Karbala International Journal of Modern Science

Volume 12 | Issue 1 Article 7

Sperm-associated antigen 6 (SPAG6) as a potential marker of sperm function in diagnosing male infertility

Ibtisam A. Al-Ali,
College of Sciences/University of Kerbala, Kerbala, Iraq, i.alali@uokerbala.edu.iq

Kawkab A. Alsaadi
College of Sciences/University of Kerbala, Kerbala, Iraq

Sperm-associated antigen 6 (SPAG6) as a potential marker of sperm function in diagnosing male infertility

Abstract

Background: While male infertility has become a significant global health concern, conventional analysis of semen has not made progress in the detection of contraindicated molecular biomarkers, such as sperm-associated antigen 6 (SPAG6), which is a protein involved in axonemal structure and flagellar motility.

Hypothesis: Expression of SPAG6 correlates with sperm motility and structural integrity. This means that its dysregulation may serve as a molecular indicator of impaired sperm performance.

Methods: A comprehensive review of recent studies was conducted with a focus on SPAG6's molecular characteristics, expression patterns in normal versus abnormal spermatozoa, and the role it plays in motility-related disorders. To assess SPAG6's diagnostic value, experimental data from immunohistochemical analyses, gene knockout models, and clinical semen samples were evaluated.

Findings: SPAG6 is consistently expressed in the central apparatus of the sperm axoneme and dysregulation correlates to abnormal flagellar architecture, often resulting in impairment of motility and asthenozoospermia. This highlights its utility as a molecular marker for diagnosis of sperm dysfunction, and its potential to guide targeted therapeutic strategies.

Conclusion: SPAG6 is a promising biomarker to evaluate sperm function. Its expression level offers greater mechanistic insights than can be achieved in conventional semen analysis. Further validation in clinical settings may establish its role in improving the precision of diagnosis to guide targeted treatment for male infertility.

Keywords

SPAG6, biomarker, sperm motility, male fertility

REVIEW ARTICLE

Sperm-associated Antigen 6 (SPAG6) as a Potential Marker of Sperm Function in Diagnosing Male Infertility

Ibtisam A. Al-Ali*, Kawkab A. Alsaadi

College of Sciences, University of Kerbala, Kerbala, Iraq

Abstract

Background: **While male infertility has become a significant global health concern, conventional analysis of semen has not made progress in the detection of contraindicated molecular biomarkers, such as sperm-associated antigen 6 (SPAG6), which is a protein involved in axonemal structure and flagellar motility.**

Hypothesis: **Expression of SPAG6 correlates with sperm motility and structural integrity. This means that its dysregulation may serve as a molecular indicator of impaired sperm performance.**

Methods: **A comprehensive review of recent studies was conducted with a focus on SPAG6's molecular characteristics, expression patterns in normal versus abnormal spermatozoa, and the role it plays in motility-related disorders. To assess SPAG6's diagnostic value, experimental data from immunohistochemical analyses, gene knockout models, and clinical semen samples were evaluated.**

Findings: **SPAG6 is consistently expressed in the central apparatus of the sperm axoneme and dysregulation correlates to abnormal flagellar architecture, often resulting in impairment of motility and asthenozoospermia. This highlights its utility as a molecular marker for diagnosis of sperm dysfunction, and its potential to guide targeted therapeutic strategies.**

Conclusion: **SPAG6 is a promising biomarker to evaluate sperm function. Its expression level offers greater mechanistic insights than can be achieved in conventional semen analysis. Further validation in clinical settings may establish its role in improving the precision of diagnosis to guide targeted treatment for male infertility.**

Keywords: SPAG6, Biomarker, Sperm motility, Male fertility

1. Introduction

For the DNA-containing male gamete to efficiently travel to the fertilization point, being the ampulla of the fallopian tubes, adequate motility is required. Sperm motility is considered a requirement for "natural conception" as transport of sperm to the oocyte is an essential preliminary step in the transfer of paternal genetic and epigenetic material to the maternal gamete. Even in cases where assisted reproductive techniques are used, such as in vitro fertilization, efficient sperm motility is important, as explained in the World Health Organization (WHO) recommendations [1].

Sperm-associated antigen 6 (SPAG6) was discovered after recognizing a cDNA sequence in the expression library of the human testis, that is homologous to the sequence encoding the PF16 protein expressed by Chlamydomonas reinhardtii [2], which is a green algae that uses two flagella for mobility. The PF16 protein is a dynamic portion of the central structure of the '9 + 2' axoneme. It is central to the structural integrity and mobility of the flagellum and is comparable to the

Received 16 April 2025; revised 5 December 2025; accepted 9 December 2025.
Available online 9 January 2026

* Corresponding author.
E-mail address: i.alali@uokerbala.edu.iq (I.A. Al-Ali).

https://doi.org/10.33640/2405-609X.3443

mammalian SPAG6 protein that serves the same purpose in the sperm tail axoneme [2,3] (Fig. 1). In their experiments on animal models, Sapiro and colleagues demonstrated that about half of the mice lacking the cDNA sequence encoding SPAG6 died of hydrocephalus within eight weeks after birth. Out of the mice that survived, the females were still able to reproduce, but the male mice were infertile. The sperm from the surviving male mice displayed significant problems with motility and this was corroborated by the appearance of structural irregularities, consisting frequently of irregular flagellar structures and detachment of the sperm head. With further observation of flagellar irregularity, it was determined that the central pair of microtubules were absent, and the outer dense fibers and fibrous sheath were disorganized. Hence, as previously mentioned, it was concluded that the SPAG6 protein is essential to the motility of sperm through morphogenesis of ependymal cilia. Furthermore, the non-sperm tissues of SPAG6 knockout mice demonstrated inadequate cilia beating within the epithelial layers where SPAG6 is otherwise expressed in typical specimens [4].

