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The Restraint of Bovine Sperm Cell Motility Increases Survival: Role of Extracellular Calcium in the Phenomena

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Abstract

Sperm cells are complex models for handling *in vitro*, their viability is limited, and their physiology is complex. The study of their properties is of great application in the animal production industry, to improve the selection of gametes, control pathologies and for the development of cryobiology protocols. It is therefore important to have viable and functional gametes. Consequently, it has been demonstrated that the increase of sperm cell mortality is related to the increase of the Reactive Oxygen Species (ROS), and ROS is secondary to normal metabolic processes of the cell, i.e., special motility. One of the processes where the mature sperm cells' main activity is the consumption of more energy, it is the flagellar movement through which high ATP consumption generates high quantities of ROS in the seminal plasma. There is evidence of strategies that lead to reduced metabolic activity for different variables (temperature, pH and other), the intention being that seminal plasma protects the sperm cells and reduces the mortality, and thus it is correct to suggest reducing mortality by reducing motility. It has to be considered that flagellar movement is a complex action that involves energy consumption, regulated by calcium. The phenomenon has not been fully characterized, but it is established that in certain mammalian models, the entry of calcium in specific channels such as CATsper or voltage-dependent channels is a signal for flagellar movement to occur. Reduce the motility of bovine spermatozoa using calcium channel blockers can increase cell survival and we hypothesized that: the general blockade of the calcium channel generated reduced the calcium entry into bovine sperm cells, restricting motility and increased survival of these cells. We propose to in the future explore whether the modulation of calcium channels in bovine sperm cells can reduce motility and increase the survival of these cells in experimental conditions, to reduce the mortality of the sample and improve laboratory manipulation.

Keywords: Sperm cells; Gametes; Seminal plasma

General Overview

"Mature sperm cells are complex cellular machines that control a series of steps and environments to reach their target, which is the oocyte, and fulfill the purpose of delivering their genetic material via fertilization. For this study, we highlight kinetic parameters of motility and the complex capacitation process, whose final step is characterized by acrosome reaction. Regarding the process of capacitation, in recent years the function of CatSper channels as regulatory elements has been demonstrated to be indirectly involved in modulating sperm motility and fertilization capacity, as well as calcium entry. Additionally, a recent study demonstrated that the CatSper channel is involved in the motility process but not so in the Acrosome Reaction (AR) [1]. Flagellar movement generates various changes such as ROS production, and these ROS increases may be able to explain the reduction of cell viability. Moreover, some sperm cell models can remain immobile for a period of time, as in the case of fish sperm cells. The activation process occurs and the cells become motile only when external signaling takes place (i.e., osmotic changes). These fish sperm cells show a long period without mortality and maintain cellular functions for days. Calcium regulation is important for the general cellular function. In mammalian sperm cells, a recent study described that there are two steps regulated by calcium entry: motility and AR. Motility generates metabolic changes and therefore our hypothesis is that regulation by calcium reduces motility and the cells' general metabolic state, leading to an increase in cell survival. Hypothesize that regulation the calcium flux, reduces the motility and the general metabolic state of the cells, leading to increased cell survival. Regulatory mechanisms, for calcium flux, are important for the conservation and manipulation of sperm cells. Because food production, especially that of animal protein, has increased in recent decades, reproductive processes must be controlled more efficiently. It is vital for the development of the food industry to study these processes, yet little is known about the cells involved and the conditions that must occur. Thus, we should study other species as a reference for the development and maintenance of sperm handling", taken from the Parodi 2014 review [2].

Sperm Cell Motility

Flagellar motion and motility generation for sperm cells is a highly demanding energetic process in which extracellular calcium has been shown to play a role at the onset of hyperactivation. Once the sperm enter the oviduct, they go through a process of called "hyperactivation" [3], characterized by high amplitude, asymmetrical beating pattern of the sperm tail (flagellum). These movements are associated with an increase in speed, a decrease in linearity and an increase in the amplitude of lateral head movement and whipping of the flagellum, all of which differ from what is observed in isolated ejaculated sperm [4,5].

