamic actin filaments and microtubules. In addition, they can interact laterally, forming bundles, which are recognized as the biologically active form of septins [94] (Fig. 2).Various factors were identified as regulators of the assembly and disassembly of the septin filaments. As mentioned previously, septin filaments can interact with membrane phospholipids, and both can influence the behavior of the other. As such, the septin can modulate the shape of the phospholipid membrane, while simultaneously, the phospholipids can control the formation of the septin filaments. Other discovered regulators of the septin filaments formation and disassembly include post-translational modifications such as phosphorylation, sumoylation, and ubiquitylation [74].

Functions of septins

Septins are categorized as a eukaryotic cytoskeleton component based on their role in different cytoskeletal-related functions. One of the leading roles of highly complex septin formations is serving as a scaffold, recruiting other proteins, and facilitating their functions. This can be demonstrated in budding yeasts, where septin filaments can arrange into an hourglass structure that

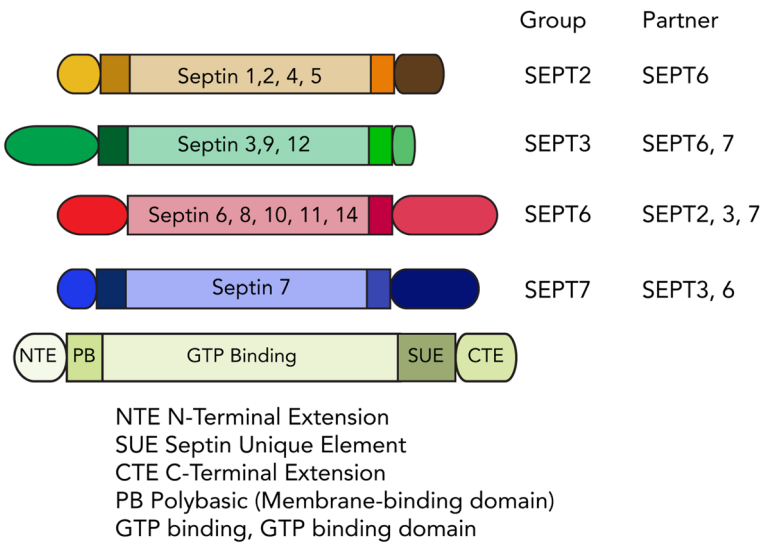

Fig. 1 Schematic description of septin domain structure, summarising septin groups and their binding partners. All septins share a conserved GTP-binding domain, a phosphoinosite-binding polybasic region (PB), and a septin unique element (SUE). The length and amino acid sequences of the N- and C-terminal extensions (NTE and CTE, respectively) vary between septin groups. Figure adapted from [77]

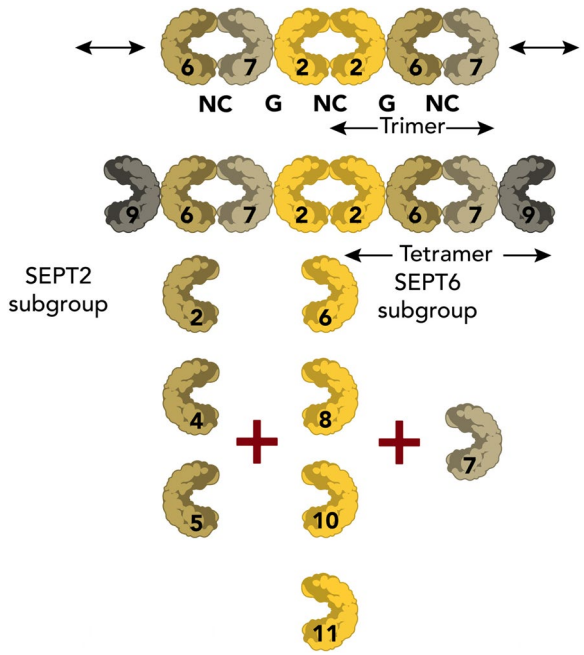

Fig. 2 A schematic description of the structure of the complex formed between septins using *SEPT2-SEPT6-SEPT7* as an example. Two copies of each septin are arranged symmetirically (*SEPT7-6–2-2–6-7*), generating a hexamer by alternating N- and C-termini (NC) and G-interface (GTP-binding domain) with individual septins exhibiting binding preference to other septins defined by the septin subgroup. Figure adapted from [77]

enables the arrangement of vital cytokinesis proteins into the division site. Similar structures have also been appreciated in mammalian cells [10, 34, 74, 81].

Septins also play vital roles in cell division, influencing chromosomal movement and spindle elongation, particularly at the mitotic midplane. They interact with the microtubule-dependent motor protein and centromere-associated protein E [113]. Additionally, septins act as a base, ensuring proper membrane rigidity and regulating cell shape and movement. Septins also play a role in the motility of T cells, which are intricately linked to the development of their navigation system. Studies have demonstrated that *SEPT7* is crucial for properly developing these components. Its absence from lymphocytes leads to alterations in the uropod's structure, causing elongation and impacting the cell's ability to sustain motility [20]. Depletion of *SEPT7* disrupts the persistent motility of T cells, allowing them to pass through narrow pores due to loss of membrane rigidity [74, 101].