In the flagellar apparatus of sperm or algae, the SPAG6/PF16 protein interacts with the C1 central microtubule of the axoneme because of protein−protein associations occurring via a series of armadillo repeat domains, which stabilizes the central arrangement [4−6]. This provides solidity of the central apparatus, being crucial for the accurate and successful improvement of the sperm flagellum [4].

Despite the increasing interest in SPAG6, there is still no comprehensive synthesis of its biological roles, clinical relevance, and diagnostic potential available in the published literature. This review aims to fill this gap by critically evaluating the current literature on SPAG6 to highlight its functional implications and potential utility in characterizing male fertility at the level of an individual. Furthermore, this review aims to guide future research in reproductive medicine by emphasizing this promising area in diagnosis and evaluation.

1.1. Gene encoding SPAG6 and its expression in reproductive tissues

The gene that encodes SPAG6 is found on chromosome 10p12.3. This protein has several alternate

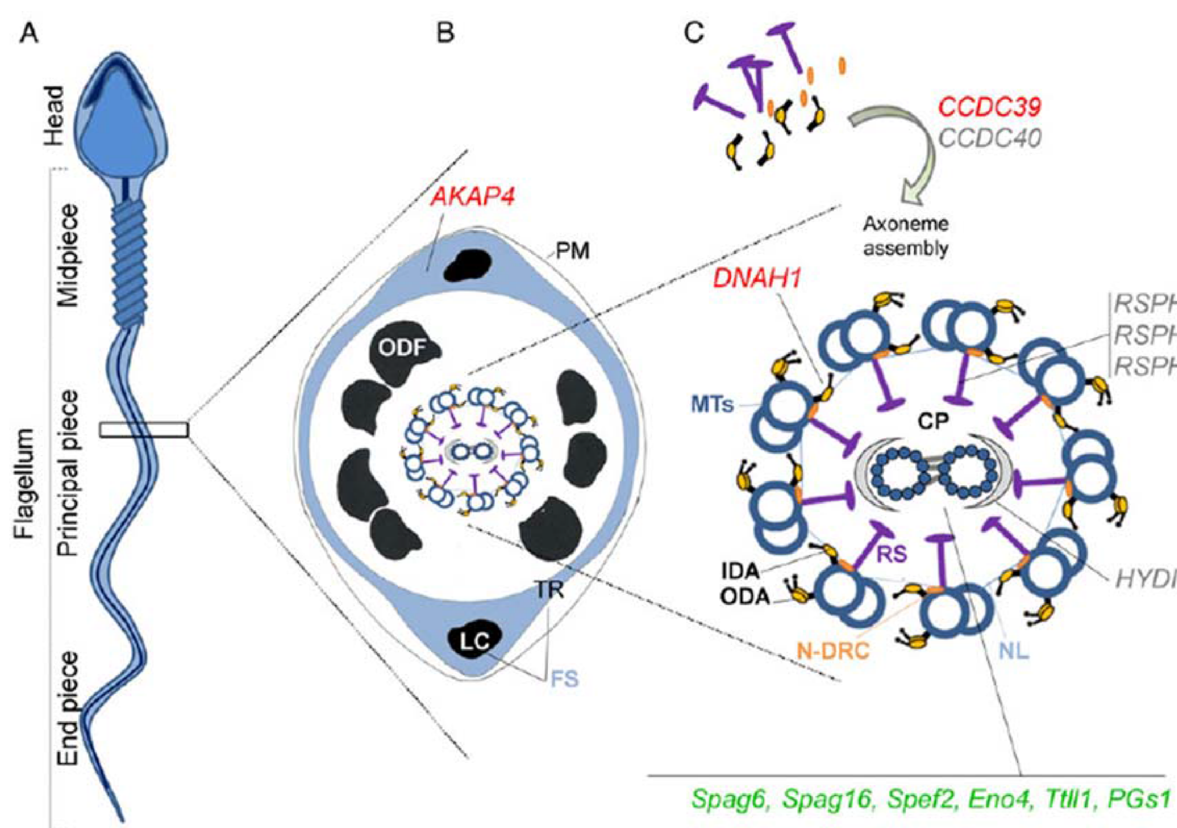

Fig. 1. Illustrates the structural components of the sperm flagellum (axoneme, fibrous sheath, dense fibers) and highlights genes associated with flagellar defects. Adapted from Coutton et al., 2015 [7].

names including CT141, repro-sa-1, RP11-301N24.4, or pf16. Although the current discussion relates to sperm motility, the protein has also been recognized as a cancer-testis antigen [2,8].

