REVIEW

The role of sperm ion channels in male infertility

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ABSTRACT

Spermatogenesis is a complex and well-orchestrated process, in which spermatogonial stem cells undergo multiple rounds of DNA replication, meiosis, and cellular differentiation to produce mature spermatozoa. Sperm ion channels play an important role in maintaining sperm physiology and motility and fertilizing ability. Aberrant functioning of these ion channels may affect sperm functions like hyperactivation, capacitation, acrosome reaction, which will affect fertilization and subsequently may lead to infertility. The present review is focused on summarizing the role of sperm ion channels in male infertility.

KEYWORDS: Male infertility, ion channels, fertilization

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INTRODUCTION

The incidence of male infertility is increasing and several genetic risk factors associated with its etiology have been identified. Freshly ejaculated spermatozoa are poorly capable of fertilizing an oocyte. Ion channels play a central role in sperm by maintaining intracellular ion concentration and regulating various physiological processes such as hyperactivation, capacitation, acrosome reaction, and fertilization. The recent reports highlight the presence of sperm associated cation channel (catsper1-4), proton ion channel (Hv1), potassium ion channel (SLO3/KCNU1), sodium channel (NaV 1.1-1.9) and members of TRP channel family, suggesting an indispensable role of ion channels in maintaining sperm physiology and fertilizing potential. Aberrant functioning of these ion channels may affect sperm functions such as hyperactivation, capacitation, acrosome reaction, which will affect fertilization and subsequently may lead to infertility.

Ion channels in male infertility

CATSPER channels

The fertilization potential of sperm is contingent on the appropriate and time-dependent acquisition of hyperactivation, chemotaxis, capacitation, and the acrosome reaction, where calcium (Ca^{2+}) is considerably involved in almost every step. Therefore, manipulation of the functions of channel proteins is closely related to Ca^{2+} influx, ultimately affecting male fertility. A large number of Ca^{2+} ion channel proteins regulating the Ca^{2+} influx in spermatozoa have been identified. Basically, there are two Ca^{2+} channels that are known to be involved in male fertility: a) the Orai1 channel, that are "store-operated" channels that get activated upon depletion of internal calcium stores, and b) the Cation Channel Spermia (CatSper) channel, which are the most widely studied Ca^{2+} ion channel proteins in mammalian sperm (Sun et al. 2017). The role of CatSper ion proteins in sperm hyperactivation and male fertility has been well characterized. The CatSper channel has a heterotetrameric structure composed of four pore-forming α (alpha) subunits (CatSper 1–4) and additional auxiliary subunits viz, CatSper β (beta), CatSper γ (gamma) and CatSper δ (delta) (Singh and Rajender, 2015). The CatSper ion channel complex is encoded by at least seven genes (Singh and Rajender, 2015). While Catsper1–3 are known to be specifically expressed in the testis, CatSper4 is enriched in the testis but also shows weak expression in placenta and lung (Sun et al. 2017). CatSper1–4 proteins are shown to be localized in the plasma membrane of the principal piece of spermatozoa where they are involved in the regulation of flagellar whipping (Sun et al. 2017). CatSper channels help in regulating the intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) in the sperm. CatSper1 and CatSper2 deficient mice display low flagellar bend amplitudes compared to the wild-type mice (Marquez et al. 2007). Interestingly, induction of the releases of Ca^{2+} from internal stores in CatSper1 and CatSper2 deficient mice resulted in increased flagellar bend and amplitude from low levels to normal pre hyperactivated levels (Sun et al. 2017).

It was demonstrated using knockout mice that CatSper1 and CatSper2 are essential for sperm hyperactivation (Carlson et al. 2009; Ren and Xia 2010). Also, it was shown that both CatSper3-/- and CatSper4-/- mice are completely infertile despite having normal sperm counts (Jin et al. 2007). Interestingly, CatSper1 knockout sperm show complete absence of CatSperβ, CatSperγ, CatSperδ, CatSper2, CatSper3, and CatSper4 on sperm plasma membrane, suggesting that all CatSper subunits are required for proper channel assembly; the absence of a single subunit may lead to the degradation of remaining CatSper proteins (Liu et al. 2007; Carlson et al. 2009; Wang et al. 2009). Similarly, another study showed that targeted disruption of murine CatSper3 or CatSper4 resulted in altered sperm hyperactivated

motility and male fertility, but did not affect spermatogenesis and initial motility (Qi et al. 2007).

CATSPER1 gene promoter sequence is enriched in potential interaction sites for the sex-determining region Y gene (SRY), which is well known protein for testis determination and differentiation. Very recently, to determine the regulation of human *CATSPER1* gene by the SRY transcription factor, a study introduced a series of deletions and mutations in the wild- type *CATSPER1* gene promoter to eliminate the SRY sites. The constructs were examined for their ability to activate transcription in a murine spermatogonial germ cell line (GC1-spg) using luciferase assays. Their results demonstrated that SRY specifically binds to different sites in the promoter sequence *CATSPER1* and has the ability to regulate its gene transcription (Olivares et al. 2018).

