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REVIEW

Calcium Channels and Modulators as Potential Therapeutic Targets for Contraceptives and Male Fertility: A Scoping Review

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Background: Calcium channels are critical regulators of spermatogenesis and sperm functions, orchestrating processes like motility, capacitation, and fertilization. Their dysfunction is linked to male infertility, contributing to approximately 50% of infertility cases globally. This scoping review synthesizes evidence on the role of calcium channels in male fertility, focusing on their physiological mechanisms and implications for reproductive health.

Methods: Following the PRISMA-ScR checklist and Arksey and O'Malley's framework, a search of PubMed, Scopus, and Web of Science identified 978 records from January 2000 to July 2025. After screening, 142 peer-reviewed studies on mammalian models were included. Data were extracted on channel types, study designs, and outcomes, categorized by the physiological functions of sperm.

Results: The review identified five channel-mediated regulators of sperm calcium, including CatSper, voltage-dependent calcium channels (VDCCs), TRP channels, inositol triphosphate (IP_3) and ryanodine receptors (IP3Rs & RyRs), and calcium-sensing receptor (CaSR), as key players in sperm motility, capacitation, and fertilization. About 19 non-channel mediators of sperm calcium were also reported.

Conclusion: Calcium channels are pivotal to male fertility and contraceptives, offering diagnostic and therapeutic potential. Future research should focus on multi-channel interactions, human-based studies, and environmental impacts to enhance fertility treatments and develop non-hormonal contraceptives.

Keywords: calcium, contraceptives, male fertility, sperm function

Introduction

Calcium (Ca^2+) channels are pivotal regulators of numerous physiological processes, including spermatogenesis and sperm functions. These specialized ion channels facilitate the influx of calcium ions across cellular membranes, orchestrating signal transduction cascades crucial for male fertility. The importance of calcium signaling in male reproduction has increasingly drawn scientific attention, with evidence underscoring its role in sperm motility, capacitation, the acrosome reaction, and fertilization.[1] Dysfunction of these channels has been linked to male infertility, prompting significant research into their structure, regulation, and mechanisms of action. For instance, exposure to calcium channel blockers and antifertility compounds such as gossypol and nimodipine has shown a significant decline in sperm motility and fertility in both animal and human models.[2,3]

Globally, male infertility contributes to about 50% of infertility cases among couples, affecting approximately 7% of all men.[4] A comprehensive meta-analysis by Boivin et al[5] estimates that 9% of couples worldwide face infertility challenges, with male factors contributing significantly. In terms of calcium channel-related dysfunction, mutations in

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CatSper genes alone have been reported in 20–30% of men with idiopathic infertility.[6] This growing concern has driven global initiatives, including the World Health Organization's (WHO) commitment to reproductive health, which recognizes male infertility as a public health issue requiring further research and intervention.

Using methodologies like in vivo animal models, electrophysiology (eg, patch clamp), gene knockouts (eg, CatSper-null mice), proteomics, transcriptomics, immunohistochemistry, flow cytometry, calcium imaging, and pharmacological inhibition, the pathophysiological relevance of calcium channels in spermatogenesis and sperm functions has been well-investigated.[7] During spermatogenesis, calcium ions influence germ cell development, meiosis, and sperm maturation through channels such as T-type (CaV3.1, CaV3.2), cation channel of sperm (CatSper), transient receptor potential (TRP), and voltage-dependent calcium channels (VDCCs).[7] Among these, CatSper is sperm-specific and essential for hyperactivation and successful fertilization. CatSper's disruption leads to defective motility and reduced fertilization capacity.[8] Gene-deficient mouse models lacking CatSper1 or CatSper4 demonstrate infertility due to failed hyperactivation.[8,9] Additionally, TRP channels, such as transient receptor potential cation channel subfamily M member 8 (TRPM8) and transient receptor potential vanilloid (TRPV) 1, contribute to temperature and chemical sensitivity during sperm navigation and activation.[10,11] Risk factors contributing to calcium channel disruption include environmental toxins (pesticides, heavy metals), heat stress, pharmaceutical exposure, and lifestyle habits like smoking and alcohol consumption. Heat stress and pesticide exposure have been found to affect CatSper and T-type channel expression, further impairing spermatogenesis.[12,13] Studies investigating male fertility in animal models have shown that reduced fertility can be associated with specific genetic pathways and molecular mechanisms, as demonstrated by genome-wide analyses of bull fertility traits.[14] Genetic mutations in CatSper genes are also more prevalent in certain ethnic populations, such as the Middle East and South Asia, suggesting regional susceptibility.[15]

Mechanistically, calcium channels regulate intracellular Ca^{2}+ homeostasis, a prerequisite for activating downstream kinases and ion exchanges that promote motility, acrosome reaction, and zona pellucida binding.[16] CatSper channels, located on the sperm flagellum, mediate Ca^{2}+ influx in response to progesterone, altering sperm behavior from progressive to hyperactivated motility.[17] T-type channels, found in spermatogenic cells, modulate germ cell differentiation and are targets of hormonal and environmental modulations.[18] Furthermore, TRP channels help the sperm navigate temperature gradients (thermotaxis), while calcium-sensing receptors (CaSR) mediate testicular calcium balance.[19]

Despite the well-established role of calcium in spermatogenesis and sperm functions, there is currently no drug being used to manage male factor infertility that targets calcium channels. Given their emerging importance in fertility regulation and as potential targets for male contraception, there is a critical need for comprehensive mapping of the current studies that have reported the role of calcium channels in spermatogenesis and sperm functions. This scoping review aimed to do this mapping, which will serve educational, research, and therapeutic purposes concerning male factor infertility.

Methodology

This scoping review was conducted in accordance with the PRISMA-ScR checklist and Arksey and O'Malley's six-stage framework. This methodological approach, recognized for its thorough yet flexible structure, is particularly suited for mapping broad scientific evidence and identifying key concepts, knowledge gaps, and types of available research.[20]

The first stage involved specifying the research question: *How do calcium channels influence mammalian spermatogenesis and sperm functions?* This question was designed to guide the entire review process and ensure consistency in article selection and analysis. In the second stage, a search strategy was developed to identify relevant literature from databases including PubMed, Scopus, and Web of Science. The search focused on studies published in English between January 1, 2000, and July 31, 2025, and exclusively included research conducted on mammals. Search terms, including "calcium channels," "spermatogenesis," "sperm motility," "capacitation," "acrosome reaction," "male infertility," and "mammals" were combined using Boolean operators for precision. In the third stage, study selection was conducted through a two-step screening process. All retrieved articles were imported into Covidence software, where duplicates were removed, and two independent reviewers screened the titles and abstracts. Eligible full-text articles were then reviewed in detail based on inclusion and exclusion criteria. Non-mammalian studies, reviews, and unrelated records (eg, theses) were excluded. No formal methodological quality or bias appraisal was conducted, consistent with scoping

review methodology. In the fourth stage, data from the included studies were extracted using a standardized data collection sheet to chart details such as first author, publication year, study population, sample size, calcium channels investigated, study design, tissue source, and key findings (Supplementary File S1). The studies were categorized by research themes and the regions where the research was conducted to map global research trends. The fifth stage focused on summarizing and synthesizing the data. The findings were mapped thematically to present the biological roles of calcium channels in sperm development and function, including their involvement in sperm motility, capacitation, and acrosome reaction. Disruptions caused by environmental toxins and pharmacological agents were also highlighted. Finally, the sixth stage involved expert consultation. Specialists in reproductive physiology and ion channel biology were engaged to validate the identified themes and clarify complex mechanisms, thereby enriching the interpretation and accuracy of the review.

Results

The scoping review followed PRISMA guidelines for article selection. From an initial pool of 978 records identified through comprehensive database searches targeting original articles on mammalian animal species (in vivo or in vitro), 757 records were excluded during the screening phase due to irrelevance and duplicity based on title and abstract. The remaining 221 full-text articles underwent eligibility assessment, resulting in the exclusion of 79 articles—18 for focusing on non-mammalian species and 61 for not being original research (eg, reviews, protocols, or dissertations). Ultimately, 142 studies were included, providing a robust synthesis of evidence (Figure 1). This rigorous process enabled the extraction of key details, including the country of origin (based on the corresponding or first author's affiliation), specific calcium channels investigated, and their implications for spermatogenesis (sperm production) and sperm function (eg, motility, capacitation, acrosome reaction).

Country Contributions

The geographical distribution of the 142 included studies highlights a broad global interest in calcium channel research related to male reproductive health, though contributions vary considerably by country. China led with 32 studies,

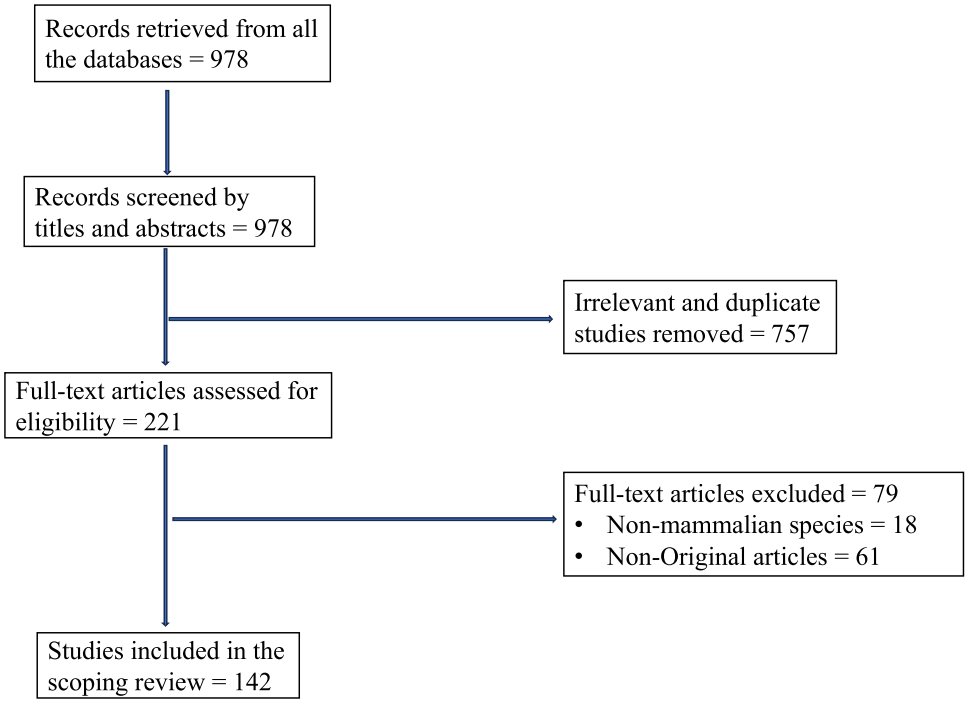

Figure 1 PRISMA-ScR flow chart illustrating the study selection process, including the number of records identified from databases, records screened and excluded, and the final number of studies included in the scoping review.

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followed by the United States with 22 and Mexico with 8. Moderate contributions were observed from India (7), Egypt (6), Iran (6), and Italy (6), while France and Spain each produced 5 studies. Germany, Nigeria, Turkey, and the United Kingdom each accounted for 4 studies, with Canada and Korea contributing 3 each. Countries such as Argentina, Austria, Brazil, Iraq, Israel, Japan, and Poland provided 2 studies apiece. Additional single-study contributions came from Australia, Denmark, Malaysia/Saudi Arabia, the Netherlands, Pakistan, Russia, Switzerland, UK/Portugal, and USA/India. This distribution illustrates both concentrated research efforts in leading countries and emerging contributions from diverse regions, reflecting a steadily expanding global awareness of the role of calcium channels in male fertility (Figure 2).

Types of Calcium Channels Involved in Spermatogenesis and Sperm Functions

The 142 studies investigated a range of calcium channels critical to spermatogenesis and sperm function, with the CatSper family being the most frequently studied due to its pivotal role in sperm hyperactivation and fertilization. Other channels, including L-type and T-type VDCCs, TRP, IP_3 & ryanodine receptors, CaSR, high-voltage activated, acrosomal/intracellular Ca^2+ stores, store-operated Ca^2+ entry, and other regulators and mediators of intracellular Ca^2+ levels were also explored for their roles in spermatogenesis, sperm motility, acrosome reaction, fertilization, and environmental responsiveness (Tables 1 and 2).

