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REVIEW ARTICLE

Physiological role of potassium channels in mammalian germ cell differentiation, maturation, and capacitation

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Abstract

Background: Ion channels are essential for differentiation and maturation of germ cells, and even for fertilization in mammals. Different types of potassium channels have been identified, which are grouped into voltage-gated channels (Kv), ligand-gated channels (K_{ligand}), inwardly rectifying channels (K_{ir}), and tandem pore domain channels (K_{2P}).

Material-Methods: The present review includes recent findings on the role of potassium channels in sperm physiology of mammals.

Results-Discussion: While most studies conducted thus far have been focused on the physiological role of voltage- (Kv1, Kv3, and Kv7) and calcium-gated channels (SLO1 and SLO3) during sperm capacitation, especially in humans and rodents, little data about the types of potassium channels present in the plasma membrane of differentiating germ cells exist. In spite of this, recent evidence suggests that the content and regulation mechanisms of these channels vary throughout spermatogenesis. Potassium channels are also essential for the regulation of sperm cell volume during epididymal maturation and for preventing premature membrane hyperpolarization. It is important to highlight that the nature, biochemical properties, localization, and regulation mechanisms of potassium channels are species-specific. In effect, while SLO3 is the main potassium channel involved in the K^+ current during sperm capacitation in rodents, different potassium channels are implicated in the K^+ outflow and, thus, plasma membrane hyperpolarization during sperm capacitation in other mammalian species, such as humans and pigs.

Conclusions: Potassium conductance is essential for male fertility, not only during sperm capacitation but throughout the spermiogenesis and epididymal maturation.

KEYWORDS
cell volume, epididymal maturation, plasma membrane potential, sperm capacitation, spermatogenesis

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1 | INTRODUCTION

Sperm differentiation and function are regulated by several ion channels, which are distributed throughout the plasma membrane, and activated and modulated by different external and internal factors (reviewed by Nowicka-Bauer and Szymczak-Cendlak,[1] and by Delgado-Bermúdez et al.[2]). While little data on the relevance of ion channels during spermatogenesis are available, several studies demonstrate that they are essential for spermatozoa movement toward the egg in lower and higher vertebrates.[3–6] In mammals, scientific evidence supports the role of bicarbonate (HCO_3^-) and calcium (Ca^{2+}) as second messengers that activate the soluble adenylate cyclase (sAC) and protein kinase A pathway (PKA) after ejaculation, which are essential for motility activation and protein tyrosine phosphorylation.[7,8] The spermatozoa plasma membrane contains a wide diversity of bicarbonate and calcium transporters differing in solute affinity, stoichiometry, and regulation mechanisms.[9–12] Moreover, HCO_3^- influx and proton (H^+) efflux are essential for alkalinization of sperm cytoplasm (pH_i) and activation of several pH-dependent channels that trigger the sequence of events associated to capacitation occurring in the upper female reproductive tract.[2,10]

Not only does sperm function after ejaculation rely upon intracellular Ca^{2+}, HCO_3^-, and H^+ levels, but also on the conductance of other ions across the plasma membrane, mainly potassium (K^+), sodium (Na^+), and chloride (Cl^-).[1,2,4,13,14] Little data, nevertheless, exist about the types of channels implicated in the transport of these ions and their physiological role during sperm differentiation, maturation, and capacitation, most of the studies having been performed in rodents. In mammals, potassium channels are the main transporters involved in the initiation of sperm motility during ejaculation and in the hyperactivation of sperm movement.[15,16] The plasma membrane of ejaculated spermatozoa is slightly polarized (−30 to −40 mV), but it hyperpolarizes during capacitation to approximately −60 mV because of an increased K^+ permeability.[17] It has been reported that around 10% of sub-fertile patients have reduced K^+ outflux or enhanced K^+ inward, thereby resulting in prolonged depolarization of the plasma membrane and defective hyperpolarization during sperm capacitation.[18] In spite of this, a clear relationship between potassium channel dysfunction and male sub-fertility is yet to be established.

In general terms, K^+ channels constitute a superfamily that include a high diversity of transporters that participate in the osmotic regulation of cell volume and the control of membrane potential.[19] According to their regulation mechanisms, K^+ channels are grouped into four major classes: voltage-gated channels (Kv), ligand-gated channels (K_{ligand}), inwardly rectifying channels (K_{ir}), and tandem pore domain channels (K_{2P})[20,21]; only K_{2P} channels are dimers, whereas the rest of channels have a tetrameric structure (Figure 1). Each monomer or α-subunit has a pore-forming domain (PD) and a voltage-sensor domain (VSD); the association of the four PDs gives rise to a central pore surrounded by VSDs.[19,20] Pore conformation is similar and well-conserved in all types of K^+ channels, being highly selective and efficient, and at least 10,000 times more permeable to K^+ than Na^+; in contrast, VSDs differ in structure according to the channel and cell type.[19,20] Besides the α-subunits, K^+ channels can also contain auxiliary subunits (β- and/or γ-subunits) that modulate their activity and even their trafficking and localization.[8,20]

Potassium channels can show three different states: resting, activated, and inactivated. Channel gating is regulated by both intracellular and extracellular signals, which can exert either a negative or positive effect in different channels and cell types (reviewed by Kuang et al.[20]). Channel closing usually occurs during the resting state, and several K^+ channels are inactivated shortly after opening (reviewed by Kuang et al.[20]).

The present review gathers recent evidence on the structure, localization, and physiological relevance of K^+ channels during spermatogenesis, epididymal maturation, and capacitation; the types of potassium channels present in the different germ cell types and their general function are summarized in Table 1. As aforementioned, most of the studies conducted thus far were performed in humans and rodents, so that little information on other mammalian species is available, despite the potential species-specific differences in the role of these channels. The types of potassium channels that have been identified in the spermatozoa of different species and their localization are represented in Figure 2.

2 | VOLTAGE-GATED POTASSIUM CHANNELS (KV CHANNELS)

The human genome contains 40 genes encoding for voltage-gated potassium channels, which are grouped into 12 subfamilies (Kv1–Kv12)[19] as Table 2 summarizes. Kv channels are tetramers of α-subunits, which usually associate with auxiliary β-subunits and/or K^+ channel tetramerization domain proteins (KCTD).[22,23]

As a general feature, plasma membrane (de)polarization determines whether Kv channels are open or closed, as they are open when the membrane is depolarized and closed when it is hyperpolarized.[24] In addition, Kv channels show great molecular and functional diversity, differing in the inactivation pattern, voltage dependence, and kinetics. While Kv1, Kv2, and Kv4 are fast inactivated channels, the triggering of Kv1, Kv4, and Kv7 channels occurs by low membrane depolarization, and high depolarization is required for Kv2 and Kv3 opening.[19] Channel diversity can arise from alternative splicing and/or heterogenic assembling.[19,25] The assemblage of Kv1, Kv7, and Kv10 α-subunits give rise to heterotetramers with different functional properties compared with the corresponding homotetramers.[19] Moreover, Kv5, Kv6, Kv8, and Kv9 are electrically silent α-subunits that co-assemble with Kv2 and Kv3 α-subunits, thus forming channel complexes with modified properties.[24,25]

All Kv α-subunits are similar in structure, with six transmembrane alpha-helices domains (TM1–TM6) being located between the cytosolic N- and C-termini. Helices TM1–TM4 form the VSD, and helices TM5–TM6 form the PD; TM5 and TM6 are joined by an extracellular phosphatase-binding loop (P-loop) that constitutes the ion selectivity filter in the functional channel (Figure 3).[26–28] The assemblage of

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FIGURE 1 Classification and general structure of potassium channels. (A) K_{ir} channels present a 2TM/P structure, which consists of two transmembrane α-helices and a connecting P-loop; this corresponds to the canonical structure of K^+ channels. (B) K_{2P} channels present a 4TM/2P structure and contain two consecutive 2TM/P sequences. (C) Kv and K_{ligand} channels present a 6TM/P structure, with four transmembrane α-helices followed by a connecting loop and a 2TM/P sequence. TM: transmembrane segment.

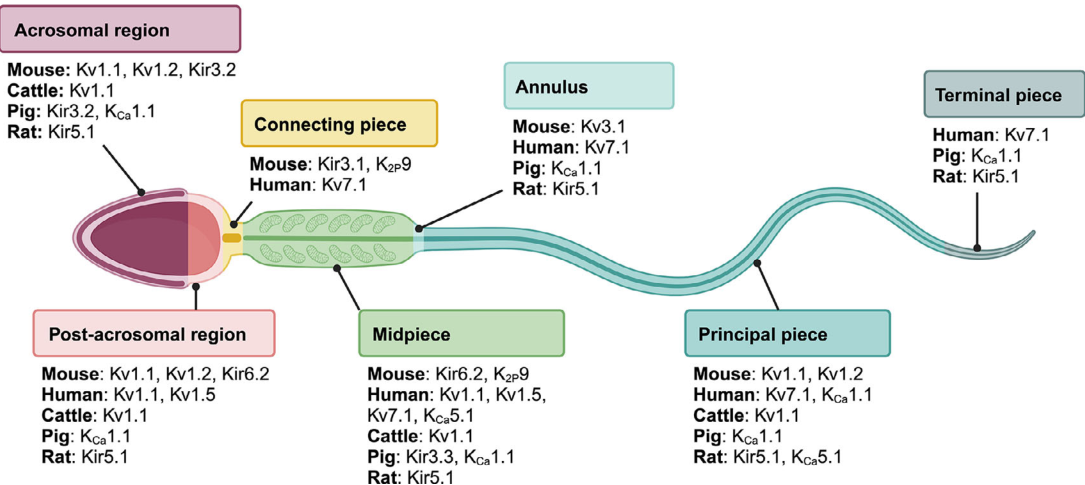

FIGURE 2 Localization of potassium channels in the spermatozoa of different mammalian species (acrosomal region, post-acrosomal region, connecting piece, midpiece, annulus, principal piece, and terminal piece). When a channel has been described to be located in the sperm head without specifying the region, it has been considered to be present in both the acrosomal and post-acrosomal regions. When a channel has been found to be located in the sperm tail without specifying the region, it has been considered to be present in the midpiece, annulus, principal piece, and terminal piece. Kv: voltage-gated K^+ channels; K_{ir}: inwardly rectifying K^+ channels; K_{Ca}: calcium-gated K^+ channels; K_{2P}: tandem pore domain K^+ channels.