Another significant physiological function of septins is forming diffusion barriers, which are essential in various cellular processes. Although their role in forming similar barriers during cell division is not fully proven, studies emphasize their significance in non-dividing cells [74]. Notably, septins form a diffusion barrier at the base of cilia, which are hair-like projections with diverse functions. For instance, *SEPT2* is known to confine and stabilize a complex of proteins known as a ciliopathy complex at the base of the cilium and maintain tubulin glutamylation. Any modifications impeding this process are associated with a ciliopathy known

as Joubert syndrome [15, 58, 74, 95]. In the context of renal epithelia, the primary cilium acts as a fluid flow sensor in the nephron. Research indicates that the absence of *SEPT2* disrupts cilium morphology, causing a shorter cilium. This disturbance further results in the dislocation of ciliary membrane proteins, impacting signal transduction and specialized cilium functions. Findings have also suggested that other septins, such as *SEPT7* and *SEPT9*, also interact with microtubules in the cilium, influencing its length [20].

A major septin in the annulus of the sperm is *SEPT4*, which, if knocked out, causes the protein compartmentalization to be lost and the sperm motility and morphology to be compromised, leading to sterility, as proven by *SEPT4*-knockout mice experiments [41]. Septins can also facilitate the growth of actin filaments on the microtubule mesh by directly interacting with both structures. This process is vital for developing and dynamics of growth cones, essential neuronal protrusions guiding axonal growth [76]. Furthermore, embryological studies involving the in vivo knockout of *SEPT4* and *SEPT14* revealed an interruption in the migration process, with neurons halting at earlier stages (ventricular and intermediate zones) and consequently failing to reach their intended destination, the cortical plate of the developing cortex [20]. Dysregulation in septin genes has been linked to neurodegenerative disorders such as Alzheimer's disease (AD), characterized by altered neuronal morphology and function [71]. Patients with AD had changed protein levels of various septins. This indicates that septins may also be early indicators of synaptic malfunction and synaptotoxicity [71].

In addition, it was demonstrated that septins regulate microtubule-dependent transport by controlling the movement of specific motor proteins and the cargo they carry. Septin also seemingly directly controls the motility of the kinesin motor protein [96, 97]. One study evaluated how *SEPT9* affected kinesin-1/KIF5 and kinesin-3/KIF1A. The kinesin-1/KIF5 motion was improved, and the kinesin-3/KIF1A motion was hindered when the septin level was decreased. Conversely, overexpression of *SEPT9* resulted in an opposite effect [96]. In an in vitro experiment, a group of researchers examined the physical interaction between septins and plus-end tracking protein-1 (EB1), a prominent regulator of microtubule mobility, to investigate the unique structure in the cytoskeletal network linked with septins. The study demonstrated the strong bonding strength between EB1 and *SEPT2*, *SEPT6*, and *SEPT7*, as shown by the published equilibrium dissociation constant studies, concluding that septins primarily control microtubules through communication with EB1 proteins [78].

Involvement of septins in the cytoskeleton and cytoskeletal dynamics

Septins have risen as critical players in maintaining cytoskeletal design, controlling cell shape, and planning different cellular functions. This is done by framing filamentous structures related to other cytoskeletal components, such as actin fibers and microtubules [50]. Septins assemble into complexes and polymers that are more stable than microtubules and actin filaments as they are nonpolar and lack the polarity aspect [4]. The assembly and preservation of actomyosin networks is evident by their interaction with Septins at specific regions of the cytoplasm and plasma membrane [97]. Experiments involving the reconstitution of biological processes in a controlled environment outside living organisms have demonstrated the direct interaction between insect septins (specifically Drosophila *Septin 1–Septin 2–Pnut*) and mammalian Septins (human *SEPT2 – SEPT6 – SEPT7* and *SEPT9*) with both actively forming and pre-assembled actin filaments. This interaction leads to the creation of various structural configurations, including curved, circular, and linear bundles.

Septins have been found to link with actin fibers at the cell cortex, contributing to the arrangement of actin-based structures just like the cortical cytoskeleton. They interact with actin-binding proteins and signaling effectors to provide feedback regulation, which allows them to control actomyosin organization and contractility [97]. Therefore, septins contribute to the mechanical solidness and organization of the cytoskeleton. Moreover, a pivotal function of septins is to control the cell shape and maintain its cellular integrity [51]. Septin fibers, composed of GTP-binding proteins, can gather into higher-order structures within particular cell regions [6]. These higher-order structures can take the shape of rings or gauze-like meshwork that encompass certain cellular areas. Examining the organization and creation of septins in different cellular settings revealed that septins frame intricate filamentous structures acting as a stage, providing essential support in shaping the cell [5]. Septins commonly interact with actin stress fibers by either directly engaging or via the facilitation of actin-binding proteins. The thinning of actin stress fiber is triggered by the depletion or relocalization of septin.