Channel-Mediated Regulators

CatSper Family

The CatSper family is a sperm-specific calcium channel essential for hyperactivated motility, capacitation, and fertilization.[21,71,72] CatSper is modulated by pH, progesterone, and certain pharmacological agents, such as nifedipine or escanbil extract,[71–73] and mutations or loss of its subunits produce asthenozoospermia and infertility.[74] Molecularly, CatSper is a multi-subunit, pH- and voltage-sensitive Ca^{2+} channel complex formed by four pore-forming α-like subunits (CatSper1–4) and multiple auxiliary/regulatory subunits (CatSper γ, δ, ε, ζ, CatSper τ/*C2CD6*, and *EFCAB9*, among

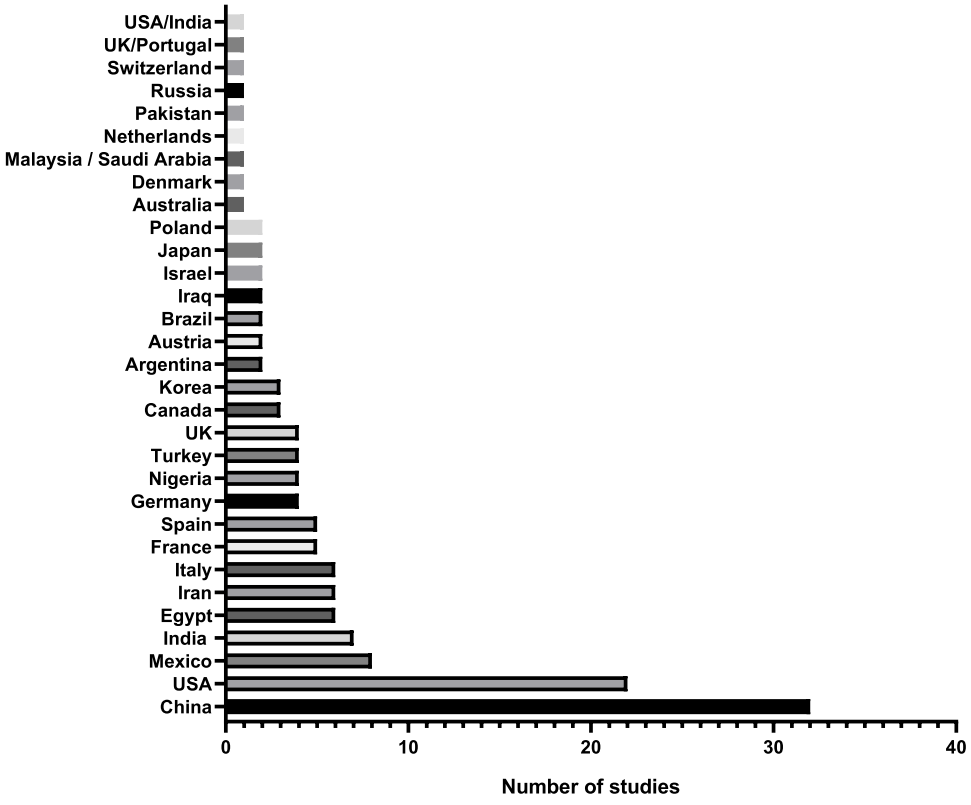

Figure 2 Studies published per country on the role of calcium channels in spermatogenesis and sperm functions.

Table 1 Channel-Mediated Calcium Regulators in Sperm

Section No	Calcium Channel Family	Physiological Functions in Sperm	Number of Articles that Reported the Channel's Effect in Sperm	Representative References
3.2.1	**CatSper Family**	Essential for hyperactivated motility, capacitation, and fertilization. Modulated by pH, progesterone, and pharmacological agents (eg, nifedipine, escanbil extract). Mutations or loss of subunits cause asthenozoospermia and infertility. Localized to the principal piece of the sperm flagellum, forming longitudinal nanodomains for Ca^2+ influx to control beat asymmetry and amplitude. Regulated by intracellular pH, membrane potential, and cAMP/PKA signaling.	24	Lobley et al,[21] Chung et al,[8] Hwang et al,[22] Marchiani et al[23]
3.2.2	**VDCCs**	Regulate motility, capacitation, and acrosome reaction. Predominantly located in the sperm neck and midpiece. L-type VDCCs are activated by depolarization and inhibited by blockers like nifedipine or amlodipine, impairing motility and fertilization. T-type channels contribute to acrosomal signaling and flagellar initiation. HVA channels (P/Q-, N-, R-type) support sustained Ca^2+ entry for capacitation and acrosome reaction.	18	Goodwin et al, [24] Escoffier et al,[7] Morakinyo et al[2]
3.2.3	**TRP Channels**	Act as polymodal sensors for motility, hyperactivation, and acrosome reaction, responding to temperature, pH, and chemical stimuli. TRPM8 (flagellum) regulates chemotaxis and thermotaxis; TRPV4 (plasma membrane) initiates hyperactivated motility; TRPC channels (TRPC3, 4, 6, 7) modulate capacitation via calcium mobilization. Modulated by ligands like menthol, DAG, and vitamin D3.	12	De Blas et al,[10] Mundt et al,[25] Ru et al,[26] Lacalle et al,[27] Governini et al[28]
3.2.4.	**IP3 & Ryanodine Receptors (IP3Rs & RyRs)**	Intracellular Ca^2+ release channels in acrosomal and mitochondrial membranes, generating calcium oscillations for capacitation and acrosome reaction. IP3Rs facilitate acrosomal exocytosis and thermotaxis; RyRs contribute to hyperactivation. Modulated by caffeine, ryanodine, and 2-APB. Dysregulation affects mitochondrial function and motility.	7	Rossato et al,[16] Bahat and Eisenbach,[29] Herrick et al,[30] Maleki et al[31]
3.2.5	**Calcium-Sensing Receptor (CaSR)**	GPCR in the sperm head and midpiece detects extracellular calcium fluctuations to regulate motility, capacitation, and acrosomal responsiveness. Modulates cAMP/PKA pathways and coordinates with CatSper/TRPCs. Dysregulation (eg, by metabolic disorders or genetic variants) impairs sperm function and fertility.	3	Qian et al,[32] Nasraldin et al[19]

Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; GPCR, G-protein coupled receptor; HVA, high-voltage-activated; IP3Rs, inositol triphosphate receptors; PKA, protein kinase A; RyRs, ryanodine receptors; TRPC, transient receptor potential channel; TRPM8, transient receptor potential cation channel subfamily M member 8; TRPV, transient receptor potential vanilloid; VDCCs, voltage-dependent calcium channels.

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Table 2 Non-Channel-Mediated Regulators of Intracellular Calcium in Sperm: Physiological Functions and Implications for Male Fertility

Section No	Calcium Regulator Family	Physiological Function in Sperm	Number of Articles that Reported the Regulator's Effect on Sperm	Representative References
3.2.2.1	Slo3 (Ca^2+-activated K+ channel)	Regulates intracellular calcium indirectly via membrane hyperpolarization, promoting calcium influx through CatSper channels. Essential for motility, hyperactivation, capacitation, and acrosome reaction.	5	Brenker et al;[33] Lavoie-Ouellet et al;[34] Bi et al;[35] de Liz Oliveira Cavalli et al;[36] Luis et al[37]
3.2.2.2	Transmembrane protein 203 (TMEM203)	Modulates cytosolic calcium concentrations, supports spermatogenesis, sperm motility, and capacitation by influencing calcium fluxes and interacting with mitochondrial regulators.	5	Shambharkar et al;[38] Bai et al;[39] Agarwal et al;[4] Hou et al[40]
3.2.2.3	Rod outer segment guanylate cyclase 1 (ROS-GC1)	Converts Ca^2+ fluctuations into cGMP signaling, regulates motility, capacitation, and acrosome reaction. Interacts with calcium-binding proteins to modulate sperm function.	5	Jankowska et al;[41] Jankowska et al;[42] Bai et al;[39] Agarwal et al[4]
3.2.2.4	Cysteine-Rich Secretory Protein 2 (CRISP2)	Modulates calcium influx via CatSper and voltage-gated calcium channels, regulates motility, capacitation, and acrosome reaction. Interacts with cAMP pathways for sperm-egg fusion.	6	Masai et al;[43] Jamsai et al;[44] Roberts et al;[45] Krähling et al;[46] Lavoie-Ouellet et al[34]
3.2.2.5	STAC3 (SH3 and Cysteine-Rich Domain-Containing Protein 3)	Regulates calcium influx, mitochondrial membrane potential, and steroidogenesis in Leydig cells. Essential for motility, capacitation, and spermatogenesis.	4	Bi et al;[35] Lavoie-Ouellet et al;[34] Agarwal et al[4]
3.2.2.6	FYN Kinase	Modulates calcium influx, actin cytoskeleton remodeling, and mitochondrial function via phosphorylation. Critical for capacitation, motility, and acrosome reaction.	4	Luo et al;[47] Bai et al;[39] Lavoie-Ouellet et al[34]
3.2.2.7	Sperm Head and Tail Associated Protein (SHTAP)	Stabilizes protein complexes for calcium flux, motility, and acrosome reaction. Essential for spermatogenesis and fertilization competence.	3	Jamsai et al;[44] Kwon et al[48]
3.2.2.8	CABCOCO1	Acts as a calcium sensor in the sperm flagellum, regulates axonemal dynein and mitochondrial function for motility and capacitation.	3	Kawashima et al;[49] Agarwal et al[4]
3.2.2.9	Tex13a	Regulates mRNA turnover and calcium signaling, modulates motility and capacitation via calcium-dependent pathways and mitochondrial function.	3	Li et al;[50] Bai et al[39]
3.2.2.10	AMP-Activated Protein Kinase (AMPK)	Regulates energy homeostasis, motility, and capacitation by modulating mitochondrial function and calcium-dependent pathways.	4	Martin-Hidalgo et al;[51] Bai et al;[39] Hou et al[40]
3.2.2.11	Vitamin D Receptor (VDR) and Metabolites	Regulates calcium homeostasis, motility, capacitation, and acrosome reaction via genomic and non-genomic pathways.	6	Oluwole et al;[52] Roussev et al;[53] Ciccone et al;[54] Aşır et al;[55] Agarwal et al;[4] Hou et al[40]
3.2.2.12	Group X Phospholipase A2 (PLA2G10)	Hydrolyzes phospholipids to generate second messengers, modulates calcium fluxes for capacitation, acrosome reaction, and motility.	4	Escoffier et al;[56] Bai et al;[39] Agarwal et al[4]
3.2.2.13	Na+/H+ Exchangers (NHE)	Maintains intracellular pH, regulates motility and capacitation by modulating calcium channel activity and mitochondrial function.	4	Gardner et al;[57] Bai et al;[39] Agarwal et al[4]
3.2.2.14	G-Protein Coupled Receptors (GPCRs) and Adenosine Receptors	Modulates cAMP and calcium signaling for motility, capacitation, and acrosome reaction via A2A receptor activation.	3	Chen et al;[58] Agarwal et al[4]

3.2.2.15	CRAC Channels (Orai/STIM), Acrosomal and Intracellular Ca^2+ Stores, and SOCE	Mediates store-operated calcium entry for capacitation, hyperactivation, and acrosome reaction. Regulates sperm-oocyte fusion.	10	Agarwal et al;[4] Kato et al;[59] Naaby-Hansen et al;[60] Dey et al;[61] Navarrete et al;[62] Wang et al;[63] Miro-Moran et al;[64] Rossato et al;[16] Sato et al[65]
3.2.2.16	Heat Shock Protein 90 (HSP90)	Stabilizes proteins involved in calcium signaling, capacitation, and acrosome reaction. Regulates motility and fertilization.	3	Li et al;[66] Bai et al[39]
3.2.2.17	Sphingomyelin Synthase 2 (SMS2)	Regulates lipid raft integrity and calcium signaling for motility, capacitation, and sperm survival.	5	Li et al;[67] Shi et al;[68] Agarwal et al[4]
3.2.2.18	Nicotine Acetylcholine Receptor Subunit α7 (CHRNA7)	Mediates calcium influx for hyperactivation, capacitation, and acrosome reaction. Regulates mitochondrial activity and motility.	3	Bray et al;[69] Bai et al;[39] Agarwal et al[4]
3.2.2.19	ClpP/ClpX Mitochondrial Proteases	Maintains mitochondrial proteostasis and calcium buffering for motility, capacitation, and fertilization.	2	Guo et al;[70] Agarwal et al[4]

Abbreviations: A2A, adenosine 2A receptor; AMPK, AMP-activated protein kinase; cAMP, cyclic adenosine monophosphate; Ca^2+, calcium ion; CABCOCO1, calcium-binding and coiled-coil domain-containing protein 1; CHRNA7, nicotine acetylcholine receptor subunit α7; ClpP/ClpX, caseinolytic protease P and X; CRAC, calcium release-activated calcium; CRISP2, cysteine-rich secretory protein 2; cGMP, cyclic guanosine monophosphate; FYN, FYN Kinase; GPCRs, G-protein-coupled receptors; HSP90, heat shock protein 90; Na+/H+, sodium/hydrogen; NHE, Na+/H+ exchangers; PLA2G10, group X phospholipase A2; ROS-GC1, rod outer segment guanylate cyclase 1; SHTAP, sperm head and tail associated protein; Slo3, calcium-activated potassium channel; SMS2, sphingomyelin synthase 2; SOCE, store-operated calcium entry; STAC3, SH3 and cysteine-rich domain-containing protein 3; Tex13a, testis-expressed 13a; TMEM203, transmembrane protein 203; VDR, vitamin D receptor.