four α-subunits gives rise to a Kv channel constituted by four peripheral VSDs that surround and attach weakly to the central pore.[29–32] Voltage-gated sodium (Nav), calcium (Cav), and proton (HVCN1) channels have also been reported to contain VSDs.[33,34] The capability of VSDs to sense voltage is conferred by positively charged amino acid residues (mainly Arg and Lys) in the TM4; the number of such residues varies from six to 13 depending on the channel type.[19,35] Interestingly, positively charged residues are separated by two hydrophobic residues, thus forming a triplet residue motif that is evolutionarily conserved and is necessary for voltage sensing.[20] The positive charges of the TM4 segment are balanced by negatively charged residues present in other transmembrane segments, mainly TM2 and TM3; moreover, electrostatic interactions between transmembrane helices favor the TM4 movement within the lipid bilayer.[19,31]

Channel activation during depolarization induces VSD conformational changes that are transferred to the channel pore, thus changing its conformation from closed to open state.[24] Noticeably, the K^+ flux in the open state is dependent on the electrochemical gradient.[20] Upon repolarization, VDS returns to its resting state, which induces channel deactivation by closing the pore.[24] Immediately after deactivation, channels can be activated again; however, if depolarization-induced activation extends beyond a few milliseconds, channels switch to the inactivated state.[24] Kv channels recover from inactivation only after spending enough time at a hyperpolarized potential.[36] Interestingly, while β-subunits can modify the kinetics of Kv activation, they do not alter the voltage dependence.[8,20,37] β-Subunits associated to Kv channels belong to the KCNE family, which consists of five members (KCNE1–KCNE5)—each encoded by

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TABLE 1 Types of potassium channels present in the plasma membrane of mammalian germ cells.

Germ cell generation	Species	Function
Spermatogonia		
Kv7.1 or KCNQ1	Rat,[52] human[52]	Cell proliferation
Kir3.1, KCNJ3. or GIRK1	Mouse[42]	Cell proliferation
Kv6.4 or KCNG4	Mouse[25]	Cell proliferation
Kir5.1 or KCNJ16	Mouse,[69] rat[74]	Unknown
K_{Ca}1.1, KCNMA1, SLO1, BK, or MaxiK	Rat[43]	Plasma membrane potential
Spermatocytes		
Kv6.4 or KCNG4	Mouse[25]	Cell proliferation
Kir3.1, KCNJ3, or GIRK1	Mouse[42]	Cell proliferation
Kir5.1 or KCNJ16	Mouse,[69] rat[74]	Unknown
K_{Ca}1.1, KCNMA1, SLO1, BK, or MaxiK	Rat[43]	Plasma membrane potential
Spermatids		
Kv1.3 or KCNA3	Rat[43,51]	Resting membrane potential Cell volume regulation
Kv6.4 or KCNG4	Mouse[25]	Unknown
Kir3.1, KCNJ3, or GIRK1	Mouse[42]	Unknown
Kir3.2, KCNJ6, or GIRK2	Mouse[70]	Unknown
Kir5.1 or KCNJ16	Mouse,[69] rat[74]	Unknown
Sperm cells		
Kv1.1 or KCNA1	Mouse,[42] human,[41] cattle[14]	Cell volume regulation Sperm capacitation
Kv1.2 or KCNA2	Mouse[42]	Cell volume regulation Sperm capacitation
Kv1.5 or KCNA5	Human[41]	Cell volume regulation Sperm capacitation
Kv3.1 or KCNC1	Mouse[42]	Cell volume regulation Sperm capacitation
Kv7.1 or KCNQ1	Human[8]	Sperm capacitation
Kir3.1, KCNJ3, or GIRK1	Mouse[42]	Sperm capacitation
Kir3.2, KCNJ6, or GIRK2	Pig,[70] mouse[70]	Sperm capacitation
Kir5.1 or KCNJ16	Rat[74]	Sperm capacitation
Kir6.2, K_{ATP}, or KCNJ11	Mouse[68]	Sperm capacitation
K_{2P}9 or KCNK9	Mouse[41]	Cell volume regulation
K_{Ca}1.1, KCNMA1, SLO1, BK, or MaxiK	Human,[123,124] pig[125,127]	Sperm capacitation
K_{Ca}5.1, KCNU1, or SLO3	Human,[45,92,108,110,112,126,134] rat,[107] mouse[105,109,137–139]	Osmotic homeostasis Sperm capacitation

a specific gene (*KCNE1–KCNE5*)—that share a similar structure, with a single transmembrane domain, an inner C-terminus, and an outer N-terminus.[37] Kv channels can also associate with other regulatory proteins, mainly with those of the KCTD family, which is constituted by 25 members that not only participate in Kv regulation but are also involved in cell apoptosis and protein ubiquitination and degradation.[23,38] KCTD are small cytoplasmic proteins (40 kDa) that have an amino acid sequence such as the cytoplasmic domain of Kv channels, and usually associate with Kv α-subunits at a 1:1 ratio[22,39]; the N-terminal domain is relatively conserved, whereas the C-terminal one shows great diversity.[39] In mouse testis, the complexes formed by KCTD19 and zinc finger protein 541 (ZFP541) localize in the nucleus of spermatocytes and round spermatids, but are absent from elongating spermatids[23]; these complexes are involved in chromatin reorganization during meiosis.[23,40] In *Kctd19*$^{-/-}$ mice, spermatocytes show meiotic arrest and undergo apoptosis, whereas spermatids are lacking.[23]

Little data exist about the types and distribution of Kv channels in differentiating and mature sperm cells (Table 1). Recent evidence suggests the presence of Kv1, Kv3, and Kv7 in mature spermatozoa;

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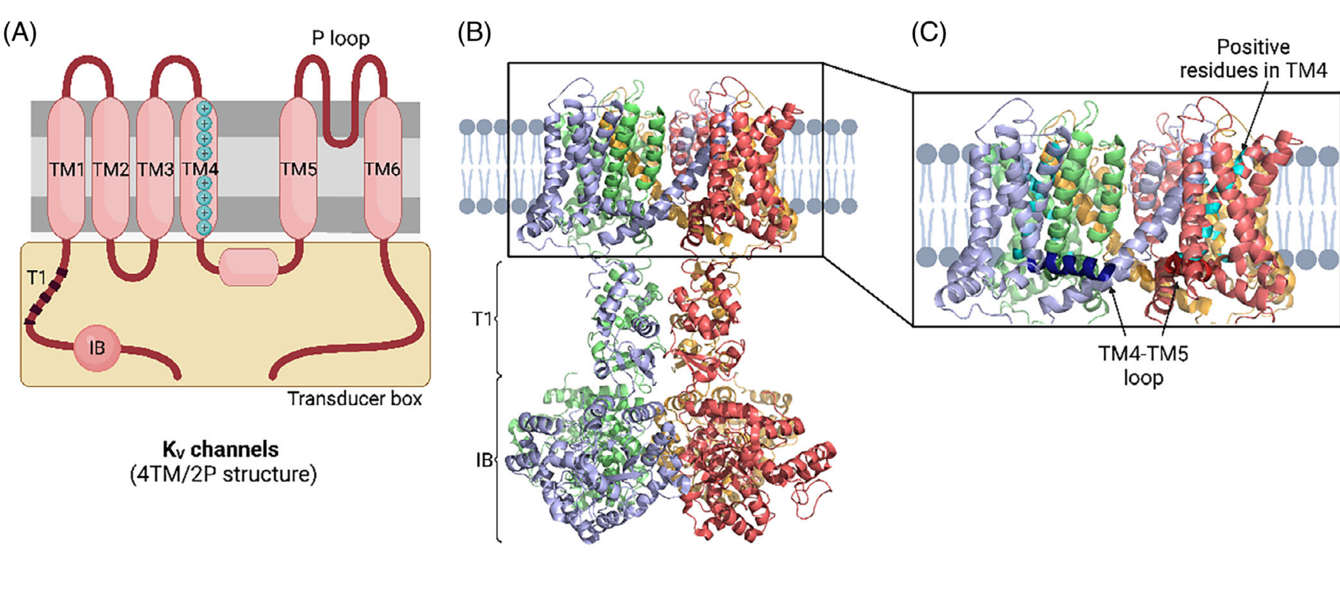