The human SPAG6 encoding gene has nine hitherto known splicing isoforms, which are designated as SPAG6-201, SPAG6-202, SPAG6-203, SPAG6-204, SPAG6-205, SPAG6-206, SPAG6-207, SPAG6-208, and SPAG6-209. They are functionally and evolutionary distinct proteins expressed in the testis and sperm. It was observed that male infertility can occur when the antigen is treated as foreign by the immune system (autoimmunity). Hence, many of these isoforms have been the subject of research that seeks potential treatments for infertility [9]. In contrast to human testes and sperm, only two isoforms are currently known for the murine SPAG6, encoded by similar genes. These include the first identified, being SPAG6-BC061194, and a related derivative identified later [10]. As previously mentioned, SPAG6 expression is not restricted to sperm and testes. It occurs in other tissues with cilia, including the lungs, nervous system, and inner ear [2,3,11].

Research is not also starting to convey that expression levels of SPAG6 above baseline may improve male fertility. For example, the notably elevated expression of SPAG6 in a line of pigs was associated with enhanced reproductive ability. Reproductive ability was measured not only by increased success in fertilization, but also by the larger litter sizes, which positively correlated to improved sperm motility after preservation [12].

Research on SPAG6 has also been applied to non-mammalian animals. For example, immunocytochemistry methods were used to determine the location of SPAG6 in chicken sperm. Then by using western blot analysis the relative amounts of SPAG6 in avian sperm samples from a rooster were determined. It was shown that sperm mobility was positively correlated, similar to mammals. When comparing between different tissues, SPAG6 was determined to be more abundant in epididymal tissues compared to testes, but its highest concentration was in vas deferens tissues. Furthermore, the levels of SPAG6 generally increased as sperm cells matured. In the sperm, the protein was concentrated at the central microtubule pair in the flagella, with lower amounts in the acrosomal region [13].

Furthermore, although the ancient and evolved forms of the mouse sperm-associated antigen 6 (MSA6) gene share sequence similarity, their biological functions in the two forms are distinct. Researchers demonstrated through comparative genetic and functional analysis in mouse models that each variant influences different aspects of sperm physiology and fertilization. This divergence of functions suggests that following gene duplication, the variants underwent either sub-functionalization or neofunctionalization. Nevertheless, MSA6-A (ancient) and MSA6-E (evolved) are not functionally redundant in either form. Each plays a unique role in the reproductive processes, offering insights into the evolution of sperm-specific genes and specific mechanisms that regulate fertility [14].

Conversely, in a latter study the expression of SPAG6 was measured and compared between fertile and sub-fertile roosters, providing apparently contradictory data. Judged by having a fertilizing efficiency below 40 %, the sub-fertile roosters had a 1.1-fold higher level of SPAG6 in their sperm compared to the fertile roosters (fertilization efficiency above 70 %) [15]. This unexpected result may be attributed to species-specific biological and technical factors. Alternative splicing in birds that produce structurally present but functionally inactive SPAG6 isoforms, lead to their accumulation without enhancing motility. Hence, this impaired protein turnover results in the persistence of non-functional SPAG6, conferring no benefit to fertility. In this regard, western blot analysis using whole-cell lysates may be used to determine where SPAG6 is being expressed, particularly by focusing on non-functional regions like the cytoplasm, rather than its active site within the axoneme. As it stands, without precise localization techniques, such assays may overestimate the biologically relevant pool of SPAG6 by including SPAG6 that is expressed in redundant regions of the cell.

1.2. Molecular and structural role of SPAG6

The mRNA associated with SPAG6 expression is comprised of ten exons and sixteen domains. The encoded protein itself possesses eight consecutive WD repeats, to enact protein−protein interfacing, which is something that is essential for brain development [16]. Because SPAG6 is a microtubule-associated protein, it plays a critical role in the formation of the cellular cytoskeleton. This is most relevant to mammalian or algal cilia and flagella as it is also key to cellular polarization [17].

In male humans with immune-intermediated infertility, SPAG6 is targeted by a class of anti-sperm autoantibodies. According to Xu et al., men with mutations on SPAG6 leading to the expression of SPAG6 variants have less normal SPAG6 in their spermatozoa. This may indicate that SPAG6 variants contribute to the development of multiple morphological abnormalities of the sperm flagella phenotype, causing both syndromic severe asthenozoospermia and the non-syndromic asthenoteratozoospermia [18]. SPAG6 expression is critical in maintaining the structural integrity and coordinated motility of motile cilia, making it relevant to fertility and cellular respiration, or any other cilia-related function. This protein plays a essential role in assembling the central pair of microtubules within the cilia's axoneme, so its absence or mutation negatively alters this structure, impairing sperm and respiratory ciliary movement. As a consequence, mutations in SPAG6 are associated with male infertility and conditions like primary ciliary dyskinesia [5].

This is corroborated by a recent study that identified genetic changes affecting both copies of the SPAG6 gene, demonstrating a link to primary ciliary dyskinesia. This not only causes male infertility, but is associated with recurring respiratory infections [19]. Additionally, mice that completely lack the SPAG6 gene (SPAG6 knock out mice) exhibit significant health issues beyond infertility, such as neonatal mortality, and the development of hydrocephalus, as previously mentioned [2,4].

While spermatozoa are transcriptionally and translationally inactive, they undertake specialized metabolic processes that enable fertilization to carry through. Beyond the mechanical aspect of sperm mobility is the various signaling cascades occurring at the metabolomic level. A salient example is cAMP/protein kinase A and phosphoinositide 3-kinase signaling cascade, which is mediated via ionic processes using $Ca2^{+}$ and $HCO3^{-}$ [20]. This phosphorylation process drives key processes such as capacitation, hyperactivation, and the regulation of flagellar beating. This reinforces energy production and other structural dynamics necessary for effective sperm motility and eventual fertilization.