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others) that assemble into a linear nanodomain along the flagellar principal piece to create a single continuous Ca^{2+} signaling apparatus.[8,22,75,76] The α-like CatSper proteins share the six-transmembrane domain architecture typical of voltage-gated cation channels, but their assembly and extensive extracellular domains are specialized for sperm function; while their auxiliary subunits form pH sensors, scaffolding, and extracellular interfaces required for proper assembly and gating.[8,9,76] Structural continuity across these subunits is essential, as loss or in-frame deletions in core CatSper subunits or defects in accessory proteins disrupt channel formation or localization and abolish normal channel activity.[8,77]

CatSper channels are localized exclusively to the principal piece of the sperm flagellum, where they form longitudinal nanodomains that concentrate Ca^{2+} entry in the flagellar membrane. This strategic placement couples local Ca^{2+} influx to the axonemal and outer dense fiber machineries that control beat asymmetry and amplitude.[8,78] Targeting of the channel complex to discrete Ca^{2+} signaling domains is mediated by accessory proteins such as CatSper τ (*C2CD6*) and scaffold subunits, which anchor CatSper to the flagellar membrane, and the disruption of these targeting elements mislocalizes the complex and impairs motility and fertility.[8,22] Expression of CatSper subunits is testis-restricted and transcripts/protein levels correlate with sperm maturation and clinical outcomes after assisted reproduction, linking localization and expression to function and fertility prognosis.[23,75]

CatSper channel activity in sperm is tightly regulated by intracellular pH, membrane potential, and various pharmacological agents, which are critical for sperm function and fertilization. Intracellular alkalinization and physiological ligands, such as progesterone in human sperm, potentiate CatSper opening, driving calcium signaling essential for hyperactivated motility and acrosome reaction.[79,80] Specific inhibitors, including small-molecule blockers and environmental or drug metabolites like AM404 (a paracetamol metabolite), disrupt CatSper-dependent calcium signaling, impairing sperm motility and fertilizing capacity.[6,81,82] Pharmacological inactivation of CatSper is a promising target for non-hormonal contraception, as it blocks hyperactivation without altering capacitation status.[83] Conversely, compounds like selenium upregulate CatSper expression in animal models, enhancing motility parameters.[84] Additionally, cAMP/PKA signaling and redox status modulate CatSper function, linking it to energy metabolism and oxidative stress responses.[85,86]

Genetic, environmental, proteomic, and transcriptomic factors collectively highlight CatSper's essential role in male fertility and its susceptibility to dysregulation.[15,77] Loss-of-function mutations or deletions in CatSper subunits (eg, CATSPER1-4, CATSPERE) result in impaired channel function, abolishing hyperactivated motility and causing infertility or subfertility in humans and mice.[9,87–89] Population genetics studies link CATSPER polymorphisms to idiopathic azoospermia and oligospermia, underscoring their clinical relevance.[15,23] Environmental stressors, such as heat stress, gamma irradiation, and obesity, alter CatSper expression and function, disrupting spermatogenesis and sperm quality.[12,90,91] Transcriptomic and proteomic analyses reveal differential expression of CatSper and related signaling molecules in low-fertility sperm and testis samples, implicating channel dysregulation in infertility.[92–96] Chemical capacitation agents (eg, YK 3–237) and environmental toxicants, including perfluorooctane sulfonate, modulate CatSper-mediated calcium flux, affecting capacitation and motility.[72,85,97] Cryopreservation and oxidative stress further impact CatSper-associated proteins, highlighting environmental and procedural influences on channel function.[98–100]

In summary, CatSper acts as the principal pathway for Ca^{2+} entry that triggers the switch from symmetric progressive beating to asymmetric, high-amplitude hyperactivated motility required for zona penetration and fertilization. This is accomplished by highly localized Ca^{2+} microdomains created by the CatSper nanostructure and regulated by pH sensors (EFCAB9), scaffolds (CatSper τ), and extracellular modulatory domains (CatSper ε), so that perturbations in any of these elements translate into altered beat patterns, capacitation responses, and fertilizing potential.[8,76,101]

VDCCs

VDCCs are heteromeric transmembrane channels consisting of α1, α2δ, β, and γ subunits, mediating calcium influx in sperm cells.[102,103] They are predominantly located in the sperm neck and midpiece, regulating motility, capacitation, and acrosome reaction.[73,104] L-type VDCCs are activated by depolarization, and their activity can be inhibited by calcium channel blockers like nifedipine or amlodipine, leading to impaired sperm motility and reduced fertilization potential.[104] Studies have demonstrated that VDCC dysfunction is linked to structural defects in the testis and altered intracellular Ca^{2}

+ oscillations, which compromise mitochondrial activity and energy metabolism.[63,88] Additionally, VDCCs interact with cAMP/PKA and PI3K/AKT/mTOR pathways, highlighting their role in sperm capacitation and intracellular signaling.[105]

L-type (CaV1) channels (the L-type voltage-dependent calcium channels) are classical high-conductance, high-voltage-activated channels whose core molecular architecture is built around a single large pore-forming α1 subunit (the CaV1 family, notably α1C/CaV1.2 and α1D/CaV1.3), which provides the voltage sensor, ion selectivity filter, and gating machinery. Its auxiliary β, α2δ, and γ subunits associate with α1 to modulate expression, trafficking, and biophysical properties.[2] Molecular evidence for L-type expression in male gametes includes the detection of L-type α1C mRNA in ejaculated human spermatozoa.[24] And proteomic/isolation studies reporting CaV1.2 protein in porcine sperm membranes from fresh, frozen, and permeabilized semen preparations.[94] Within the spermatozoon, L-type channels are principally localized to the plasma membrane overlying the flagellum and portions of the head in species examined, placing them in the ideal position to control head-tail Ca^{2+} signaling that underpins capacitation, flagellar beat patterns, and progressive motility.[24,106,107] The L-type channels are well known to be sensitive to classical dihydropyridine calcium-channel blockers (CCBs) such as nifedipine and amlodipine, and to phenylalkylamines and benzothiazepines; these agents reduce L-type mediated Ca^{2+} influx and thus blunt downstream Ca^{2+}-dependent sperm responses.[2]

Molecular and electrophysiological studies implicate L-type currents in the regulation of capacitation and hyperactivation,[108] while in vivo and toxicological studies link therapeutic CCB exposure to impaired spermatogenesis and altered sperm parameters. For example, chronic amlodipine administration produced reductions in testicular histology quality, sperm count, morphology, and motility in adult Wistar rats,[108] and broader investigations of CCBs report anti-reproductive effects in rodents, consistent with blockade of L-type function.[2] Clinical-toxicologic comparisons indicate that commonly prescribed cardiovascular drugs that target L-type channels can have measurable reproductive side effects in laboratory models and warrant careful interpretation for human fertility contexts.[109] Together, the molecular, localization, and pharmacology data[2,24,106–109] show that intact L-type CaV function is central for the Ca^{2+} signals required for normal sperm motility and fertilizing capability, and that their inhibition produces clear deleterious effects on spermatogenesis and mature sperm function.

T-type (CaV3) calcium channels (CaV3.1, CaV3.2, and CaV3.3) are small-conductance, low-voltage-activated channels with an α1 pore-forming subunit distinct from the CaV1/2 families. They activate near the resting membrane potential and show rapid inactivation and characteristic kinetics that make them effective at shaping low-threshold Ca^{2+} transients and burst firing in excitable cells. In the male reproductive tract and germ line, molecular and genetic evidence have robustly identified CaV3.1 and CaV3.2 expression in sperm and spermatogenic cells.[7] Localization studies place CaV3 channels in regions critical for acrosomal signaling and flagellar initiation — the acrosomal domain and proximal flagellum — positioning them to mediate the brief, local Ca^{2+} rises that trigger acrosome exocytosis and influence beat frequency.[7,107] The pharmacology of T-type channels differs from L-type channels, as the former's currents are sensitive to a distinct set of modulators and inhibitors, including certain endogenous regulators, steroid modulators, and a subset of small molecules.[18]

VDCCs (L-, T-, N-type families) are canonical pore-forming α1 subunits with auxiliary subunits expressed variably in testis and somatic cells. In sperm, evidence points to VDCC-like currents on the plasma membrane and in intracellular membranes that influence motility, acrosome stability, and maturation.[100,110] Proteomic/transcriptomic profiling in multiple species[93,94,111] finds VDCC subunit transcripts/proteins associated with fertile versus subfertile phenotypes. Pharmacologic modulation of VDCCs (eg, pregabalin binding the $\alpha_2\delta$ auxiliary subunit) produces testicular and sperm structural/function changes in rodent models,[112,113] suggesting that perturbation of VDCC function affects spermatogenesis and sperm quality. Epidemiologic analyses showing medication associations with low semen quality also point toward drug-channel interactions as a contributor to reduced sperm parameters.[114] Inflammation and toxicant exposure perturb VDCC-mediated signaling, while antioxidants and L-arginine show protective modulation in animal models.[115] Genetic deletion of CaV3.1 or CaV3.2 in mice alters acrosome reaction probability and motility parameters, indicating these channels contribute to both capacitation-associated exocytosis and hyperactivation.[7] Pharmacological studies on spermatogenic cells show that antifertility natural products target T-type currents — gossypol is reported to inhibit T-type Ca^{2+} currents in mouse spermatogenic cells, resulting in reduced Ca^{2+} signaling and impaired cell function.[116] Also, modulators such as raloxifene were demonstrated to suppress T-type Ca^{2+} currents in mouse spermatogenic cells,

highlighting that clinically used agents can indirectly influence germ cell electrophysiology.[18] Intracellular signaling pathways also regulate T-type currents in the germ line; for example, activation of the growth-hormone-secretagogue (ghrelin) receptor inhibits T-type currents via a pertussis toxin-sensitive protein kinase C pathway in mouse spermatogenic cells, showing receptor-coupled control of T-type channel availability.[117] Inhibition or genetic loss of these channels impairs acrosome reaction and diminishes motility patterns necessary for zona pellucida penetration, and several studies correlate T-type blockade with reductions in fertility indices in animal models.[7,18,39,117] Thus, T-type CaV channels act as sensitive low-threshold Ca^{2+} gates in sperm physiology, as their precise timing and localization make them indispensable for acrosomal exocytosis and the initiation and modulation of flagellar activity, and both pharmacological inhibitors and genetic ablation produce measurable deficits in sperm function and spermatogenic cell excitability.

The CaV2.x are high-voltage-activated (HVA) calcium channels that include P/Q-type (CaV2.1), N-type (CaV2.2), and R-type (CaV2.3) families, followed by L-type (CaV1), whose α1 subunits confer high-threshold activation and substantial Ca^{2+} conductance. Structurally, they share the multi-domain α1 architecture plus auxiliary β and α2δ subunits that modulate channel trafficking and kinetics. In spermatozoa, evidence for HVA channel participation is somewhat heterogeneous across species, but functional assays and toxin/venom perturbations implicate HVA conductance in capacitation and the acrosome reaction. Biochemical isolation and imaging studies have detected HVA-type proteins (including CaV1.2) in sperm membranes,[106] and patch-clamp recordings under physiological ionic conditions reveal multiple pharmacologically distinct cationic currents consistent with HVA channel activity in human sperm.[107] Natural toxins that target HVA channels provide functional proof, as a fraction derived from the sea anemone *Lebrunia neglecta* reduced boar sperm capacitation, an effect the authors attribute, at least in part, to interference with HVA calcium channels that are required for capacitation-associated Ca2+ signaling.[118]

Broad pharmacological blockade of HVA channels — for example, via plant-derived antifertility compounds such as those from *Tripterygium wilfordii* — has been shown to inhibit Ca^{2+} currents in spermatogenic cells and correlate with impaired spermatogenesis.[116] Clinically relevant drugs that target VDCCs more generally (for instance, amlodipine) also produce testicular histological changes and depressed sperm quality in rodents, consistent with the notion that blocking HVA currents during development and maturation compromises spermatogenesis and the production of functional sperm.[2,108,109] Mechanistically, HVA channels contribute to sustained Ca^{2+} entry necessary for longer-lasting signaling events such as the cascades leading to protein tyrosine phosphorylation during capacitation, and their disruption leads to deficits in hyperactivation, acrosome responsiveness, and ultimately, fertilization capacity. Taken together, studies on molecular identification, toxin perturbation, and pharmacological inhibition[2,106,108,116,118] indicate that HVA channels — whether classical L-type or other HVA family members — provide critical sustained Ca^{2+} fluxes for capacitation and fertilizing events, and their alterations produce clear adverse effects on sperm function and spermatogenic integrity.