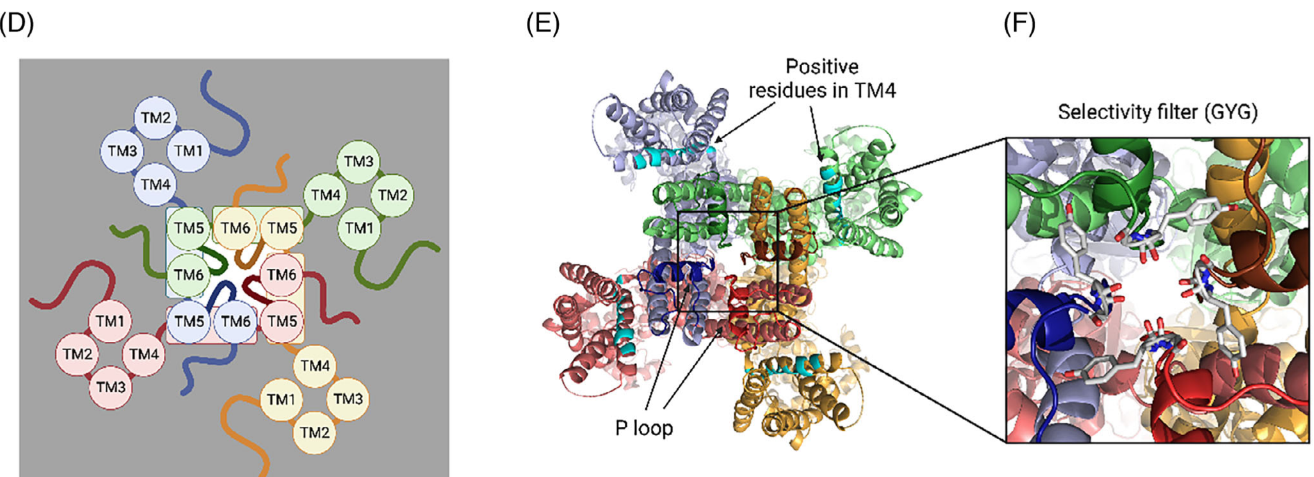

FIGURE 3 Structural characteristics of voltage-gated K^+ channels (Kv). (A) Voltage-gated K^+ channels present a large N-terminal domain, which includes the tetramerization domain (T1) and the inactivation ball (IB). The transmembrane segment TM4 presents a series of positively charged residues that form a voltage-sensing domain (VSD). The loop between TM4 and TM5 is the inactivation-ball-binding loop. (B) Voltage-gated K^+ channels arrange in tetramers, and the regions corresponding to T1 and IB form a large intracellular domain. (C) Transmembrane domains from different monomers overlap. Darker residues highlight the TM4–5 connecting loop. Positively charged residues from the VSD in TM4 are highlighted (light blue). (D) The tetrameric structure forms a central pore that involves TM5–6 and the connecting loop between TM4 and TM5 (squares) of each monomer, whereas TM1–4 are not directly involved in the structure of the central pore. (E) P-loops (darker residues) are oriented toward the central pore, and the VSD in TM4 (light blue) is found outside of the pore. (F) Each P-loop presents a GYG sequence, which contributes to the selectivity filter (PDB reference 7EJ1). TM: transmembrane segment.

however, differences exist in the activity and localization according to the species. Kv1 subfamily includes eight members (Kv1.1–Kv1.8, or KCNA1–KCNA 8) and Kv3 four (Kv3.1–Kv3.4, or KCNC1–KCNC4); thus far, immunolocalization approaches have allowed for the elucidation of the presence of Kv1.1, Kv1.2, Kv1.5, and Kv3.1 in the plasma membrane of mature sperm cells from mammals, and the absence of Kv1.4, Kv4.2, and Kv4.3 subunits in human spermatozoa.[41] In mice,[42] humans,[41] and cattle,[14] Kv1.1 (KCNA1) channels (56 kDa), as revealed by immunostaining, localize over the sperm head and flagellum. In mouse spermatozoa, Kv1.2 (KCNA2) channels show positive immunoreactivity in the sperm head and principal piece, and Kv3.1 (KCNC1) in the annulus.[42] Kv1.5 (KCNA5) channels (76 kDa) are immunolocalized in the post-acrosomal region and midpiece of human spermatozoa.[41] The specific role of each channel type is unknown, but Kv channels contribute, together with other K^+ channels, in the volume regulation of sperm cells during epididymal maturation and after ejaculation,[41] and in the K^+ outflow that triggers plasma membrane hyperpolarization and acrosome exocytosis during sperm capacitation.[8,14,43] Extracellular K^+ concentration increases progressively throughout the epididymal duct, which favors a rise in the intracellular levels of K^+ in sperm cells; this increase of inner K^+ is associated to a decrease of sperm volume.[41] High extracellular K^+ levels

TABLE 2 Diversity of voltage-gated potassium (Kv) channels and the coding genes in mammals.

Kv channels	Coding gene
Kv1.1 or KCNA1	*Kcna1/KCNA1*
Kv1.2 or KCNA2	*Kcna2/KCNA2*
Kv1.3 or KCNA3	*Kcna3/KCNA3*
Kv1.4 or KCNA4	*Kcna4/KCNA4*
Kv1.5 or KCNA5	*Kcna5/KCNA5*
Kv1.6 or KCNA6	*Kcna6/KCNA6*
Kv1.7 or KCNA7	*Kcna7/KCNA7*
Kv1.8 or KCNA10	*Kcna10/KCNA10*
Kv2.1 or KCNB1	*Kcnb1/KCNB1*
Kv2.2 or KCNB2	*Kcnb2/KCNB2*
Kv3.1 or KCNC1	*Kcnc1/KCNC1*
Kv3.2 or KCNC2	*Kcnc2/KCNC2*
Kv3.3 or KCNC3	*Kcnc3/KCNC3*
Kv3.4 or KCNC4	*Kcnc4/KCNC4*
Kv4.1 or KCND1	*Kcnd1/KCND1*
Kv4.2 or KCND2	*Kcnd2/KCND2*
Kv4.3 or KCND3	*Kcnd3/KCND3*
Kv5.1 or KCNF1	*Kcnf1/KCNF1*
Kv6.1 or KCNG1	*Kcng1/KCNG1*
Kv6.2 or KCNG2	*Kcng2/KCNG2*
Kv6.3 or KCNG3	*Kcng3/KCNG3*
Kv6.4 or KCNG4	*Kcng4/KCNG4*
Kv7.1 or KCNQ1	*Kcnq1/KCNQ1*
Kv7.2 or KCNQ2	*Kcnq2/KCNQ2*
Kv7.3 or KCNQ3	*Kcnq3/KCNQ3*
Kv7.4 or KCNQ4	*Kcnq4/KCNQ4*
Kv7.5 or KCNQ5	*Kcnq5/KCNQ5*
Kv8.1 or KCNV1	*Kcnv1/KCNV1*
Kv8.2 or KCNV2	*Kcnv2/KCNV2*
Kv9.1 or KCNS1	*Kcns1/KCNS1*
Kv9.2 or KCNS2	*Kcns2/KCNS2*
Kv9.3 or KCNS3	*Kcns3/KCNS3*
Kv10.1 or KCNH1	*Kcnh1/KCNH1*
Kv10.2 or KCNH5	*Kcnh5/KCNH5*
Kv11.1 or KCNH2	*Kcnh2/KCNH2*
Kv11.2 or KCNH6	*Kcnh6/KCNH6*
Kv11.3 or KCNH6	*Kcnh7/KCNH7*
Kv12.1 or KCNH8	*Kcnh8/KCNH8*
Kv12.2 or KCNH3	*Kcnh3/KCNH3*

Abbreviations: KCNA, potassium voltage-gated channel subfamily A members; KCNB, potassium voltage-gated channel subfamily B members; KCNC, potassium voltage-gated channel subfamily C members; KCND, potassium voltage-gated channel subfamily D members; KCNF, potassium voltage-gated channel subfamily F members; KCNG, potassium voltage-gated channel subfamily G members; KCNH, potassium voltage-gated channel subfamily H members; KCNQ, potassium voltage-gated channel subfamily Q members; KCNS, potassium voltage-gated channel subfamily S members; KCNV, potassium voltage-gated channel subfamily V members (adapted from González et al.[19]).

also prevent premature membrane hyperpolarization and, thus, sperm capacitation.[44,45]

Kv7 subfamily is constituted by five members (Kv7.1–Kv7.5, or KCNQ1–KCNQ5). Kv7.1 (KCNQ1, or KvLQT1) is expressed in a wide range of human tissues and cells,[8,32] and can interact with different auxiliary subunits that are tissue specific.[46,47] Kv7.1 are tetrameric channels of low conductance; each α-subunit contains six transmembrane segments (TM1–TM6) and four intracellular C-terminal helices (HA-HD).[28,34,48] α-Subunits can further co-assemble with auxiliary β-subunits, mainly from the KCNE family.[46,49] In human spermatozoa, Kv7.1 (70 kDa) distributes throughout the plasma membrane as evidenced by indirect immunofluorescence, and associates with KCNE1 (15 kDa) in the neck and the tail.[8] Both proteins contribute to K^+ efflux and, hence, to membrane hyperpolarization during sperm capacitation, as well as to protein phosphorylation, motility hyperactivation, and acrosome exocytosis.[8] Kv7.1 activity is regulated by calmodulin, a cytosolic Ca^{2+}-binding protein that interacts with intracellular helices HA and HB; therefore, low inner Ca^{2+} levels are associated to Kv7.1 inactivation.[48] In sperm cells, calmodulin locates in the head and flagellum, and activates additional phosphorylation cascades regulated by calmodulin kinase that are also essential for both hyperactivation of sperm motility and acrosomal exocytosis.[10,50] Pharmacological blockade of Kv channels in capacitated spermatozoa inhibits K^+ efflux, thus resulting in prolonged membrane depolarization, impaired hyperpolarization, and acrosome exocytosis.[7,8,14] In humans, however, this inhibition of Kv channels only alters sperm capacitation partially, because of the compensatory effect of other K^+ channels, mainly SLO1 and SLO3.[8,45]

Most studies on the presence and role of Kv channels in differentiating male germ cells have been performed in rodents. In rats, patch clamping and immunofluorescence showed the presence of Kv1.3 (KCNA3) channels in the plasma membrane of round and elongating spermatids, where they seem to be involved in the regulation of the resting membrane potential, intracellular levels of Ca^{2+} and K^+, and cell volume during spermiogenesis.[43,51] In mouse, adult transgenic males with *Kcng4* deletion are sterile, and exhibit a significantly reduced sperm concentration, motility, and morphology, with sperm cells having smaller heads and shorter tails than the control.[25] These data support that Kv6.4 (KCNG4) has an essential role during germ cell proliferation and spermiogenesis.[25] In the rat testis, in situ hybridization assays allowed for the identification of both Kv7.1 (KCNQ1) and auxiliary KCNE1 subunits in spermatogonia, but not in meiotic or post-meiotic germ cells or in somatic testicular cells, thus suggesting that they are involved in the proliferation of undifferentiated germ cells.[52] Interestingly, neither Kv7.1 nor KCNE1 protein subunits are present in patients with Sertoli cells-only syndrome,[52] whereas the massive proliferation of spermatogonia occurring in patients affected by seminoma, a slow-growing testicular cancer, is associated to an increased expression of both Kv7.1 (KCNQ1) and KCNE1,[52] as well as of other K^+ channels.[53] Moreover, human asthenospermia can be associated to an abnormal methylation of the *Kcnq1* gene, which leads to inappropriate gene transcription that can be transmitted to the embryo.[54]

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TABLE 3 Diversity of inwardly rectifying potassium (K_{ir}) channels and the coding genes in mammals.

