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Ca²⁺ homeostasis and male fertility: a target for a new male contraceptive system

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ABSTRACT

Ca²⁺ is a key secondary messenger that determines sperm motility patterns. Mammalian sperm undergo capacitation, a process to acquire fertilizing ability, in the female reproductive tract. Capacitated sperm change their flagellar waveform to develop hyperactivated motility, which is crucial for successful sperm navigation to the eggs and fertilization. The sperm-specific channel, CATSPER, and an ATPase transporter, PMCA4, serve as major paths for Ca²⁺ influx and efflux, respectively, in sperm. The ionic paths coordinate Ca²⁺ homeostasis in the sperm, and their loss-of-function impairs sperm motility, to cause male infertility. In this review, we summarize the physiological significance of these two Ca²⁺ gates and suggest their potential applications in novel male contraceptives.

Introduction

In mammals, ejaculated sperm migrate toward the fertilizing site along the female reproductive tract. They sense environmental factors to acquire the fertilizing ability during their journey to the eggs in a process termed capacitation (Austin 1951; Chang 1951). Capacitated sperm develop a unique motility pattern called hyperactivated motility, which is characterized by the asymmetric beating of their flagella with increased amplitude (Figure 1; Suarez et al. 1993; Yanagimachi 1969). This hyperactivated motility enables sperm to pass through a luminal structure filled with a viscous fluid such as the utero-tubal junction (UTJ) to successfully reach the fertilizing site, the ampulla (Chung et al. 2014; DeMott et al. 1995). In addition, sperm hyperactivation is crucial to penetrate the glycoprotein barrier of eggs, the zona pellucida, for successful fertilization (Ren et al. 2001; Stauss et al. 1995). Therefore, defective hyperactivated motility impairs male fertility in mammals.

Environmental factors in the female reproductive tract trigger Ca²⁺ signaling pathways in sperm to develop hyperactivated motility. Albumin in the female reproductive tract causes cholesterol efflux from the sperm membrane (Aitken and Nixon 2013), which enhances sperm capacitation by altering membrane fluidity and making membrane proteins ready to function. In addition, high levels of bicarbonate and increasing pH condition toward the fertilizing site alkalinize

sperm intracellular pH (pH$_i$) in the female reproductive tract (Lishko et al. 2012; Vyklicka and Lishko 2020). These upstream events coordinate the introduction of Ca²⁺ into sperm cells via the sperm-specific Ca²⁺ channel, CATSPER, during sperm capacitation (Hwang and Chung 2023). Simultaneously, PMCA4, a Ca²⁺ ATPase pump that extrudes intracellular Ca²⁺ from sperm, is crucial for normal and hyperactivated sperm motility (Withers et al. 2006). Previous studies have demonstrated that genetic alterations in the genes encoding CATSPER subunits or PMCA4 result in male infertility with defective sperm hyperactivation in mammals (Wang et al. 2021). The importance of these two Ca²⁺ paths – CATSPER and PMCA4 – which transport Ca²⁺ in opposite directions, highlights their coordination in maintaining Ca²⁺ homeostasis, which is crucial for hyperactivated sperm motility and male fertility.

Around 50% of pregnancies are unintended globally (Bearak et al. 2020), which is another reproductive health issue together with infertility. Unintended pregnancy would result in negative outcomes including health problems and social issues like early termination of career and education for both men and women (Horvath and Schreiber 2017; Nickels and Yan 2024). In particular, over half of the unwanted pregnancies end in abortion (Nickels and Yan 2024), which strongly demands mixed contraception for successful family planning. Currently, women mainly bear the responsibility for
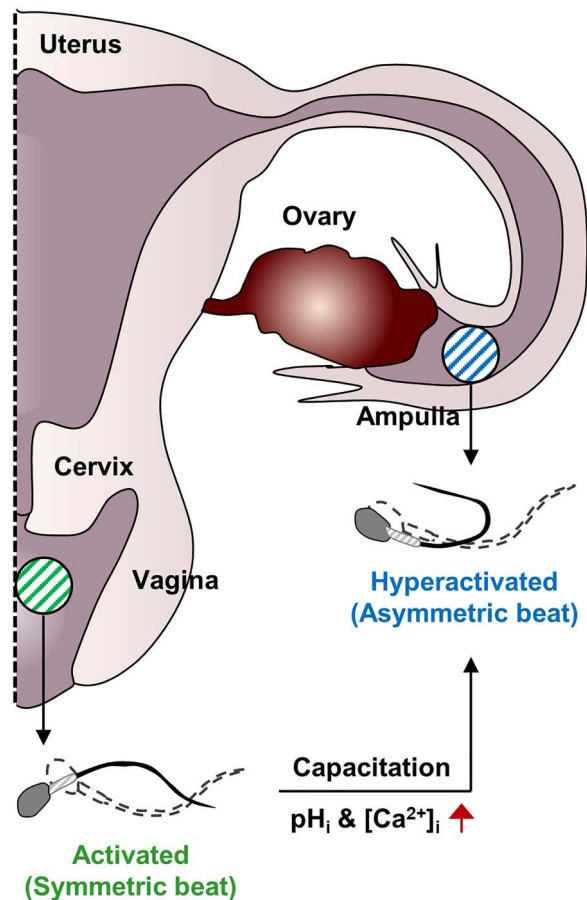
Figure 1. Mammalian sperm develop hyperactivated motility during their migration to the fertilizing site in the female reproductive tract. Ejaculated mammalian sperm initiate to swim (activated) with symmetrical flagellar beating. They start to migrate toward the fertilizing site, ampulla, and undergo the capacitation process, which provides fertilizing ability to sperm. During capacitation, intracellular pH (pH_i) and Ca^{2+} concentration ($[Ca^{2+}]_i$) are increased, enabling sperm to develop hyperactivated motility. Sperm can reach the ampulla, by developing this motility pattern with asymmetric flagellar beating in the female reproductive tract.

contraception, whereas men only rely on two methods of contraception: condom and vasectomy (Amory 2023). Given the accumulating knowledge on male reproduction at the molecular level, the demand for novel male contraceptive development continues to grow.

In this review, we introduce the current understanding for the CATSPER channel and PMCA4 to manage Ca^{2+} homeostasis and hyperactivated motility in mammalian sperm, and their potential application to the targets for male contraceptives.

Ca^{2+} signaling in sperm hyperactivation

In the female reproductive tract, mammalian sperm undergo changes in physiological characteristics to

acquire fertilizing ability, a process called capacitation (Austin 1951; Chang 1951). One of the most outstanding changes is that sperm start to beat their flagella asymmetrically with an increased amplitude (Yanagimachi 1969, 1970). This unique flagellar beating changes the sperm swimming pattern, so-called hyperactivated motility, giving them sufficient force to pass through the viscous environment (DeMott et al. 1995) and penetrate the glycoprotein barrier of the eggs, the zona pellucida (Stauss et al. 1995). Therefore, hyperactivated motility is essential for successful sperm navigation to the eggs and fertilization.

The development of hyperactivated motility is regulated by Ca^{2+} (Ho et al. 2002; Lindemann and Goltz 1988; Suarez et al. 1987; Yanagimachi 1982), which transduces cell signals in diverse systems. Previous studies have demonstrated that mammalian sperm fail to develop hyperactivated motility during in vitro capacitation in the absence of Ca^{2+} supplementation (Yanagimachi 1982). Mouse sperm treated with A23187, a Ca^{2+} ionophore, can develop hyperactivated motility (Suarez et al. 1987). In addition, the tail of demembranated rat and bull sperm, of which membrane is dissolved by mild non-ionic detergent, generate a huge curvature upon Ca^{2+} exposure (Ho et al. 2002; Lindemann and Goltz 1988). Furthermore, other studies have revealed that sperm developing hyperactivated motility have increased intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) both in vitro and in vivo (Babcock and Pfeiffer 1987; Suarez et al. 1993). All the results clearly demonstrate that Ca^{2+} is the key component to trigger hyperactivated motility in mammalian sperm.

Although now it is well known that $[Ca^{2+}]_i$ increase is essential for the development of hyperactivated motility in mammalian sperm, how the increased $[Ca^{2+}]_i$ regulates downstream signaling in capacitated sperm remains unclear. A few studies have demonstrated that defective Ca^{2+} increase potentiates PKA signaling and global tyrosine phosphorylation excessively (Chung et al. 2014; Navarrete et al. 2015). Although this negative correlation indicates that Ca^{2+} signaling could be linked to various phosphorylation mechanisms in capacitating sperm, how the post-translational modifications and Ca^{2+} signaling coordinate flagellar movement has not been comprehensively elucidated.