TRP

The TRP channels represent a diverse family of non-selective cation channels with distinct molecular structures that allow them to act as polymodal sensors for sperm physiology, particularly in the flagellum and acrosome.[28,119] Structurally, TRP channels share a common architecture of six transmembrane (TM) segments (S1–S6) with a pore-forming loop between S5 and S6, which permits calcium and other cations to flow into the cell. The N- and C-terminal cytoplasmic domains carry regulatory elements, such as ankyrin repeat motifs and calmodulin binding sites, that modulate gating properties and channel–protein interactions. These domains confer functional versatility, allowing TRP channels to respond to temperature, osmolarity, chemical ligands, and membrane potential changes. TRPM8, for instance, is a cold and menthol-sensitive ion channel, while TRPV4 is known for thermal sensitivity and osmoregulation, both critical features for sperm adaptation to the female reproductive tract.[10,25,120] They respond to temperature, pH, and chemical stimuli, mediating Ca^{2}+ influx crucial for motility, hyperactivation, and the acrosome reaction.[86,121] Their modulators, such as Ginkgo biloba extract, have been reported to restore Ca^{2}+ homeostasis and improve sperm morphology and motility under oxidative stress or toxicant exposure.[121,122] The TRP channels act in concert with CatSper and VDCCs to regulate intracellular Ca^{2}+ oscillations, acrosomal responsiveness, and energy metabolism.[123,124]

Dysregulation of TRP channels, due to environmental toxins or genetic variants, reduces sperm fertilization competence and contributes to male infertility.[125,126]

In terms of localization, TRP channels are distributed across multiple regions of spermatozoa, reflecting their diverse roles in regulating fertility. TRPM8 has been found in the principal piece and flagellum of human sperm, where it contributes to motility and chemotaxis.[10] TRPV4 is localized in the sperm plasma membrane, acting as a thermal sensor crucial for initiating hyperactivated motility at physiological temperatures.[25] Members of the transient receptor potential channel (TRPC) subfamily, including TRPC3, TRPC4, TRPC6, and TRPC7, have been identified in boar spermatozoa during bicarbonate-triggered capacitation, with mRNA expression increasing in response to capacitation signals.[27] Furthermore, TRPC3 specifically has been linked to sperm motility regulation through calcium mobilization, playing a pivotal role in the capacitation process.[26] Such spatial expression suggests TRP channels are not uniformly distributed but rather tailored to the functional requirements of motility, capacitation, and acrosome reaction.

TRP channels are subject to modulation by both natural ligands and external agents. TRPM8 is activated by cooling and menthol, with antagonists such as capsazepine blocking its activity, underscoring its role as a thermo- and chemosensor.[10,120] TRPV4, in contrast, is activated by warm temperatures, osmotic stress, and endogenous ligands, including arachidonic acid derivatives, making it a broad-spectrum environmental sensor.[25] TRPC channels are modulated by diacylglycerol (DAG) and phospholipase C (PLC)-linked pathways, aligning their function with receptor-operated calcium entry, which is crucial for sperm capacitation.[26] Interestingly, vitamin D3 and its metabolites have recently been implicated in modulating TRP channel function through receptor-mediated mechanisms, suggesting an indirect hormonal regulatory pathway that links nutrition and endocrine balance to TRP-mediated calcium signaling in sperm.[52] These findings highlight the complex interplay of environmental and biochemical cues that fine-tune TRP activity in reproductive processes.

Transcriptomics and proteomics identify TRP family members or their pathway associates in low-quality semen and aging/dietary models,[92,127,128] consistent with functional roles. Functional work in mammals and chiropteran models demonstrates stimulus-linked Ca^2+ fluxes and membrane potential changes consistent with TRP contribution to acute Ca^2+.[72] Environmental toxicants (cadmium) and metabolic stressors influence intracellular Ca^2+ and autophagy pathways in germ cells, implicating TRP-mediated Ca^2+ entry as an upstream event in cellular stress responses.[129]

Several studies have demonstrated the impact of TRP channel modulation on spermatogenesis and sperm function. TRPM8 activity has been shown to influence chemotaxis and thermotaxis in human sperm, with its inhibition leading to reduced directional motility, thereby impairing the ability of sperm to locate the oocyte.[10] Comparative analyses across species revealed a correlation between TRPM8 expression and vertebrate evolution, suggesting that its role in sperm motility has been conserved as an adaptive mechanism to improve fertilization efficiency.[120] TRPV4-mediated thermosensitivity has been directly linked to the induction of hyperactivated motility in human sperm, a prerequisite for zona pellucida penetration, and its dysfunction could compromise fertilization potential.[25] In porcine models, TRPC channels were significantly upregulated during capacitation, indicating their importance in calcium influx and sperm activation.[27] More specifically, TRPC3 was found to regulate protein tyrosine phosphorylation, a biochemical hallmark of capacitation, as well as motility patterns, reinforcing its central role in fertilization success.[26] Collectively, these findings position TRP channels as an essential regulator of male fertility, with their modulation affecting processes ranging from spermatogenesis and capacitation to motility and chemotaxis.

IP_3 & Ryanodine Receptors

The inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) are two critical families of intracellular calcium channels that regulate calcium release from internal stores, particularly the acrosome and redundant nuclear envelope in sperm. They are intracellular Ca^2+ release channels localized to acrosomal and mitochondrial membranes in sperm. Structurally, IP_3Rs are large tetrameric channels located on the membranes of intracellular Ca^2+ stores, composed of an N-terminal ligand-binding domain, a regulatory domain, and a C-terminal transmembrane pore-forming region. These receptors are activated by binding to inositol 1,4,5-trisphosphate (IP_3), a second messenger generated via phospholipase C activity. On the other hand, RyRs are also tetrameric channels with large cytoplasmic domains and transmembrane segments forming the pore. They share structural similarities with IP_3Rs, reflecting their evolutionary

relationship, and are activated primarily by Ca^2+ itself in a calcium-induced calcium release (CICR) mechanism.[30] Together, they both generate intracellular calcium oscillations essential for capacitation and acrosome reaction.[85,126] Modulators, including caffeine and ryanodine, influence channel activity and sperm fertilization potential. Dysfunction in these channels alters mitochondrial function and motility, predisposing sperm to premature acrosome reactions or reduced fertilization.[63,130] Furthermore, oxidative stress, heavy metals, and pharmacological agents can dysregulate these receptors, emphasizing their vulnerability and key regulatory roles in spermatogenesis and sperm function.[126,131]

In spermatozoa, IP_3Rs and RyRs are strategically localized to support specialized functions. The IP_3Rs are predominantly found in the acrosome, which serves as a major intracellular calcium store, and their activation facilitates acrosomal exocytosis, a critical step for sperm penetration of the zona pellucida.[16] In human sperm, IP_3Rs also play a role in thermotaxis, guiding sperm toward the oocyte by sensing temperature gradients, an essential mechanism for fertilization.[29] Similarly, RyRs have been detected in the acrosome and other intracellular stores of sperm, contributing to calcium release necessary for hyperactivation and the acrosome reaction.[30] This localization ensures that both receptor types regulate calcium dynamics crucial for sperm motility, capacitation, and fertilization competence.

Pharmacological modulation of these channels highlights their importance in sperm function. Inhibitors such as 2-aminoethoxydiphenyl borate (2-APB) can block IP_3Rs' activity, disrupting calcium signaling. RyRs are modulated by ryanodine, which can act as both an agonist and antagonist, depending on concentration, and by caffeine, which sensitizes the receptor to Ca^2+. Such agents have been used experimentally to demonstrate the role of these channels in sperm physiology. Functional sperm studies reporting capacitation-associated [Ca^2+]i transients and acrosome reaction defects implicate these receptors indirectly via altered store release and cross-talk with plasma membrane channels.[74,132] Genetic loci linked to impaired acrosome reaction (eg, *FKBP6* in stallions) and acrosomal biogenesis proteins imply that dysregulation of intracellular Ca^2+ release machinery can underlie failed exocytosis and fertilization.[132] Consequently, several studies have demonstrated the impact of IP_3R and RyR modulation on spermatogenesis and sperm function. Bahat and Eisenbach[29] showed that blocking IP_3R activity significantly impaired human sperm thermotaxis, underscoring its essential role in guiding sperm to the oocyte. Herrick et al[30] provided evidence that the acrosome functions as a calcium store regulated by both IP_3Rs and RyRs, linking these channels to successful acrosomal exocytosis. Rossato et al[16] further highlighted that depletion of intracellular calcium stores through these receptors triggers the acrosome reaction, which is indispensable for sperm-oocyte fusion. At the genetic level, Maleki et al[31] identified *ITPR1*, which encodes the IP_3R1 isoform, as a novel target of hsa-miR-34b-5p in men with non-obstructive azoospermia. Their findings suggest that dysregulation of IP_3R1 contributes to impaired spermatogenesis via calcium and apoptosis pathway cross-talk, linking receptor modulation to male infertility. Collectively, these studies demonstrate that IP_3Rs and RyRs are not only essential for sperm function but also influence broader spermatogenic processes, making them key targets for understanding and addressing male infertility.

CaSR

The CaSR is a G-protein-coupled receptor (GPCR) belonging to the family C GPCRs, characterized by a large extracellular Venus flytrap domain, a seven-transmembrane region, and an intracellular C-terminal tail located in the sperm head and midpiece, detecting extracellular calcium fluctuations and regulating sperm motility, capacitation, and acrosomal responsiveness.[12,79] It modulates intracellular signaling cascades (PLC → IP_3 → intracellular Ca^2+ release) and has been detected in reproductive tissues.[39] Its molecular structure is optimized to detect and respond to changes in extracellular calcium concentrations, thereby influencing intracellular signaling cascades. The Venus flytrap domain is responsible for calcium binding, while the transmembrane and intracellular domains mediate downstream effects through coupling with G-proteins and activation of secondary messenger pathways. This structural organization underpins its ability to modulate physiological functions within reproductive tissues, particularly in sperm and spermatogenic cells.[12,79]

Within the sperm, CaSR is expressed in both mature spermatozoa and spermatogenic cells, suggesting a crucial role in multiple stages of male reproduction. Immunohistochemical and comparative expression studies have demonstrated the presence of CaSR on the plasma membrane of sperm cells, as well as in developing spermatogenic cells in the testes. These findings indicate that CaSR not only influences the functional capacity of ejaculated sperm but also participates in

earlier stages of spermatogenesis, linking extracellular calcium sensing to cellular maturation and differentiation processes.[19] Thus, its localization highlights its dual importance in sperm function and testicular development.

CaSR activity is modulated by a variety of agonists and antagonists that either enhance or inhibit its sensitivity to extracellular calcium. Agonists such as calcium and calcimimetics stimulate intracellular Ca^2+ signaling, whereas antagonists impair sperm function.[86] Classical agonists include calcium ions and polycationic molecules, which promote receptor activation and subsequent intracellular signaling. Other agonists, such as vitamins D and C, at supraphysiological levels, have been reported to indirectly affect CaSR pathways, thereby influencing spermatogenesis. Conversely, calcilytics act as antagonists, reducing CaSR activity and impairing calcium signaling in reproductive tissues. These modulatory agents provide insight into how nutritional and pharmacological factors can regulate CaSR activity and, by extension, sperm function and fertility outcomes.[19]

Studies indicate that CaSR modulates cAMP/PKA pathways and coordinates with CatSper and TRP channels to optimize calcium influx and energy metabolism during sperm capacitation.[64,91] Dysregulation of CaSR, due to metabolic disorders, obesity, or genetic variants, compromises sperm motility and fertilization outcomes.[88,91] Its functional relevance is further supported by knockout studies showing severe subfertility linked to impaired sperm hyperactivation and acrosomal reaction.[88] Several studies linking extracellular milieu changes, drug exposures, or metabolic disease to testicular dysfunction point to a role for CaSR-mediated signaling in testicular homeostasis.[133,134] For example, diabetes-related testicular dysfunction ameliorated by dapagliflozin involves modulation of oxidative/inflammatory pathways and may secondarily affect Ca^2+ signaling axes that include CaSR.[134] Indirect transcriptomic/proteomic datasets that show altered expression of calcium-sensing and downstream signaling components in poor-quality semen suggest that CaSR pathways may be dysregulated in subfertility.[92,93]

Several studies have reported the effect of CaSR modulation on spermatogenesis and sperm functions. More recent experimental evidence has shown that abnormal modulation of CaSR can impair spermatogenesis. For example, Nasraldin et al[19] demonstrated that hypervitaminosis C and D induced disturbances in spermatogenesis by altering CDKN1B expression, a cell cycle regulator, suggesting that excessive CaSR activation contributes to impaired germ cell development. Furthermore, Qian et al[32] compared CaSR expression in normal and abnormal sperm, revealing that reduced expression and dysfunction of the receptor were associated with impaired sperm motility and morphological abnormalities. These studies collectively indicate that both overactivation and downregulation of CaSR pathways can negatively influence male fertility, making it a key molecular target for understanding and managing reproductive dysfunction.