K_{ir} channel	Coding gene
K_{ir}1.1 or KCNJ1	*Kcnj1/KCNJ1*
K_{ir}2.1 or KCNJ2	*Kcnj2/KCNJ2*
K_{ir}2.2 or KCNJ12	*Kcnj12/KCNJ12*
K_{ir}2.3 or KCNJ4	*Kcnj4/KCNJ4*
K_{ir}2.4 or KCNJ14	*Kcnj14/KCNJ14*
K_{ir}3.1 or KCNJ3 or GIRK1	*Kcnj3/KCNJ3*
K_{ir}3.2 or KCNJ6 or GIRK2	*Kcnj6/KCNJ6*
K_{ir}3.3 or KCNJ9 or GIRK3	*Kcnj9/KCNJ9*
K_{ir}3.4 or KCNJ5	*Kcnj5/KCNJ5*
K_{ir}4.1 or KCNJ10	*Kcnj10/KCNJ10*
K_{ir}4.2 or KCNJ15	*Kcnj15/KCNJ15*
K_{ir}5.1 or KCNJ16	*Kcnj16/KCNJ16*
K_{ir}6.1 or KCNJ8	*Kcnj8/KCNJ8*
K_{ir}6.2 or KCNJ11	*Kcnj11/KCNJ11*
K_{ir}7.1 or KCNJ13	*Kcnj13/KCNJ13*

Abbreviations: GIRK, G protein-coupled inwardly rectifying potassium channel; KCNJ, potassium inwardly rectifying channels subfamily J members (adapted from González et al.[19] and Poli et al.[15]).

3 | INWARDLY RECTIFYING POTASSIUM CHANNELS (K_{ir} CHANNELS)

Inwardly rectifying potassium channels (K_{ir} channels), also known as KCNJ, are involved in the maintenance of the resting membrane potential by the inward conductance of K^+.[55] They are currently grouped into seven subfamilies (K_{ir}1–K_{ir}7; Table 3), the subunits being encoded by 15 different genes (*KCNJ1–KCNJ16*).[15,19,56] K_{ir} channels are activated by membrane hyperpolarization rather than depolarization, thus favoring K^+ influx into the cell, and are modulated by different mediators, including ions, phospholipids, and proteins.[56]

K_{ir} channels have either a homo- or a heterotretameric structure, with the pore extending beyond the transmembrane region to the cytosol, two inner N- and C-termini, and two gates formed by the P-loop and the G-loop, respectively (Figure 4).[19,56] All subunits have two transmembrane spanning helices (TM1 and TM2) that are linked by the P-loop, which works as an extracellular ion selectivity filter.[57,58] This outer selectivity filter constitutes the narrowest part of the pore and contains four binding sites for K^+ ions.[57] The cytosolic C-terminal domain has several β-sheets and extends the ion conduction pathway; it contains different binding sites for regulatory mediators, mainly Mg^{2+} and polyamines,[56] which are evolutionary conserved and critical to inward rectification,[59–61] K_{ir} channels also contain a narrow G-loop in the apex of the cytosolic domain, which has an intrinsic flexibility and adopts different conformations in the resting, activated, and inactivated states.[57,60,61] The G-loop acts as a lower gate and can also modulate the upper gate.[59,62] Interestingly, activation of K_{ir} channels depends on the binding of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to the interface region between the pore and cytosolic domains,[63] thus inducing translational movements toward transmembrane domains and conformational changes in the cytosolic domain.[64] K_{ir} gating can also be modulated by ATP/ADP ratio, channel phosphorylation, and G-proteins.[19,58] Furthermore, the mechanism of inward rectification by K_{ir} requires intracellular Mg^{2+} and polyamines, which occlude the outward flow of K^+ by binding to the cytoplasmic side of the pore.[55,57]

K_{ir} family includes K_{ATP} channels, which are weak inward rectifiers[57] and act as metabolic sensors that regulate plasma membrane potential based on the intracellular ATP/ADP balance.[65] In effect, K_{ATP} channels are inhibited by intracellular ATP and activated by ADP[65,66]; therefore, a rise in ATP/ADP ratio results in channel closing and thus in cell depolarization.[66,67] K_{ATP} channels are formed by a tetramer of K_{ir}6.1 and/or K_{ir}6.2 subunits plus four subunits of sulfonylurea receptor (SUR1 and/or SUR2), a member of the ATP-binding cassette (ABC) family that confers a specific sensitivity to ATP/ADP variations.[65,68] The assemblage of all these subunits gives rise to an octameric channel, where K_{ir}6 subunits form the pore and are surrounded by SUR receptor subunits.[66,67]

The types and relevance of K_{ir} channels in spermatozoa remain largely unknown (Table 1). In rodents, patch-clamp analyses have demonstrated the presence of K_{ir} channels in the plasma membrane of differentiating and mature sperm cells.[52,69] K_{ir}3 subfamily is constituted by four members (Table 3): K_{ir}3.1 (KCNJ3 or GIRK1), K_{ir}3.2 (KCNJ6 or GIRK2), K_{ir}3.3 (KCNJ9 or GIRK3), and K_{ir}3.4 (KCNJ5).[19] K_{ir}3 are G-protein-gated, inwardly rectifying potassium channels (GIRK), which assemble forming either homo- or heterotetramers that regulate the membrane potential of several cell types.[42,55] In mouse germ cells, K_{ir}3.1 (KNCJ3/GIRK1) and K_{ir}3.2 (KCNJ6/GIRK2) form homotetrameric channels of around 40 kDa.[70] Also in this species, positive immunoreactivity of K_{ir}3.1 (KCNJ3/GIRK1) is observed in spermatogonia and primary spermatocytes, and in the connecting piece of epididymal and mature spermatozoa.[42] In reverse transcription qPCR analyses, *Kcnj3* transcripts were only detected in types A and B spermatogonia, but not in fully differentiated sperm cells, thus suggesting that gene transcription only takes place at early stages of spermatogenesis[70]; nevertheless, its specific role in cell proliferation has not been described. Immunolocalization approaches showed the presence of K_{ir}3.2 protein (KCNJ6/GIRK2) in the caudal manchette of mouse spermatids and in the acrosomal region of testicular and mature sperm; in contrast, *Kcnj6* transcripts are only found in spermatids.[70] In mammals, K_{ir}3.2 channels are involved in spermiogenesis, sperm capacitation, and acrosomal exocytosis,[70] and the activity of these channels during spermiogenesis is under the control of G-protein, Na^+, and $PI(4,5)P_2$.[71] Yet, *Kcnj6* null mice are fertile, maybe because of a compensatory effect of other GIRK channels.[70] In pig spermatozoa, whereas K_{ir}3.2 channels are immunolocalized in the acrosomal region and have a molecular weight of 39 kDa, the amount of K_{ir}3.3 proteins is low and the immunolocalization is restricted to the midpiece; in contrast, K_{ir}3.1 subunits have not been identified in the spermatozoa of this species.[70]

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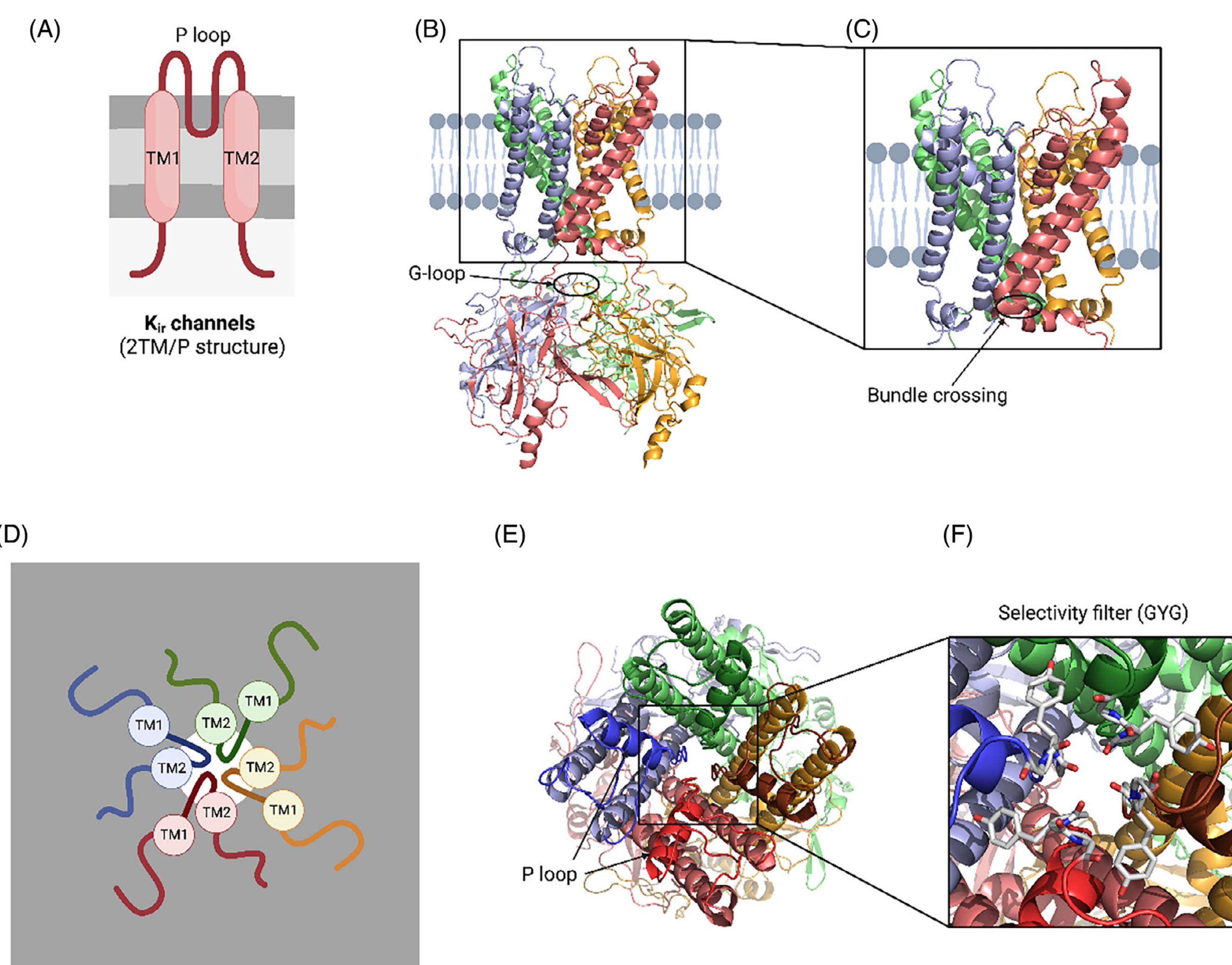