Gates for Ca^{2+} homeostasis in sperm

Extracellular Ca^{2+} in the female reproductive tract is the original source of increased $[Ca^{2+}]_i$ in sperm to develop hyperactivated motility. Sperm increase $[Ca^{2+}]_i$ by introducing extracellular Ca^{2+} via the Ca^{2+} entry path to develop hyperactivated motility. However, excessive

$[Ca^{2+}]_i$ could make sperm immotile transiently by inhibiting flagellar beating (Sanchez-Cardenas et al. 2018; Suarez et al. 1987). This means that continued Ca^{2+} influx and the resulting excessive $[Ca^{2+}]_i$ would prevent sperm from swimming and hyperactivation. Therefore, both the influx and efflux of Ca^{2+} in the sperm should be balanced to maintain a range of $[Ca^{2+}]_i$ for flagellar beating and hyperactivated motility.

Catsper – the primary Ca^{2+} entry path in sperm

Various ion channels such as voltage-gated channels and transient receptor potential (TRP) channels have been suggested as the major Ca^{2+} entry sites for sperm hyperactivation. However, currently, the sperm-specific Ca^{2+} channel, CATSPER, is known as the primary channel to introduce Ca^{2+} into mammalian sperm.

Molecular characteristics

The CATSPER channel is a multiprotein complex composed of at least 13 subunits (Figure 2(A); Hwang and Chung 2023). CATSPER1-4 are six transmembrane (TM) proteins that carry a voltage-sensing domain and form a heterotetrameric pore in a way reminiscent of voltage-gated K^+ channels (Qi et al. 2007; Quill et al. 2001; Ren et al. 2001). Interestingly, all pore subunits carry a coiled-coil domain at their C-terminus, which could be involved in the interaction within the subunits to form pore (Lobley et al. 2003). Four auxiliary subunits, CATSPERβ, γ, δ, and ε, are predicted to be type I TM proteins with a huge extracellular domain (Chung et al. 2011, 2017; Liu et al. 2007; Wang et al. 2009). Recently resolved atomic structure of the mouse CATSPER channel complex revealed that the four auxiliary subunits form a canopy structure above the pore in sperm (Lin et al. 2021; Zhao et al. 2022). Two proteins, CATSPERζ and EFCAB9, are cytosolic subunits which form a binary complex to modulate CATSPER channel activity as the pH-dependent Ca^{2+} sensor (Chung et al. 2017; Hwang et al. 2019). These 10 subunits are considered to form one functional CATSPER channel complex. Interestingly, recent cryo-electron microscopy (cryo-EM) studies have revealed that individual CATSPER channels link each other and arrange to zig-zag row (Lin et al. 2021; Zhao et al. 2022) to form quadrilinear CATSPER nanodomains along the principal piece of sperm tail (Figure 2(B,C); Chung et al. 2014; Hwang et al. 2019). Two newly identified TM subunits, CATSPERη and CATSPERθ, which were previously annotated to Tmem262 and Tmem249, respectively, are suggested to link each CATSPER channel (Huang et al. 2023; Lin et al. 2021). The last identified component is an anionic transporter Slco6c1 (Lin et al. 2021). This subunit is composed of 12 TMs with Kazal domain and is located on the side of CATSPERε as a wing-like structure in the complex (Figure 2(B)).

Physiological significance

After identifying the first subunit, the physiological significance of the CATSPER channel has been demonstrated in mice and humans. Currently, *Catsper1-*, *2-*, *3-*, *4-*, *d-*. *z-*, *q-*, and *Efcab9*-null mouse models have been reported (Chung et al. 2011, 2017; Huang et al. 2023; Hwang et al. 2019; Qi et al. 2007; Quill et al. 2003; Ren et al. 2001). Among them, male mice with the genetic alteration of TM subunits are 100% infertile. The males do not show defective spermatogenesis and their sperm are morphologically normal. However, sperm from the mouse models lacking TM subunits do not carry the functional CATSPER channel (Chung et al. 2011; Huang et al. 2023; Qi et al. 2007) and fail to develop hyperactivated motility. Therefore, sperm lacking the CATSPER channel are unable to swim against flow (rheotaxis), pass the UTJ, and penetrate zona pellucida (Miki and Clapham 2013; Ren et al. 2001). Sperm from the male mice lacking one of the TM subunits express the other subunits in the testes (Chung et al. 2017). However, the remaining subunits in the testes fail to assemble and to localize at the sperm tail, which results in the absence of entire CATSPER subunits in mature sperm (Chung et al. 2017; Hwang et al. 2019; Qi et al. 2007). This extremely strong interdependency within the subunits highlights that the CATSPER channel complex should be assembled precisely to traffic successfully to the mature sperm tail during germ cell development in mice.

By contrast, *Catsperz-* and *Efcab9*-null males are subfertile (Chung et al. 2017; Hwang et al. 2019). These two cytosolic subunits are interdependent and directly interact to form a binary complex in mature sperm. The absence of either CATSPERζ or EFCAB9 remains only ~20% of the functional CATSPER channel with fragmented arrangement along the tail. Interestingly, sperm lacking CATSPERζ-EFCAB9 have a tail with a rigid proximal region and fail to develop hyperactivated motility. This could be due to the altered Ca^{2+} entry into the sperm, which is reviewed below. Recent studies have revealed that a CATSPER-associated protein, CATSPERτ, manages flagellar targeting of the assembled CATSPER component (Hwang et al. 2022; Yang et al. 2022). Loss-of-function of the CATSPERτ does not affect CATSPER channel assembly. However, CATSPERτ-deficient sperm only have less than 10% of the functional CATSPER channel with the defective organization of the CATSPER nanodomain (Hwang et al. 2022). In line with
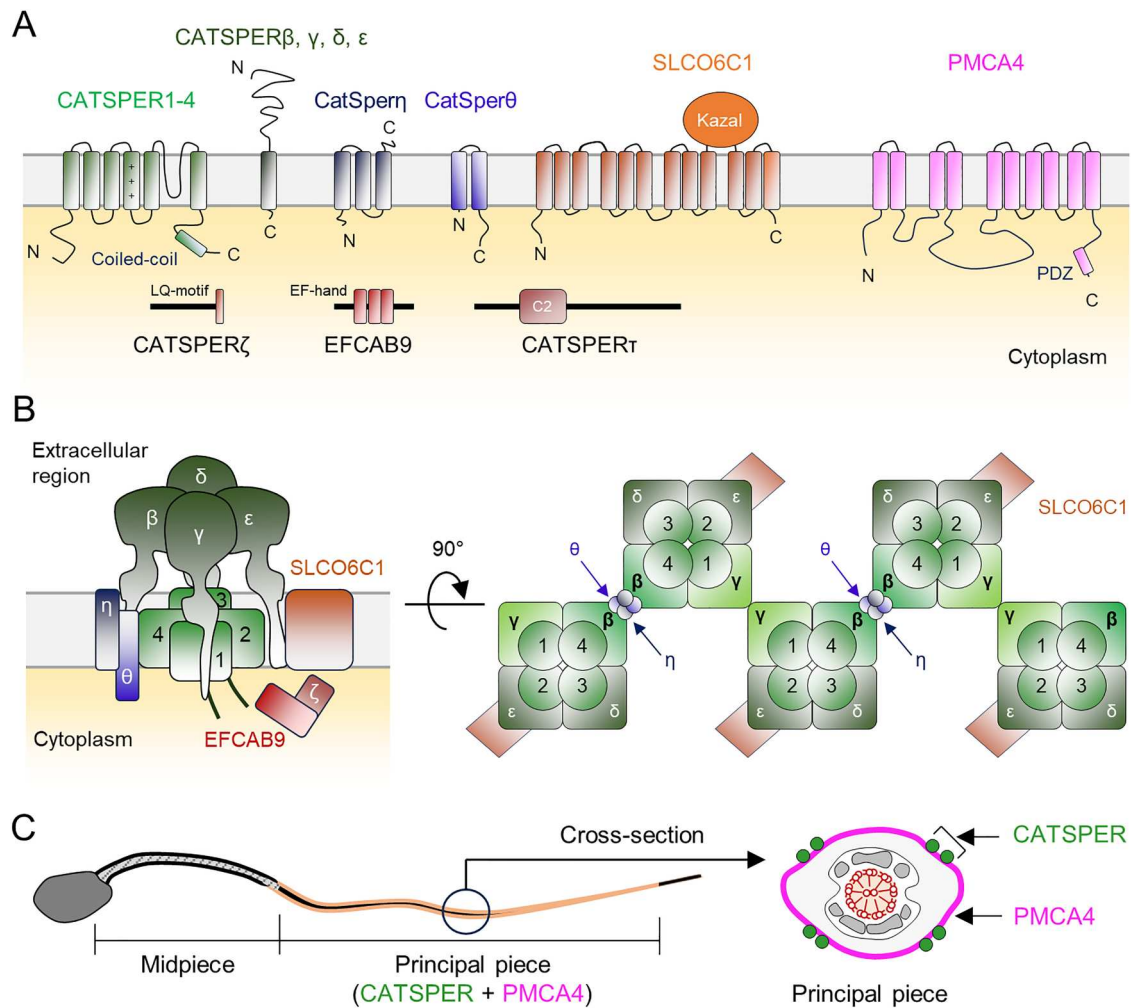
Figure 2. CATSPER channel and PMCA4 are the major Ca^{2+} paths localizing at the principal piece of sperm flagella. (A–B) CATSPER channel is composed of multiple subunits that are connected to form a unique nanodomain structure. (A) Topologies of transmembrane and cytosolic CATSPER subunits, a CATSPER-associated protein, CATSPERτ, and PMCA4. Functional domains are marked. (B) Assembled CATSPER channel complex (*left*) and its arrangement on the sperm tail (*right*). (C) CATSPER and PMCA4 localize at the principal piece of sperm flagella. Shown are top-down (*left*) and cross-section (*right*) views of the sperm tail. Two rows of CATSPER nanodomain (green) distribute quadrilaterally, which is distinguished from the PMCA4 distribution (magenta).

the studies, the CATSPER nanodomain structure is well preserved in sperm to develop hyperactivated motility (Chung et al. 2014) and to reach the fertilizing site (Ded et al. 2020). These results indicate that there could be a threshold amount of CATSPER channel for normal sperm hyperactivation, which also requires the intact quadrilinear nanodomain structure.