Other ion channels in sperm

Among other ion channels, K^+ ion channels are important in maintaining capacitation-associated hyperpolarization (Zeng et al. 2013). The SLO3 potassium channel is a closely related paralogue of the high conductance calcium-activated potassium channel, SLO1. SLO3 is expressed mainly in testis whereas SLO1 is expressed in many tissues and in many phyla. It was found that the genetic removal of the SLO3 gene alone is sufficient to completely remove hyperpolarization in capacitating conditions in mouse (Santi et al. 2010). It has also been shown that genetic deletion of SLO3 abolishes pH-dependent K^+ currents in mouse spermatozoa (Zeng et al. 2013). Voltage-gated sodium channels (VGSCs) also play an important role in the generation of depolarization and maintaining action potential. VGSCs are well characterized in human sperm (Pinto et al. 2009); however, no mutations of sodium channels in infertile humans have been reported yet. The importance of transient receptor potential ion channel (TRP) in male infertility using mouse model has also been demonstrated (Weissgerber et al. 2012).

Cystic fibrosis transmembrane conductance regulator (CFTR) is a channel protein involved in conductance of Cl^- and HCO^{3-}. The process of capacitation has been shown to be largely dependent of extracellular HCO^{3-} (Demarco et al. 2003). It was shown that the use of a CFTR inhibitor reduced the number of capacitated sperm, suggesting its role in sperm capacitation. Also, sperm from the heterozygous $Cftr^{+/-}$ mice showed a reduced ability to undergo capacitation compared with those of the wild type littermates (Xu et al, 2007). Thus, the interplay between a variety of ion channels in sperm is the major event involved in capacitation, hyperpolarization, and activation leading to fertilization. Calcium is thought to play a key role in maintaining the intracellular pH and proper functioning of ion channels. The estimation of calcium ion concentration in semen can be of considerable interest. It has been found that the calcium ion concentration in seminal plasma was less in the patients, which exhibited hypo-motile sperm as compared to the patients having normal motile sperm (Banjoko and Adeseolu, 2013).

Hv1 is a proton voltage-gated ion channel involved in alkalanization of sperm cytoplasm and thus hyperactivation. As Hv1 and CatSper channels are located in same sub cellular domain, the combined action of Hv1 and CatSper channels should regulate intracellular alkalinisation required for sperm activation in the female reproductive tract (Lishko and Kirichok 2010). Recently a group investigated the expression of CatSper 1, 2, 3, 4, Proton Voltage-Gated Ion Channel1 *(Hv1)*, Potassium Channel Subfamily U1 *(KCNU1*), and transmembrane protein (*TMEM16A)* in sperm from infertile men with phenotypes such as, asthenozoospermia, oligozoospermia, oligo-astheno-teratozoospermia and teratozoospermia and compared it with normozoospermic controls. The analysis detected that the expression of *CatSper1*, *4*, *HV1*, *KCNU1*, and *TMEM16A* gene was higher in the oligozoospermic group as compared to the controls. However, the expression of *CatSper1*, *2*,

3, 4, KCNU1, and *TMEM16A* gene was lower in the asthenozoospermic group as compared to the controls. The study suggested that ion channels

may play a significant role in sperm motility (Carkci et al. 2017). However, there is no direct study conducted to establish the roles of these sperm ion channels in male fertility and its association in pathogenic condition like infertility in humans. Therefore, it is essential to study the role of sperm ion channels in male infertile patients (Figure1).