Both motile cilia and sperm flagella share the evolutionarily conserved cytoskeletal structure, which is the microtubule-based axoneme. This core component is made up of a central pair of microtubules and nine peripheral doublet microtubules. The collection of adjoining structural components includes radial spokes, dynein arms, and nexin links. The motion of flagella is driven by dynein molecular motors that cause sliding of outer-doublet microtubules. The molecular motor power is provided by protein phosphorylation at the radial spoke/central pair apparatus. This leads to the flagellar bending, which is the characteristic wiggling tail of a spermatozoa [21]. Hence, any alterations to the genes that code for axonemal proteins will disrupt the organization of cilia and flagella. The disorganization is referred to as primary ciliary dyskinesia, and is symptomatically known as male infertility [7].

SPAG6 functions with microtubules and organizes the cytoskeletal section of cells by the process of tubulin acetylation [22]. It possibly performs this function in the acrosomal region, the cytoplasmic vesicles, and the microtubule-based manchette of spermatids, thereby preserving structural integrity and/or functions. The process is likely to involve post-translational modifications of microtubules, as well as the transferal of proteins from the cytoplasm to the acrosome.

Sperm motility is not the only fertility related effect of decreased levels of SPAG6, as it was observed to decrease overall sperm count in mice, which may be partly attributable to the dysfunction of the acrosome and/or manchette [23]. Likewise, the crucial mechanical characteristic of SPAG6, being the eight armadillo-type repeats, carries out structural or regulatory roles by cooperating with other central pair proteins [23,24]. Notably, SPAG6's interactions with the additional central pair protein components, SPAG16 and SPAG17, regulate the function of the axoneme [16,22]. In a genetic study of male infertility where it was linked to primary ciliary dyskinesia (PCD), mutations on the SPAG6 and RSPH3 genes were found and shown to be correlated to sperm motility complications [19].

Furthermore, a recent study showed that SPAG6 and its paralog SPAG6L jointly regulate spermiogenesis and male fertility in mice. SPAG6L deficiency causes infertility without changing the appearance of sperm, whereas SPAG6 knockout alone does not affect fertility. Mutations on both cause a compounding effect that leads to severe sperm defects. The effects include abnormal chromatin, acrosome malformation, and abnormal flagellar structure. Both proteins interact with SPINK2 and influence AKAP4 and SPAG16L levels, thereby affecting the fibrous sheath and central apparatus. Interestingly, SPAG6 expression leads to inhibition of tubulin acetylation, whereas SPAG6L expression promotes it, indicating that the two are involving in modulation of processes related to microtubule formation. Hence, they work together to coordinate sperm development and fertility through reciprocating mechanisms [25].

1.3. SPAG6 as a possible diagnostic biomarker for evaluating sperm quality and motility

The current WHO-standard for semen analysis uses parameters such as the concentration of sperm, its motility, morphology, and vitality [26]. While this is useful for identifying general fertility status, it is not yet specific enough to detect molecular or ultrastructural defects in sperm flagella, which may be useful for directing interventional strategies. Hence, SPAG6 assessment takes the diagnosis to elevate clinical practice to the next level. While in the spermatid phase of spermatozoa biogenesis, the onward development of the highly structured flagellum (sperm tail) [27], is highly dependent on expression of SPAG6, as the structural integrity of the flagellum is essential for the movement of sperm flagella [23]. Studies have conclusively demonstrated that SPAG6 can be used as a biomarker for evaluating both sperm quality and motility. Poor expression of the SPAG6 gene will inevitably reduce sperm motility and increase rates of programmed cell death. Ren et al. investigated the roles of the SPAG6 and PPP1CC genes in sperm quality of pigs, using shRNA technology driven by germ cell promoters. They revealed that inhibiting these genes reduced sperm motility and enhanced apoptosis rates [28].

SPAG6 knockout mice demonstrate disrupted ciliary structure and function of spermatozoa, and defects in the central microtubules of the "9 + 2" axoneme were observed. It also resulted in ciliary drift and impaired ciliary function in the trachea and bronchi of air passageways [4,29]. While this result has been consistent and relatively reliable in the study of mammals, the presence of SPAG6 within the reproductive tract of roosters (avian sperm) was less predictive of fertility issues. SPAG6 levels were higher in the epididymis and vas deferens and increased with sperm maturation, however sperm motility was not correlated to the measured levels [13].

Other proteins that are addition to SPAG6, such as SPAG16 and AKAP4, are also known to have critical roles in sperm motility and male infertility. They also contribute to the structural and regulatory organization of the sperm flagellum. For instance, SPAG16 encodes components of the radial spoke complex, which is required for coordinating the rhythmic beating of the flagellum. This was confirmed by Zhang et al. (2006) who demonstrated that mutations on SPAG16 indeed reduced sperm motility and compromised fertility, even if the perceived structure of the flagellum appeared relatively unchanged [30].