In humans, genetic aberrations in *CATSPER1*, *2*, and *E* have been reported (Avenarius et al. 2009; Avidan et al. 2003; Brown et al. 2018; Luo et al. 2019). Similar to the mouse models, all males with mutations of the *CATSPER* genes are infertile. Among them, unsuccessful Ca^{2+} entry and impaired hyperactivated motility of ejaculated sperm have been demonstrated in patients carrying a *CATSPER2* mutation (Schiffer et al. 2020; Smith et al. 2013). Despite the shared physiological

significance, a previous study reported that certain phenotypes are distinguished within humans and mice (Schiffer et al. 2020). In humans, *CATSPER2*-mutant sperm still possess other CATSPER subunits with the quadrilateral distribution along the principal piece, despite the absence of a functionally assembled CATSPER complex. In addition, the CATSPER-dependent rheotactic sperm swimming is not observed in humans. These differences suggest species-specific mechanisms for CATSPER trafficking and Ca^{2+} signaling to modulate sperm swimming.

Functional regulation

Like other voltage-gated channels, the heterotetrameric pore of the CATSPER channel carries voltage-sensing domains (Qi et al. 2007; Quill et al. 2001; Ren et al.

2001). However, the CATSPER is only weakly voltage-dependent. Interestingly, the voltage sensitivity is drastically increased by elevated pH_i in mouse sperm (Kirichok et al. 2006). Thus, intracellular alkalinization facilitates activation of the CATSPER channel in mouse sperm, which is supported by increasing $[Ca^{2+}]_i$ and pH_i both in capacitated sperm (Babcock and Pfeiffer 1987). Although the factor to activate CATSPER channel has been well demonstrated, the molecular mechanisms underlying the CATSPER activation have not been well understood. Recently, Hwang et al., reported that EFCAB9-CATSPERζ binary complex is the pH-dependent Ca^{2+} sensor for modulating CATSPER channel activity in mouse sperm (Hwang et al. 2019). Initially, EFCAB9 and CATSPERζ tightly bind each other, and the interaction presumably block the pore to prevent aberrant Ca^{2+} influx via the CATSPER channel in non-capacitated sperm with acidic pH_i. Then, in capacitating sperm, these two cytosolic subunits start to be dissociated by intracellular alkalinization, which might open the pore to introduce the extracellular Ca^{2+} into the sperm. Finally, the introduced Ca^{2+} could bind to the EF-hand domains of the EFCAB9 to further potentiate the CATSPER channel by its conformational change. Considering the mammalian-specific preservation of the CATSPERζ and its conserved interaction to EFCAB9 in marsupials (Hwang et al. 2021), the activation mechanism is expected to be conserved in mammals.

Intracellular alkalinization also potentiates CATSPER channel in human sperm (Lishko et al. 2010). However, the CATSPER requires steroidal hormones, progesterone and prostaglandin, to be fully activated in human sperm (Lishko et al. 2011; Strünker et al. 2011). This ligand-mediated CATSPER activation is also supported by unsuccessful activation of CATSPER in sperm from CATSPER2-mutant patients (Schiffer et al. 2020; Smith et al. 2013; Young et al. 2024). The difference between human and mouse sperm could be originated from sequence variability of the CATSPER components and/or from species-specific ligand receptors, which are functionally linked to the CATSPER channel, such as α/β hydrolase domain-containing protein 2 (ABHD2) in humans (Miller et al. 2016). The different factors and underlying mechanisms of CATSPER activation in different species need to be understood further (Hwang 2023b).

Pmca4 – the Ca^{2+} extrusion path in sperm

Ca^{2+} influx is the key trigger to develop hyperactivated motility in mammalian sperm. However, treating a Ca^{2+} ionophore, A23187, which introduces excessive extracellular Ca^{2+} into sperm, suppresses their flagellar beating and swimming (Sanchez-Cardenas et al. 2018; Suarez et al. 1987). This indicates that the threshold range of $[Ca^{2+}]_i$ would be crucial for both activated and hyperactivated motility in sperm. Currently, a plasma membrane Ca^{2+}-ATPase pump, PMCA4, is known for the opposite path to the CATSPER channel by extruding Ca^{2+} from the cytoplasm to maintain Ca^{2+} homeostasis in mammalian sperm (Figure 2(A); Okunade et al. 2004; Schuh et al. 2004).

Molecular characteristics

PMCA4 is a P-type ATPase which transports Ca^{2+} from cytoplasm to outside of the cells by hydrolyzing ATP. Like other Ca^{2+}-ATPase pumps, PMCA4 is a 10 TM protein, and the cytoplasmic domains carry various sites to regulate its function (Withers et al. 2006). Especially, a cytoplasmic domain between S4 and S5 carries an ATP-binding and a catalytic site with an aspartyl-phosphate intermediate site generated from ATP hydrolysis. In addition, there are two Mg^{2+} binding sites, which facilitate its enzymatic activity (Post et al. 2010). There are multiple phosphorylation sites that also regulate PMCA4 activity (Enyedi et al. 1996). Cytosolic C-terminus of the PMCA4 is the domain where the multiple proteins interact. PMCA4 carries two calmodulin (CaM)-binding sites (Enyedi et al. 1994; Falchetto et al. 1991), which serve as autoinhibitory roles in other Ca^{2+} ATPase pumps by hiding ATP binding site in S4-S5 cytosolic domain at the low $[Ca^{2+}]_i$ condition (Withers et al. 2006). Furthermore, the c-terminus functions as a ligand for PDZ domain (Kim et al. 1998), which interacts with PDZ-containing proteins such as Ca^{2+}/CaM-dependent membrane-associated kinase, CASK (Schuh et al. 2003), and nitric oxide synthase (NOS; Duan et al. 2013). A very recent study reported that sperm PMCA4 can interact with a cysteine-rich secretory protein, CRISP4 (Miya et al. 2023) during epididymal maturation.

Physiological significance

PMCA4 expresses ubiquitously like PMCA1 of which depletion causes embryonic lethality in preimplantation embryos (Prasad et al. 2007). However, PMCA4 deficiency seems not to cause severe gross abnormalities (Okunade et al. 2004; Schuh et al. 2004), despite its function in the cardiovascular system such as pulmonary arterial remodeling, cardiac contraction, and cardiac hypertrophy (Deng et al. 2021; Mohamed et al. 2011, 2016). Pmca4-null mice grow normally without severe physiological defects (Schuh et al. 2004). One study observed defective contraction of the portal vain, which could be due to the apoptosis of the smooth muscle cells, but this result was not consistent

within mouse stains (Okunade et al. 2004). Interestingly, *Pmca4*-null mice are infertile only in males and their sperm motility is severely compromised. Although sperm lacking PMCA4 can be motile, their swimming velocity is significantly lower than that of normal sperm. Especially, $[Ca^{2+}]_i$ in PMCA4-deficient sperm is much higher than that in normal sperm after capacitation (Schuh et al. 2004), which elucidates that Ca^{2+} overload causes their defective swimming ability. Transient exposure of the A23187 rescues hyperactivated motility in sperm lacking CATSPER channel, but not in PMCA4-deficient sperm (Navarrete et al. 2016), which supports the importance of the PMCA4 in Ca^{2+} homeostasis for sperm hyperactivation. Thus, PMCA4 is indispensable for normal sperm motility and male fertility to maintain Ca^{2+} homeostasis in capacitated sperm to develop hyperactivated motility. Considering that PMCA4 localizes specifically along the principal piece with the cylindric arrangement (Figure 2 (C); Okunade et al. 2004; Schuh et al. 2004), PMCA4 and CATSPER channel could coordinate Ca^{2+} homeostasis by inducing local enrichment and polarized distribution of intracellular Ca^{2+} in sperm tail.