Septins engage with actin on fungal cell membranes through an indirect interaction facilitated by myosin II. This connection is established through the myosin-binding factor Bni540–43. Additionally, septins interact with actin through protein complexes that include the formin Bnr1, as well as Bin/amphiphysin/Rvs domain and ezrin/radixin/moesin family proteins [97]. In addition to the interaction of septins with actinomycins, evidence revealed that myosin, which plays a role in

muscle contraction, also contributes to septin-mediated cytoskeletal elements [109]. Despite their leading role in muscle cells, myosin is also associated with assisting septins in regulating necessary cellular forms like cell motility, cytokinesis, and tissue morphogenesis.

In addition to actin and myosin, intermediate fibers are critical players within the cytoskeletal framework, contributing to the mechanical quality of cells [84]. They frame an assorted family of proteins, counting keratins, vimentin, neurofilaments, and lamins, each with capacities in distinctive cell types and tissues. Unlike actin fibers, intermediate filaments are more steady and less energetic. Intermediate fibers are found in different cellular compartments, including the cytoplasm, core, and cell–cell intersections, playing a significant role in maintaining cell shape, resisting mechanical stretch, and supporting cellular structures. For example, in epithelial cells, intermediate fibers called keratins help shape and arrange the cytoplasm. The affiliation of septin fibers with intermediate filaments provides a link between the cytoskeleton and cell–cell intersections. This connection is pivotal for supporting the tissues and the appropriate functioning of epithelial layers, permitting septins to participate in cell attachment, cell migration, and tissue morphogenesis forms, where intermediate filaments are known to play a vital role [30]. To this degree, given the increasing evidence of association of the septin family of proteins with actin, myosin and numerous intermediate filaments, multiple avenues of investigation have begun to find associations between this family of proteins and specific cell types involved in reproduction, mainly in spermatogenesis.

Septins at spermatogenesis

Spermatogenesis is the process whereby the male gamete called sperm is produced, involving mitotic, meiotic, and spermiogenesis (physical transformation) phases. The entirety of mammalian spermatogenesis unfolds through 12 distinct stages within the seminiferous tubules of the testes, with spermiogenesis involving the formation of the sperm annulus within spermatids. This process begins when the annulus accumulates at the lower end of the nucleus in stage I spermatids, and gradually moves down the sperm tail finally reaching the junction between the midpiece and principal piece [92]. Male infertility can result from various factors, including problematic sperm production, hormonal imbalances, immune system deficits, ejaculation dysfunction, environmental exposures, and genetic mutations [64]. Within germ cells, structures called intercellular bridges undergo a transformation into a stable form, which is crucial for fertility. The role of septins in these intercellular bridges seems to be consistent across different species. In Drosophila, three septins (*Pnut, septin1*, and *septin2*) create a structure between the intercellular bridge of male and female germ cells. Similarly in mice, *SEPT2*, *SEPT7*, and *SEPT9* are located within the intercellular bridge in male germ cells. Loss of this septin-containing bridge increased germ cell death [63, 64], suggesting an important role for septins within spermatogenesis [62].

Septins can also affect flagellar proteins, which are important for generating energy in the annular region. Their ring-like structure provides a circular force that helps propel sperm forward in the female reproductive tract. As a primary cytoskeletal protein, septins are remarkable at forming filaments, impairment of which in mice led to the production of immotile sperm, hindering natural conception [106]. However, the core structure and mechanism of septin-related complexes in sperm are still unknown and require further investigation [63]. *SEPT4* is a vital part of the annulus, with mice exhibiting a *SEPT4*-null mutation exhibiting significantly impaired ability to reproduce [41, 64]. The absence of *SEPT4* resulted in annulus defects, leading to immotile sperm due to defective tails [41, 53, 102], perhaps due to the requirement of the annulus/*SEPT4* ring in organizing the fibrous sheath of the annulus [41].

SEPT12 forms filament-like structures in isolated mouse germ cells, while in fully-developed sperm cells, *SEPT12* is detectable in the head, neck, and midpiece, with minimal amounts in the tail [62–64]. Sperm samples from humans with conditions like hypospermatogenesis, maturation arrest, and asthenozoospermia showed decreased *SEPT12*. Chimeric male mice with no *SEPT12* exhibited lower testis weights, sperm counts, and sperm motility than those with functional *SEPT12*. Some mice displayed abnormalities in the seminiferous epithelium, mirroring the hypospermatogenesis-like phenotype observed in humans [64]. Although the impact on reproduction is significantly great when there isa deficiency in *SEPT4*, even only haploinsufficiency of *SEPT12* can lead to severe abnormalities in both mature and immature germ cells during spermiogenesis in mice [64]. Indeed, Kuo et al. [57] identified two novel mutations from separate infertile patients in the GTPase domain of *SEPT12* that although heterozygous, were suggested to alter the protein structure, with one mutation reducing GTP hydrolytic activity, with the other interfering with GTP binding. Interestingly, both patients with these mutations exhibited abnormal sperm motility and morphology (oligoasthenozoosperma and asthenoteratozoospermia). However, it should be noted that this was observed in only 2 patients from a total of 160 infertile patients screened, suggesting such occurences are relatively rare. However, this is not to say that there were no septin defects in these patients as only patient DNA was

examined for *SEPT12* rather than protein levels in sperm. It would be worth examining other septin genes from such patients and/or examining sperm septin protein levels in relation to sperm parameters and other important sperm conditions.