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Various physiological stimuli such as calcium, cAMP, bicarbonate and metabolic substrates have been used for the initiation or maintenance of motility hyperactivation *in vitro*. In hamster sperm, calcium can be added to the medium to maintain hyperactivation and keep the sperm cells swimming [6]. Understanding of the various waves affecting motility is important for the comprehension of other phenomena, as in gamete selection, pathology control, the development of cryobiological protocols and overall viability of the sperm cells [2]. How this process is triggered has not been completely characterized, but some models have established the role of specific channels such as the CatSper, for example [7], or voltage-dependent calcium channels for maintaining motility [8]. The manipulation of calcium channel and its impact on the handling and conservation of sperm cells and has not been fully assessed. Calcium wave modeling and analysis has been performed on some sperm cells and suggests that depolarizing changes of the membrane may also induce calcium entry, which could be interpreted as a different signal from hyperactivation, mediated by CATsper. The complex model of sperm cell motility shows dependency on calcium and generates several compounds for live sperm cells' motility [9]. After ejaculation, once the sperm mixes with seminal plasma which exhibits a pH<7.0, there is an alkalizing effect on sperm cytoplasm [10], inducing sperm motility. Another important factor in sperm motility activation is an increase in cAMP levels, which is reported to activate protein kinase A (PKA) and cause phosphorylation on axonemal proteins Nevertheless, even though sperm may be motile, the fertilization capacity can be minimal and is reversed when the sperm undergo capacitation [11,12]. Once human spermatozoa have penetrated the cervical barrier and entered the uterine cavity, upon finding the oviductal epithelium they initiate the process of sperm capacitation, and calcium is key for triggering these phenomena [2]. In biological terms, capacitation can be seen as a priming process by which spermatozoa attain a level of heightened responsiveness to signals emanating from the cumulus–oocyte complex. One of the changes that signals the attainment of a capacitated state is the expression of hyperactivated motility [13]. This particular form of movement is characterized by the development of high-velocity, large-amplitude asymmetrical flagellar waves, and is thought to facilitate detachment of spermatozoa from the oviductal epithelium and penetration of the *Zona pellucida* (ZP), and is part of the complex function of capacitation [14].

Sperm Cell Mortality and Function

Handling procedures result in alterations in sperm that can cause premature sperm capacitation [15]. This process leads to the acrosome reaction and decreases the sperm's useful life. Loss of fertilization capacity results from the presence of large amounts of Reactive Oxygen Species (ROS) following ejaculation. Kirchhoff [16] and Alvarez [17] indicated that sperm produce and export ROS generated mainly by the mitochondria to the extracellular environment, secondary to the flagellar activity of the cells. The loss of sperm function, i.e., the fertilization capacity, results from the presence of high levels of ROS, either following ejaculation or secondary to high levels of motility. Kirchhoff [16] and Alvarez [17] have indicated that sperm produce and export ROS to the extracellular environment, in their majority generated by mitochondria and the product of the monovalent reduction of molecular oxygen during oxidative phosphorylation [16,17]. Mammalian sperm cells are described as having a one-hour period of viability and function with high motility and metabolism. In porcine sperm, when the temperature is reduced, conservation increases sample preservation time [18]. Further, in fowl, temperature regulates calcium influx [19], a process mediated by the channel TRPM8. These lines of evidence suggest the importance of temperature control during *in vitro* manipulation of sperm cells, which is correlated with changes during travel through the oviduct. Temperature is a key factor in sperm cell function and can be controlled *in vitro*. Thus, natural changes can be observed in the oviduct when the sperm cells are swimming toward the oocyte. In example the temperature are important for maintaining motility and increases the mortality in porcine sample [20] and changes in pH are important for generation of calcium signaling, in porcine sample for example [21]. Several process occurred to the spermatozoa, for example, survival regulation occurs in the epididymis and cell maturation [22]. In stallion semen, conservation of the mitochondrial function by exogenous molecules (antioxidants), is used to preserve viability [23] and can change same protein state phosphorylation for intracellular signaling, for example in porcine sperm [24]. Other models present evidence that the phosphorylation state can change calcium flux and induce capacitation, particularly in humans; and the function of PKA activity induces increased calcium entry [25]. For these reasons, recent reports have described how the extracellular solution and seminal plasm can regulate and prolong the function of the spermatozoa models [26]. The reduction of ROS to increase antioxidant molecules has a positive effect on the samples [27] and different solutions, with antioxidant may be employed to observe spermatozoa function *in vitro* conservation [28].