Functional regulation

As described above, PMCA4 carries CaM binding sites. At low $[Ca^{2+}]_i$ condition, CaM binds to the C-terminus of the PMCA4 to prevent enzymatic catalysis for the Ca^{2+} extrusion. Once $[Ca^{2+}]_i$ increases, CaM binds to the free Ca^{2+} and is released from the PMCA4 to make the pump functional (Withers et al. 2006). In addition, Mg^{2+} also increases PMCA4 function (Post et al. 2010). Recent studies report that PMCA4 could be functionally associated with nitric oxide signaling and phosphorylation (Andrews et al. 2015; Aravindan et al. 2012; Olli et al. 2018). PMCA4 interacts with the nitric oxide synthase via its PDZ ligand, and the interaction is stronger in capacitated sperm rather than non-capacitated sperm in humans (Andrews et al. 2015). Like neuronal cells and endothelial cells where PMCA4 suppresses NOS activity (Holton et al. 2010; Schuh et al. 2001), PMCA4-deficiency increases NOS activity, especially in capacitated sperm, to elevate peroxinite levels to cause apoptosis in sperm (Olli et al. 2018). Considering PMCA4 recruits NOS near the plasma membrane to form cGMP microdomain in the heart (Mohamed et al. 2011), PMCA4 could regulate nitric oxide signaling and cGMP-mediated signaling in sperm. In addition, CASK also interacts with PMCA4 via PDZ domain in sperm (Aravindan et al. 2012). This result suggests that PMCA4 could be involved in downstream phosphorylation events via CASK in sperm. Currently, detailed PMCA4-mediated signaling pathways associated with nitric oxide and phosphorylation are unclear. Yet, the signaling pathways

seem to be physiologically significant as PMCA4 and its interacting components could be supplied through secreted exosomes at their pass in the male and female reproductive tract (Al-Dossary et al. 2013; Martin-DeLeon 2015; Patel et al. 2013).

Ca^{2+} signaling and male contraception

Growing interest for male contraceptives

Although low birth rates are one of the biggest social issues in many developed countries including South Korea (Hwang 2023a), many couples and individuals have been suffering from unwanted pregnancies globally. Surprisingly, unintended pregnancy, which is another major issue for reproductive health in opposite to infertility and low-birth rate issues, happens around 213 million times annually (Nickels and Yan 2024). This causes health issues, such as low birth weight and increased risk for pre-termination of the birth, and social issues including problems in mentality, finance, and career opportunities for the family members (Nickels and Yan 2024). More strikingly, up to 66% of cases of unwanted pregnancies are eventually ended up in abortion, which also causes health issues for mothers (Bearak et al. 2020; Finer and Zolna 2016).

Many couples have their child based on family planning due to the negative outcomes of unintended pregnancy these days. The need for family planning also requires the development of novel contraceptive systems (Nickels and Yan 2024). Currently, contraception methods are dominantly oriented to the woman. Multiple methods are available for contraception in women such as hormonal pills, intrauterine devices, intravaginal rings, tubal ligation, spermicides, and physical barriers. By contrast, condoms and vasectomy are the only two methods for male contraception, which makes women mainly responsible for contraception (Campo-Engelstein 2012).

Interestingly, previous studies reveal that this discrepancy is not simply due to men's unwillingness to use novel contraceptive systems. Although there are some variations, a certain proportion of men are interested in new male contraceptives (Glasier 2010; Nguyen and Jacobsohn 2023). From 34 to 82% of the men are willing to participate in clinical trials for novel contraceptives, depending on their societies (Nickels and Yan 2024). Additionally, women strongly support the concept of their partners using male contraceptives (Reynolds-Wright et al. 2021). These studies clearly indicate that there is a common demand to develop novel male contraceptives in both men and women. The social demand is also well reflected by the predicted

market sizes of male contraceptives, which are over 12 million in the United States and are over 400 million dollars worldwide (Dorman and Bishai 2012; Nickels and Yan 2024).

Targets and strategies to develop male contraceptives

Indeed, there have been continuous trials to develop male contraceptives. Hormonal regulation is critical for normal spermatogenesis (Ahn et al. 2022; Sheng et al. 2024). Thus, increasing testosterone level which suppresses gonadotrophic secretion from the pituitary and hypothalamus, has been applied for a strategy of male contraception (Long et al. 2021). Despite its significant effects to reduce sperm counts and the successful inhibition of male fertility (Nieschlag 2010; Wang and Swerdloff 2022), continued clinical trials using hormonal contraception have reported various side effects, such as depression and mood swings (Behre et al. 2016). The limits for hormonal application request the development of novel male contraceptives.

Hormonal contraceptives aim to deplete male germ cells like non-obstructive azoospermia patients lacking mature sperm in their ejaculates. However, different from the female germ cells, to eliminate the entire sperm in the male gonad is barely feasible due to their huge and rapid generation during spermatogenesis (Griswold 2016). Many proteins are essential for early spermatogenesis, and disrupting the targets might remove mature sperm significantly. However, their isoforms are also shared in diverse somatic cells (Suzuki et al. 2019; Zhang et al. 2017), indicating that targeting these candidates could cause various side effects in other tissues. In addition, developed sperm cells, which overcome the intended disruption of early spermatogenesis, could have chromosomal abnormalities to result in birth defects (Nickels and Yan 2024). Furthermore, the depletion of all male germ cells could be irreversible which is not suitable for future family planning. Notably, numerous infertile males still produce mature sperm with functional defects (Curi et al. 2003). Thus, accumulating studies have focused on targeting the late stage of spermatogenesis after finishing meiosis, when the corresponding haploid germ cells, round spermatids, express various sperm-specific proteins (Matzuk and Lamb 2008). Mouse model studies well demonstrate that disrupting the candidates expressed during the late spermatogenesis step can induce 100% male infertility without gross abnormalities, including sperm generation (Wang et al. 2021). Therefore, gene products expressed exclusively in post-meiotic germ cells, such as round or elongating spermatids and even mature

sperm, are suggested as high-priority targets for male contraceptives (Yan 2009).

Current progresses on non-hormonal male contraceptives

Several proteins have been suggested as targets for pharmacological male contraceptives. Especially, recent two studies have made impressive progresses in developing male contraceptives by targeting post-meiotic components to disrupt sperm function (Balbach et al. 2023; Chang et al. 2021). Chang et al., reported triptonide, which is isolated from a natural plant, *Tripterygium wilfordii,* as a non-hormonal male contraceptive (Chang et al. 2021). Oral intake of triptonide results in male infertility by impairing sperm morphology and motility in mice and monkeys. Long-term intake does not affect severe defects in both animal models except for male infertility. Surprisingly, male mice and monkeys can rescue their fertility after stopping to take triptonide in several weeks, which indicates that the chemical can be applicable for non-hormonal and reversible male contraceptives. Triptonide appears to function by binding to plakoglobin to alter its interaction with the SPEM1 during spermiogenesis. Balbach et al., introduced a new allosteric inhibitor for soluble adenylyl cyclase (sAC) as an on-demand male contraceptive (Balbach et al. 2023). sAC generates cyclic AMP by binding to bicarbonate which is abundant in female reproductive tract (~25 mM) to initiate signaling for capacitation and motility activation of ejaculated sperm (Buffone et al. 2014; Wennemuth et al. 2003). Thus, functional defects of the sAC cause male infertility in humans and mice (Akbari et al. 2019; Esposito et al. 2004). The small molecule, TDI-11861, suppresses sAC function more efficiently than its predecessor, TDI-10229 (Balbach et al. 2021). TDI-11861 does not significantly affect the functions of other kinases and membrane proteins with low cytotoxicity, which is further supported by the normal behavior without gross abnormalities in mice taking it. Oral delivery of TDI-11861 can be effective at least after 1 h by blocking enzymatic activity significantly, which impairs epididymal sperm motility. In addition, the ejaculated sperm from males treated with TDI-11861 by intraperitoneal injection are immotile. Surprisingly, epididymal sperm are almost immotile only after 15 min of TDI-11861 injection, which can be recovered in 24 h. Furthermore, around 90% of males can rescue their fertility after 1–5 days from a single injection of TDI-11861. The rapid effect to cause male infertility and its successful recovery highlight that the sAC inhibitor can be applicable as an on-demand male contraceptive. Together with the studies, other molecules, such as

ATP1A4, GAPDHS, EPPIN, SLO3, and CATSPER, are expected for the target to develop male contraceptives (Mariani et al. 2023).

Potential male contraceptives targeting sperm Ca^{2+} paths

Previous studies demonstrated that Ca^{2+} homeosis to maintain its threshold level is crucial for sperm motility and their hyperactivation. As described above, the absence of the Ca^{2+}-entry path, CATSPER, causes male infertility with defective sperm hyperactivation in humans and mice (Hwang and Chung 2023). Simultaneously, the genetic alteration of the PMCA4 also causes male infertility with defective sperm motility in mice (Okunade et al. 2004; Schuh et al. 2004). The shared defective motility suggests that inhibiting either CATSPER or PMCA4 will set the sperm $[Ca^{2+}]_i$ out of the threshold range, which impairs sperm hyperactivation and male fertility (Figure 3). Especially, the infertility phenotypes without gross abnormalities highlight that targeting both paths could minimize the side effects. In addition, as both are membrane proteins in mature sperm, targeting them could make acute response and successful recovery of the sperm hyperactivation like sAC inhibitor (Balbach et al. 2023). Moreover, considering that transient exposure to A23187 can develop hyperactivated motility by bypassing the other signaling pathways (Tateno et al. 2013), altering Ca^{2+} homeostasis by targeting the Ca^{2+} paths could be direct approach to induce defective sperm hyperactivation and male infertility.