FIGURE 4 Structural characteristics of inwardly rectifying K^+ channels (K_{ir}). (A) Inwardly rectifying K^+ channels present a basic structure that consists of two transmembrane segments linked by a connecting P-loop. (B) Inwardly rectifying K^+ channels arrange in tetramers that present a large intracellular domain that controls the access to the transmembrane pore. Four cytoplasmic loops form a girdle around the central pore, the G-loop, which contributes to the modulation of inward rectification. (C) Transmembrane domains from different monomers overlap, and at the cytosolic end of the transmembrane pore, the TM2 segments cross to form a bundle crossing, which also contributes to the modulation of channel gating. (D) The tetrameric structure forms a central pore that involves TM2 segments from the different monomers. (E) P-loops (darker residues) are oriented toward the central pore. (F) Each P-loop presents a GYG sequence, which contributes to the selectivity filter (PDB reference 7ZDZ). TM: transmembrane segment.

In most tissues, $K_{ir}5.1$ controls K^+ fluxes during acid-basic changes, and regulates cellular processes that are pH dependent.[15] Acidification of pH_i inhibits $K_{ir}5.1$ leading to plasma membrane depolarization, whereas pH_i alkalinization results in membrane hyperpolarization[72,73]; to note, these channels are insensitive to extracellular pH (pH_o).[15] $K_{ir}5.1$ can form homo- or heterotetramers by co-assembling with either $K_{ir}4.1$ or $K_{ir}4.2$ subunits; heterotetrameric channels have an increased pH_i sensitivity within the physiological range.[15] In rodents, patch-clamp and immunocytochemistry approaches showed the presence of $K_{ir}5.1$ (KCNJ16) channels in the plasma membrane of spermatogonia, spermatocytes, and spermatids, as well as throughout the cephalic and tail regions of sperm cells.[69,74] In murine spermatozoa, $K_{ir}5.1$ only forms homotetramers, as $K_{ir}5.1/K_{ir}4.1$ and $K_{ir}5.1/K_{ir}4.2$ heterotetramers are absent.[15] In contrast, the previous identification of *$K_{ir}4.1$* and *$K_{ir}4.2$* transcripts in human spermatozoa suggests the presence of $K_{ir}5.1/K_{ir}4.1$ and $K_{ir}5.1/K_{ir}4.2$ heterotetramers, although immunodetection of protein subunits has not been reported.[15] The physiological role of $K_{ir}5.1$ channels in differentiating mature germ cells is still unknown. In mice, deletion of *Kcnj16* is associated with smaller testes and a greater percentage of sperm cells with folded tails as compared with intact males,[15] thus suggesting a relevant role during spermatogenesis. Interestingly, $K_{ir}5.1$ channels are inactivated in mature spermatozoa stored in the epididymal cauda because of the slightly acidic pH_i (~6.8)[75]; in contrast, during sperm capacitation, pH_i alkalinization appears to increase $K_{ir}5.1$ activity, thus contributing to membrane hyperpolarization.[15]

Few studies based on patch-clamping and indirect immunofluorescence reported the presence of K_{ATP} channels in spermatogenic

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TABLE 4 Diversity of tandem pore domain potassium (K_{2P}) channels and the coding genes in mammals.

K_{2P} channel	Coding gene
K_{2P}1 or KCNK1	*Kcnk1/KCNK1*
K_{2P}2 or KCNK2	*Kcnk2/KCNK2*
K_{2P}3 or KCNK3	*Kcnk3/KCNK3*
K_{2P}4 or KCNJ4	*Kcnj4/KCNK4*
K_{2P}5 or KCNK5	*Kcnk5/KCNK5*
K_{2P}6 or KCNK6	*Kcnk6/KCNK6*
K_{2P}7 or KCNK7	*Kcnk7/KCNK7*
K_{2P}9 or KCNK9	*Kcnk9/KCNK9*
K_{2P}10 or KCNK10	*Kcnk10/KCNK10*
K_{2P}12 or KCNK12	*Kcnk12/KCNK12*
K_{2P}13 or KCNK13	*Kcnk13/KCNK13*
K_{2P}15 or KCNK15	*Kcnk15/KCNK15*
K_{2P}16 or KCNK16	*Kcnk16/KCNK16*
K_{2P}17 or KCNK17	*Kcnk17/KCNK17*
K_{2P}18 or KCNK18	*Kcnk18/KCNK18*

Abbreviation: KCNK, potassium channel subfamily K members (adapted from Kuang et al.[20]).

cells, despite being considered the most relevant K_{ir} channels during spermatogenesis.[68] K_{ir}6.2 and SUR2 monomers have been immunodetected in epididymal spermatozoa from rodents.[76] Patch-clamp and immunocytochemistry analyses also allowed for the identification of K_{ir}6.2 and SUR1 in the post-acrosomal region and the midpiece of mouse spermatozoa, whereas SUR2 was found to be distributed along the principal piece and, to a lesser extent, in the connecting piece and midpiece.[68] In this species, K_{ATP} is known to participate in plasma membrane hyperpolarization; during sperm capacitation, the increase in pH_i favors K_{ATP} opening, and the decrease in ATP content extends the opening time.[68] Moreover, K_{ATP} channel subunits have also been immunolocalized in Sertoli cells and Leydig cells.[77] In Sertoli cells, testosterone stimulus is associated to K_{ATP} closing and further depolarization of the plasma membrane.[78,79]

4 | TANDEM PORE DOMAIN POTASSIUM CHANNELS

K_{2P} family includes 15 different channel types (K_{2P}1–K_{2P}18), each encoded by a single gene (*Kcnk1–18*; Table 4), which are abundant in both excitable and non-excitable cells.[20] In contrast to other types of K^+ channels, K_{2P} are usually opened with the main gating site being in the extracellular domain.[80,81] The channel activity is under control of several mediators, including ions, pH_i, osmolytes, lipids, and proteins.[19,82] Under physiological conditions, K_{2P} opening does not rely upon plasma membrane potential,[82] but rather depends on its stretch and fatty acid content.[19] In spite of this, and as far as authors are aware, the effect of fatty acid composition of the plasma membrane on channel function has not been interrogated.

In mammals, all types of K_{2P} channels present a dimeric structure, forming either homo- or heterodimers; each subunit has four transmembrane α-helix segments (TM1–TM4), two P-loops (P1 and P2) between TM1 and TM2 and between TM3 and TM4, respectively, and a short N- and a long C-termini located in the cytosol (Figure 5).[81,83–85] The P1 is a large extracellular loop that forms an overhead cap structure, whereas the inner N- and C-termini contain different motifs that can regulate channel activity by phosphorylation and protein interaction.[85] Dimerization requires the interaction between TM1 and P1 domains of both subunits, and gives rise to channels with a single and central K^+ selective pore.[81,83]

The presence and distribution of K_{2P} channels in germ cells has been scarcely studied (Table 1). In mice, *K2p9* transcripts were detected in post-meiotic germ cells using microarrays,[86,87] and immunostaining allowed for the detection of K_{2P}9 subunits in the connecting piece and midpiece of mature spermatozoa.[41] Abundance of K^+ channels in the connecting piece supports the hypothesis that the cytoplasmic droplet has an essential role in fluid exchange and volume regulation during sperm maturation and ejaculation.[41,88]

TABLE 5 Diversity of ligand-gated potassium (K_{ligand}) channels and the coding genes in mammals.

K_{ligand} channel	Coding gene
K_{Ca}1.1 or KCNMA1 or SLO1 or BK or MaxiK	*Kcnma1/KCNMA1*
K_{Ca}2.1 or KCNN1 or SK_{Ca}1	*Kcnn1/KCNN1*
K_{Ca}2.2 or KCNN2 or SK_{Ca}2	*Kcnn2/KCNN2*
K_{Ca}2.3 or KCNN3 or SK_{Ca}3	*Kcnn3/KCNN3*
K_{Ca}3.1 or KCNN4 or IK_{Ca}1	*Kcnn4/KCNN4*
K_{Ca}5.1 or KCNU1 or SLO3	*Kcnu1/KCNU1*
K_{Na}1.1 or KCNT1 or SLO2.2 or Slack	*Kcnt1/KCNT1*
K_{Na}1.2 or KCNT2 or SLO2.1 or Slick	*Kcnt2/KCNT2*

Abbreviations: BK, big potassium channel; K_{Ca}, Ca^{2+}-dependent potassium channel; KCNMA1, potassium calcium-activated channel subfamily M member 1; KCNN, potassium calcium-activated channel subfamily N members; KCNT, potassium calcium-activated channel subfamily T members; KCNU1, potassium calcium-activated channel subfamily U member 1; K_{Na}, Na^+-dependent potassium channel (adapted from González et al.,[19] Kaczmarek et al.,[13] and Vicens et al.[89]).