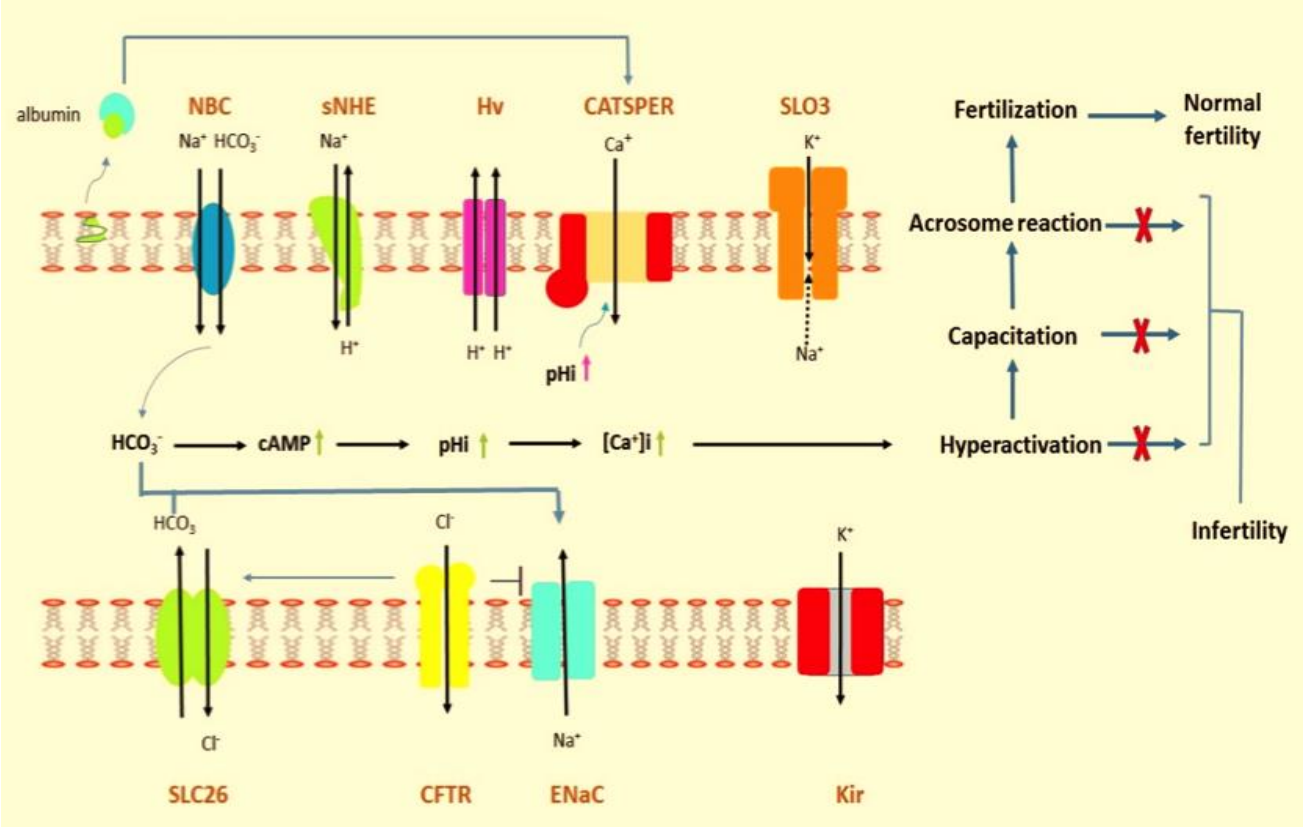

Figure 1. Schematic diagram showing interplay of various ion channels in sperm leading to successful fertilization, an impairment of which may lead to male infertility.

CATSPER gene variants and male infertility

A single nucleotide polymorphism (SNP) refers to a DNA sequence variation resulting from single nucleotide change at the genome level (Shu et al. 2015). SNPs in the *CATSPER* gene family, particularly, *CATSPER1* and *CATSPER2* have been shown to correlate with human male infertility (Avenarius et al. 2009). Furthermore, a study identified ~70 kb deletion in chromosome 15q15, which removed a part of *CATSPER2* gene in three siblings of a French family who suffered from asthenoteratozoospermia and nonsyndromic deafness in addition to Congenital Dyserythropoietic Anemia type I (CDAI). This study was the first report of a human autosomal gene defect associated with non-syndromic male infertility (Avidan et al. 2003).

Recently, a study reported that rs1893316 polymorphism in *CATSPER1* is significantly associated with idiopathic asthenozoospermia risk (Shu et al. 2015). The loss of *CATSPER2* and STRC in contiguous gene deletion syndromes are associated with Deafness-infertility syndrome (DIS)

characterized by deafness and infertility (Zhang et al. 2007).

CatSper ion channels as targets for contraception

Since CatSper ion channels are polymodal sensors that are extensively involved in the regulation of physiological events required for sperm functions, they serve as one of the potential targets for human contraceptive development (Choi et al. 2006; Garside et al. 2013; Singh and Rajender 2015). An interesting study was performed by Li et al. to investigate the contraceptive potential of CatSper1 in mice and human sperm using *in-vitro* approach. The results reflected a significant reduction in sperm motility after incubation of

Sperm with anti-CatSper1 IgG antibody. This study highlighted the significance of using CatSper1 as a potential target for immunocontraception (Li et al. 2009). Similarly, CatSper channel blockers such as HC-056456, have been shown to prevent hyperactivated motility in sperm (Carlson et al. 2009). Contemplating the unique structure and expression of CatSper ion channels, a specific blocker is anticipated to show minimal side effects on other tissues. It also opens up a new arena for exploring other sperm specific ion channels as targets for infertility treatment and development of contraceptives.

Conclusion and future directions

There are several reports on the importance of ion channels in sperm. However, to the best of our knowledge, there is no report till date about what type of ion channels are expressed in human sperm and what are their statuses in the infertile men with normal sperm count. Are their aberrant expressions associated with male infertility? The understanding of the differential expression profile and localization of sperm ion channels in infertile patients will help in better understanding of sperm function and relative contribution of ion channels in the development of infertile phenotype. The information will also help in the treatment and counseling of unexplained infertile patients with normal sperm count before they opt for assisted reproduction.

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Conflict of interest

The author has declared no conflict of interests.

Authors' contributions

VS conceived, designed and drafted the manuscript all through.

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