Although the CATSPER and PMCA4 could be promising targets, still there are hurdles to develop non-hormonal male contraceptives targeting these Ca^{2+} paths. Heterologous reconstruction of the CATSPER channel complex is currently not successful, which makes it difficult to apply drug screening. Recently resolved
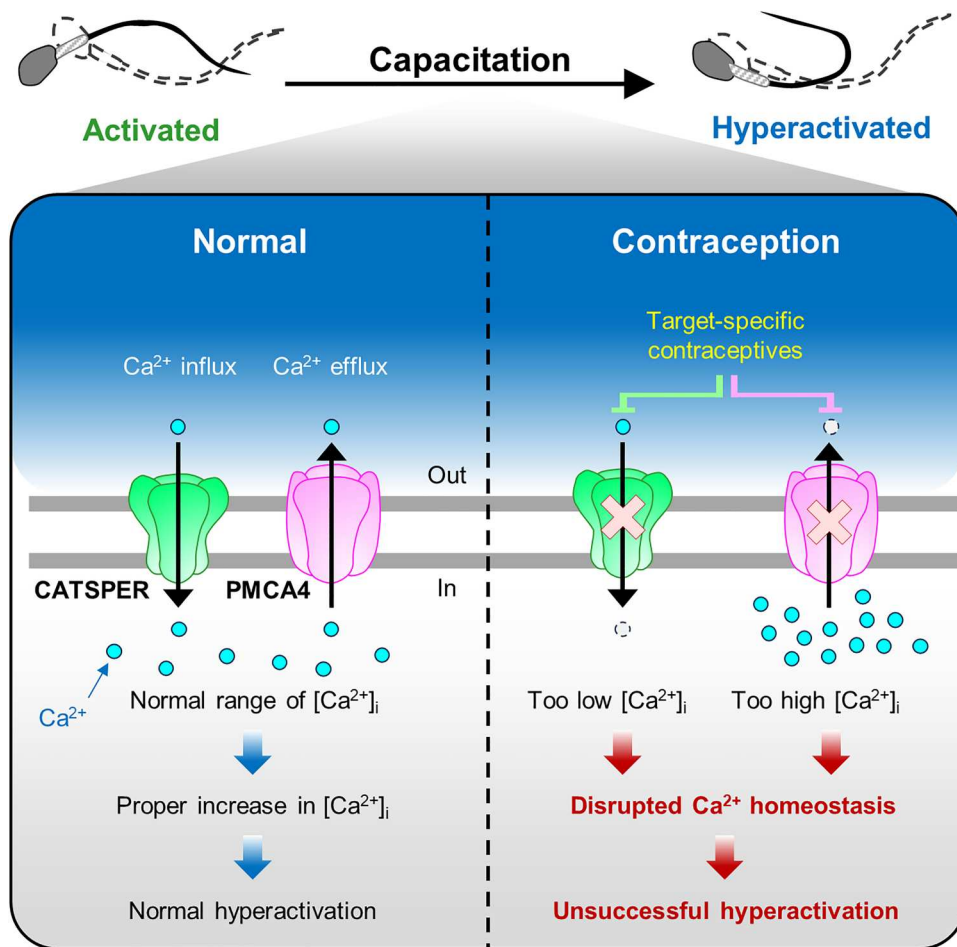

Figure 3. Targeting sperm Ca^{2+} paths could be applied to develop male contraceptives by disrupting Ca^{2+} homeostasis in sperm. A cartoon shows the potential application of male contraceptives targeting paths for influx (CATSPER) and efflux (PMCA4) of Ca^{2+} ions. Male contraceptives targeting either CATSPER or PMCA4 will inhibit influx or efflux of Ca^{2+}, respectively, which impairs intracellular Ca^{2+} level ($[Ca^{2+}]_i$) in capacitating sperm. The disrupted Ca^{2+} homeostasis will prevent sperm from developing hyperactivated motility to cause male infertility. Blue circles and two gray lines indicate Ca^{2+} ions and plasma membranes, respectively.

atomic structure of the mouse CATSPER could provide potential inhibitors for the channel (Lin et al. 2021), that of the human CATSPER should be also resolved considering its activation mechanisms different from mouse CATSPER (Kirichok et al. 2006; Lishko et al. 2011; Strünker et al. 2011). For PMCA4, although the mice lacking the Ca^{2+} pump seem to be physiologically normal, there could be potential defects considering its universal tissue expression (Prasad et al. 2007). In addition, a supplement of the PMCA4 through the exosome in the reproductive tract (Al-Dossary et al. 2013; Martin-DeLeon 2015) can affect the efficacy of contraceptives targeting the Ca^{2+} pump. Therefore, overcoming the hurdles is essential to develop male contraceptives targeting sperm Ca^{2+} paths. Alternatively, sperm-specific molecules which participate in either functional regulation of the paths or their downstream signaling pathways could be targeted to develop novel male contraceptives.

Conclusion

Importance of the Ca^{2+} homeostasis in sperm motility and male fertility has been well demonstrated for around a half century. The recent development of the techniques for mouse genetics, sequencing, and cryo-EM further supported our understanding of Ca^{2+} homeostasis and sperm hyperactivation in molecular level. However, how Ca^{2+} signaling is transduced to trigger the mechanical changes of sperm flagella in hyperactivated motility is still not well understood. Therefore, unraveling the missing part will further improve our knowledge of Ca^{2+} signaling specific for hyperactivated motility in mammalian sperm, which will also contribute to developing novel male contraceptives.

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References

Ahn JS, Won J-H, Kim D-Y, Jung S-E, Kim B-J, Kim J-M, Ryu B-Y. 2022. Transcriptome alterations in spermatogonial stem cells exposed to bisphenol A. Animal Cells Syst (Seoul). 26:70–83. doi:10.1080/19768354.2022.2061592.

Aitken RJ, Nixon B. 2013. Sperm capacitation: a distant landscape glimpsed but unexplored. Mol Hum Reprod. 19:785–793. doi:10.1093/molehr/gat067.

Akbari A, Pipitone GB, Anvar Z, Jaafarinia M, Ferrari M, Carrera P, Totonchi M. 2019. ADCY10 frameshift variant leading to severe recessive asthenozoospermia and segregating with absorptive hypercalciuria. Hum Reprod. 34:1155–1164. doi:10.1093/humrep/dez048.

Al-Dossary AA, Strehler EE, Martin-DeLeon PA. 2013. Expression and secretion of plasma membrane Ca2+-ATPase 4a (PMCA4a) during murine estrus: association with oviductal exosomes and uptake in sperm. PLoS One. 8:e80181. doi:10.1371/journal.pone.0080181.

Amory JK. 2023. Male contraception. Proceedings of the Seminars in Reproductive Medicine. New York, NY: Thieme Medical Publishers.

Andrews RE, Galileo DS, Martin-DeLeon PA. 2015. Plasma membrane Ca2+-ATPase 4: interaction with constitutive nitric oxide synthases in human sperm and prostasomes which carry Ca2+/CaM-dependent serine kinase. MHR: Basic Sci Reprod Med. 21:832–843. doi:10.1093/molehr/gav049.

Aravindan RG, Fomin VP, Naik UP, Modelski MJ, Naik MU, Galileo DS, Duncan RL, Martin-DeLeon PA. 2012. CASK interacts with PMCA4b and JAM-A on the mouse sperm flagellum to regulate Ca2 + homeostasis and motility. J Cell Physiol. 227:3138–3150. doi:10.1002/jcp.24000.

Austin C. 1951. Observations on the penetration of the sperm into the mammalian egg. Aust J Biol Sci. 4:581–596. doi:10.1071/BI9510581.

Avenarius MR, Hildebrand MS, Zhang Y, Meyer NC, Smith LL, Kahrizi K, Najmabadi H, Smith RJ. 2009 Apr. Human male infertility caused by mutations in the CATSPER1 channel protein. Am J Hum Genet. 84:505–510. Epub 20090402. doi:10.1016/j.ajhg.2009.03.004.

Avidan N, Tamary H, Dgany O, Cattan D, Pariente A, Thulliez M, Borot N, Moati L, Barthelme A, Shalmon L, et al. 2003 Jul. CATSPER2, a human autosomal nonsyndromic male infertility gene. Eur J Hum Genet. 11:497–502. doi:10.1038/sj.ejhg.5200991.