5 | LIGAND-GATED POTASSIUM CHANNELS

K_{ligand} channels can be either Ca^{2+}-dependent (K_{Ca}) or Na^+-dependent (K_{Na}), and their conductance large, intermediate, or small.[13,89] K_{Ca} channels are grouped into three different families (Table 5): (i) K_{Ca}1 family, which includes a single member (K_{Ca}1.1, SLO1, BK or MaxiK channel) encoded by *KCNMA1*; it is a large conductance channel and not only is sensitive to Ca^{2+} but it also depends on membrane voltage; (ii) K_{Ca}2 family, which includes three small conductance channels (K_{Ca}2.1 or SK_{Ca}1, K_{Ca}2.2 or SK_{Ca}2, and K_{Ca}2.3 or SK_{Ca}3), encoded by

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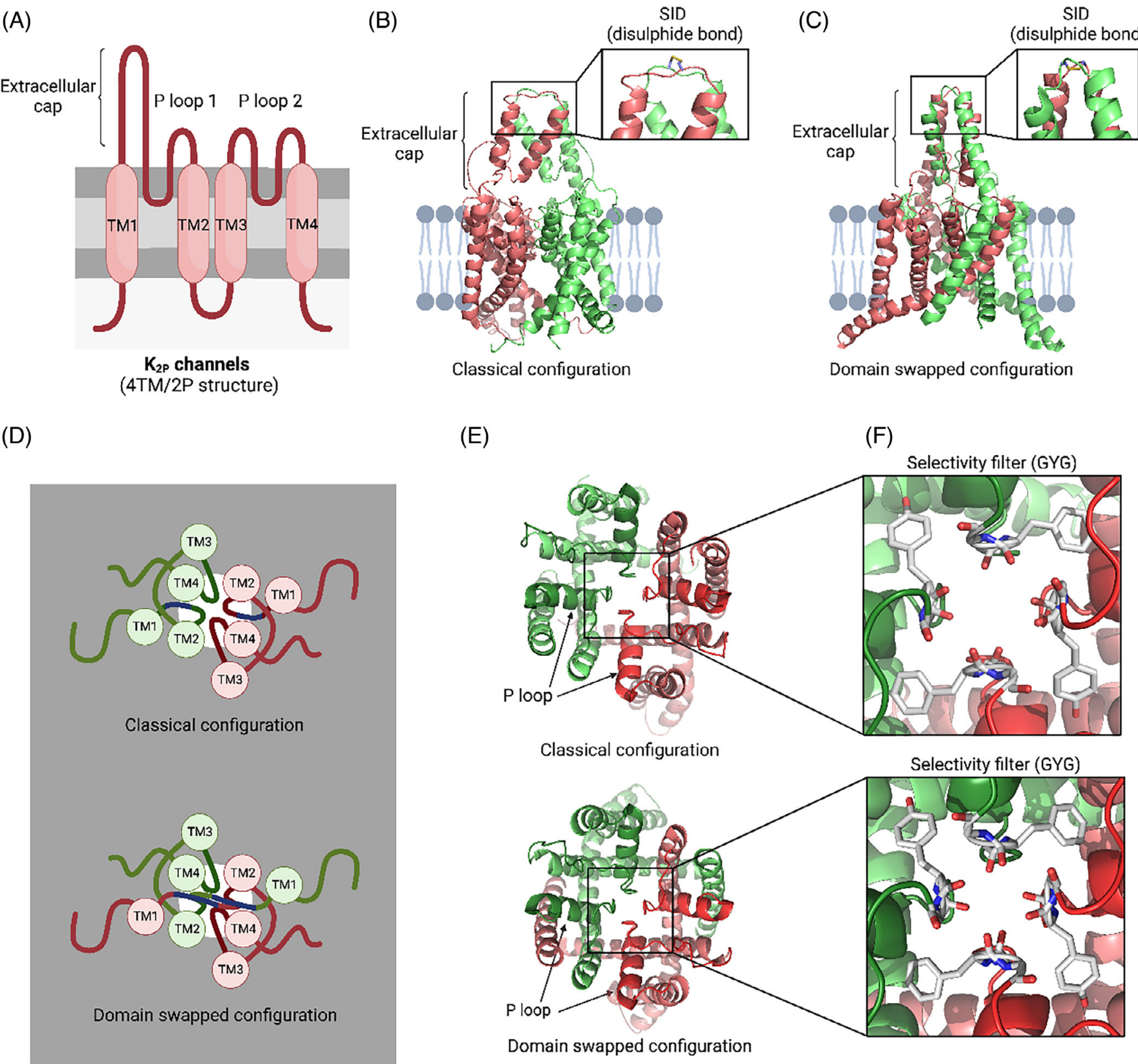

FIGURE 5 Structural characteristics of tandem pore domain K^+ channels (K_{2P}). (A) Tandem pore domain K^+ channels present two P-loops. The linker between the TM1 and the first P-loop is prominent, and forms the extracellular cap. (B and C) Tandem pore domain K^+ channels arrange in dimers. Self-interacting-domain (SID) allows dimerization through the formation of disulfide bonds between cysteine residues (Cys69) that are present at the extracellular cap. Two different conformations of the TM segments have been identified: the classical configuration (B), and the domain-swapped configuration (C). (D) The tetrameric structure forms a central pore that involves TM2 and TM4, whereas TM1 and TM3 are not directly involved in the structure of the central pore. In the classical configuration, TM segments from one monomer do not overlap with the other, and the outer helix from each TM1-P-loop 1 linker interacts with the inner helix from the same monomer. Meanwhile, in the domain-swapped configuration, the TM1 segments present swapped positions, and the outer helix from each TM1-P-loop 1 linker interacts with the inner helix from the other monomer. P-loops (darker residues) are oriented toward the central pore. The SID (blue residues) is present at the TM1-P-loop 1 linker, in the extracellular cap. (E) P-loops (darker residues) are oriented toward the central pore. (F) Each P-loop presents a GYG sequence that contributes to the selectivity filter (PDB references 3UM7 [classical configuration] and 4BW5 [swapped domain configuration]). TM: transmembrane segment.

KCNN1-KCNN3 genes that are insensitive to membrane voltage, and the $K_{Ca}3.1$, SK4, or $IK_{Ca}1$ channel, of intermediate conductance and encoded by *KCNN4*; and (iii) $K_{Ca}5$ family, formed by a single channel ($K_{Ca}5.1$, KCNU1, or SLO3) encoded by *KCNU1* gene, which is voltage- and pH-dependent and presents high conductance.[13,19,89] To date, two different K_{Na} channels have been identified (Table 5): $K_{Na}1.1$, Slack or SLO2.2; and $K_{Na}1.2$, Slick or SLO2.1, encoded by *KCNT1* and *KCNT2*, respectively; the regulation of these large conductance channels does not only rely on Na^+ inner levels, but also on intracellular Cl^- levels.[13,89] It is important to highlight that in the literature,

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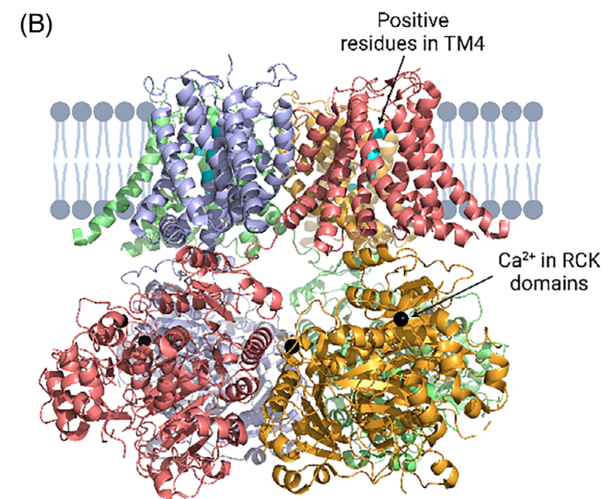
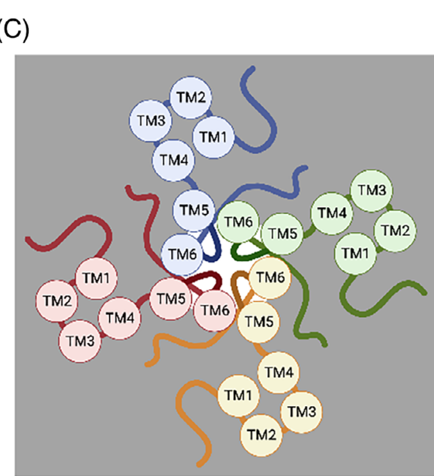
(A)
P loop
TM0 TM1 TM2 TM3 TM4 TM5 TM6
S7 RCK1 S8
S9 RCK2 S10





