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Sperm Na^+ K^+-ATPase and Ca^{2+}-ATPase Activities: A Potential Predictive Parameter of Sperm Motility Disorder in Infertile Men

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ABSTRACT

Current highlight on the etiology of male infertility is disorder of sperm motility in which caused by ion homeostasis imbalance involving the ubiquitous multifunctional transmembrane protein, Na^+K^+-ATPase and Ca^{2+}-ATPase enzymes. The emphasis of this review is evaluating the sperm Na^+K^+-ATPase and Ca^{2+}ATPase activity as predictive parameters of sperm motility disorder. To this purpose, a computerized search of PubMed database was performed and obtained data were reviewed in this paper. The retrieved studies were laboratory experiments involving human and mice sperm as the subjects. Na^+ K^+-ATPase and Ca^{2+}-ATPase play an essential role in sperm motility by controlling ion homeostasis. Na^+ K^+-ATPase maintains the intracellular pH by transporting 3 Na^+ out and 2 K^+ into the cell, whereas Ca^{2+}-ATPase extrudes Ca^{2+} from the cell. The impairment of these enzymes and its isoforms, Na^+ K^+-ATPase $\alpha 4$ and PMCA4 expression were proved to decrease sperm motility.

Keywords: Male infertility, Sperm motility, Na^+ K^+-ATPase, Ca^{2+}ATPase.

INTRODUCTION

Male infertility is an inability of an individual to make his partner conceive which is due to the inexistence of abnormal semen analysis parameters after one year having regular insecure intercourse. For a successfully obtaining pregnancy, there are several things that must be occurred. At first, the male has to produce a healthy sperm which depends on how the reproductive organ grow and formed during puberty. At least one of the testicles must function properly for achieving pregnancy. Moreover, endocrine homeostasis such as androgen production has to be maintained in order to trigger sperm production.

The sperm must be carried out into the semen and sufficient enough to function properly in achieving fertility. In case the number of sperm in the semen is less than normal, thus it may reduce the chances of the sperm fertilizing the partner's oocyte. Lastly, the sperm must be functional and motile

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properly, in the case of abnormal sperm motility termed asthenozoospermia, it may fail to reach or even penetrate the partner's oocyte. In addition, the etiology involves an array of biochemical and functional defects.[1]

The complex series of sperm motility is involving mounting of proteins and ions in appropriate concentration that critical for molecular mechanism thus generating the flagella sperm movement.[2] Among them, Na^+ K^+-ATPase and Ca^{2+}ATPase are an enzyme which essential in maintaining the gradient membrane and conducting sperm motility such as activation and hyperactivation.

The Na^+ K^+-ATPase is the transmembrane protein that responsible for controlling ion homeostasis involving an active transport of Na and K ions across the plasma membranes of most cells,[3] including sperm.[4] Calcium, one of an important cellular second messenger, is essential for sperm motility which initially controlled by the activity of the plasma membrane Ca^{2+}-ATPase (PMCA), by extruding Ca^{2+} from the cells against the gradient membrane. Disruption of these both process, Na^+ K^+-ATPase, and Ca^{2+}-ATPase homeostasis activities, may contribute to sperm motility failure and probably driving to infertility in male.[5]

Sperm Na^+ K^+-ATPase and Ca^{2+}-ATPase as a Potential Predictive Parameter of Sperm Motility Disorder

Na^+ K^+-ATPase Role in Sperm Motility

Na^+ K^+-ATPase as an ion transporter involves in the active exchange of intracellular Na^+ for extracellular K^+ in sperm in order to generate Na^+ and K^+ gradients which essential for maintaining cell ion homeostasis, cell membrane resting potential at -70mV, cell volume, and the transport of solutes pass through the cell membrane. Na^+ K^+-ATPase is constituted by heterodimer of two catalytic molecular variants, termed ± and ² subunits, which involved in the ATP hydrolysis of Na^+ K^+-ATPase.[1]

Data reported that four ± and three ² subunits were identified as isoform of Na^+ K^+-ATPase in mammal.[6] The subunit α polypeptide has been extensively investigated in the role of the ATP hydrolysis and ion-translocation functions of Na^+ K^+-ATPase. Kocak-Toker *et al*.,[4] have provided evidence for the existence of α4 along with ±1 isoform are expressed in the mid-piece of the flagella sperm. By forming active complexes, α4 subunit along with β1 and β3 subunits, the catalytically active of Na^+ K^+-ATPase is being produced.