Non-Channel Mediated Regulators

Slo3 (Ca^2+-Activated K+ Channel)

Slo3 is a sperm-specific potassium channel that plays a fundamental role in regulating intracellular calcium indirectly through membrane hyperpolarization. Structurally, Slo3 consists of a tetrameric α-subunit forming the channel pore, with voltage-sensing domains that respond to changes in membrane potential and Ca^2+-dependent gating mechanisms.[33] Its localization is highly restricted to the principal piece of the sperm flagellum, a region critical for motility and hyperactivation. The hyperpolarization mediated by Slo3 promotes calcium influx via CatSper channels, linking K+ efflux to intracellular calcium dynamics.[33] Pharmacologically, Slo3 can be modulated by selective K+ channel blockers, which reduce hyperpolarization, consequently impairing calcium entry and sperm motility, and preventing the progesterone-induced acrosome reaction.[33] Functional studies in human sperm and murine models demonstrate that Slo3 deficiency or inhibition leads to reduced progressive motility, compromised capacitation, and infertility, highlighting its essential regulatory role.[33] Additionally, Slo3 interacts with other modulators, including calmodulin-binding proteins, which fine-tune calcium-dependent signaling cascades, ensuring proper mitochondrial activity and energy supply for flagellar movement[34,35] Experimental evidence further indicates that environmental toxins or pharmacological agents that disrupt Slo3 function indirectly alter intracellular calcium oscillations, resulting in impaired fertilization capacity and defective spermatogenesis.[36,37] The integration of Slo3 activity with other sperm ion channels, including CatSper and T-type calcium channels, forms a tightly regulated network that governs motility patterns and acrosomal exocytosis, essential for successful fertilization.[33] Overall, Slo3 acts as a pivotal regulator of sperm intracellular calcium, linking

potassium conductance to calcium homeostasis, capacitation, and reproductive competence, and its modulation represents a promising target for both therapeutic intervention in infertility and male contraceptive strategies.

Transmembrane Protein 203 (TMEM203)

TMEM203 is a transmembrane protein identified as a novel regulator of intracellular calcium homeostasis in sperm and testicular cells. The protein exhibits multiple transmembrane domains with cytoplasmic loops that facilitate interactions with calcium-signaling machinery, including endoplasmic reticulum calcium stores and plasma membrane calcium channels.[38] TMEM203 expression is detected in spermatogonia, spermatocytes, and mature spermatozoa, indicating a role throughout spermatogenesis and sperm maturation. Functional studies show that TMEM203 modulates cytosolic calcium concentrations by influencing both basal and stimulated calcium fluxes, thereby supporting spermatogenic progression and proper sperm function.[38] Genetic knockout of TMEM203 results in severe disruption of calcium homeostasis, impaired spermatogenesis, reduced sperm counts, and decreased fertilization rates, confirming its essential role.[38] Pharmacologically, although specific agonists or antagonists for TMEM203 are not yet characterized, indirect modulation via calcium channel blockers or activators affects its regulatory function, demonstrating its reliance on broader calcium signaling networks.[39] Additionally, TMEM203 interacts with mitochondrial regulators and calcium-binding proteins, ensuring energy-dependent calcium signaling critical for motility and capacitation.[4,35] Environmental stressors, such as oxidative stress, have been shown to affect TMEM203-mediated calcium homeostasis, leading to defective sperm morphology and function.[36] Collectively, TMEM203 represents a key intracellular calcium modulator whose proper function is indispensable for spermatogenic integrity, sperm motility, and fertility outcomes, making it a potential target for understanding idiopathic male infertility and developing fertility-enhancing interventions.

Rod Outer Segment Guanylate Cyclase 1 (ROS-GC1)

Rod Outer Segment Guanylate Cyclase 1 (ROS-GC1) is a calcium-modulated, membrane-bound enzyme critical for sperm calcium signaling, motility regulation, and spermatogenesis. Structurally, ROS-GC1 is a single-pass transmembrane protein featuring an extracellular domain, a kinase-like domain, a dimerization domain, and an intracellular catalytic domain responsible for cyclic GMP (cGMP) synthesis.[41,42] It is predominantly localized in the sperm head, midpiece, and flagellum, regions essential for acrosomal exocytosis, energy-dependent motility, and hyperactivation during fertilization.[42] ROS-GC1 acts as a calcium sensor, converting intracellular Ca^{2}+ fluctuations into cGMP signaling, which regulates ion channels and motility machinery critical for sperm capacitation and fertilization competence.

The enzyme's activity is tightly regulated by calcium-binding proteins, notably guanylate cyclase-activating proteins (GCAPs) and S100B, which modulate its function based on intracellular calcium concentrations.[41,42] Elevated Ca^{2}+ levels trigger conformational changes in GCAPs, enhancing ROS-GC1 activity, whereas abnormally low calcium or disrupted GCAP function impairs cGMP production, leading to defective acrosome reactions and reduced sperm motility.[39,42] ROS-GC1 also interacts with intracellular modulators like calmodulin and protein phosphatases, fine-tuning calcium-dependent signaling pathways essential for sperm capacitation and zona pellucida binding.[34]

Environmental and pharmacological stressors, such as oxidative stress or calcium channel inhibitors, disrupt ROS-GC1 function, causing motility abnormalities and subfertility.[36,37] Mechanistically, ROS-GC1-mediated cGMP synthesis activates protein kinase G (PKG) pathways, which modulate flagellar beating patterns and mitochondrial function, ensuring energy homeostasis for spermatogenesis and fertilization.[4] Its coordination with other calcium channels and transporters forms an integrated network controlling hyperactivation and capacitation. Experimental studies highlight that ROS-GC1 dysregulation impairs acrosome reactions and sperm motility, contributing to male infertility in rodent models and human cases.[4] Given its pivotal role, ROS-GC1 is a promising target for understanding idiopathic infertility and developing novel therapeutic strategies to enhance male fertility outcomes.

Cysteine-Rich Secretory Protein 2 (CRISP2)

CRISP2 is a member of the CRISP family of proteins, which are characterized by their conserved cysteine-rich domains capable of binding divalent cations such as calcium. Structurally, CRISP2 consists of an N-terminal pathogenesis-related domain and a C-terminal cysteine-rich domain that contributes to its calcium-binding and regulatory properties.[43,44] In

sperm, CRISP2 is predominantly localized in the acrosome, sperm tail, and connecting piece, indicating its involvement in both acrosomal function and flagellar motility.[43,44] The protein acts as an intracellular regulator of calcium by modulating calcium influx through ion channels, particularly CatSper and voltage-gated calcium channels, thereby influencing calcium-dependent processes in sperm.[45] CRISP2 function is modulated by both agonists and antagonists that affect calcium signaling; for instance, interactions with extracellular modulators such as progesterone can enhance CRISP2-mediated calcium flux, whereas specific inhibitors of calcium channels can suppress its regulatory effects.[43,45] Experimental studies have reported that loss or dysfunction of CRISP2 leads to impaired sperm motility, defective capacitation, and reduced acrosome reaction, which collectively compromise fertilization potential.[43,44] Additionally, CRISP2 interacts with cAMP-dependent pathways and other calcium-binding proteins to ensure proper intracellular calcium oscillations necessary for hyperactivation and sperm-egg fusion.[34,46] Dysregulation of CRISP2, whether by genetic mutations or environmental stressors, has been associated with male subfertility and infertility in both animal models and human studies.[43,45] Overall, CRISP2 serves as a critical modulator of intracellular calcium in sperm, linking calcium homeostasis to essential physiological processes such as motility, capacitation, and acrosomal exocytosis, making it a key target for understanding male reproductive dysfunction and potential therapeutic interventions.

STAC3 (SH3 and Cysteine-Rich Domain-Containing Protein 3)

STAC3 is a signaling protein that contains an SH3 domain and a cysteine-rich domain, which are critical for its interaction with calcium channels and regulation of intracellular calcium signaling.[35] Its molecular structure allows it to modulate excitation-contraction coupling by stabilizing calcium channel activity, specifically in muscle cells, and in sperm, it plays a similar role in regulating calcium influx essential for motility and capacitation.[35] In sperm, STAC3 is localized primarily in the midpiece and flagellum, areas enriched with mitochondria, which supports its role in coordinating calcium signaling with energy metabolism to sustain motility.[35] STAC3 influences calcium channel function by interacting with voltage-gated calcium channels and possibly with CatSper channels, acting as a modulator that ensures proper calcium entry during capacitation and hyperactivation.[35] Experimental evidence demonstrates that STAC3 deficiency disrupts mitochondrial membrane potential, impairs calcium homeostasis, and reduces steroidogenic activity in Leydig cells, leading to compromised spermatogenesis and reduced sperm quality.[35] Furthermore, STAC3 modulates calcium-dependent signaling cascades linked to protein phosphorylation and energy metabolism, highlighting its dual role in both calcium regulation and sperm energetic capacity.[35] Pharmacological or genetic perturbation of STAC3 leads to abnormal sperm motility patterns, impaired capacitation, and reduced fertilization success, emphasizing its critical function in male fertility.[35] In summary, STAC3 acts as a key intracellular calcium regulator in sperm, linking calcium influx to mitochondrial function and motility, and its proper function is indispensable for spermatogenesis, capacitation, and overall male reproductive potential. Its modulation presents a promising target for understanding and treating male subfertility associated with calcium dysregulation.

FYN Kinase

FYN kinase is a non-receptor tyrosine kinase of the Src family that plays a critical role in sperm function and spermatogenesis.[47] Structurally, FYN kinase comprises an SH3 domain, an SH2 domain, and a tyrosine kinase catalytic domain, which facilitates substrate recognition and phosphorylation, thereby modulating intracellular signaling cascades.[47] In sperm, FYN kinase is predominantly localized to the sperm head and flagellum, areas essential for capacitation, motility, and the acrosome reaction.[47] This localization allows FYN to interact with membrane proteins and ion channels, influencing calcium influx critical for sperm activation. FYN kinase is modulated indirectly through signaling molecules; for instance, calcium ionophores and progesterone can enhance its phosphorylation activity, while tyrosine kinase inhibitors, such as PP2, act as antagonists, reducing its function.[39,47] Experimental studies have demonstrated that Fyn-null mice exhibit defective spermatogenesis characterized by abnormal sperm morphology, reduced motility, and impaired fertilization capacity, indicating its vital role in sperm maturation and function.[47] Mechanistically, FYN kinase mediates its effects by phosphorylating substrates involved in the regulation of actin cytoskeleton remodeling, mitochondrial function, and calcium channel activity, thereby facilitating processes such as hyperactivation and capacitation.[47] Disruption of FYN signaling leads to defective calcium-dependent events in sperm,

including impaired acrosome reaction and motility deficits, ultimately compromising male fertility.[39,47] Collectively, FYN kinase integrates multiple signaling pathways within spermatozoa, ensuring proper spermatogenic progression and functional competence, making it a key target for understanding male infertility.[47]

Sperm Head and Tail Associated Protein (SHTAP)

SHTAP is a recently characterized protein crucial for spermatogenesis and sperm function. Structurally, SHTAP is a coiled-coil protein with domains that enable protein-protein interactions, particularly with cysteine-rich secretory proteins such as CRISP2, which are essential for sperm maturation and motility.[44] This structural configuration facilitates its dual localization in both the sperm head and tail, suggesting roles in acrosomal stability and flagellar motility. Specifically, SHTAP is predominantly expressed in spermatogenic cells during the late stages of spermiogenesis and is retained in mature spermatozoa, localizing to the head and along the principal piece of the flagellum, aligning with sites critical for motility and fertilization competence.[44,48]

Currently, there are no well-defined small-molecule agonists or antagonists directly targeting SHTAP; its activity is modulated indirectly through interactions with other sperm proteins, such as CRISP2, which influence calcium signaling pathways and capacitation.[44] Functional studies in mice have demonstrated that SHTAP disruption impairs sperm motility and morphology, leading to reduced fertilization rates, indicating its essential role in spermatogenesis and post-testicular sperm function.[44,48] Mechanistically, SHTAP contributes to the regulation of calcium-dependent processes in sperm, including flagellar bending and acrosome reaction, by stabilizing protein complexes that mediate intracellular calcium flux, mitochondrial activity, and energy production necessary for hyperactivated motility.[44] These findings highlight SHTAP as a pivotal scaffold protein orchestrating both structural and signaling pathways in sperm, emphasizing its potential as a biomarker for male infertility and a target for understanding defects in sperm motility and fertilization capacity.