Furthermore, AKAP4 (A-kinase anchor protein 4) is key to the structural formation of the fibrous sheath surrounding the sperm tail, by anchoring protein kinase A (PKA) and facilitating intracellular signaling pathways that regulate motility. Mice deficient in AKAP4 also displayed profoundly impaired sperm motility which led to infertility [31]. While both SPAG16 and AKAP4 are critical to sperm function, SPAG6 stands out because of its direct contribution to the structural integrity of the flagellum, particularly because it is within the central apparatus of the axoneme. The SPAG6 gene encodes for a protein that localizes to the central pair microtubules, which is an area critical for the mechanical coordination of flagellar movement. Hence, the more direct way of identifying the underlying structural defects, even when conventional semen parameters appear borderline or normal, is by assessing SPAG6 expression via transcriptomic profiling, immunostaining, or Western blot [18,23].

One of the challenges faced by incorporation of molecular analysis of SPAG6 into sperm motility diagnosis is optimizing the yield and sensitivity of molecular assays. Hence, the specific method should be standardized with various outcomes in mind, such as efficient extraction of nucleic acids or proteins, carefully designed primers targeting SPAG6-specific sequences, and well-calibrated thermal cycling parameters [32]. If proteomic analyses is a more logistically viable option, it is necessary to develop high-affinity antibodies and finely tuned buffer compositions [23]. It is also important to build guidelines specific to the biological source and timing of sample collection, because various factors can influence SPAG6 expression levels, such as when testicular tissue is in active spermatogenesis [25]. Various aspects of the analysis, such as those mentioned, influence the guidelines on interpreting SPAG6 levels at the clinical level.

1.4. Potential advantages and disadvantages of utilizing SPAG6 as a biomarker

SPAG6 analysis is expected to be a better tool than traditional markers such as DNA fragmentation index (DFI), acrosome integrity, and reactive oxygen species (ROS) levels (Table 1). While these markers are useful indicators of sperm health, they are not specific for motility-related dysfunctions and hence, do not go the extra step in characterizing the dynamic interplay between cytoskeletal components and flagellar propulsion. Because SPAG6 is directly involved in microtubule stabilization and axonemal coordination functions, it is a biomarker of function this is central to sperm motility and fertilization.

Due to its diagnostic value in assessing sperm flagellum motility and structural integrity, SPAG6 may be used as a marker when evaluating male

Table 1. Cross examination between SPAG6 and proven Sperm Function markers.

Sperm function Biomarker	Function	Limitations in Application	Applications	References
Acrosome Reaction (AR)	The acrosome reaction enables sperm to penetrate the zona pellucida by releasing enzymes, an essential stage for fertilization to happen.	Acrosin is critical in the later steps of fertilization.	Acrosin main role is its vital, Versatile function in mammalian fertilization, operating as a proteolytic enzyme and binding protein enabling sperm to cross zona pellucida, an essential pathway for reproductive achievement.	[33,34]
Protamine 1 (PRM1) and Protamine 2 (PRM2)	PRM1 and PRM2 are essential for chromatin condensation and DNA packaging	Protamine evaluation demonstrates limited clinical usage due to assay variability, genetic variety, and its indirect association with sperm abnormalities.	Connects directly to sperm structural organization, motility and morphological aspects.	[35–37]
Reactive Oxygen Species	Used as an indicator of oxidative stress.	Non-specific and varying; affected by environmental and physiological reasons, making it challenging to distinguish a singular cause for raised levels or specify a universally appropriate normal range.	Essential signaling intermediaries that regulate protein action and cellular routes.	[38–40]
DNA Fragmentation Index	Assesses sperm DNA integrity and fragmentation.	Elevated DNA fragmentation index may not directly associate with sperm motility or fertilization capacity, although higher levels are usually associated with declined motility and, in specific cases, decreased fertilization achievement.	Precisely measuring sperm genetic integrity. It assists clarifying unexplained infertility, predicts Assisted Reproductive Technology results and miscarriage threat and informs treatment options like using testicular sperm or lifestyle changes to improve success.	[41–43]
SPAG6	Regulates axonemal structure, motility, and spermatogenesis.	In the process of evaluation, Insufficient clinical evidence	Extensive indicator with capability for early analysis and therapeutic directing.	[17,44]

infertility in mammals [4]. Second, SPAG6 expression increases as sperm maturation takes place, where it is mainly localized in the flagellar axoneme. Hence, in addition to motility, SPAG6 levels allow prediction of the stage of sperm development [13]. Furthermore, research shows that males with mutations in the SPAG6 and RSPH3 genes is a marker for asthenoteratospermia, yet positive pregnancy results can be achieved with intracytoplasmic sperm injection [19].

However, the specificity of SPAG6 and possible variations of expression create barriers to its tangibility as a male fertility biomarker. Indeed, research has linked SPAG6 to infertility and defects in sperm motility, but its expression may change among people or circumstances, such as age, health, or other lifestyle factors, which could generate inconsistent results and reduce predictability. Furthermore, sperm mobility is affected by the amount of SPAG6. Knockout research has proven that SPAG6 is essential for appropriate sperm motility, yet mice with one competent copy of the SPAG6 gene had higher sperm mobility than those with two competent copies [23]. This demonstrates that sperm mobility is not efficiently predicted by SPAG6 abundance alone, and this may also explain the apparent contradictory results in the study of roosters. Finally, since sperm morphology and overall semen quality are critical in fertility, SPAG6 possibly will not offer a thorough estimation of male fertility. This means that it is not wise to depend only on SPAG6, but rather, to use it together with other biomarkers to discern male reproductive health. Instead, a compounding approach that combines metrics on SPAG6 with markers with others like SPAG16 may offer a more thorough diagnosis. Because both genes are crucial for sperm flagellar structure and motility, evaluation of their co-expression is likely to improve the accuracy of genetic screening. This will aid in the detection of infertility causes and enable the clinician to adopt more tailored treatment strategies. Nevertheless, clinicians still require more comprehensive directives on how to use the data, which can only be determined by broader clinical validation through large-scale multicenter studies.