Numerous researches investigated the role and correlation between Na^+ K^+-ATPase and sperm motility as presented in Table 1. (Table 1) The experimental researches were carried out by using human and mouse sperm. Jimenez et al[1] reported that the inhibition of Na^+ K^+-ATPase α4 isoform by ouabain was sufficient to decrease the motility of sperm significantly. Other researches have strengthened the findings that there was a significant relation between Na^+ K^+-ATPase activity and sperm motility performance.[4, 5, 7] The findings in unison agreed that Na^+ K^+-ATPase has a crucial role in the functioning of sperm thus worked properly.

The mechanism of Na^+ K^+-ATPase in sperm motility were wary which as demonstrated in Table 2. (Table 2) The Na^+ K^+-ATPase has a role in the active transport of Na^+ and K^+ across the plasma membranes by catalyzing the exchange of cytoplasmic Na^+ and extracellular K^+ movement in 3:2 ratio.[3] Afterward, another conducted research, by Hamamah and Gatti,[9] proved that Na^+ H^+ exchangers regulated the exchange of intracellular H^+ out of the cell and extracellular Na^+ into the cell toward intracellular pH regulation, abolishing excess acid from the cells. Wong *et al*.,[10] proposed that H^+ efflux was related to Na^+ influx, as performed during the initiation of motility H^+ were extruded from sperm. The alteration of intracellular pH to acidic state was leading to sperm motility reduction.

The authors assessed that functional sperm rely on the regulation of Na^+ K^+-ATPase activity and its isoform in order to support and maintain membrane potential, motility changes, and hyperactivation during capacitation, all of which play a crucial role in male fertility. In addition, based on previously research, there is a possibility to utilize Na^+ K^+-ATPase α4 isoform as a potential biomarker for male infertility, afterward considered as a promising agent of male contraception.

Ca^{2+}-ATPase Role in Sperm Motility

In achieving of successful fertilization, the sperm have to pass through reproductive tract and capacitation prior to penetrate the oocyte. Following

turn the sperm interact with the zona pellucida and release acrosome material. Calcium is suggested as an important ion to exert a sperm function in all of the process. Calcium as one of important second messenger is essential in wary of sperm functions, one of which is associated with sperm motility particularly activation and hyper-activation. In sperm, calcium ion homeostasis is under highly controlled of calcium pump, located in head and tail of sperm, which is due to the activity of the plasma membrane Ca^{2+}-ATPase (PMCA).[2]

Numerous researches investigated the role of Ca^{2+}-ATPase in sperm motility as presented in Table 3. (Table 3) Vignini *et al*.,[12] investigated that calcium ion homeostasis in physiological cell function which responsible for calcium pump is performed by Ca^{2+}-ATPase, which is an ATPase has a role in extrusion Ca^{2+} out of the cell.[13] PMCA4 was found in the principal piece of flagellum and has a certain errand in sperm motility and hyperactivity.[14] The inhibition by quercetin, as an inhibitor of PMCA, drives to reduction of sperm motility.[15] and furthermore probably caused infertility in men.[16] Another finding showed that incubation of sperm using cadmium, compound which found in seminal plasma of smoker, associated with significant disminish of sperm motility afflicting Ca^{2+}-ATPase and axonemal dynein-ATPase.[2]

The mechanism of Ca^{2+}-ATPase in sperm motility is demonstrated in Table 4. Poburko *et al*.,[17] described that PMCA activated as a Ca^{2+}/H^{+} counter transport with a 1:1 stoichiometric proportion producing a large amount of calcium protons extrusion into the mitochondrial matrix and resulting in pH decrease. Whereas, Boczek *et al*.,[18] explained that calcium ion homeostasis was also linking to cell metabolism by mitochondria. The physiological and pathological calcium signals were modulated by the activity of mitochondria, i.e. by buffering the intracellular calcium ions and by synchronizing the Ca^{2+}-dependent activation or inhibition of numerous processes Chalmers *et al*.,[19] By acting