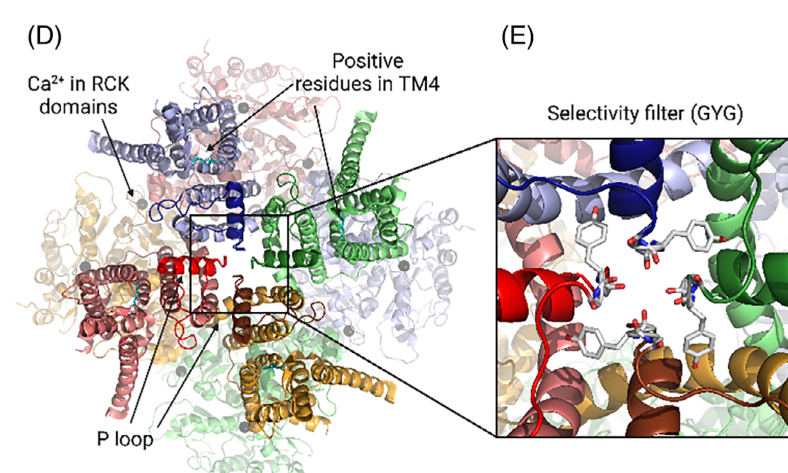


FIGURE 6 Structural characteristics of ligand-gated K^+ channels (K_{ligand}). (A) Ligand-gated K^+ channels present an additional TM segment (TM0). The transmembrane segment TM4 presents a series of positively charged residues that form a voltage-sensing domain (VSD). The loop between TM4 and TM5 is the inactivation-ball-binding loop. They also present a large C-terminal domain that presents binding sites for their ligands. In this case, a calcium-gated K^+ channel (SLO1) is represented, which presents two regulators of K^+ conductance (RCK) domains, each of them formed by two segments (S). (B) Ligand-gated K^+ channels arrange in tetramers. Positively charged residues from the VSD in TM4 are highlighted (light blue). Each RCK domain from calcium-gated K^+ channels present a binding site for calcium. (C) Transmembrane domains from different monomers overlap. Darker residues highlight the TM4–5 connecting loop. (D) The tetrameric structure forms a central pore that involves TM5–6 of each monomer, whereas TM1–4 are not directly involved in the structure of the central pore. (E) P-loops (darker residues) are oriented toward the central pore, and the VSD in TM4 (light blue) is found outside of the pore. (F) Each P-loop presents a GYG sequence, which contributes to the selectivity filter (PDB reference 6V38). TM: transmembrane segment.

K_{Na} channels are often classified erroneously as $K_{Ca}4.1$ and $K_{Ca}4.2$. According to Kaczmarek,[90] the term Slack means "Sequence like a calcium-activated K^+ channel," and Slick means "Sequence like an intermediate conductance K^+ channel."

K_{ligand} channels have great structural similarities with Kv, and they arise from the assemblage of four α-subunits forming either homo- or heterotretamers; active channels also contain auxiliary β- and γ-subunits.[91–93] α-Subunits can have either two or six TM domains, or even additional TMs, and an extensive C-terminus domain that acts as a binding site for different intracellular factors that modulate channel gating (Figure 6).[20,94–96] The active sites of the cytosolic domain differ between α-subunits and so its properties for interacting with different regulatory signals; in most channels, not only do cytosolic domains sense the variations in ligand levels, but they can also act as receptor centers for different messengers, including cAMP,[29] $NADP^+$,[97] and ATP.[90] K_{Ca} channels participate in many biological processes including generation of action potentials, modulation of the tone of blood vessels, and release of hormones and neurotransmitters.[98,99] In the central nervous system, K_{Ca} can co-assemble with multiple types of Ca_v channels to form macromolecular complexes that regulate and

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modify K_{Ca} activity.[98,100] β-Subunits of K_{Ca} channels have two transmembrane segments connected by an extracellular loop; the N- and C-termini are placed in the cytoplasm.[22] These subunits increase the Ca^{2+} sensitivity, and can either increase or decrease the voltage dependence of K_{Ca} channels; moreover, it has also been suggested that they participate in K_{Ca} channel trafficking toward the plasma membrane.[22]

5.1 | Calcium-gated potassium channels (K_{Ca} channels)

SLO1 (K_{Ca}1.1) and SLO3 (K_{Ca}5.1) are considered the main K_{Ca} channels regulating K^+ currents in spermatozoa, and hence the inner osmolality and membrane potential.[101] Both channels show marked functional differences, but they are evolutionary related arising from the duplication of an ancestral *SLO* gene.[102] SLO1 channels are highly conserved, widely expressed Ca^{2+}-activated channels that participate in excitatory signals in both neural and non-neural cells; nevertheless, differences exist between cell types in the kinetic behavior, pharmacological sensitivity, and regulation mechanisms of SLO1.[89,103] As K_{Ca}1.1 subunits are encoded by a single gene, differences between cells regarding SLO1 are related to both alternative splicing of mRNA and association with specific auxiliary subunits.[13,104] In contrast, SLO3 channels show a reduced Ca^{2+} sensitivity as compared with SLO1, which could be because of a fast evolution of Ca^{2+}-binding sites; they express mainly in differentiating and mature sperm cells and play important roles in sperm physiology and male fertility.[17,105–108] Interestingly, gating properties of SLO3 channels vary between species, probably because of a rapid sequence divergence, mainly in the cytosolic domain that result in a great site diversity for intracellular sensors implicated in gating regulation.[89,107,109,110] Specific SLO3 gating has been reported in some mammalian species; in mice, SLO3 channels are activated by intracellular alkalinization,[111] whereas in humans they are triggered by both an increase in Ca^{2+} levels and alkaline pH_i.[45,110,112]

Functional K_{Ca}1.1, BK, or SLO1 channels arise from the assemblage of four α-subunits that usually associate with auxiliary β- and γ-subunits.[93] Each α-subunit consists of seven transmembrane domains, six canonical (TM1–TM6) and one additional (TM0) before TM1, and a P-loop between TM5 and TM6 (Figure 6).[19] TM0 domain contacts and interacts with VSDs (TM1–TM4), thus modulating the balance between resting and activated states of the channel.[113] The sensitivity to membrane voltage is conferred by charged residues of TM2, TM3, and especially, TM4 domains, which contain Arg or Lys residues at every third position that respond to changes in membrane voltage even at low intracellular Ca^{2+} levels[19,114]; moreover, TM1, TM2, and TM3 have an essential role in the physical association with auxiliary β-subunits.[19] Ca^{2+} binding to the eight RCK domains of a K_{Ca}1.1 tetramer results in a conformational change that extends to transmembrane domains through TM6.[13,19] Noticeably, activation by increased Ca^{2+} levels requires the shift of voltage dependence to progressively more negative potentials.[13] The N-terminus localizes outside the cell, and the large cytosolic C-terminus contains two regulators of the conductance of potassium (RCK1 and RCK2), which act as binding sites for Ca^{2+} and Mg^{2+} ions.[90,115–117] In the tetrameric structure, the C-termini assemble forming a gating ring.[1]

SLO1 channels also contain auxiliary β- and γ-subunits, which regulate their gating.[118] β-Subunits (β1–β4), encoded by four different genes (*KCNMB1–KCNMB4*), locate between the VSD of two adjacent α-subunits[118,119]; this association between α- and β-subunits increases the sensitivity of the channel to Ca^{2+}.[19] Each β-subunit has two transmembrane domains (TM1 and TM2); the N-terminus contains a short N-loop that precedes TM1, whereas the short C-terminus extends beyond TM2.[119] Gamma-subunits belong to the family of leucine-rich repeat containing membrane proteins (LRRC), which have an extracellular N-terminal domain with a signal sequence, a single transmembrane segment, and a short C-cytosolic domain with 17—24 amino acid residues.[37,104] Different LRRC proteins modulate SLO1 sensitivity to membrane potential and Ca^{2+}, mainly LRRC26, LRRC52, LRRC55, LRTM1, and LRTM2.[120] Moreover, it has been reported that LINGO1, which belongs to the subfamily of leucine-rich repeat and Ig domain-containing proteins (LINGO1–LINGO4), can also act as an auxiliary subunit that mediates fast SLO1 current inactivation.[121] The stoichiometry is variable, so the number of β- and γ-subunits associated to a single SLO1 channel can vary from one to four.[118,122]

In rats, SLO1 channels form a 122 kDa band, and localize mainly in primary spermatocytes and to a lesser extent in spermatogonia as evidenced by patch-clamping and immunocytochemistry; in contrast, spermatids and mature spermatozoa are devoid of these channels.[43] As previously indicated, the composition of Kv and K_{ir} channels also differs between differentiating germ cells; these differences in potassium channel content suggest that cell proliferation and differentiation during spermatogenesis is associated with changes in the plasma membrane potential of germ cells.[43] In human spermatozoa, SLO1 has a molecular weight of 110—130 kDa and, as immunostaining revealed, localizes in the principal piece close to CatSper channels, both channel types being functionally related to K^+ and Ca^{2+} conductance through the sperm tail.[123,124] In pigs, immunofluorescence demonstrated that SLO1 channels (80 kDa) are widely distributed throughout the sperm tail and the post-acrosomal region, and they seem to be implicated in the mobilization of inner Ca^{2+} stores of the post-acrosomal region during sperm capacitation.[125] In both human[126] and pig spermatozoa,[125,127] K^+ conductance throughout the sperm flagellum does not only rely on SLO1, but also on other K^+ channels. In Leydig cells, SLO1 channels exert a relevant role in steroidogenesis, favoring K^+ efflux and plasma membrane hyperpolarization upon luteinizing hormone (LH) stimulus.[128]

K_{Ca}2 and K_{Ca}3 α-subunits contain six α-helix transmembrane domains (TM1–TM6); the pore-forming domain localizes in TM5 and TM6, both segments being joined by a P-loop; intracellular N- and C-termini account for channel diversity.[19] The TM4 domain has few positively charged residues, which explains why these channels are insensitive to changes of membrane potential.[13] K_{Ca}2 and K_{Ca}3 channels associate constitutively to calmodulin, which confers them a great sensitivity to small changes in intracellular Ca^{2+} levels[129]; the calmodulin-binding domain localizes adjacent to TM6.[19] Moreover, K_{Ca}2.2 and K_{Ca}2.3 channels are part of a multiprotein complex formed

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by calmodulin, casein kinase 2, and protein phosphatase 2A.[130,131] Casein kinase 2 can phosphorylate threonine residues of calmodulin, thus reducing Ca^{2+} sensitivity and inducing a fast channel closing after a transient increase of intracellular Ca^{2+} levels; the activation of protein phosphatase 2A reverses this effect.[130,131] While calmodulin has an essential regulatory role during sperm capacitation (reviewed by Delgado-Bermúdez et al.[2]), the presence of $K_{Ca}2$ and $K_{Ca}3$ in differentiating and mature sperm cells has not, to the best of the authors' knowledge, been reported.