CABCOCO1

CABCOCO1 is a recently characterized coiled-coil protein with calcium-binding activity, predominantly localized in the sperm flagellum, suggesting a specialized role in sperm motility and structural integrity.[49] Structurally, CABCOCO1 contains canonical coiled-coil motifs facilitating protein-protein interactions and multiple calcium-binding domains, enabling it to act as a calcium sensor within the sperm tail.[49] This molecular configuration allows the channel to respond dynamically to intracellular calcium fluctuations, which are critical for flagellar beating and hyperactivation during capacitation. CABCOCO1 is primarily expressed along the axoneme of the sperm flagellum, positioning it strategically to regulate localized calcium signaling essential for motility.[49] Functional modulation of CABCOCO1 involves calcium ions as agonists, enhancing its conformational activity, while experimental chelators or calcium channel blockers can attenuate its function, reducing flagellar activity.[39,49] Several studies have highlighted that disruptions in CABCOCO1 expression or activity lead to impaired spermatogenesis and diminished sperm motility, implicating its role in male fertility.[49] Mechanistically, CABCOCO1 mediates its effects through calcium-dependent regulation of axonemal dynein and mitochondrial function, thereby integrating energy production with motility patterns required for successful fertilization.[4,49] This channel also interacts with other sperm ion transporters, coordinating calcium influx with downstream signaling pathways, including cAMP-PKA cascades, to facilitate capacitation and acrosome reaction.[49] The convergence of structural specialization, precise localization, and calcium-mediated modulation underscores CABCOCO1 as a critical determinant of sperm functional competence and a potential target for fertility assessment and therapeutic intervention.[49]

Tex13a

Tex13a is a testis-expressed protein critically involved in spermatogenesis and sperm motility, primarily through its regulation of mRNA turnover and calcium signaling pathways. Structurally, Tex13a contains RNA-binding domains that facilitate its interaction with specific mRNA transcripts, influencing their stability and translation.[50] Within sperm cells, Tex13a is predominantly localized in the cytoplasm of spermatids and in the midpiece of mature spermatozoa, positioning it strategically to modulate processes linked to energy metabolism and motility.[50] Although Tex13a itself

is not a classical ion channel, its function is tightly linked to calcium homeostasis, and it indirectly affects calcium channel activity, thereby modulating intracellular Ca^2+ concentrations critical for sperm hyperactivation and capacitation.[50]

Agonists and antagonists of calcium channels, such as progesterone and specific pharmacological blockers, can influence Tex13a-mediated pathways by altering Ca^2+ influx, which in turn affects sperm motility and acrosome reaction.[39,50] Experimental studies have shown that Tex13a deficiency or dysregulation impairs sperm motility and reduces fertilization potential, likely through disrupted Ca^2+-dependent signaling cascades and defective mRNA turnover essential for the translation of motility-related proteins.[50] Mechanistically, Tex13a contributes to the regulation of intracellular calcium oscillations by stabilizing transcripts encoding calcium-handling proteins and interacting with signaling networks that control flagellar beating and energy production.[50] This regulatory role ensures proper mitochondrial function and ATP availability in sperm, thereby optimizing motility and fertilization capacity. Overall, Tex13a acts as a pivotal modulator linking mRNA dynamics with calcium-dependent signaling pathways, demonstrating its essential role in male reproductive physiology.[50]

AMP-Activated Protein Kinase (AMPK)

AMPK is a highly conserved serine/threonine kinase that functions as a central energy sensor in eukaryotic cells, including spermatozoa.[51] Structurally, AMPK exists as a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits, with the γ subunit containing binding sites for AMP, ADP, and ATP, which modulate the kinase activity according to cellular energy status.[40,51] In sperm, AMPK localizes predominantly in the midpiece, where mitochondria are concentrated, but is also detected in the flagellum and acrosomal region, reflecting its role in energy homeostasis, critical for motility and capacitation.[39,51] AMPK activity can be modulated by pharmacological agents and physiological cues. Activators include AICAR and metformin, which mimic energy stress by increasing AMP:ATP ratios, whereas inhibitors such as compound C directly block the kinase activity.[51] These modulators influence sperm functions, including motility, mitochondrial membrane potential, and capacitation. For instance, AMPK activation enhances ATP production in the midpiece, supporting hyperactivated motility, and regulates reactive oxygen species, which are essential for capacitation signaling.[40]

Several studies have demonstrated that AMPK plays a pivotal role in spermatogenesis and sperm function. Martin-Hidalgo et al[51] reported that AMPK deficiency in mouse sperm reduces motility and fertilization capacity, while Bai et al[39] showed that AMPK interacts with Ca^2+ signaling pathways to modulate acrosome reactions. Mechanistically, AMPK regulates key downstream effectors such as mitochondrial biogenesis, glycolytic flux, and phosphorylation of proteins involved in motility and capacitation.[40,51] Moreover, AMPK-mediated phosphorylation of target proteins ensures energy balance under stress conditions, preventing premature apoptosis in spermatogenic cells.[40] In summary, AMPK acts as a metabolic regulator in sperm, linking energy status to motility, capacitation, and fertilization, with pharmacological modulation demonstrating its potential as a target for improving male fertility.[40,51]

Vitamin D Receptor (VDR) and Metabolites

VDR is a nuclear receptor that binds the active form of vitamin D, 1,25-dihydroxyvitamin D3, regulating gene transcription involved in calcium homeostasis and male reproductive function.[52,53] Structurally, VDR comprises a DNA-binding domain with two zinc fingers, a ligand-binding domain that accommodates vitamin D metabolites, and a hinge region facilitating receptor dimerization and interaction with co-regulators.[52] In spermatozoa, VDR localizes predominantly in the head, particularly in the post-acrosomal region, and in the midpiece, suggesting roles in capacitation, motility, and acrosome reaction.[53,54]

VDR activity is modulated by agonists such as 1,25(OH)2D3, which enhance calcium influx and support sperm motility, and by antagonists or vitamin D deficiency, which reduce receptor-mediated signaling, impairing spermatogenesis and sperm function.[39,55] Several studies report that VDR activation improves sperm motility, mitochondrial function, and fertilization potential by regulating calcium-dependent pathways, mitochondrial membrane potential, and expression of genes critical for spermatogenesis.[4,52,53] Conversely, insufficient vitamin D or VDR dysfunction correlates with reduced sperm count, motility, and abnormal morphology, underscoring its essential role in male fertility.[54,55]

Mechanistically, VDR mediates genomic effects through transcriptional regulation of calcium-handling proteins, mitochondrial enzymes, and antioxidant pathways, while non-genomic effects include rapid modulation of intracellular calcium and activation of signaling cascades such as PI3K/AKT and cAMP/PKA pathways that drive capacitation and acrosome reaction.[52,53] These dual mechanisms enable VDR to maintain sperm functionality and overall male reproductive health, highlighting its critical integrative role in calcium-mediated and metabolic processes essential for fertilization.

Group X Phospholipase A2 (PLA2G10)

PLA2G10 is a secreted enzyme with a conserved catalytic domain characterized by a His-Asp dyad and Ca^2+-binding loop, enabling hydrolysis of phospholipids at the sn-2 position to release arachidonic acid and lysophospholipids.[56] Structurally, PLA2G10 belongs to the secreted phospholipase A2 (sPLA2) family, with a small molecular weight (~14 kDa) and a compact fold stabilized by disulfide bonds, facilitating interactions with membranes and sperm surface receptors.[56] In sperm, PLA2G10 is localized predominantly in the acrosomal region, positioning it to regulate acrosome reaction and membrane remodeling during capacitation.[39,56]

PLA2G10 activity can be modulated by specific inhibitors such as indoxam and scalaradial, which block its enzymatic activity and reduce downstream signaling, while physiological agonists include calcium ions and phospholipid substrates that enhance catalysis.[56] Modulation of PLA2G10 affects sperm function, as studies in mice demonstrate that PLA2G10-deficient sperm show impaired acrosome reaction and reduced fertilization capability, highlighting its essential role in sperm-egg fusion.[56] Human studies suggest similar pathways where PLA2 activity contributes to capacitation, motility, and membrane fluidity.[4,39]

Mechanistically, PLA2G10 hydrolyzes membrane phospholipids to generate lysophospholipids and free fatty acids, which serve as second messengers activating protein kinase pathways and modulating ion channels, particularly calcium fluxes required for capacitation and acrosome reaction.[39,56] This lipid-mediated signaling facilitates membrane destabilization and exocytosis, essential for sperm-oocyte fusion. Moreover, PLA2G10-derived metabolites can interact with reactive oxygen species and mitochondrial pathways, influencing sperm energy metabolism and motility.[4,39] Therefore, PLA2G10 and its metabolites act as critical regulators of male fertility by linking lipid metabolism to calcium-dependent signaling in spermatozoa.

Na+/H+ Exchangers (NHE)

The NHEs are integral membrane proteins that maintain intracellular pH and ion homeostasis by exchanging intracellular H+ for extracellular Na+. Structurally, NHEs possess 10–12 transmembrane α-helices forming the ion translocation pathway and a cytoplasmic C-terminal regulatory domain that interacts with intracellular signaling molecules, allowing pH-sensitive modulation.[57] In spermatozoa, NHE isoforms, particularly NHE1, NHE5, and sperm-specific NHE (sNHE), are predominantly localized to the flagellum and midpiece, critical regions for motility and energy production.[39,57] These transporters are regulated by various agonists, including cyclic nucleotides like cAMP, which activate sNHE to promote hyperactivation during capacitation, while pharmacological inhibitors such as amiloride and its derivatives serve as antagonists that block Na+/H+ exchange, resulting in reduced sperm motility and impaired acrosome reaction.[39]

Studies have demonstrated that modulation of NHE activity significantly affects spermatogenesis and sperm function. For instance, knockout or pharmacological inhibition of sNHE in mice leads to defective sperm motility, reduced mitochondrial membrane potential, and impaired fertility.[57] Mechanistically, NHEs contribute to sperm function by regulating intracellular pH, which is essential for activating dynein ATPase in flagellar axonemes, thereby sustaining motility.[39] Additionally, NHE-mediated proton extrusion influences the activity of voltage-gated Ca^2+ channels, indirectly modulating capacitation and acrosomal exocytosis. Disruption of NHE activity alters the ionic microenvironment of sperm, leading to mitochondrial dysfunction and decreased ATP production, ultimately compromising fertilization potential.[4,57] Overall, NHEs act as pivotal regulators of sperm bioenergetics and capacitation, linking ionic homeostasis with functional competence.[57]

G-Protein Coupled Receptors (GPCRs) and Adenosine Receptors

The GPCRs, particularly adenosine receptors of the A2A subtype, are integral membrane proteins characterized by seven transmembrane α-helices connected by three extracellular and three intracellular loops, with an extracellular N-terminus and intracellular C-terminus. These receptors transduce extracellular signals into intracellular responses via coupling with G proteins, primarily Gs, which activate adenylate cyclase, increasing intracellular cAMP levels.[58] In human sperm, A2A receptors are predominantly localized in the sperm head and midpiece, areas critical for motility and fertilization processes, indicating their involvement in capacitation, acrosome reaction, and mitochondrial regulation.[58] Agonists such as adenosine and selective A2A receptor agonists enhance sperm motility and viability, whereas antagonists like SCH58261 inhibit these functions, highlighting their modulatory capacity.[58] Studies have demonstrated that activation of A2A receptors stimulates cAMP-dependent PKA signaling, which enhances sperm motility, hyperactivation, and acrosomal responsiveness, ultimately facilitating fertilization.[58] Furthermore, adenosine receptor signaling has been linked to mitochondrial bioenergetics in sperm, where receptor activation maintains mitochondrial membrane potential and ATP production necessary for flagellar movement.[58] The mechanistic role of A2A receptors involves modulation of intracellular calcium levels via cAMP-mediated phosphorylation of downstream effectors, contributing to capacitation-related calcium influx and acrosome reaction initiation.[58] Collectively, these findings establish that GPCRs, specifically adenosine A2A receptors, act as critical modulators of sperm physiology, influencing motility, energy metabolism, and fertilization potential through precise intracellular signaling pathways. Their dysregulation can, therefore, compromise male fertility, as observed in studies of infertile men and animal models.[4,58]

CRAC Channels (Orai/STIM), Acrosomal and Intracellular Ca^2+ Stores, and Store-Operated Calcium Entry (SOCE)

Calcium-release-activated calcium (CRAC) channels, primarily composed of Orai proteins and regulated by stromal interaction molecule (STIM) sensors, mediate store-operated calcium entry (SOCE) to replenish acrosomal and intracellular Ca^2+ stores, forming an integrated system critical for sperm calcium homeostasis. This system orchestrates sperm capacitation, hyperactivation, acrosome reaction, motility, and sperm-oocyte fusion, with disruptions impairing male fertility.