Sperm Capacitation

Fertilization is a unique and amazing process involving fusion of two morphologically distinct cells, the sperm and the oocyte. This process begins when the sperm begins to penetrate the oocyte envelope and plasma membrane, and ends with the exchange of maternal and paternal chromosomes, representing the formation of the zygote. The sperm must undergo functional changes since its genesis and subsequent maturation takes place during the epididymal transit. During capacitation, the sperm cell undergoes a series of biochemical and biophysical changes at the level of the cell membrane, cytoplasm and nucleus, which results in changes in motility patterns. The seminal plasma provides many molecules for spermatozoa survival and induces activation when the spermatozoa reach the oocyte and perform the acrosome reaction prior to the fertilization process [29]. Thus, displacement of capacitation-inducing factors in the sperm membrane, such as antioxidants, metal ions and peptides, increases the removal of cholesterol from the membrane and raises calcium levels through the activation of Ca channels and CatSper, leading to an increase in cAMP levels and phosphorylation of tyrosine residues [30,31]. The entire capacitation process, including hyperactivation, must be regulated by the entry of calcium to the cells, is described as one single step but our group suggested in a review, more complex process and includes entry of the calcium, increase of motility and Acrosome Reaction (AR) for a calcium wave [21], that the calcium wave concept was incorporated (Figure 1) and it was suggested that with a one calcium entry and motility increase and a second wave for AR, the process can be manipulated and the cells kept healthy [32,33]. The AR allows sperm to penetrate the physical barriers posed by the oocyte and, thus enabling delivery of genomic content. The capacitation process leads to the acrosome reaction and causes a decrease in the spermatozoa lifespan. A premature state of capacitation can be induced through sperm dilutions, which are a component of various treatments, and potentially lead to the removal of adsorbed proteins and other compounds present in the seminal plasma that are necessary to maintain sperm viability. Studies have shown that the addition of adequate amounts of seminal plasma in equine models can protect sperm by providing membrane stability when sperm are subjected to cryopreservation processes [34], high dilutions [35,36] or separation via flow cytometry [37].