A good example of such an uninvestigated relationship in sperm is association of septins with sperm DNA fragmentation (SDF). SDF is increasingly considered a leading cause underlying male infertility and subfertility, with elevated sperm DNA fragmentation associated with lower chances of successful natural conception and increased chances of recurrent pregnancy loss. SDF is also associated with increased miscarriage and lower pregnancy rates following fertility treatment [2]. Interestingly, Kremer et al. [56] identified that the *SEPT2-SEPT6-SEPT7* complex is involved in DNA damage repair pathways via nuclear NCK, with knockdown of the *SEPT2-SEPT6-SEPT7* complex resulting in increased DNA fragmentation in such cells [52]. Concurrently, Hara et al. [35] indicated that depletion of *SEPT8* in murine retina photoreceptor cells was concomitant with increased nuclear DNA fragmentation. However, given such well characterised links between septins and sperm defects, and septins and DNA fragmentation, such correlations in human sperm.

SEPT14, a relatively recent addition to the septin family, interacts with *SEPT9* in human testes [63, 103], exhibiting colocalization within the testes. Two heterozygous missense mutations in the coding region of *SEPT14* corresponded to abnormal head morphology. In fertile men with non-obstructive azoospermia, an incurable disorder linked to spermatogenic failure, *SEPT14* was evaluated, and men were divided into three groups: TEST (+) patients with hypospermatogenesis, TEST (-) group with maturation arrest (MA), and TEST (-) group with Sertoli cell-only syndrome. The highest levels of *SEPT14* were observed only in sperm from the TEST (+) group, indicating that this protein is associated with the occurrence of spermatogenesis [103]. A similar mutation was also identified in a similar patient with azoospermia in *SEPT12* within the GTPase domain and predicted to be 'probably damaging' although this was not investigated further [27].

Collectively, this paints a highly regulated picture of septin dynamics in mammalian spermatogenesis, with specific septin interactions and regulatory factors underlying the correct progression of this complex process (Fig. 3). Indeed, most investigations linking septins and spermatogenesis have revolved around *SEPT12* given its testis-specific nature. This is also not limited to mutations as modifications such as phosphorylation of *SEPT12* was also shown to inhibit filament formation, resulting in abnormal sperm structure and loss of male fertility [92]. However, beyond spermatogenesis, an often overlooked process in the context of fertilisation are maturation steps the sperm undergoes before fertilization; namely – the sperm acrosome reaction (involving rapid depolymerization of the apical acrosomal cap of the

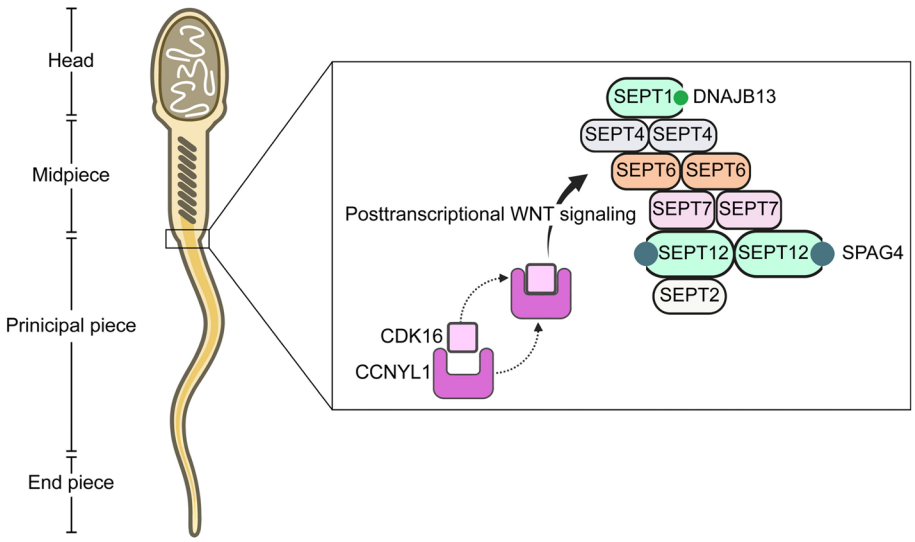

Fig. 3 Schematic illustration of a human sperm illustrating the septin-based annulus. The sperm head and tail are joined via the connecting piece, while the annulus (region highlighted by the black box) connects the midpiece and the sperm flagellum. The annulus is a complex between *SEPT1*, *SEPT2*, *SEPT4*, *SEPT6*, *SEPT7*, and *SEPT12*, a complex which is associated with cochaperones DNAJB13 (on *SEPT1*) and SPAG4 (on *SEPT12*). The CCNYL1/CDK16 complex also determines the structure and function of the annulus by acting via WNT signalling on *SEPT4* clustering in the epididymis. Figure adapted from [14]