Babcock DF, Pfeiffer DR. 1987 Nov 5. Independent elevation of cytosolic [Ca2+] and pH of mammalian sperm by voltage-dependent and pH-sensitive mechanisms. J Biol Chem. 262:15041–15047. doi:10.1016/S0021-9258(18)48135-5.

Balbach M, Ghanem L, Rossetti T, Kaur N, Ritagliati C, Ferreira J, Krapf D, Puga Molina LC, Santi CM, Hansen JN. 2021. Soluble adenylyl cyclase inhibition prevents human sperm functions essential for fertilization. Mol Hum Reprod. 27:gaab054. doi:10.1093/molehr/gaab054.

Balbach M, Rossetti T, Ferreira J, Ghanem L, Ritagliati C, Myers RW, Huggins DJ, Steegborn C, Miranda IC, Meinke PT. 2023. On-demand male contraception via acute inhibition of soluble adenylyl cyclase. Nat Commun. 14:637. doi:10.1038/s41467-023-36119-6.

Bearak J, Popinchalk A, Ganatra B, Moller A-B, Tunçalp Ö, Beavin C, Kwok L, Alkema L. 2020. Unintended pregnancy and abortion by income, region, and the legal status of abortion: estimates from a comprehensive model for 1990–2019. Lancet Glob Health. 8:e1152–e1161. doi:10.1016/S2214-109X(20)30315-6.

Behre HM, Zitzmann M, Anderson RA, Handelsman DJ, Lestari SW, McLachlan RI, Meriggiola MC, Misro MM, Noe G, Wu FC. 2016. Efficacy and safety of an injectable combination

hormonal contraceptive for men. J Clin Endocrinol Metab. 101:4779–4788. doi:10.1210/jc.2016-2141.

Brown SG, Miller MR, Lishko PV, Lester DH, Publicover SJ, Barratt CLR, Martins Da Silva S. 2018 Oct 1. Homozygous in-frame deletion in CATSPERE in a man producing spermatozoa with loss of CatSper function and compromised fertilizing capacity. Hum Reprod. 33:1812–1816. doi:10.1093/humrep/dey278.

Buffone MG, Wertheimer EV, Visconti PE, Krapf D. 2014. Central role of soluble adenylyl cyclase and cAMP in sperm physiology. Biochim Biophys Acta (BBA)-Mol Basis Dis. 1842:2610–2620. doi:10.1016/j.bbadis.2014.07.013.

Campo-Engelstein L. 2012. Contraceptive justice: why we need a male pill. AMA J Ethics. 14:146–151. doi:10.1001/virtualmentor.2012.14.2.msoc1-1202.

Chang M. 1951. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. Nature. 168:697–698. doi:10.1038/168697b0.

Chang Z, Qin W, Zheng H, Schegg K, Han L, Liu X, Wang Y, Wang Z, McSwiggin H, Peng H. 2021. Triptonide is a reversible non-hormonal male contraceptive agent in mice and non-human primates. Nat Commun. 12:1253. doi:10.1038/s41467-021-21517-5.

Chung JJ, Miki K, Kim D, Shim SH, Shi HF, Hwang JY, Cai X, Iseri Y, Zhuang X, Clapham DE. 2017 Feb 23. Catsperzeta regulates the structural continuity of sperm Ca(2+) signaling domains and is required for normal fertility. Elife. 6: e23082. Epub 2017/02/23.

Chung JJ, Navarro B, Krapivinsky G, Krapivinsky L, Clapham DE. 2011 Jan 11. A novel gene required for male fertility and functional CATSPER channel formation in spermatozoa. Nat Commun. 2:153. Epub 2011/01/13. doi:10.1038/ncomms1153.

Chung JJ, Shim SH, Everley RA, Gygi SP, Zhuang X, Clapham DE. 2014. Structurally distinct Ca2 + signaling domains of sperm flagella orchestrate tyrosine phosphorylation and motility. Cell. 157:808–822. doi:10.1016/j.cell.2014.02.056.

Curi S, Ariagno J, Chenlo P, Mendeluk GR, Pugliese M, Sardi Segovia L, Repetto H, Blanco A. 2003. Asthenozoospermia: analysis of a large population. Arch Androl. 49:343–349. doi:10.1080/01485010390219656.

Ded L, Hwang JY, Miki K, Shi HF, Chung JJ. 2020. 3D in situ imaging of the female reproductive tract reveals molecular signatures of fertilizing spermatozoa in mice. Elife. 9: e62043. doi:10.7554/eLife.62043.

DeMott RP, Lefebvre R, Suarez SS. 1995 Jun. Carbohydrates mediate the adherence of hamster sperm to oviductal epithelium. Biol Reprod. 52:1395–1403. doi:10.1095/biolreprod52.6.1395.

Deng L, Chen J, Wang T, Chen B, Yang L, Liao J, Chen Y, Wang J, Tang H, Yi J. 2021. PDGF/MEK/ERK axis represses Ca2 + clearance via decreasing the abundance of plasma membrane Ca2 + pump PMCA4 in pulmonary arterial smooth muscle cells. Am J Physiol-Cell Physiol. 320:C66–C79. doi:10.1152/ajpcell.00290.2020.

Dorman E, Bishai D. 2012. Demand for male contraception. Expert Rev Pharmacoecon Outcomes Res. 12:605–613. doi:10.1586/erp.12.52.

Duan W, Zhou J, Li W, Zhou T, Chen Q, Yang F, Wei T. 2013. Plasma membrane calcium ATPase 4b inhibits nitric oxide generation through calcium-induced dynamic interaction with neuronal nitric oxide synthase. Protein Cell. 4:286–298. doi:10.1007/s13238-013-2116-z.

Enyedi A, Verma AK, Filoteo AG, Penniston JT. 1996. Protein kinase C activates the plasma membrane Ca2 + pump isoform 4b by phosphorylation of an inhibitory region downstream of the calmodulin-binding domain. J Biol Chem. 271:32461–32467. doi:10.1074/jbc.271.50.32461.

Enyedi A, Verma AK, Heim R, Adamo HP, Filoteo AG, Strehler EE, Penniston JT. 1994. The Ca2 + affinity of the plasma membrane Ca2 + pump is controlled by alternative splicing. J Biol Chem. 269:41–43. doi:10.1016/S0021-9258(17)42307-6.

Esposito G, Jaiswal BS, Xie F, Krajnc-Franken MA, Robben TJ, Strik AM, Kuil C, Philipsen RL, van Duin M, Conti M. 2004. Mice deficient for soluble adenylyl cyclase are infertile because of a severe sperm-motility defect. Proc Natl Acad Sci USA. 101:2993–2998. doi:10.1073/pnas.0400050101.

Falchetto R, Vorherr T, Brunner J, Carafoli E. 1991. The plasma membrane Ca2 + pump contains a site that interacts with its calmodulin-binding domain. J Biol Chem. 266:2930–2936. doi:10.1016/S0021-9258(18)49937-1.

Finer LB, Zolna MR. 2016. Declines in unintended pregnancy in the United States, 2008–2011. N Engl J Med. 374:843–852. doi:10.1056/NEJMsa1506575.

Glasier A. 2010. Acceptability of contraception for men: a review. Contraception. 82:453–456. doi:10.1016/j.contraception.2010.03.016.

Griswold MD. 2016. Spermatogenesis: the commitment to meiosis. Physiol Rev. 96:1–17. doi:10.1152/physrev.00013.2015.

Ho H-C, Granish KA, Suarez SS. 2002. Hyperactivated motility of bull sperm is triggered at the axoneme by Ca2 + and not cAMP. Dev Biol. 250:208–217. doi:10.1006/dbio.2002.0797.

Holton M, Mohamed TM, Oceandy D, Wang W, Lamas S, Emerson M, Neyses L, Armesilla AL. 2010. Endothelial nitric oxide synthase activity is inhibited by the plasma membrane calcium ATPase in human endothelial cells. Cardiovasc Res. 87:440–448. doi:10.1093/cvr/cvq077.

Horvath S, Schreiber CA. 2017. Unintended pregnancy, induced abortion, and mental health. Curr Psychiatry Rep. 19:1–6. doi:10.1007/s11920-017-0832-4.

Huang X, Miyata H, Wang H, Mori G, Iida-Norita R, Ikawa M, Percudani R, Chung JJ. 2023 Sep 26. A CUG-initiated CATSPERtheta functions in the CatSper channel assembly and serves as a checkpoint for flagellar trafficking. Proc Natl Acad Sci USA. 120:e2304409120. Epub 20230919. doi:10.1073/pnas.2304409120.

Hwang J. 2023a. Later, fewer, none? Recent trends in cohort fertility in South Korea. Demography. 60:563–582. doi:10.1215/00703370-10585316.

Hwang JY. 2023b. Sperm hyperactivation and the CatSper channel: current understanding and future contribution of domestic animals. J Anim Sci Technol. doi:10.5187/jast.2023.e133.

Hwang JY, Chung JJ. 2023 May 1. Catsper calcium channels: 20 years On. Physiology (Bethesda). 38:0. Epub 20221213.