Table 1: Role of Na^{+} K^{+}-ATPase in sperm motility

Na^{+} K^{+}-ATPase role in sperm motility	Reference
Ion homeostasis is controlled by the activity of Na^{+} K^{+}-ATPase defining the sperm motility.	Jimenez T, et al., 2011 [1]
Inhibition of Na^{+} K^{+} activity poses a significant reduction in sperm motility	Koçak-Toker N, et al., 2002 [4]
Ouabain incubation of mouse and human sperm performed selective inhibition to α4 and sufficient to significantly decrease sperm motility	Koçak-Toker N, et al., 2002 [4]; Woo AL, et al., 2000 [5]; Sanchez G, et al., 2006 [7]; Jimenez T, et al., 2012 [8]
Na^{+} K^{+}-ATPase α4 isoform plays a crucial role in sperm motility	Sanchez G et al., 2006 [7]

Table 2: Mechanism of Na^{+} K^{+}-ATPase in sperm motility

Na^{+} K^{+}-ATPase mechanism in sperm motility	Reference
Catalyze the exchange of cytoplasmic Na+ for extracellular K^{+} in 3:2 ratio.	Kaplan JH, 2002 [3]
NHE exchanges H^{+} out of the cell for extracellular Na^{+} and regulate intracellular pH.	Hamamah S and Gatti JL, 1998 [9]
Impairment of intracellular pH to an acidic state reduces sperm motility	Wong PY, et al. 1981 [10]; Giroux-Widemann V, et al., 1991 [11]

Table 3: Role of Ca^{2+}-ATPase in sperm motility

Ca^{2+}-ATPase role in sperm motility	Reference
Ca^{2+}-ATPase responsible for maintaining calcium ion homeostasis in the cell	Vignini, et al., 2009 [12]
The plasma membrane Ca^{2+} pump (PMCA) was an ATPase that extrudes Ca^{2+} out of the cell	Salvador, et al., 1998 [13]
Quercetin incubation of human sperm could reduce sperm motility	Williams, et al., 2003 [15]
Cadmium incubation diminishes Ca^{2+}-ATPase activity of human sperm and reduce sperm motility	Da Costa et al, 2015 [2]
PMCA4 had a certain errand in sperm motility and hyperactivity and PMCA4 dysfunction caused infertility in men.	Schuh, et al., 2004 [14]; Prasad, et al., 2004 [16]

Table 4: Mechanism of Ca^{2+}-ATPase in sperm motility

Ca^{2+}-ATPase mechanism in sperm motility	Reference
Activation of Ca^{2+}/H+ transport by PMCA generating Ca^{2+} extrusion into the mitochondrial matrix and resulting in pH decrease.	Poburko, et al., 2011 [17]
Calcium ion homeostasis was linking to cell metabolism by mitochondria.	Boczek, et al., 2014 [18]
The physiological and pathological calcium signals were modulated by the activity of mitochondria	Chalmers, et al., 2008 [19]; McKenzie, et al., 2004 [20]
Mitochondria altered the promulgation of calcium ion transport, and enable the restocking of intracellular calcium ion stores.	Malli R, et al., 2003 [21]

as a transient calcium ion buffers, the mitochondria will alter the promulgation of calcium ion transport, modify the activity of plasma membrane channels and transporters and enable the restocking of intracellular calcium ion stores.[20, 21]

The authors assessed that Ca^{2+}-ATPase involves in controlling the intracellular calcium concentration as well as a major parameter which part of the mechanism regulating sperm motility. Ca^{2+}-ATPase could be used as a potential biomarker for analyzing either genetic or environmental causes of male infertility. In addition, exposure of inhibitory agents against Ca^{2+}-ATPase proved in decreasing sperm motility and further leading to infertility. In the future, the development of inhibitory agents for targeting the Ca^{2+}-ATPase by mimicking the effect of gene deletion on sperm motility may act as contraceptive drugs, yet the further research is required.

CONCLUSION

In conclusion, Na^+, K^+-ATPase and Ca^{2+}-ATPase have a role in ion homeostasis which is required in the physiological function of cell including sperm especially for sperm motility. Furthermore, Na^+ K^+-ATPase has a potential as biomarker of male infertility, thus considered as a promising agent of male contraception. On the other hand, Ca^{2+}-ATPase as calcium pump which responsible for calcium homeostasis has a role in initiation of motility leading to acrosome reaction. The impairment of Na^+ K^+-ATPase and Ca^{2+}-ATPase activities and the

expression of Na^+ K^+-ATPase ±4 and PMCA4 may be considered as predictive parameters of sperm motility disorder.

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