2. Conclusion

Recent research into reproductive biology is starting to demonstrate that Sperm-Associated Antigen 6 (SPAG6) is not only important in the structural and functional integrity of spermatozoa, but it is involved in fertility issues. SPAG6 is specifically involved in the organization of the sperm flagellum, particularly by maintaining axonemal stability and facilitating effective motility. Experimental models employing gene knockout and targeted mutations have consistently demonstrated that the absence or disruption of SPAG6 leads to pronounced defects in sperm motility and flagellar architecture. Hence, there is a direct association between SPAG6 deficiency and asthenozoospermia. Immunohistochemical staining and transcriptomic profiling have confirmed that the testis-specific expression of SPAG6 peaks during the critical phases of spermatogenesis. Taken together, analysis of SPAG6 expression has great potential as a functionally relevant biomarker, aimed at evaluating sperm function and male fertility.

Ethics information

This article is a review of previously published studies. No new human or animal data were collected, and therefore ethics approval was not required.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest, financial or otherwise.

References

[1] World Health Organization, WHO Laboratory Manual for the Examination and Processing of Human Semen, fifth ed., WHO Press, Geneva. 2010 https://doi.org/10.1016/s2173-5786(10)70143-8.

[2] L.I. Neilson, P.A. Schneider, P.G. Van Deerlin, M. Kiriakidou, D.A. Driscoll, M.C. Pellegrini, S. Millinder, K.K. Yamamoto, C.K. French, J.F. Strauss III, cDNA cloning and characterization of a human sperm antigen (SPAG6) with homology to the product of the Chlamydomonas PF16 locus, Genomics 60 (1999) 272—280, https://doi.org/10.1006/geno.1999.5914.

[3] R. Sapiro, L.M. Tarantino, F. Velazquez, M. Kiriakidou, N.B. Hecht, M. Bucan, J.F. Strauss III, Sperm antigen 6 is the murine homologue of the Chlamydomonas reinhardtii central apparatus protein encoded by the PF16 locus, Biol. Reprod. 62 (2000) 511—518, https://doi.org/10.1095/biolreprod62.3.511.

[4] R. Sapiro, I. Kostetskii, P. Olds-Clarke, G.L. Gerton, G.L. Radice, J.F. Strauss III, Male infertility, impaired sperm motility, and hydrocephalus in mice deficient in sperm-associated antigen 6, Mol. Cell. Biol. (2002) 6298—6305, https://doi.org/10.1128/mcb.22.17.6298-6305.2002.

[5] M.E. Teves, P.R. Sears, W. Li, Z. Zhang, W. Tang, L. van Reesema, R.M. Costanzo, C.W. Davis, M.R. Knowles, J.F. Strauss III, Sperm-associated antigen 6 (SPAG6) deficiency and defects in ciliogenesis and cilia function: polarity, density, and beat, PLoS One 9 (2014) e107271, https://doi.org/10.1371/journal.pone.0107271.

[6] E.F. Smith, P.A. Lefebvre, PF16 encodes a protein with armadillo repeats and localizes to a single microtubule of the central apparatus in Chlamydomonas flagella, J. Cell Biol. 132 (1996) 359—370, https://doi.org/10.1083/jcb.132.3.359.

[7] C. Coutton, J. Escoffier, G. Martinez, C. Arnoult, P.F. Ray, Teratozoospermia: spotlight on the main genetic actors in the human, Hum. Reprod. Update 21 (2015) 455—485, https://doi.org/10.1093/humupd/dmv020.

[8] K. Silina, P. Zayakin, Z. Kalnina, L. Ivanova, I. Meistere, E. Endzeliņš, A. Abols, A. Stengrevics, M. Leja, K. Ducena, Sperm-associated antigens as targets for cancer immunotherapy: expression pattern and humoral immune response in cancer patients, J. Immunother. 34 (2011) 28—44, https://doi.org/10.1097/cji.0b013e3181fb64fa.

[9] R.K. Naz, X. Zhu, Molecular cloning and sequencing of cDNA encoding for human FA-1 antigen, Mol. Reprod. Dev. 63 (2002) 256—268, https://doi.org/10.1002/mrd.90010.

[10] H. Qiu, A. Gołas, P. Grzmil, L. Wojnowski, Lineage-specific duplications of Muroidea Faim and Spag6 genes and atypical accelerated evolution of the parental Spag6 gene, J. Mol. Evol. 77 (2013) 119—129, https://doi.org/10.1007/s00239-013-9585-9.

[11] X. Hu, R. Yan, X. Cheng, L. Song, W. Zhang, K. Li, S. Zhao, The function of sperm-associated antigen 6 in neuronal proliferation and differentiation, J. Mol. Histol. 47 (2016) 531—540, https://doi.org/10.1007/s10735-016-9694-z.

[12] X. Liu, L. Zhen, J. Fu, L. Wang, Q. Yang, P. Li, Y. Li, Quantitative proteomic profiling indicates the difference in reproductive efficiency between Meishan and Duroc boar spermatozoa, Theriogenology 116 (2018) 71—82, https://doi.org/10.1016/j.theriogenology.2018.04.025.