Hwang JY, Mannowetz N, Zhang Y, Everley RA, Gygi SP, Bewersdorf J, Lishko PV, Chung JJ. 2019 May 30. Dual sensing of physiologic pH and calcium by EFCAB9 regulates sperm motility. Cell. 177:1480–1494 e1419. Epub 2019/05/06. doi:10.1016/j.cell.2019.03.047.

Hwang JY, Maziarz J, Wagner GP, Chung JJ. 2021 Apr 29. Molecular evolution of CatSper in mammals and function

of sperm hyperactivation in gray short-tailed opossum. Cells. 10(5):1047. doi:10.3390/cells10051047.

Hwang JY, Wang H, Lu Y, Ikawa M, Chung JJ. 2022 Jan 18. C2cd6-encoded CatSpertau targets sperm calcium channel to Ca(2+) signaling domains in the flagellar membrane. Cell Rep. 38:110226. Epub 20220107. doi:10.1016/j.celrep.2021.110226

Kim E, DeMarco SJ, Marfatia SM, Chishti AH, Sheng M, Strehler EE. 1998. Plasma membrane Ca2+ ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (PSD-95/Dlg/ZO-1) domains. J Biol Chem. 273:1591–1595. doi:10.1074/jbc.273.3.1591.

Kirichok Y, Navarro B, Clapham DE. 2006 Feb 9. Whole-cell patch-clamp measurements of spermatozoa reveal an alkaline-activated Ca2+ channel. Nature. 439:737–740. doi:10.1038/nature04417.

Lin S, Ke M, Zhang Y, Yan Z, Wu J. 2021. Structure of a mammalian sperm cation channel complex. Nature. 595:746–750. doi:10.1038/s41586-021-03742-6.

Lindemann CB, Goltz JS. 1988. Calcium regulation of flagellar curvature and swimming pattern in triton X-100–extracted rat sperm. Cell Motil Cytoskeleton. 10:420–431. doi:10.1002/cm.970100309.

Lishko PV, Botchkina IL, Fedorenko A, Kirichok Y. 2010. Acid extrusion from human spermatozoa is mediated by flagellar voltage-gated proton channel. Cell. 140:327–337. doi:10.1016/j.cell.2009.12.053.

Lishko PV, Botchkina IL, Kirichok Y. 2011. Progesterone activates the principal Ca2+ channel of human sperm. Nature. 471:387–391. doi:10.1038/nature09767.

Lishko PV, Kirichok Y, Ren D, Navarro B, Chung J-J, Clapham DE. 2012. The control of male fertility by spermatozoan ion channels. Annu Rev Physiol. 74:453–475. doi:10.1146/annurev-physiol-020911-153258.

Liu J, Xia J, Cho K-H, Clapham DE, Ren D. 2007. Catsperβ, a novel transmembrane protein in the CatSper channel complex. J Biol Chem. 282:18945–18952. doi:10.1074/jbc.M701083200.

Lobley A, Pierron V, Reynolds L, Allen L, Michalovich D. 2003. Identification of human and mouse CatSper3 and CatSper4 genes: characterisation of a common interaction domain and evidence for expression in testis. Reprod Biol Endocrinol. 1:1–15. doi:10.1186/1477-7827-1-53.

Long JE, Lee MS, Blithe DL. 2021. Update on novel hormonal and nonhormonal male contraceptive development. J Clin Endocrinol Metab. 106:e2381–e2392. doi:10.1210/clinem/dgab034.

Luo T, Chen HY, Zou QX, Wang T, Cheng YM, Wang HF, Wang F, Jin ZL, Chen Y, Weng SQ, et al. 2019 Mar 1. A novel copy number variation in CATSPER2 causes idiopathic male infertility with normal semen parameters. Hum Reprod. 34:414–423. doi:10.1093/humrep/dey377.

Mariani NA, Silva JV, Fardilha M, Silva EJ. 2023. Advances in non-hormonal male contraception targeting sperm motility. Hum Reprod Update. 29(5):545–569. doi:10.1093/humupd/dmad008.

Martin-DeLeon PA. 2015. Epididymosomes: transfer of fertility-modulating proteins to the sperm surface. Asian J Androl. 17:720. doi:10.4103/1008-682X.155538.

Matzuk MM, Lamb DJ. 2008. The biology of infertility: research advances and clinical challenges. Nat Med. 14:1197–1213. doi:10.1038/nm.f.1895.

Miki K, Clapham DE. 2013. Rheotaxis guides mammalian sperm. Curr Biol. 23:443–452. doi:10.1016/j.cub.2013.02.007.

Miller MR, Mannowetz N, Iavarone AT, Safavi R, Gracheva EO, Smith JF, Hill RZ, Bautista DM, Kirichok Y, Lishko PV. 2016. Unconventional endocannabinoid signaling governs sperm activation via the sex hormone progesterone. Science. 352:555–559. doi:10.1126/science.aad6887.

Miya V, Kumar C, Breed AA, Idicula-Thomas S, Pathak BR. 2023. Mammalian cysteine-rich secretory proteins interact with plasma membrane Ca2 + exporter PMCA4b. Andrology. doi:10.1111/andr.13549.

Mohamed TM, Abou-Leisa R, Stafford N, Maqsood A, Zi M, Prehar S, Baudoin-Stanley F, Wang X, Neyses L, Cartwright EJ. 2016. The plasma membrane calcium ATPase 4 signalling in cardiac fibroblasts mediates cardiomyocyte hypertrophy. Nat Commun. 7:11074. doi:10.1038/ncomms11074.

Mohamed TM, Oceandy D, Zi M, Prehar S, Alatwi N, Wang Y, Shaheen MA, Abou-Leisa R, Schelcher C, Hegab Z. 2011. Plasma membrane calcium pump (PMCA4)-neuronal nitric-oxide synthase complex regulates cardiac contractility through modulation of a compartmentalized cyclic nucleotide microdomain. J Biol Chem. 286:41520–41529. doi:10.1074/jbc.M111.290411.

Navarrete FA, Alvau A, Lee HC, Levin LR, Buck J, Leon PM-D, Santi CM, Krapf D, Mager J, Fissore RA. 2016. Transient exposure to calcium ionophore enables in vitro fertilization in sterile mouse models. Sci Rep. 6:33589. doi:10.1038/srep33589.

Navarrete FA, Garcia-Vazquez FA, Alvau A, Escoffier J, Krapf D, Sanchez-Cardenas C, Salicioni AM, Darszon A, Visconti PE. 2015 Aug. Biphasic role of calcium in mouse sperm capacitation signaling pathways. J Cell Physiol. 230:1758–1769. doi:10.1002/jcp.24873.

Nguyen BT, Jacobsohn TL. 2023. Men's willingness to use novel male contraception is linked to gender-equitable attitudes: results from an exploratory online survey. Contraception. 123:110001. doi:10.1016/j.contraception.2023.110001.

Nickels L, Yan W. 2024. Nonhormonal male contraceptive development—strategies for progress. Pharmacol Rev. 76:37–48. doi:10.1124/pharmrev.122.000787.

Nieschlag E. 2010. Clinical trials in male hormonal contraception. Contraception. 82:457–470. doi:10.1016/j.contraception.2010.03.020.

Okunade GW, Miller ML, Pyne GJ, Sutliff RL, O'Connor KT, Neumann JC, Andringa A, Miller DA, Prasad V, Doetschman T. 2004. Targeted ablation of plasma membrane Ca2+-ATPase (PMCA) 1 and 4 indicates a major housekeeping function for PMCA1 and a critical role in hyperactivated sperm motility and male fertility for PMCA4. J Biol Chem. 279:33742–33750. doi:10.1074/jbc.M404628200.

Olli KE, Li K, Galileo DS, Martin-DeLeon PA. 2018. Plasma membrane calcium ATPase 4 (PMCA4) co-ordinates calcium and nitric oxide signaling in regulating murine sperm functional activity. J Cell Physiol. 233:11–22. doi:10.1002/jcp.25882.

Patel R, Al-Dossary AA, Stabley DL, Barone C, Galileo DS, Strehler EE, Martin-DeLeon PA. 2013. Plasma membrane Ca2+-ATPase 4 in murine epididymis: secretion of splice variants in the luminal fluid and a role in sperm maturation. Biol Reprod. 89(6):1–11.

Post H, Schwarz A, Brandenburger T, Aumüller G, Wilhelm B. 2010. Arrangement of PMCA4 in bovine sperm membrane

fractions. Int J Androl. 33:775–783. doi:10.1111/j.1365-2605.2009.01022.x.

Prasad V, Okunade G, Liu L, Paul RJ, Shull GE. 2007. Distinct phenotypes among plasma membrane Ca2+-ATPase knock-out mice. Ann N Y Acad Sci. 1099:276–286. doi:10.1196/annals.1387.029.