sperm head to release digestive enzymes involved at for zona pellucida digestion) and sperm capacitation (a series of complex physiological and biochemical modifications inside the female reproductive tract that activated signal transduction pathways leading to actin polymerization on the sperm membrane). In both instances, actin reorganization is underlined by sperm Ca^{2+} influx. Intriguingly, as discussed before, Wang et al. [108] found that *SEPT12* phosphorylation and *SEPT4* activity are required to facilitate effective sperm capacitation in mice, suggesting a larger potential arena for septins in sperm beyond spermatogenesis.

Septins and the regulation of calcium homeostasis – potential avenues for investigation in fertilizing oocytes

Fertilization in mammals is a multistep process in which gamete fusion results in the generation of a genetically unique individual [1]. This interaction initiates a signal transduction cascade where the oocyte is converted into a diploid zygote that allows for the initiation of oocyte activation [24, 86]. Oocyte activation is an important step for the development of an embryo as it is a process that involves a series of events that allow for a mature metaphase II-arrested oocyte to transition into an early, developing embryo [86, 98]. Fertilization elevates cytosolic calcium (Ca^{2+}) levels in oocytes, initiating the signal transduction cascade needed for activation [39, 55, 107]. Upon gamete fusion, a sperm-specific phospholipase C (PLC) isozyme, termed PLCzeta (PLCζ), mediates cytosolic Ca^{2+} elevations (Ca^{2+} oscillations in mammals) by hydrolyzing Phosphatidyl inositol-4,5-bis phosphate (PIP_2) into inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. IP_3 binds to IP_3 receptors (IP3Rs) on the ER, eliciting Ca^{2+} release for several hours [44, 79, 90]. While Ca^{2+} regulation is known to alter physiological processes resulting in conditions including heart disease [45, 80, 104], abnormal Ca^{2+} release profiles may also affect embryogenic development via cell cycle progression, a key component of oocyte activation [21–23, 43, 46, 47, 73, 99].

Intracellular Ca^{2+} is not the only mechanism at play during fertilization and oocyte activation. The decreasing ER Ca^{2+} levels are sensed by ER-membrane localized Stromal Interaction Molecules (STIM) [66], releasing Ca^{2+} from the luminal EF-hand domain of STIM, causing STIM oligomerization and its translocation to ER regions in close proximity to the plasma membrane (PM) called the ER-PM junctions [18, 65, 112]. STIM proteins at the ER-PM junctions physically interact with the Ca^{2+}-selective Orai channel located on the plasma membrane, which opens upon ER Ca2 + depletion and STIM translocation, facilitating extracellular Ca^{2+} entry. STIM and Orai proteins are key components of SOCE, which regulates Ca^{2+} influx in response to depleting ER store Ca^{2+} within cells [38]. Upon ER Ca^{2+} store depletion, STIM undergoes conformational change, activating Orai proteins, which are Ca^{2+} channels in the plasma membrane, facilitating Ca^{2+} store replenishing in the ER via the Sarco/endoplasmic reticulum Ca^{2+}-ATPase and further STIM channels on the ER. This regulated reuptake of Ca^{2+} into the ER maintains Ca^{2+} homeostasis throughout oocyte activation. In mice, other channels also carry extracellular Ca^{2+} from the plasma membrane, including the low-voltage-activated T-type calcium channel 3.2, the transient receptor potential vanilloid member 3, and TRP melastatin 7 [107].

Septins seem to play a regulatory role in intracellular Ca^{2+} homeostasis via such mechanisms. With SOCE seemingly heavily influenced by septins. Indeed, loss of *SEPT2*, *SEPT4*, and *SEPT5* significantly impaired SOCE in Jurkat T-cells (Sonia [91]). SOCE is a tightly regulated process, and alongside positive regulators such as STIM/Orai, negative regulators of SOCE such as SOCE-associated Regulatory Factor can destabilize STIM1/Orai1 complexes [18, 83]. Experiments in Drosophila neurons indicated that reduced *septin7* supported SOCE via Orai, suggesting that *septin7* functions as a negative regulator of the Drosophila Orai channel in neurons [19]. Given such roles of septins alongside their unique membrane-interacting properties, Deb and Hasan [18] proposed that in resting cells, septin filaments help in sequestering dOrai in lipid domains that prevent STIM/Orai interactions and Orai opening. Perhaps septin filaments may also aid in maintaining PIP_2 organization in the plasma membrane, enabling Orai opening when required and perhaps playing a role in regional coordination of Ca^{2+} release in concert with other signalling pathways [18, 48]. Indeed, as discussed previously, septins are a key player in the regulation of the STIM/Orai complex, indicating a significant potential role in the regulation of intracellular Ca^{2+} during oocyte activation (S. [91]), and perhaps beyond. Given the key role played by septins in such Ca^{2+}-release/regulation pathways, it is worth ascertaining the role of septins in specific cells types where Ca^{2+} release at large levels occurs – namely within the fertilising oocyte (predominantly within mammals).