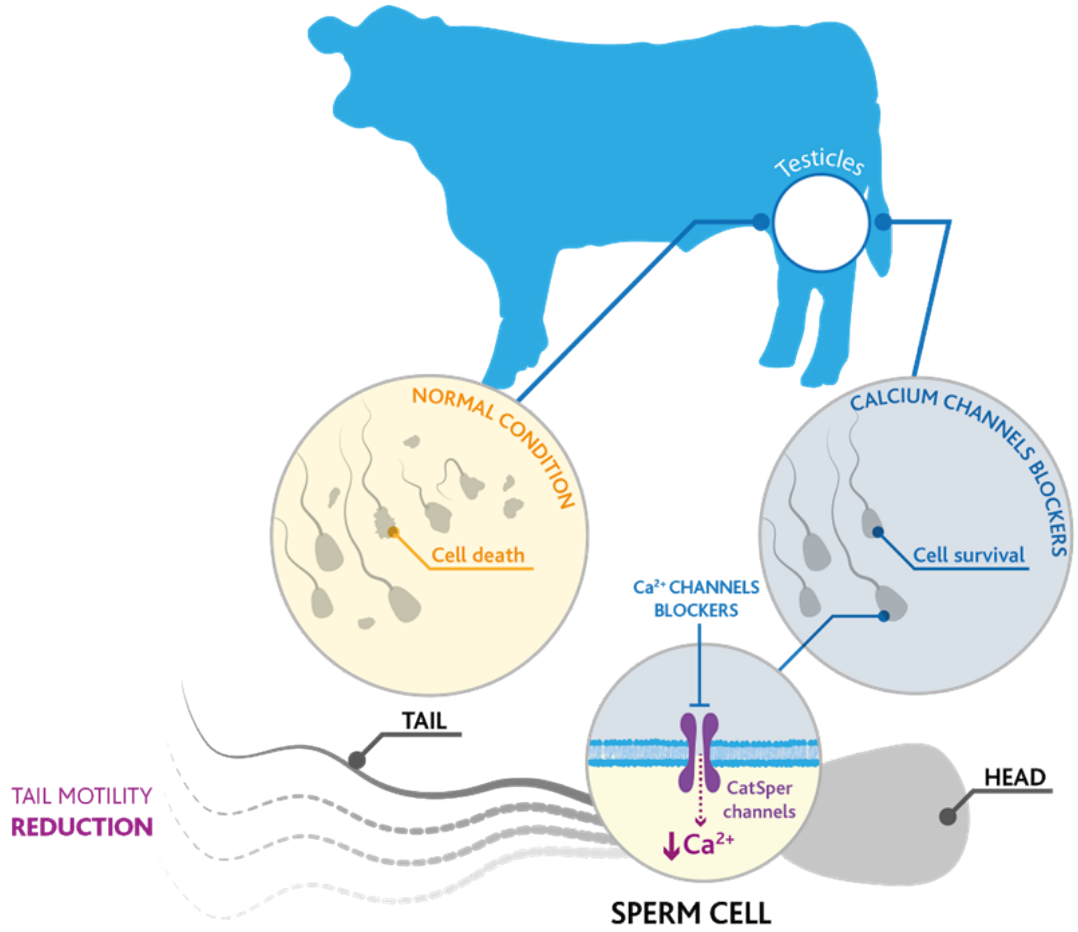

Figure 1: Motility reduction. The model summary the idea of the reduction of the motility by calcium channel blockers can reduce the mortality of the sperm cells under laboratory condition.

Calcium and Spermatozoa Function

We described previously that mammalian sperm acquire fertilizing ability through a process called capacitation. At the molecular level capacitation is a complex process involving the cAMP-dependent pathway, intracellular pH, calcium, and an increase in tyrosine phosphorylation. How these signaling systems interact during capacitation is not well understood [38]. A recent paper presented the biphasic effects of calcium in the spermatozoa function, in particular in the regulation of cAMP-dependent signaling. Using nominal zero calcium, spermatozoa incubated in this medium did not undergo PKA activation or the increase in tyrosine phosphorylation calcium. However, chelation with EGTA (ethyleneglycol acid-bis-(β-aminoethyl ether) N', N', N', N' tetra acetic acid) induced both cAMP-dependent phosphorylation and increased tyrosine phosphorylation and this suggests that calcium ions regulate sperm cAMP and tyrosine phosphorylation pathways in a biphasic manner [38] Some factors can induce a calcium wave and induce a separate calcium deposit, for example in boar semen [39] and hyperpolarization or when used bicarbonate activation signaling [40]. However, depolarization can induce calcium change and activation of the spermatozoa [41], which suggests that the regulation of calcium is important for the normal function of spermatozoa in general. The precedents of calcium are complex and depend on which stimuli are used, but are important described the exactly mechanism of regulation of this ion for understand the beginning of activation of spermatozoa. Regulation of the calcium and motility of the spermatozoa is a complex model that is not completely described and in example recent study on boar semen show differences in storage for regulated AR, supporting the idea that calcium and its effects effect depend on the storage used [42].