[13] Z. Jarrell, M. Ahammad, K. Sweeney, J. Wilson, A. Benson, Characterization of sperm-associated antigen 6 expression in the reproductive tract of the domestic rooster (Gallus domesticus) and its impact on sperm mobility, Poult. Sci. 99 (2020) 6188—6195, https://doi.org/10.1016/j.psj.2020.08.009.

[14] Y.T. Yap, W. Li, Q. Zhou, S. Haj-Diab, D.D. Chowdhury, A. Vaishnav, P. Harding, D.C. Williams Jr., B.F. Edwards, J.F. Strauss III, The ancient and evolved mouse sperm-associated antigen 6 genes have different biologic functions in vivo, Cells 11 (2022) 336, https://doi.org/10.3390/cells11030336.

[15] L. Soler, V. Labas, A. Thelie, I. Grasseau, A.-P. Teixeira-Gomes, E. Blesbois, Intact cell MALDI-TOF MS on sperm: a molecular test for male fertility diagnosis, Mol. Cell. Proteomics 15 (2016) 1998—2010, https://doi.org/10.1016/j.anireprosci.2016.03.051.

[16] Z. Zhang, R. Sapiro, D. Kapfhamer, M. Bucan, J. Bray, V. Chennathukuzhi, P. McNamara, A. Curtis, M. Zhang, E.J. Blanchette-Mackie, A sperm-associated WD repeat protein orthologous to Chlamydomonas PF20 associates with Spag6, the mammalian orthologue of Chlamydomonas PF16, Mol. Cell. Biol. 22 (2002) 7993—8004, https://doi.org/10.1128/mcb.22.22.7993-8004.2002.

[17] L.F. Cooley, M.E. El Shikh, W. Li, R.C. Keim, Z. Zhang, J.F. Strauss, Z. Zhang, D.H. Conrad, Impaired immunological synapse in sperm associated antigen 6 (SPAG6) deficient mice, Sci. Rep. 6 (2016) 25840, https://doi.org/10.1038/srep25840.

[18] C. Xu, D. Tang, Z. Shao, H. Geng, Y. Gao, K. Li, Q. Tan, G. Wang, C. Wang, H. Wu, Homozygous SPAG6 variants can induce nonsyndromic asthenoteratozoospermia with severe