Septins have also been shown to fundamentally interact with specific phospholipids in the lipid bilayer, through which septins can be organized into filaments, particularly via PIP_2-containing lipid monolayers in yeast [7, 28]. Septins are also capable of localizing actin filaments to the plasma membrane, affecting actin binding to the membrane, and can even modify actin polymerization [33]. It has also been hypothesized that perhaps septins anchor mitochondria to membrane-derived intracellular organelles such as endoplasmic reticulum or Golgi [28],

considering that membrane proteins of these organelles interact with mitochondria [32].

Studies collectively suggest that septins play a role in recruiting and translocating STIM1 to ER-PM junctions, consequently triggering the activation and redistribution of the Orai1 channel in the PM [67]. Even before ER Ca^{2+} store depletion, septins are necessary for the proper organization of ORAI1 in the plasma membrane. They facilitate the later stages of STIM1 approach to ER-plasma membrane junctions and the formation of stable ORAI1 clusters following store depletion. Upon stimulation, septins redistribute within the plasma membrane, aligning temporally with both STIM1 translocation and the formation of ORAI1 clusters. Additionally, septins delineate a lipid microdomain surrounding the STIM-ORAI complex, which correlates with the stability of the STIM-ORAI complex.

SOCE is initiated by the assembly of Orai1 with STIM proteins at ER-PM junctions. PM PIP_2 interacts strongly with *SEPT4*, potentially exerting a significant role in modulating the interaction between Orai1 and STIM1. Depletion of PIP_2 or knockdown of *SEPT4* reduces the recruitment of CDC42 to the ER-PM region, while knockdown of *SEPT4* or CDC42 + ARP2 disrupted actin organization and STIM1 clustering, leading to attenuation of Orai1 recruitment to STIM1 puncta, SOCE, and NFAT translocation to the nucleus. These findings suggested that PIP_2 and *SEPT4* coordinate actin remodeling within ER-PM junctions, thereby affecting Orai1/STIM1 clustering and regulating SOCE and downstream Ca^{2+}-dependent effector functions. [17]. Septins define not only cellular regions involved in specific signaling processes but also plasma membrane microdomains underlying numerous others signaling events (S. [91]). Given the importance of septins in regulating Ca^{2+} via STIM/Orai in various cell types and the importance of Ca^{2+} regulation at fertilization and oocyte activation, it is worth ascertaining the potential role that septins may play in this fundamentally important biological process. However, not much information is currently forthcoming in the literature regarding the role of septins within oocytes, particularly during a specific integral series of processes at fertilisation that involves large levels of intracellular Ca^{2+} at fertilisation; oocyte activation.

An intriguing case was described by H. Chen et al. (11, 12) who reported another heterozygous mutation of *SEPT12* in an infertile male whose sperm also exhibited oocyte activation failure. In the fertility clinic, such cases can potentially be resolved (although not always efficiently) via a process called assisted oocyte activation (AOA), involving use of chemicals called Ca^{2+} ionophores that artificially induce intracellular Ca^{2+} elevations. Intriguingly, the sperm morphology and motility of this male patient were within normal ranges (opposed to previous cases where *SEPT12* mutations resulted in sperm abnormalities). Homozygous *SEPT12* knockout mice generated using CRISPR/Cas methodology by H. Chen et al. (11, 12) indicated impaired spermatogenesis and infertility following breeding experiments with WT females. However, heterozygous mice for this *SEPT12* mutation (mimicking the human patient condition) were fertile rather than infertile, where sperm was made and was able to result in pups following breeding with WT females. Interestingly, examination of PLCζ (the sperm factor responsible for oocyte intracellular Ca^{2+} release and oocyte activation) indicated a complete absence in homozygous *SEPT12* knockout mice, but present in heterozygous knockout mice (albeit reduced compared to WT). This perhaps suggests that sperm septin defects may not necessarily be limited to spermatogenic failure/impairment or abnormal sperm morphology, but may also be related to other downstream affects in the oocyte and early embryo, although such comparative studies still need to be performed in humans.

Septins and their potential influence on the dynamic fertilising cytoskeleton

Septins are associated with various protein families, such as E-cadherin and beta-catenin which assist in maintaining cell junction integrity [6]. They locate the lateral PM during the formation of epithelial cysts, where they recruit actin filaments, connect E-cadherin to beta-catenin, and promote adhesion junction and apicobasal polarity. By interacting with cytoskeletal proteins, septins may be a major contributor to the structural integrity and direction of the cytoskeleton. In addition, findings have also highlighted septins' control over the binding of molecular motors to cargoes or microtubules, while also functioning as scaffolding for the binding of dynein-dynactin to lysosomes in retrograde transport [49].