Plasma Membrane and Ion Channels

The plasma membrane is a lipoprotein interface that acts as a permeability barrier, allowing the cell to maintain a different composition in the intracellular medium from that of the extracellular medium. The most abundant components of the plasma membrane are phospholipids and proteins, which together form the fluid mosaic pattern [43]. Resting potential is a particular state of the membrane potential in which the sum of ion currents through the membrane is zero, due to the presence of transmembrane electrochemical gradients resulting from selective permeability to ions and secondary various structures, such as transmembrane channels, pumps and ion exchangers. From the resting potential, the excitation of cells can generate an action potential that allows the cell to respond to different stimuli. During this process, each ion tends to draw the membrane

potential toward its own electrochemical equilibrium potential, by the Nernst Equation [44]. Ionic currents through channels not only determine transmembrane bioelectric phenomena related to the membrane potential but also modulate enzyme activity, metabolism and cellular genetics. Specifically, in sperm cells, the transmembrane ionic currents and their potential, among other factors, regulate the intracellular concentration of calcium and the genesis of second messengers. These factors are essential for fertilization-associated processes, such as sperm motility, capacitation and the Acrosome Reaction (AR) as present in the Parodi review [21].

The study of ion channels is therefore extremely valuable for understanding the electrophysiological processes and biological responses of both excitable cells and isolated cells. In particular, determining the roles of these channels in the mammalian sperm membrane is essential for understanding the processes involved in fertilization. The main tool for investigating the characteristics and distribution of ion channels in the plasma membrane is the patch-clamp technique [45,46], a high-resolution method currently used to determine the electrophysiological and pharmacological properties of the cell structure.

Kv Currents Identified in Sperm

A previous study revealed the presence of different types of potassium channels with varying localizations in relation to sperm morphology [47,48]. An example is the delayed rectifier K^+ type channel found in rat spermatogenic cells, which shows a trend that is independent of extracellular calcium and sensitive to blockade by tetraethylammonium chloride, TEA [47]. Based on these characteristics, we identified an inward rectifier K^+ channel referred to as Kir [48,49]. This channel is also regulated by the intracellular pH, with an acidic intracellular pH (6.3) inhibiting the current in spermatogenic cells, while a rising intracellular pH (7.4) significantly increases conductance in these cells. We further identified a third type of K^+ channel, designated mSlo3, which was cloned in rat spermatogenic cells and has been expressed in *Xenopus laevis* oocytes for biophysical analyses. Recent research using electrophysiological methods enabled detection of an output current from the sperm midpiece that is sensitive to TEA [50], and described the depolarization process regulating calcium entry.

Calcium Voltage Channels (CaV) Regulation during Capacitation

During capacitation, ionic channels are susceptible to being activated when a change occurs in the configuration of these channels and they are mediated by a change in the membrane potential. In untrained rat and bovine sperm, the membrane potential is between -10 and -50 mV [51,52]. Low voltage calcium is inactivated at these voltages and therefore does not respond to depolarizing stimuli. Analysis of the membrane potential of rat spermatozoa showed that only cells that maintain hyperpolarization are able to generate an increased flow of calcium secondary to contact with the ZP (likely secondary CaVs) and carry out the RA [53]. Capacitation, resulting in hyperpolarization, changes the configuration of the CAV in a manner that is open to the agonist-mediated ion flow only at a specific stage, thus avoiding early RA. Studies in sperm conducted using electrophysiological methods have demonstrated the role of calcium channel functional keys in the capacitation process, which are dependent on the membrane potential [52,54]. However, the complete mechanism underlying this phenomenon and its regulation via calcium entry is not completely understood. In this context, it was recently suggested that calcium entry occurs via depolarization and the regulation of motility, with a second entry event occurring due to pH regulation and depolarization, and this second calcium influx is mediated the AR [55]. These findings have led to new models in which not only the type of CatSper channel is responsible for this phenomenon [7], and which have further enabled the electrophysiological investigation of new phenomena such as depolarization, that are also involved in the regulation of these voltage-dependent calcium channels. The general hypotheses are present in Figure 1.