MMAF, Reprod. Biol. Endocrinol. 20 (2022) 41, https://doi.org/10.1186/s12958-022-00916-3.
[19] H. Wu, J. Wang, H. Cheng, Y. Gao, W. Liu, Z. Zhang, H. Jiang, W. Li, F. Zhu, M. Lv, Patients with severe asthenoteratospermia carrying SPAG6 or RSPH3 mutations have a positive pregnancy outcome following intracytoplasmic sperm injection, J. Assist. Reprod. Genet. 37 (2020) 829−840, https://doi.org/10.1007/s10815-020-01721-w.
[20] R. Dcunha, R.S. Hussein, H. Ananda, S. Kumari, S.K. Adiga, N. Kannan, Y. Zhao, G. Kalthur, Current insights and latest updates in sperm motility and associated applications in assisted reproduction, Reprod. Sci. (2022) 1−19, https://doi.org/10.1007/s43032-020-00408-y.
[21] K. Inaba, Molecular architecture of the sperm flagella: molecules for motility and signaling, Zool. Sci. 20 (2003) 1043−1056, https://doi.org/10.2108/zsj.20.1043.
[22] Z. Zhang, B.H. Jones, W. Tang, S.B. Moss, Z. Wei, C. Ho, M. Pollack, E. Horowitz, J. Bennett, M.E. Baker, Dissecting the axoneme interactome: the mammalian orthologue of Chlamydomonas PF6 interacts with sperm-associated antigen 6, the mammalian orthologue of Chlamydomonas PF16, Mol. Cell. Proteomics 4 (2005) 914−923, https://doi.org/10.1074/mcp.M400177-MCP200.
[23] Y. Liu, L. Zhang, W. Li, Q. Huang, S. Yuan, Y. Li, J. Liu, S. Zhang, G. Pin, S. Song, The sperm-associated antigen 6 interactome and its role in spermatogenesis, Reproduction 158 (2019) 181−197, https://doi.org/10.1530/rep-18-0522.
[24] E.F. Smith, P.A. Lefebvre, The role of central apparatus components in flagellar motility and microtubule assembly, Cell Motil. Cytoskeleton 38 (1997) 1−8, 10.1002/(SICI)1097-0169(1997)38:1<1::AID-CM1>3.0.CO;2-C.
[25] Y. Liu, W. Li, T. Li, C. Zheng, C. Niu, A. Schmitt, Y.T. Yap, M. Abdulghani, S. Yuan, C. Melander, Two Spag6 genes control sperm formation and male fertility in mice, bioRxiv (2025), https://doi.org/10.1101/2025.07.18.665465, 2025.07.18.665465.
[26] World Health Organization, WHO Laboratory Manual for the Examination and Processing of Human Semen, WHO. 2021. https://www.who.int/publications/i/item/9789240030787.
[27] T. Lehtiniemi, N. Kotaja, The genetics of postmeiotic male germ cell differentiation from round spermatids to mature sperm, in: Genetics of Human Infertility, Karger Publishers. 2017, pp. 101−115, https://doi.org/10.1159/000477281.
[28] H. Ren, Y. Zhang, Y. Bi, H. Wang, G. Fang, P. Zhao, Target silencing of porcine SPAG6 and PPP1CC by shRNA attenuated sperm motility, Theriogenology 219 (2024) 138−146, https://doi.org/10.1016/j.theriogenology.2024.02.030.
[29] Z. Zhang, W. Tang, R. Zhou, X. Shen, Z. Wei, A.M. Patel, J.T. Povlishock, J. Bennett, J.F. Strauss III, Accelerated mortality from hydrocephalus and pneumonia in mice with a combined deficiency of SPAG6 and SPAG16L reveals a functional interrelationship between the two central apparatus proteins, Cell Motil. Cytoskeleton 64 (2007) 360−376, https://doi.org/10.1002/cm.20189.
[30] Z. Zhang, I. Kostetskii, W. Tang, L. Haig-Ladewig, R. Sapiro, Z. Wei, A.M. Patel, J. Bennett, G.L. Gerton, S.B. Moss, Deficiency of SPAG16L causes male infertility associated with impaired sperm motility, Biol. Reprod. 74 (2006) 751−759, https://doi.org/10.1095/biolreprod.105.049254.
[31] K. Miki, W.D. Willis, P.R. Brown, E.H. Goulding, K.D. Fulcher, E.M. Eddy, Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility, Dev. Biol. 248 (2002) 331−342, https://doi.org/10.1006/dbio.2002.0728.
[32] M.R. Khan, A. Akbari, T.J. Nicholas, H. Castillo-Madeen, M. Ajmal, T.U. Haq, M. Laan, A.R. Quinlan, J.S. Ahuja, A.A. Shah, Genome sequencing of Pakistani families with male infertility identifies deleterious genotypes in SPAG6, CCDC9, TKTL1, TUBA3C, and M1AP, Andrology 13 (2025) 1093−1104, https://doi.org/10.1111/andr.13570.
[33] S.S. Suarez, J.W. Pollard, Capacitation, the acrosome reaction, and motility in mammalian sperm, in: Controls of Sperm Motility, 2020, pp. 77−90, https://doi.org/10.1201/9780429281884-7.
[34] H.-T. Mao, W.-X. Yang, Modes of acrosin functioning during fertilization, Gene 526 (2013) 75−79, https://doi.org/10.1016/j.gene.2013.05.058.
[35] S. Ren, X. Chen, X. Tian, D. Yang, Y. Dong, F. Chen, X. Fang, The expression, function, and utilization of Protamine1: a literature review, Transl. Cancer Res. 10 (2021) 4947−4957, https://doi.org/10.21037/tcr-21-1582.
[36] B.P. Pardede, M. Agil, I. Supriatna, Protamine and other proteins in sperm and seminal plasma as molecular markers of bull fertility, Vet. World 13 (2020) 556−562, https://doi.org/10.14202/vetworld.2020.556-562.
[37] M. Akmal, Aulanni'am, M.A. Widodo, S.B. Sumitro, B.B. Purnomo, The important role of protamine in spermatogenesis and quality of sperm: a mini review, Asian Pac. J. Reprod. 5 (2016) 357−360, https://doi.org/10.1016/j.apjr.2016.07.013.
[38] S.T. Homa, W. Vessey, A. Perez-Miranda, T. Riyait, A. Agarwal, Reactive oxygen species (ROS) in human semen: determination of a reference range, J. Assist. Reprod. Genet. 32 (2015) 757−764, https://doi.org/10.1007/s10815-015-0454-x.
[39] H. Pavuluri, Z. Bakhtiary, M.K. Panner Selvam, W.J.G. Hellstrom, Oxidative stress-associated male infertility: current diagnostic and therapeutic approaches, Medicina 60 (2024) 1008, https://doi.org/10.3390/medicina60061008.
[40] P.D. Ray, B.-W. Huang, Y. Tsuji, Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling, Cell Signal 24 (2012) 981−990, https://doi.org/10.1016/j.cellsig.2012.01.008.
[41] A. Agarwal, A. Majzoub, S. Baskaran, M.K. Panner Selvam, C.L. Cho, R. Henkel, R. Finelli, K. Leisegang, P. Sengupta, C. Barbarosie, Sperm DNA fragmentation: a new guideline for clinicians, World J. Mens Health 38 (2020) 412−471, https://doi.org/10.5534/wjmh.200128.
[42] A.T. Alahmar, R. Singh, A. Palani, Sperm DNA fragmentation in reproductive medicine: a review, J. Hum. Reprod. Sci. 15 (2022) 206−218, https://doi.org/10.4103/jhrs.jhrs_82_22.
[43] B. Yang, L. Xia, R. Deng, L. Wu, Z. Zhang, X. Wu, T. Ding, Y. Zhao, J. Huang, Z. Huang, Impact of sperm DNA fragmentation index on assisted reproductive outcomes: a retrospective analysis, Front. Endocrinol. 15 (2025) 1530972, https://doi.org/10.3389/fendo.2024.1530972.
[44] R. Sapiro, I. Kostetskii, P. Olds-Clarke, G.L. Gerton, G.L. Radice, J.F. Strauss III, Male infertility, impaired sperm motility, and hydrocephalus in mice deficient in sperm-associated antigen 6, Mol. Cell. Biol. 22 (2002) 6298−6305, https://doi.org/10.1128/mcb.22.17.6298-6305.2002.