Spermatozoa store Ca^2+ primarily in the acrosome, an organelle in the anterior sperm head, and the redundant nuclear envelope, which act as reservoirs for mobilizable Ca^2+ regulated by calcium-binding proteins such as calmodulin-like and annexin-related molecules.[59,60] SOCE, triggered by depletion of these stores, relies on STIM proteins sensing reduced Ca^2+ levels in acrosome-equivalent structures and interacting with hexameric Orai pores on the plasma membrane to facilitate highly selective extracellular Ca^2+ influx.[4,61,62] Localized mainly in the acrosomal region and flagellum, the Orai/STIM complex integrates with other channels, including CatSper, voltage-dependent Ca^2+ channels (VDCCs), and transient receptor potential (TRP) channels, to regulate precise Ca^2+ signaling.[62–64]

Activation of this system occurs through store depletion, agonists like progesterone, or intracellular signaling cascades, with zona pellucida binding mobilizing acrosomal Ca^2+ for enzymatic release and membrane fusion during oocyte penetration.[16,39] SOCE sustains these processes by restoring intracellular Ca^2+ levels, driving oscillations that activate downstream pathways such as protein kinase A (PKA) and phospholipase C, which support mitochondrial energy production, flagellar beat, chemotaxis, and acrosomal exocytosis.[4,36,62] Secreted phospholipase A_2 further enhances epididymal maturation and acrosomal integrity through Ca^2+ modulation.[65]

Pharmacological agents modulate this system significantly. Thapsigargin, a sarco/endoplasmic reticulum Ca^2+-ATPase (SERCA) inhibitor, depletes intracellular stores, triggering premature SOCE and acrosome reactions that may reduce fertilization potential.[16] Inhibitors like SKF-96365 and 2-aminoethoxydiphenyl borate (2-APB) block Orai function, stabilizing stores and preventing premature exocytosis.[39,61] Agents such as caffeine, ulipristal acetate, and demethylzeylasteral alter Ca^2+ release, affecting acrosomal status and sperm function.[39,85,135] Environmental stressors, including oxidative stress, heavy metals, and cryopreservation, disrupt Ca^2+ handling, leading to DNA damage, reduced motility, and impaired fertility.[36,123,125]

Dysregulation of Orai/STIM or SOCE impairs spermatogenesis and sperm quality. Reduced Orai/STIM expression in infertility models like varicocele is associated with defective capacitation, hyperactivation, and acrosome reaction.[4,62]

Proteomic studies identify altered Ca^2+-handling proteins in low-fertility semen, suggesting their potential as fertility biomarkers.[93,136] Animal models further link SOCE impairment to mitochondrial dysfunction and failed fertilization.[4]

In summary, CRAC channels (Orai/STIM), acrosomal and intracellular Ca^2+ stores, and SOCE form a cohesive network essential for sperm maturation, motility, and fertilization. Pharmacological or pathological disruptions compromise these processes, directly impacting male reproductive outcomes.[4,61,62]

Heat Shock Protein 90 (HSP90)

Heat Shock Protein 90 (HSP90) is a highly conserved molecular chaperone that plays essential roles in maintaining protein homeostasis, folding, and stabilization of client proteins, including those involved in spermatogenesis and sperm function.[66] Structurally, HSP90 is a dimeric ATP-dependent protein composed of an N-terminal ATP-binding domain, a middle domain responsible for client protein interaction, and a C-terminal dimerization domain, which allows it to form functional homodimers crucial for its chaperone activity.[66] In sperm, HSP90 is predominantly localized in the head and midpiece regions, which are critical for capacitation, motility, and acrosome reaction, reflecting its involvement in both structural and signaling functions within the spermatozoa.[39,66]

HSP90 activity can be modulated by specific inhibitors such as geldanamycin and celastrol, which bind to its N-terminal ATP-binding domain, disrupting client protein folding and downstream signaling pathways, thereby impairing sperm functions.[39,66] Conversely, molecular chaperones and co-chaperones, including HSP70 and p23, can enhance HSP90 activity, supporting proper protein folding and calcium homeostasis in sperm.[66] Several studies have demonstrated that modulation of HSP90 affects intracellular calcium concentration, protein tyrosine phosphorylation, and progesterone-responsive sperm functions, including motility and the acrosome reaction, ultimately influencing male fertility.[39,66] Mechanistically, HSP90 regulates spermatogenesis and sperm function by stabilizing key signaling proteins and ion channels that control calcium influx, capacitation, and the acrosome reaction. It also interacts with steroid receptor complexes and kinases, linking stress responses to reproductive competence.[39,66] By maintaining protein integrity under physiological and stress conditions, HSP90 ensures proper sperm maturation, motility, and fertilization potential.[39,66] Its disruption leads to impaired calcium signaling, reduced tyrosine phosphorylation, and defective sperm function, highlighting its critical regulatory role in male reproduction.

Sphingomyelin Synthase 2 (SMS2)

The SMS2 is an integral membrane enzyme responsible for catalyzing the conversion of ceramide and phosphatidylcholine to sphingomyelin and diacylglycerol, playing a critical role in lipid homeostasis.[67] Structurally, SMS2 comprises multiple transmembrane domains, with active sites oriented toward the cytoplasmic leaflet, enabling interactions with lipid substrates and modulation of membrane microdomains.[67,68] In spermatozoa, SMS2 is predominantly localized in the plasma membrane of the sperm head and tail, regions crucial for motility, acrosome reaction, and fertilization.[67] Its distribution allows SMS2 to influence membrane fluidity and the formation of lipid rafts, essential for signal transduction during capacitation and fertilization.[4,67]

Modulation of SMS2 activity affects sperm function. While specific agonists directly targeting SMS2 in sperm are limited, pharmacological interventions altering sphingomyelin metabolism, such as sphingomyelinase inhibitors or ceramide analogs, indirectly influence SMS2 function.[67] Conversely, inhibitors of SMS2 reduce sphingomyelin synthesis, resulting in increased apoptosis and impaired motility, indicating its protective role in sperm viability.[67,137] Studies demonstrate that SMS2 deficiency or downregulation leads to disrupted mitochondrial function, increased reactive oxygen species, and impaired sperm motility and capacitation, ultimately compromising spermatogenesis and male fertility.[4,39,67] Mechanistically, SMS2 contributes to sperm physiology by regulating lipid raft integrity, which facilitates ion channel clustering and signal transduction necessary for acrosome reaction and hyperactivation. Additionally, SMS2-mediated sphingomyelin synthesis maintains membrane stability and prevents premature apoptosis by modulating ceramide levels, thereby sustaining sperm survival during transit and fertilization.[67] Therefore, SMS2 is critical for maintaining the lipid environment that supports both the structural and functional integrity of spermatozoa.

Nicotine Acetylcholine Receptor Subunit α7 (CHRNA7)

The Nicotinic Acetylcholine Receptor Subunit α7 (CHRNA7) is a ligand-gated ion channel belonging to the pentameric family of nicotinic acetylcholine receptors (nAChRs), characterized by five identical α7 subunits forming a central pore selective for cations, particularly calcium (Ca^2+).[69] Each subunit comprises an extracellular ligand-binding domain, four transmembrane helices (M1–M4), and an intracellular loop between M3 and M4 that regulates channel trafficking and interactions with intracellular proteins.[69] This architecture allows rapid Ca^2+ influx upon binding of agonists, influencing intracellular signaling cascades. In sperm, CHRNA7 is localized predominantly in the midpiece and flagellum, regions critical for motility and energy metabolism.[69] Activation of CHRNA7 by agonists such as acetylcholine or selective compounds like PNU-282987 enhances Ca^2+ entry, which is essential for sperm hyperactivation, capacitation, and the acrosome reaction.[39] Conversely, antagonists, including α-bungarotoxin, inhibit these processes by blocking channel-mediated Ca^2+ influx.[69]

Functional studies demonstrate that genetic deletion or pharmacological inhibition of CHRNA7 results in impaired sperm motility and reduced fertilization capacity, underscoring its critical role in spermatogenesis and sperm function.[69] Mechanistically, CHRNA7-mediated Ca^2+ influx regulates downstream pathways such as PKA and calcium/calmodulin-dependent kinases (CaMK), which modulate mitochondrial activity, flagellar beating, and acrosomal exocytosis.[39] Additionally, CHRNA7 contributes to the modulation of reactive oxygen species and mitochondrial membrane potential, which are essential for maintaining sperm viability and fertilizing potential.[4,69] Collectively, CHRNA7 functions as a pivotal regulator of sperm physiology through Ca^2+-dependent signaling, with its modulation directly impacting male fertility.

ClpP/ClpX Mitochondrial Proteases

ClpP and ClpX are essential mitochondrial proteases critical for sperm function and male fertility. ClpP forms a barrel-shaped tetradecameric proteolytic complex composed of two heptameric rings, while ClpX, an ATP-dependent chaperone, assembles into hexameric rings to recognize, unfold, and translocate protein substrates into ClpP for degradation.[70] These proteases are primarily localized within the mitochondrial matrix of spermatogenic cells and mature sperm, where they maintain mitochondrial proteostasis, regulate protein quality control, and preserve bioenergetic functions.[4,70] Pharmacological modulation of ClpP/ClpX activity is possible; activators like acyldepsipeptides enhance ClpP proteolytic activity, while inhibitors, such as β-lactones, suppress its function, indirectly impairing ClpX-mediated substrate processing.[70] Dysfunction or inhibition of ClpP/ClpX causes mitochondrial stress, reduced ATP production, and impaired spermatogenesis, leading to abnormal sperm motility, morphology, and reduced fertilization capacity.[70,137]

ClpP/ClpX proteases regulate spermatogenesis and sperm function by maintaining mitochondrial protein homeostasis, preventing the accumulation of misfolded proteins, and modulating signaling pathways like mTORC1, which is vital for germ cell differentiation and energy metabolism.[70] Importantly, mitochondria in sperm play a key role in calcium homeostasis, which is essential for processes such as sperm motility, capacitation, acrosome reaction, and gamete fusion.[4] Mitochondria act as calcium buffers, regulating intracellular calcium levels by sequestering and releasing calcium ions in response to cellular demands. ClpP/ClpX deficiency disrupts mitochondrial functions, including membrane potential and respiratory chain activity, which impairs the mitochondria's ability to effectively buffer calcium.[4,70] This disruption leads to dysregulated calcium signaling, contributing to defective sperm capacitation, reduced motility, and impaired fertilization potential.[4] Additionally, ClpP/ClpX dysfunction elevates reactive oxygen species and induces apoptosis in spermatogenic cells, further exacerbating mitochondrial stress and compromising calcium regulation.[4,70]

In summary, ClpP/ClpX proteases are indispensable for maintaining mitochondrial integrity and bioenergetics, which directly support calcium homeostasis in sperm. Their dysfunction disrupts mitochondrial calcium buffering, leading to impaired sperm function and male infertility.[4,70]

Recommendations for Calcium Channel Research in Spermatogenesis and Sperm Functions

Despite extensive research on sperm calcium channels, significant knowledge gaps persist, particularly regarding their precise roles in human fertility and the underlying molecular mechanisms that regulate channel activity. While studies have established the critical involvement of CatSper, T-type, L-type, and TRP channels in sperm motility, hyperactivation, and the acrosome reaction,[16,79,81] the spatiotemporal dynamics of calcium influx in human sperm under both physiological and pathological conditions remain incompletely understood. Most current studies rely on animal models or in vitro assays, which may not fully capture the complexities of human sperm physiology. Therefore, future investigations should prioritize human-relevant experimental systems, including ex vivo analysis of sperm from diverse populations, to validate translational relevance.