CatSper Channels

Four members of the CatSper (i.e., the English acronym for cationic sperm) channels have been described (CatSper1-4) in murine sperm [56,57]. These channels consist of 6 transmembrane domains (6TM1) that are voltage-dependent and calcium-permeable and appear to be found only in sperm cells. CatSper1 and 2 channels have been reported to be essential for sperm hyperactivation and fertility. However, reports concerning these channels still mainly result from studies of humans and mice [58]. These channels are describe modulate by progesterone and mostly explain the physiological change in the mammalian sperm as described by Darszon in his 2011 review [59]; however in 2014 the models are more complex and new actors are part of the sperm cells' functional regulation [21]. The mutant sperm cells cannot fertilize the eggs with an intact zone pellucid but can fertilize eggs whose outer layers have been enzymatically removed [57], suggesting changes in some cell functions. Male mice lacking CatSper2 are also infertile due to a lack of the hyperactivated motility required for penetration of the egg's extracellular matrix [60]. In a study of humans, sub fertile men with deficient sperm motility showed significantly reduced expression of CatSper1 [61]. Little is known about CatSper3 and CatSper4, but they appear to be involved in supporting sperm cell functions [58]. However, CatSper Channels explain in the entire model the sperm activity? It is accepted that CatSper channels' isoforms are responsible for cellular functions in the sperm, but more events need to coordinate for the process of fecundation — the AR, membrane stability, calcium signaling, mitochondrial function and more. However, these are not described in all models and there are other electrical phenomena can cooperate in the cellular events described in sperm by Darszon in his 2011 review [59], which presents a complete table of ion channels, indicating the presence of calcium channel voltage dependent and CatSper, but only in humans and murines [59]. This indicates a lack of complete localization of these channels, and of other mechanisms that may also alter intracellular calcium levels.

Regulation of Calcium in Spermatozoa

Calcium influx in nonexcitable cells regulates such diverse processes as exocytosis, contraction, enzyme control, gene regulation, cell proliferation, and apoptosis. The dominant Calcium entry pathway in these cells is mediated by the calcium channel and the store-operated one [62], intracellular complex in the spermatozoa, the way of calcium are store in these cells, regulated the function in the activation of the spermatozoa [63]. There is strong evidence that in sperm, though they lack an endoplasmatic reticulum, intracellular calcium stores are present which accumulate calcium through an adenosine-triphosphate (ATP)-dependent calcium pump [64] and the explain for intracellular flux calcium are part explain for acrosome store. Thus, through this mechanism, sperm are able to control acrosomal exocytosis. Since it is known that AR can be induced by the release of calcium from the internal calcium store, it has been suggested that these stores must be localized in the acrosomal region of the sperm head. Because the IP3-receptors are placed in the same region, this organelle seems to act as the calcium store [64] and is mobilized by IP3. The release of

Ca^{2+} from these internal stores elevates intracellular calcium to induce the AR in capacitated as well as in non-capacitated sperm, but only in the presence of extracellular calcium [65]. The mitochondrion is another way to explain the intracellular calcium store in spermatozoa [66]. The calcium is moved in faster manner by plasm membrane ATP-calcium depended pump, sodium/calcium antiport and calcium channel of mitochondrion [67]. Moreover, in general we still find a describe calcium channels in these organelles, mitochondrial calcium uptake is regulated by the Mitochondrial Calcium Uniporter (MCU), at least one non-MCU calcium channel and possibly a mitochondrial ryanodine receptor and two more mechanisms can mediate calcium outward efflux, the sodium dependent channels (mNCX) and the sodium independent calcium efflux [68].

Concluding Remarks

We suggest that complex regulation of calcium in the spermatozoa and extracellular calcium have important effects on the function of spermatozoa capacitation, however the differing storage of intracellular calcium needs to be controlled in order to develop a model that can explain how reduction of the function, particularly motility, by altered calcium movement can increase the survival of bovine spermatozoa. And generate the future question for research, does the general blockade of the calcium channel generate reduced the calcium entry into bovine sperm cells, restrain motility and increase survival of these cells?

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