Qi H, Moran MM, Navarro B, Chong JA, Krapivinsky G, Krapivinsky L, Kirichok Y, Ramsey IS, Quill TA, Clapham DE. 2007 Jan 23. All four CatSper ion channel proteins are required for male fertility and sperm cell hyperactivated motility. Proc Natl Acad Sci USA. 104:1219–1223. Epub 2007/01/18. doi:10.1073/pnas.0610286104.

Quill TA, Ren D, Clapham DE, Garbers DL. 2001. A voltage-gated ion channel expressed specifically in spermatozoa. Proc Natl Acad Sci USA. 98:12527–12531. doi:10.1073/pnas.221454998.

Quill TA, Sugden SA, Rossi KL, Doolittle LK, Hammer RE, Garbers DL. 2003. Hyperactivated sperm motility driven by CatSper2 is required for fertilization. Proc Natl Acad Sci USA. 100:14869–14874. doi:10.1073/pnas.2136654100.

Ren D, Navarro B, Perez G, Jackson AC, Hsu S, Shi Q, Tilly JL, Clapham DE. 2001 Oct 11. A sperm ion channel required for sperm motility and male fertility. Nature. 413:603–609. Epub 2001/10/12. doi:10.1038/35098027

Reynolds-Wright JJ, Cameron NJ, Anderson RA. 2021. Will men use novel male contraceptive methods and will women trust them? A systematic review. J Sex Res. 58:838–849. doi:10.1080/00224499.2021.1905764.

Sanchez-Cardenas C, Montoya F, Navarrete FA, Hernandez-Cruz A, Corkidi G, Visconti PE, Darszon A. 2018 Nov 1. Intracellular Ca2 + threshold reversibly switches flagellar beat off and on. Biol Reprod. 99:1010–1021. doi:10.1093/biolre/ioy132.

Schiffer C, Rieger S, Brenker C, Young S, Hamzeh H, Wachten D, Tuttelmann F, Ropke A, Kaupp UB, Wang T, et al. 2020 Feb 17. Rotational motion and rheotaxis of human sperm do not require functional CatSper channels and transmembrane Ca(2+) signaling. EMBO J 39:e102363. Epub 20200119. doi:10.15252/embj.2019102363

Schuh K, Cartwright EJ, Jankevics E, Bundschu K, Liebermann J, Williams JC, Armesilla AL, Emerson M, Oceandy D, Knobeloch K-P. 2004. Plasma membrane Ca2+ ATPase 4 is required for sperm motility and male fertility. J Biol Chem. 279:28220–28226. doi:10.1074/jbc.M312599200.

Schuh K, Uldrijan S, Gambaryan S, Roethlein N, Neyses L. 2003. Interaction of the plasma membrane Ca2 + pump 4b/Cl with the Ca2+/calmodulin-dependent membrane-associated kinase CASK. J Biol Chem. 278:9778–9783. doi:10.1074/jbc.M212507200.

Schuh K, Uldrijan S, Telkamp M, Rothlein N, Neyses L. 2001. The plasmamembrane calmodulin–dependent calcium pump: a major regulator of nitric oxide synthase I. J Cell Biol. 155:201–206. doi:10.1083/jcb.200104131.

Sheng W, Li Y, Liu L, Li B, Sun T, Zhu C, He Q. 2024. Tortoise-shell glue ameliorates male infertility through the hypo-thalamic-pituitary-gonadal axis in obese rats. Animal Cells Syst (Seoul). 28:110–122. doi:10.1080/19768354.2024.2329684.

Smith JF, Syritsyna O, Fellous M, Serres C, Mannowetz N, Kirichok Y, Lishko PV. 2013 Apr 23. Disruption of the princi-pal, progesterone-activated sperm Ca2 + channel in a CatSper2-deficient infertile patient. Proc Natl Acad Sci USA. 110:6823–6828. Epub 20130325. doi:10.1073/pnas.1216588110.

Stauss CR, Votta TJ, Suarez SS. 1995. Sperm motility hyperacti-vation facilitates penetration of the hamster zona pellucida. Biol Reprod. 53:1280–1285. doi:10.1095/biolreprod53.6.1280.

Strünker T, Goodwin N, Brenker C, Kashikar ND, Weyand I, Seifert R, Kaupp UB. 2011 Mar 17. The CatSper channel med-iates progesterone-induced Ca2 + influx in human sperm. Nature. 471:382–386. doi:10.1038/nature09769.

Suarez SS, Varosi SM, Dai X. 1993 May 15. Intracellular calcium increases with hyperactivation in intact, moving hamster sperm and oscillates with the flagellar beat cycle. Proc Natl Acad Sci USA. 90:4660–4664. Epub 1993/05/15. doi:10.1073/pnas.90.10.4660.

Suarez SS, Vincenti L, Ceglia MW. 1987 Nov. Hyperactivated motility induced in mouse sperm by calcium ionophore A23187 is reversible. J Exp Zool. 244:331–336. doi:10.1002/jez.1402440218.

Suzuki S, Diaz VD, Hermann BP. 2019. What has single-cell RNA-seq taught us about mammalian spermatogenesis? Biol Reprod. 101:617–634. doi:10.1093/biolre/ioz088.

Tateno H, Krapf D, Hino T, Sánchez-Cárdenas C, Darszon A, Yanagimachi R, Visconti PE. 2013. Ca2+ ionophore A23187 can make mouse spermatozoa capable of fertilizing in vitro without activation of cAMP-dependent phosphoryl-ation pathways. Proc Natl Acad Sci USA. 110:18543–18548.

Vyklicka L, Lishko PV. 2020 Apr. Dissecting the signaling path-ways involved in the function of sperm flagellum. Curr Opin Cell Biol. 63:154–161. Epub 2020/02/26. doi:10.1016/j.ceb.2020.01.015

Wang C, Swerdloff RS. 2022. Approaches to contraceptive methods for Men. In: McQueen D, editor. Oxford research encyclopedia of global public health. Oxford University Press; p. 1–42.

Wang H, Liu J, Cho KH, Ren D. 2009 Sep. A novel, single, trans-membrane protein CATSPERG is associated with CATSPER1 channel protein. Biol Reprod. 81:539–544. doi:10.1095/biolreprod.109.077107.

Wang H, McGoldrick LL, Chung JJ. 2021 Jan. Sperm ion chan-nels and transporters in male fertility and infertility. Nat Rev Urol. 18:46–66. Epub 20201119. doi:10.1038/s41585-020-00390-9

Wennemuth G, Carlson AE, Harper AJ, Babcock DF. 2003. Bicarbonate actions on flagellar and Ca2+-channel responses: initial events in sperm activation. Development. 130(7):1317–1326. doi:10.1242/dev.00353.

Withers S, Cartwright EJ, Neyses L. 2006. Sperm phenotype of mice carrying a gene deletion for the plasma membrane calcium/calmodulin dependent ATPase 4. Mol Cell Endocrinol. 250:93–97. doi:10.1016/j.mce.2005.12.028.

Yan W. 2009. Male infertility caused by spermiogenic defects: lessons from gene knockouts. Mol Cell Endocrinol. 306:24–32. doi:10.1016/j.mce.2009.03.003.

Yanagimachi R. 1969. In vitro capacitation of hamster sperma-tozoa by follicular fluid. Reproduction. 18:275–286. doi:10.1530/jrf.0.0180275.

Yanagimachi R. 1970 Oct. The movement of golden hamster spermatozoa before and after capacitation. J Reprod Fertil. 23:193–196. Epub 1970/10/01. doi:10.1530/jrf.0.0230193.

Yanagimachi R. 1982. Requirement of extracellular calcium ions for various stages of fertilization and fertilization-related

phenomena in the hamster. Gamete Res. 5:323–344. doi:10.1002/mrd.1120050404.

Yang F, Gracia Gervasi M, Orta G, Tourzani DA, De la Vega-Beltran JL, Ruthel G, Darszon A, Visconti PE, Wang PJ. 2022 Jan 15. C2CD6 regulates targeting and organization of the CatSper calcium channel complex in sperm flagella. Development. 149(2):dev199988. doi:10.1242/dev.199988.

Young S, Schiffer C, Wagner A, Patz J, Potapenko A, Herrmann L, Nordhoff V, Pock T, Krallmann C, Stallmeyer B, et al. 2024 Jan 2. Human fertilization in vivo and in vitro requires the CatSper channel to initiate sperm hyperactivation. J Clin Invest. 134(1):e173564. doi:10.1172/JCI173564.

Zhang Y, Tang C, Yu T, Zhang R, Zheng H, Yan W. 2017. MicroRNAs control mRNA fate by compartmentalization based on 3′ UTR length in male germ cells. Genome Biol. 18:1–18. doi:10.1186/s13059-016-1139-1.

Zhao Y, Wang H, Wiesehoefer C, Shah NB, Reetz E, Hwang JY, Huang X, Wang TE, Lishko PV, Davies KM, et al. 2022 Jun 17. 3D structure and in situ arrangements of CatSper channel in the sperm flagellum. Nat Commun. 13:3439. doi:10.1038/s41467-022-31050-8.