Another notable gap is the limited understanding of the interaction between different calcium channel families within sperm. Although CatSper channels are recognized as the primary mediators of calcium influx during hyperactivation, potential cross-talk with T-type and L-type channels remains poorly characterized. Elucidating these interactions could clarify whether synergistic or compensatory mechanisms exist when one channel is inhibited, offering insights for therapeutic interventions.[2,12] Advanced imaging techniques, combined with electrophysiological approaches, could map real-time calcium fluxes and channel localization at subcellular resolution, providing a more holistic understanding of calcium signaling networks in sperm.[37]

Environmental and pharmacological modulators of calcium channels also represent a critical research gap. While some studies suggest that heat stress, toxicants, or common medications such as paracetamol affect CatSper and other calcium channels,[12,81] the dose-response relationships and long-term reproductive consequences remain largely undefined. Future research should integrate toxicological and pharmacokinetic assessments with functional assays to determine threshold exposures that compromise sperm function. Such studies could inform public health guidelines and clinical recommendations for men planning fertility.

Moreover, the genetic variability of calcium channels across human populations is underexplored. Mutations or polymorphisms in CatSper subunits and other channel components may contribute to idiopathic male infertility.[3,79] Large-scale genomic studies, coupled with functional validation, are needed to identify clinically relevant variants, enabling precision medicine approaches in male fertility diagnostics and treatment. In parallel, epigenetic regulation of calcium channel expression in sperm remains largely unknown. Investigating how environmental factors or lifestyle influences modify channel expression through DNA methylation, histone modification, or non-coding RNAs could reveal novel targets for fertility enhancement or contraception.[16,37]

There is also a scarcity of integrative studies examining how calcium channels coordinate with other signaling pathways, such as cAMP-dependent processes, reactive oxygen species, and membrane potential dynamics, to regulate sperm capacitation and fertilization competence.[2,81] Multi-omics approaches, including proteomics and metabolomics, combined with calcium imaging, could provide a systems-level understanding of sperm physiology, uncovering previously unrecognized regulatory nodes. These approaches may also help distinguish between normal physiological variability and pathophysiological dysfunction in infertile men.

Finally, the translational application of calcium channel research into therapeutic and contraceptive interventions requires further exploration. While the modulation of CatSper and other channels shows promise for male contraception, the safety, reversibility, and population-level efficacy of such strategies are yet to be fully evaluated.[3,81] Similarly, strategies to restore or enhance calcium channel function in infertile men—potentially through targeted pharmacological activators, gene therapy, or lifestyle interventions—remain largely conceptual. Well-designed preclinical studies, followed by carefully monitored clinical trials, are necessary to bridge this gap.

Clinical and Therapeutic Implications

There is currently no fertility or contraceptive drug that specifically targets the calcium channels. Conventional male contraceptives rely primarily on hormonal approaches (eg, testosterone or progestin analogs) to suppress spermatogenesis, while pro-fertility treatments target hormonal deficiencies, oxidative stress, or lifestyle factors. Direct

pharmacological modulation of sperm calcium channels has not yet been fully translated into routine clinical use, although preclinical studies provide compelling evidence for their potential. L-type VDCC blockers such as amlodipine and nimodipine have been observed to induce reversible infertility in rodents by disrupting sperm motility and capacitation, suggesting a non-hormonal contraceptive mechanism.[108,109] Similarly, herbal compounds like Aegle marmelos, Tripterygium wilfordii derivatives, and demethylzeylasteral inhibit calcium-mediated sperm functions, offering experimental contraceptive applications.[39,137] Conversely, pro-fertility strategies targeting calcium channels are emerging, including vitamin D supplementation to enhance CatSper-mediated calcium influx, myoinositol to modulate sperm capacitation, and *Eurycoma longifolia* to stabilize intracellular calcium homeostasis.[138,139] These approaches indicate a shift toward ion channel–targeted therapeutics, yet most remain in experimental stages.

Targeting individual calcium channels offers distinct clinical advantages. CatSper family channels (CatSper1–4, CatSperε, CatSperζ, CatSperτ) are pivotal for sperm hyperactivation, motility, and fertilization. Agonists enhancing CatSper function can improve motility in asthenozoospermic patients, whereas selective inhibitors provide a precise, non-hormonal contraceptive option with minimal systemic side effects.[82,83] L-type VDCCs (CaV1.x) modulate capacitation and acrosome reaction; pharmacological modulation can support assisted reproductive technologies (ART) by enhancing fertilization efficiency.[140] T-type VDCCs (CaV3.1, CaV3.2) regulate acrosomal calcium release, suggesting that inhibitors like gossypol can function as contraceptives, while activators may correct subfertility.[39] TRP channels (TRPM8, TRPV4) mediate thermosensitive calcium signaling; their modulation could optimize sperm motility and thermotaxis, improving ART outcomes.[10,120] IP_3 and ryanodine receptors, which coordinate intracellular calcium release, can be targeted to enhance fertilization competency and capacitation. CaSR and store-operated Ca^2+ entry channels (Orai-STIM axis) provide avenues for correcting intracellular calcium deficits, particularly in infertile patients with idiopathic suboptimal sperm function.[7] High-voltage-activated (non-L-type) channels and acrosomal calcium stores are critical for hyperactivation and exocytosis, indicating that their modulation could refine ART protocols.[64] Regulators and modulators such as TMEM203, Slo3, HSP90, ITPR1, and related chemicals present innovative therapeutic targets to stabilize calcium homeostasis, protect against oxidative stress, or correct genetic deficits.[22,33,141]

The novelty of calcium channel–targeted therapy lies in its specificity and functional precision. Current contraceptives suppress global spermatogenesis, often causing systemic side effects and requiring long recovery periods. Pro-fertility drugs generally address hormonal or metabolic deficiencies but fail to correct channel-specific defects, such as CatSper mutations linked to azoospermia or capacitation failure.[15,77] Calcium channel modulators act directly on sperm function, enabling reversible, rapid, and targeted interventions without systemic hormonal alterations. They also allow fine-tuning of capacitation, hyperactivation, and acrosome reaction, directly addressing the molecular mechanisms underlying infertility. Furthermore, integration with ART, genetic screening, and environmental mitigation strategies enhances precision medicine approaches, ensuring personalized and effective outcomes. By modulating sperm-specific calcium channels, these interventions could revolutionize male contraception and fertility therapy, bridging gaps left by conventional pharmacological or hormonal treatments.

Study Strengths and Limitations

A key strength of this review lies in its comprehensive and integrative scope, which distinguishes it from earlier reviews on calcium channel regulation in sperm biology. Previous works, such as those by Yang et al[142] focused primarily on narrative descriptions of calcium channels in humans and mice, while Lee and Hwang[143] emphasized calcium homeostasis from a contraceptive perspective. Studies by Oviedo et al[144] and Bai and Shi[145] provided valuable overviews of CatSper channels and broad physiological roles of calcium channels, respectively, but they were limited in species coverage and lacked systematic synthesis across diverse methodological approaches. In contrast, our review draws from 142 studies spanning animal models, clinical investigations, electrophysiological analyses, and omics-based research, thereby offering a more extensive evidence base. By integrating cross-species findings, highlighting both well-studied and underexplored calcium channel subtypes, and emphasizing translational implications for male infertility and contraception, this study provides a more holistic and clinically relevant perspective. Moreover, our approach critically evaluates methodological inconsistencies and identifies research gaps, thereby moving beyond descriptive summaries

to propose concrete directions for future work. This broader, evidence-driven synthesis underscores the novelty and added value of our review compared to earlier literature.[142–144]

A central limitation of this review is the disproportionate reliance on animal studies compared with human-based investigations. Many studies employed rodents or other non-human models to explore calcium channel mechanisms in sperm physiology.[12] While these models are essential for mechanistic insights, they cannot fully replicate the complexity of human reproduction. The translation of findings from animals to human infertility, therefore, remains uncertain, reducing the direct clinical relevance of much of the evidence. Increased emphasis on human-based studies, including prospective cohorts and clinical trials, is necessary to bridge this translational gap.

Another limitation stems from considerable heterogeneity in methodologies. For instance, electrophysiological techniques such as patch-clamp vary widely in ionic conditions, recording modes, and pharmacological interventions, which complicates cross-study comparisons.[107] Similarly, studies assessing calcium flux or sperm motility employed inconsistent measurement endpoints. Such methodological variability hinders the synthesis of findings into a coherent understanding of calcium channel regulation in sperm and limits the reproducibility of results. The establishment of standardized protocols would substantially strengthen evidence integration. Although this review synthesized findings across 142 studies, it did not include a meta-analysis. The absence of pooled statistical data limits the ability to determine effect sizes or perform subgroup analyses by species, infertility type, or intervention. A quantitative meta-analysis would have enhanced the robustness of conclusions, allowed the ranking of calcium channels by clinical significance, and provided greater statistical power to detect subtle effects that individual studies may overlook.

Deficiencies in the reviewed literature also restricted the strength of conclusions. Several studies relied on small sample sizes, limiting statistical generalizability.[87] Others adopted retrospective designs, such as pharmacovigilance reports, which constrain causal inference.[146] Moreover, while omics-based studies have identified potential associations between calcium channel dysregulation and infertility, many lacked experimental validation to confirm causality.[4] Case reports describing rare CatSper mutations added valuable mechanistic insights but were inherently limited in representativeness.[89] Furthermore, many studies adopted a reductionist approach by focusing on individual channel subtypes, such as TRPM8 or IP_3 receptors, without considering broader interactions among calcium channels and other signaling pathways.[10,29] This fragmented perspective overlooks the integrative signaling networks that coordinate sperm motility, capacitation, and fertilization. Multi-omics and systems biology approaches would better capture the complexity of calcium channel regulation in sperm biology.

Another challenge lies in the limited demographic representation of study populations. For example, findings derived from Iranian or Chinese cohorts[15,147] may not generalize to other global populations due to genetic, environmental, or lifestyle differences. Infertility is a multifactorial condition, and calcium channel regulation may vary across populations. Multicenter studies involving diverse cohorts are therefore required to enhance the external validity of future research. Some interventional studies employed high doses of pharmacological agents, herbal extracts, or environmental toxins when assessing calcium channel modulation.[137] These exposures may not reflect clinically relevant or environmentally realistic scenarios. Such limitations restrict the translational applicability of results to real-world fertility management or contraceptive strategies. Future research should prioritize dose-response investigations within physiologically relevant ranges.

The reviewed studies largely provided cross-sectional snapshots of calcium channel expression or activity. Few longitudinal studies followed changes in channel regulation across spermatogenesis or in response to therapeutic interventions.[147] Longitudinal data are essential for understanding temporal dynamics, particularly how sustained modulation of calcium channels affects fertility outcomes over time. Finally, publication bias may have influenced the evidence base. Studies reporting significant associations between calcium channels and fertility outcomes are more likely to be published than those with null or inconclusive results. This bias could exaggerate the perceived importance of certain channels while underrepresenting contradictory evidence. Without access to unpublished or negative data, the conclusions of this review remain vulnerable to distortion.

Conclusion and Future Directions

This review provides shreds of evidence that calcium channels are indispensable regulators of spermatogenesis, motility, capacitation, and fertilization. Their dysregulation, whether through genetic variants, toxic exposures, or pharmacological interference, emerges as a unifying mechanism behind diverse forms of male infertility. The cumulative evidence suggests that calcium channels not only function as essential biological gatekeepers but also represent promising targets for diagnostic and therapeutic interventions for male contraception and infertility, beyond the widely researched mediators of oxidative stress and antioxidant systems.[148–154]

Future research should focus on translating mechanistic insights into clinical practice. First, genetic and proteomic discoveries need to be integrated into standardized diagnostic platforms that allow early detection of channelopathies contributing to infertility. Genetic screening for CatSper or other calcium channel variants could personalize patient management, while proteomic biomarkers may guide treatment selection and predict ART success. Second, drug development efforts should prioritize sperm-specific targets such as CatSper, whose selective expression offers opportunities for non-hormonal contraceptives and fertility-enhancing agents with minimal systemic side effects. Beyond drug design, protective interventions such as antioxidants and natural compounds deserve exploration as adjuncts to mitigate environmental and pharmacological insults to calcium signaling. Third, methodological progress is critical. Standardized assays ranging from patch-clamp electrophysiology to advanced imaging are necessary to ensure reproducibility and comparability across laboratories. Coupling these approaches with multi-omics platforms and systems biology will enable a holistic view of calcium channel regulation, revealing cross-talk with mitochondrial function, oxidative stress pathways, and hormonal control. Finally, human-centric studies must replace the current dependence on animal models. Large, diverse cohorts combined with longitudinal designs will clarify how calcium channel modulation affects fertility outcomes over time. Interdisciplinary research, blending molecular biology, toxicology, genetics, and clinical andrology, will ultimately transform calcium channel biology into actionable strategies that improve reproductive health and expand contraceptive options.

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