Oocyte cytoplasmic cytoskeletal dynamics have been firmly established to exert a significant role in the competency and eventual success of fertilization, oocyte activation, and pre-implantation embryogenesis. Indeed, even before these processes, effective reorganization of relevant cytoplasmic components such as the organelles and plasma membrane occurs during oocyte maturation in mammals, the efficacy of which is directly pertinent to enhancing Ca^{2+} release at fertilization [88]. In mice, cortical ER clustering at metaphase II oocytes is primarily mediated by microfilaments [26], while in vitro matured (IVM) human oocytes (whose oocyte activation and embryogenic competency is significantly lower than in vivo matured oocytes) exhibited a severely reduced thickness of cortical actin networks compared to the naturally matured counterparts [25], which may explain the

compromised efficacy of the Ca^{2+} response and developmental competency observed in IVM [70, 88], while actin disruption also seemed to underly excessive clustering of organelles underlying abnormal oocyte morphology in human patients [100].

Indeed, it also seems that the zygotic cytoskeletal environment also exerts an effect upon Ca^{2+} release at fertilization directly. Cytoskeletal disrupted starfish eggs via heparin injection exhibited abnormal Ca^{2+} release following sperm injection, failing to prevent polyspermy [61, 85, 87]. While these mechanisms are still poorly understood in mammals, it does seem that Ca^{2+} and actin cytoskeleton dynamics are also closely linked in such oocytes, given that in mice, calcium release patterns corresponded almost exactly to rhythmic cytoplasmic actin contractions, which could also predict developmental potential [3]. Of note are findings that the association of *SEPT2 – SEPT6 – SEPT7* with actin filaments in vitro requires anillin, an actin-binding protein. The structural arrangement of Septins on actin filaments within cells, in the form of oligomers or filamentous polymers, influences microtubule dynamics [97].

The septins Cdc3, Cdc10, Cdc11, and Shs1 in a cell are organized in a manner that frames the localization of formin Bnr1, a protein that promotes the elongation of pre-existing filament, and Hof1, a domain protein that binds actin. This scaffolding depends on the protein kinase Gin4, which allows the even distribution and organization of actin filaments [97]. It would be immensely interesting to investigate the association of such septin families in the context of cytoplasmic reorganisation in fertilising oocytes or zygotes to ascertain the potential improtant role that septins could be playing in this very dynamic stage of pre-implantation embryogenesis, particularly given the potential diagnostic role carried by observing cytoplasmic dynamics in relation to human embryogenesis [3].

The actin-binding sequence of *SEPT9* has been identified within its amino-terminal basic domain. *SEPT9* interacts with actin surface domains, which are also bound by the ATP-bound myosin V subfragment 1 and the actin-severing protein cofilin. Septin interaction with actin is also mediated by anillin, non-muscle myosin II, and effectors of the small GTPases Cdc42 and Rho [97]. Therefore, septins can directly influence the organization of actin filaments through physical crosslinking, bundling, and bending, and indirectly with actin-binding proteins. *SEPT9* (also seems critical for progression past MI in mouse maturing oocytes, where abrogation of *SEPT9* prevented MI arrest alleviation and also disruption of chromosome kinetochores and spindles [13], suggesting a potential role in the distribution of chromosomes in the first polar body. This activity could be rescued by injection of *SEPT9* RNA, suggesting an important role for *SEPT9* in potentially underlying Meiosis I failure in mammalian oocytes [13]. A similar role for *SEPT4* was also suggested, as deleting this septin resulted in failure to progress to the MI stage in mouse oocytes (L. [11, 12]) (Fig. 4).

While *SEPT7* seemingly regulates the cellular cytoskeleton, *SEPT7* depletion in oocytes via siRNA microinjection abrogated spindle arrangement and