The $K_{Ca}5$ family is constituted of a single channel type ($K_{Ca}5.1$, KCNU1, or SLO3) encoded by the *KCNU1* gene. Despite purportedly being present exclusively in the testis and sperm cells of mammals,[92,105,126] recent evidence demonstrated the presence of SLO3 in fish, bird, and reptile testis,[102] and in rat brain, kidney, and eye.[132] $K_{Ca}5.1$ is a voltage-dependent, large-conductance channel that shows a high homology of sequence and structure with $K_{Ca}1.1$[92]; moreover, it is also pH sensitive, being activated by alkalinization and suppressed by acidification.[17,111,133]

$K_{Ca}5.1$ α-subunits contain seven transmembrane segments (TM0–TM6) and an extracellular N-terminus with a voltage sensor; the residues from 330 to 1062 of the C-terminus are implicated in the formation of the gating ring, which include the regulators RCK1 and RCK2.[92,134] Differences in the sequence and length of amino acid residues in the RCK1 domain have been suggested to explain the disparities in voltage sensitivity and kinetics of SLO3 between mouse and bovine spermatozoa,[19,109] whereas variations in RCK2 could be related to differences in the active sites of cytosolic domains.[95] Moreover, adaptive mutations in the *RCK2* domain have been hypothesized to underlie the differences between species in terms of the pH_i sensitivity of SLO3 channels.[89] In mouse, the mutant SLO3 protein resulting from the substitution of His by Arg at position 715 (H715R) has low stability and reduced ability to interact with other proteins because of the changes in hydrogen-binding networks.[108] In contrast, the variant allele *C382R*, which substitutes a Cys by an Arg, increases the sensitivity of SLO3 to both inner Ca^{2+} and pH_i.[110] Moreover, mouse SLO3 has high affinity to PI(4,5)P2, which upregulates its activity.[135] Interestingly, SLO3 can associate with the β-subunit BKβ4 or KCNMB4[91] and γ-subunits LRRC52 and LRRC56 in spermatogenic mouse cells.[1] Specifically, LRRC52 is a testis-specific subunit that induces SLO3 gating, and whose expression is strongly dependent on the presence of SLO3 in the plasma membrane.[91,110]

Whereas SLO3 channels in mouse spermatozoa have a molecular weight of 120 kDa and show an intense K^+ conductance in the principal piece,[105,109,110,136] they are a little bit heavier (130–140 kDa) in human spermatozoa where, as immunostaining indicates, they localize throughout the sperm tail (130–140 kDa), especially in the midpiece.[108,134] In humans, SLO1 and SLO3 can assemble forming homo- and heterotetramers, thus increasing the functional and regulation diversity of these channels.[45] In mouse, however, SLO3 is considered as the main K^+ channel involved in KSper current during sperm capacitation; in effect, the activation of $K_{Ca}5.1$ by the alkaline environment in the oviduct around ovulation leads to sperm membrane hyperpolarization prior to fertilization.[13,108,137] Interestingly, K^+ permeability of SLO3 increases three times during capacitation of spermatozoa, whereas Na^+ and Cl^- currents remain nearly invariable.[138] In spite of this, not only does SLO3 gating during sperm capacitation result in membrane hyperpolarization, but also in the activation of both sodium/proton exchangers (NHE), thus favoring pH_i alkalinization,[139,140] and CatSper channels, which are implicated in the rise of cytosolic Ca^{2+} levels.[139] This involvement of K^+ channels in the control of pH_i has also been described in somatic cells.[141] It is worth noting that pH_i alkalinization and CatSper activation appears to differ between humans and mice; in humans, the pH_i increase during sperm capacitation requiring the gating of both NHE and HCVN1 channels.[142]

In rodents, mutations or absence of the *Kcnu1* gene is associated with the male infertility; therefore, spermatozoa from *Kcnu1* null mice show impaired progressive motility and defective membrane hyperpolarization, which lead to an abnormal inactivation of CatSper and other voltage-gated channels, thus resulting in defective acrosome exocytosis.[105,126] *Slo3*-deficient mice also show aberrant sperm morphology, probably because of the impaired osmotic homeostasis.[105] Even *Lrrc52* knockout mice are sub-fertile, probably because the absence of LRRC52 γ-subunits entails that SLO3 channels require higher positive voltages and pH_i to induce KSper currents.[137] In rodents, the disturbances in SLO3 activity are poorly compensated by other K^+ channels.[15] In contrast to what is found in mice where SLO3 are the main channels implicated in KSper currents; Kv, SLO1, and SLO3 are involved in sperm physiology in humans.[4,8,108] Thus, infertile patients with intact *Kcnu1* and *Kcnma1* genes can show alterations in the KSper current.[18] On the other hand, *Kcnu1* mutations have little impact on sperm quality, notwithstanding they may result in male sub-fertility because of a defective hyperpolarization of the plasma membrane in capacitated spermatozoa,[133] which in turn entails an inability to interact with the oocyte.[108,126,136] Interestingly, male *Kcnu1* mutations do not affect embryo development in mice or humans after ICSI treatment,[108,134] thus highlighting the relevance of the interaction between spermatozoa and oocyte plasma membranes during fertilization. Finally, scientific evidence indicates that $K_{Ca}5.1$ channels are functionally different between mouse and human spermatozoa, and that in humans they are more sensitive to internal Ca^{2+} levels and less to pH_i than in rodents.[45,92,106,110,112] In humans, it has been suggested that the main K^+ channel implicated in K^+ flux is SLO1 ($K_{Ca}1.1$).[4,123] In pigs, different pharmacological assays suggested that sperm capacitation and acrosome exocytosis require the activation of separate K^+ channels, including K_{Ca}, Kv, and K_{2P}.[125,127]

5.2 | Sodium-gated potassium channels (K_{Na} channels)

$K_{Na}1.1$ channels (SLO2.2) are activated by changes in Na^+ and Cl^- concentrations.[94,96,143] α-Subunits have six transmembrane domains (TM1–TM6); the TM4 domain does not contain the repeated basic amino acid motif characteristic of $K_{Ca}1.1$, so that $K_{Na}1.1$ has a reduced sensitivity to voltage changes.[144] The cytoplasmic C-terminus also has two RCK domains (RCK1 and RCK2); in the tetrameric structure,

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constriction of all RCK domains forms a cytoplasmic gating ring that closes the channel pore.[143] RCK domains can be activated by PI(4,5)P2[145] and nicotinamide adenine dinucleotide (NAD),[146] whereas ATP induces the closing of the channel.[19] RCK2 domain constitutes the binding site of Na^+ ions, and its mutations are associated with a decrease in channel sensitivity to Na^+.[147] The activity of $K_{Na}1.1$ channels can also be regulated by different proteins, such as protein kinase C, which phosphorylates serine residues of the linker region between TM6 and RCK domains, thus resulting in an increased K^+ current amplitude,[148] and the fragile X mental retardation protein (FMRP), which interacts with the C-domain thus increasing the opening time.[149–151]

$K_{Na}1.1$ isoforms arise from alternative splicing of RNA; one of the best known is the Slack-B isoform, which has a long cytoplasmic N-terminal domain and associates with $K_{Na}1.2$, thus forming heteromers.[13] Slack-B can also interact with $K_{Ca}1.1$ proteins, thereby providing new channels with different functional properties.[13] $K_{Na}1.1$ channels are expressed in the central and peripheral nervous systems, as well as in the testis and kidney.[152–154] The specific localization of these channels in proliferating and differentiating sperm cells is, nevertheless, unknown. On the other hand, $K_{Na}1.2$ channels are very close to $K_{Na}1.1$, as sequence homology is 74% with major differences being observed in the C-terminus.[94] In contrast to $K_{Na}1.1$, $K_{Na}1.2$ channels are sensitive to small changes in cell volume, being strongly gated by cell swelling and inhibited by shrinkage.[155] In addition, $K_{Na}1.2$ channels are widely distributed throughout the nervous system and in a variety of non-neuronal cells like the cardiac ones[94,96,154,156]; yet their presence in immature and mature sperm cells has not been reported.

6 | CONCLUSIONS

KSper currents are essential for male fertility, as they are involved in germ cell differentiation and maturation, and in sperm capacitation. Little data, nonetheless, exist on the types of K^+ channels present in male germ cells, and most of the studies are conducted in rodents and humans, the results being non-comparable between these two species. Scientific evidence shows the physiological relevance of Kv and K_{ir} channels in the regulation of ionic balance, membrane potential, and cell volume during spermatogenesis; epididymal maturation; and sperm capacitation. Interestingly, the types of Kv and K_{ir} channels differ between differentiating and mature sperm cells; however, the specific role of each channel type remains unknown, as well as the physiological relevance and functional relationship between them. Remarkably, most studies of K^+ channels are focused on the role of SLO1 and SLO3 during sperm capacitation. In rodents, SLO3 is essential for K^+ outflux and membrane hyperpolarization, whereas in humans and pigs, K^+ conductance is mediated by Kv, SLO1, and SLO3. Considering the relevance of K^+ conductance for male fertility, further research to identify the types of K^+ channels present in differentiating and mature sperm cells, and their physiological role is much warranted.

AUTHOR CONTRIBUTIONS

Elisabeth Pinart conceived the review article, prepared tables, and wrote the original draft. Ariadna Delgado-Bermúdez drew the figures. Marc Yeste and Sergi Bonet made a critical revision of the manuscript. All authors approved the submitted version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest regarding this review article.

DATA AVAILABILITY STATEMENT

There are no new data associated with this article.

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