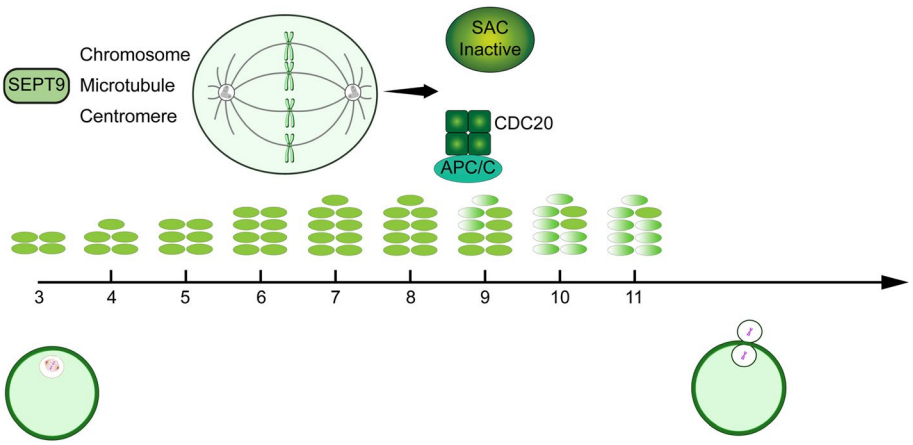

Fig. 4 Schematic illustration of how *SEPT9* could exert a regulatory role during the Metaphase I (MI) to Anaphase I (AI) transition in mouse oocytes by influencing the stability of kinetochore-microtubule connections. In wild type mice, *SEPT9* allowed CCNB1 (green) degradation, allowing the MI to AI transition followed by the first polar body extrusion. However, depleting *SEPT9* disrupted CCNB1 degradation by sustained activation of the spindle assembly checkpoint (SAC) and downregulating the activity of the anaphase promoting complex (APC/C^{CDC20}). The sustained SAC activation was driven by unstable kinetochore-microtubule connections in *SEPT9* -depleted oocytes, which arrested at the MI stage and did not extrude the first polar body. Figure adapted from [13]

the emission of the first polar body (PB1). Cells with inhibited *SEPT7* exhibited a decreased tension at the kinetochores of chromosomes aligned at the opposite poles of a bipolar spindle, indicating that *SEPT7* depletion in the cell underlies increased spindle defects and decreased polar body emission [60] in addition to cytoskeletal dynamics at fertilisation/early embryogenesis. Conversely, *SEPT7* overexpression in mouse oocytes interfered with chromosome alignment and the regulation of α-tubulin recruitment to spindles, consequently influencing the emission of the second polar body following fertilisation [60], suggesting that *SEPT7* overexpression interrupted chromosome arrangement, hence affecting the emission of the second polar body, underlying abnormal meiosis II completion. Furthermore, *SEPT1* in the oocyte was found to be localized along the complete meiotic spindle, seemingly exerting a role in chromosome segregation and spindle assembly [113]. Injection of oocytes with *SEPT1* siRNA resulted in damaged spindles, leading to chromosomal misalignment. Upon assessing whether *SEPT1* -depleted oocytes could continue to maturation, polar body emission was only slightly decreased, with 50% of the oocytes completing meiosis I [113].

One of the major reasons underlying low pregnancy rates following fertility treatment are currently considered to be chromosomal abnormalities underlying pregnancy loss and birth defects [69, 105]. Of most concern are chromosomal defects arising during meiosis, as this will impart such abnormalities in all embryonic cells. The incidence of such aneuploidies is also correlative with increasing maternal age, where 65–70% of women post-35 years of age exhibiting such defects [9, 75], and can originate from errors during the first meiotic division (MI), the second meiotic division (MII) or from both [105]. However, the specific causes underlying aneuploidies in oocytes remains a subject requiring significant investigation, especially relating to the mechanisms underlying chromatid segregation. Indeed, Verdyck et al. [105] found that the predominant cause underlying aneuploidy was precocious separation of sister chromatids (PSSC) in MI (~ 49% of cases in their cohort), followed by reverse segregation or non-disjunction of chromatids following MII (~ 36% of cases). Given that septins seem to exert significant effect upon the efficacy of cytokinesis and its successful segregation of chromatids, it is rather surprising that more investigations have not been performed examining septins in the context of embryo aneuploidies, particularly when considering that a sizeable portion of aneuploidies in embryos remain unexplained due to incomplete knowledge of the mechanisms governing this phenomenon [105].

Conclusion and future perspectives

In conclusion, the review highlighted the importance of septins in processes like spermatogenesis and oocyte activation. Septins play a significant role in maintaining cell shape, supporting cellular structures, and regulating intracellular Ca^{2+} levels, impacting fertility and embryonic development. Specifically, knock out of *SEPT4*, *SEPT12* and *SEPT14* resulted in mice with impaired annulus, sperm motility or count. These studies provide evidence that septins might be key elements in mammalian infertility and understanding the mechanisms could help in developing novel treatments. However, despite the growing network of evidence present to suggest a dynamic role of septins throughout the mammalian reproductive process, very few studies have been devoted to ascertaining the extent and nature of this. Indeed, while the role of septins has been investigated thoroughly in the context of spermatogenesis, this requires more in depth investigation at further sperm maturation levels (namely the acrosome reaction and capacitation), particularly in the context of sperm clinical parameters such as sperm motility and morphology, and perhaps its correlation with molecular markers of fertilisation such as PLCζ. Furthermore, while some preliminary studies have been performed regarding the role of septins in mammalian oocytes, these seem predominantly related to oocyte maturation and completion of the first meiosis. There is a considerable amount of work that could be directly relevant to embryogenesis and clinical treatment fidelity by examining the role of septins in the context of early fertilisation and subsequent pre-implantation embryogenesis. Further investigations are necessary to unveil the unknown mechanisms by which septins regulate these immensely important processes, the answers to which may also tremendously benefit not only basic knowledge underlying cell and reproductive biology, but also in the treatment of infertility.

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