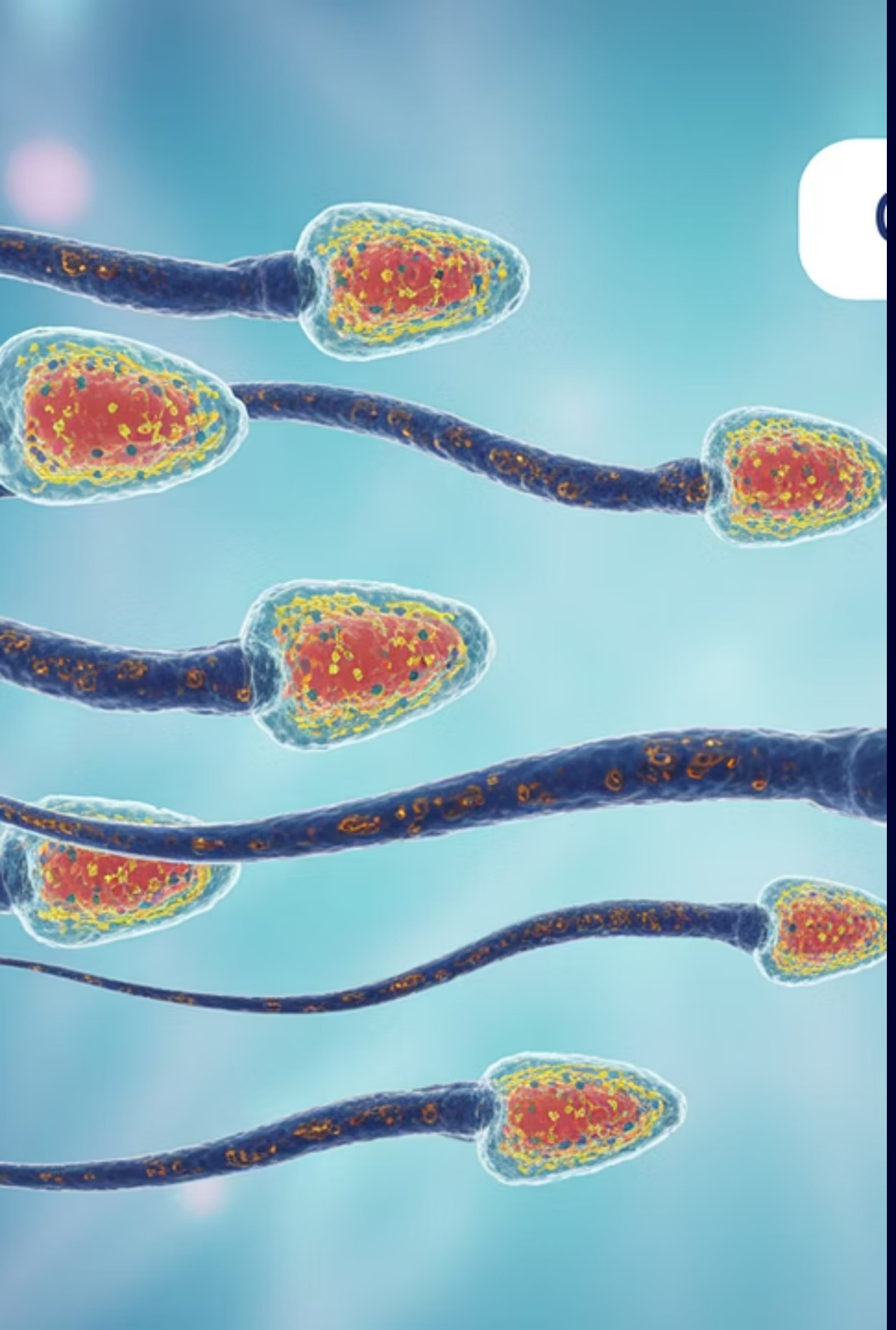


Sperm Capacitation: Molecular Mechanisms and Physiological Significance

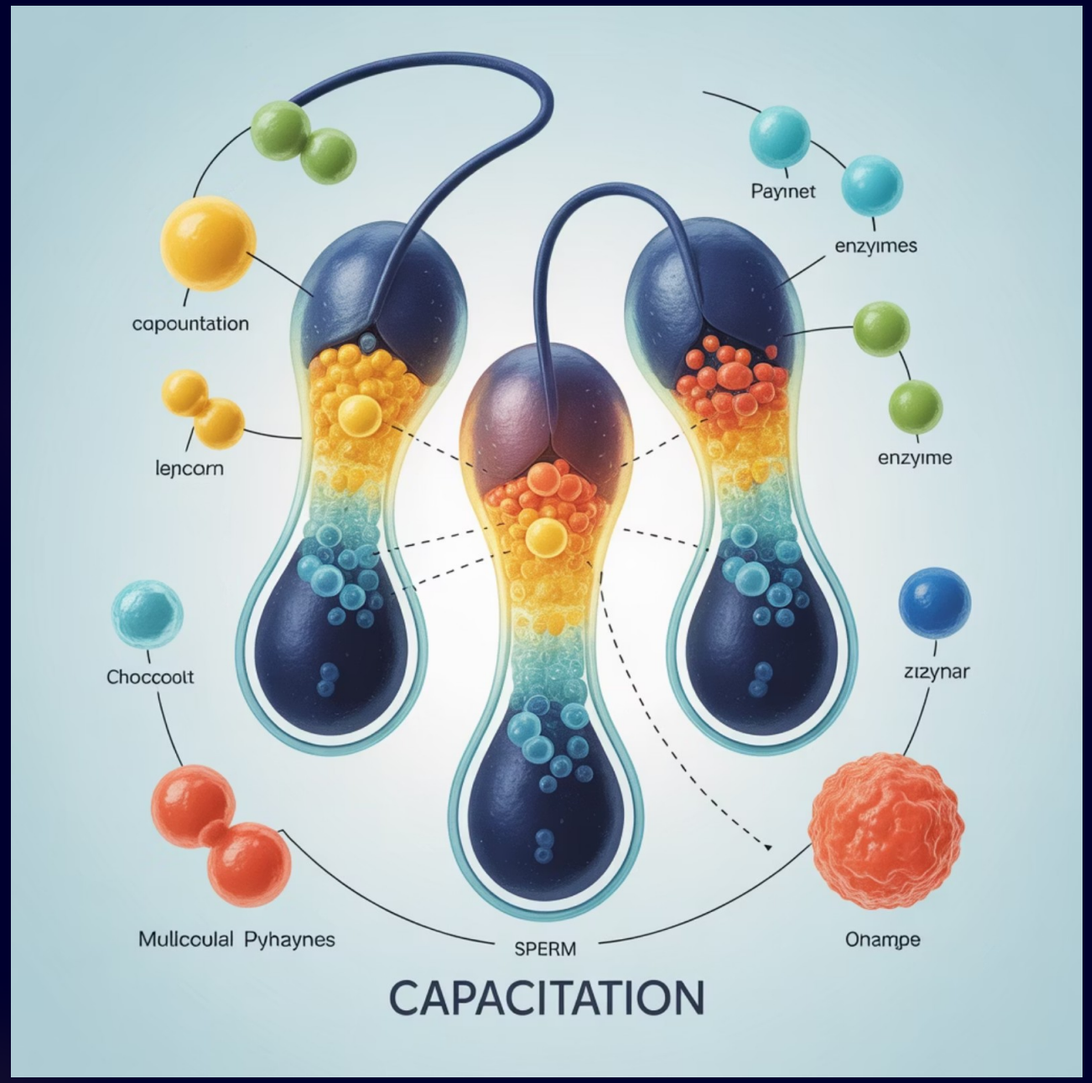
In the early 1950s, Austin and Chang independently described the changes that are required for the sperm to fertilize oocytes in vivo. These changes were originally grouped under name of "capacitation" and were the first step in the development of in vitro fertilization (IVF) in humans. Following these initial and fundamental findings, a remarkable number of observations led to characterization of the molecular steps behind this process. The discovery of certain sperm-specific molecules and the possibility to record ion currents through patch-clamp approaches helped to integrate the initial biochemical observation with the activity of ion channels.

by Fertility Guidance Technologies

The Significance of Capacitation in Reproduction

Capacitation is a biochemical transformation that sperm must undergo to acquire the ability to penetrate the zona pellucida and fertilize the egg. This process is of particular importance in the male gamete due to the fact that sperm are transcriptionally inactive. Therefore, sperm must control all these changes that occur during their transit through the male and female reproductive tracts by complex signaling cascades that include post-translational modifications.

While capacitation naturally occurs within the female reproductive tract, in assisted reproductive technologies (ART) such as intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI), we mimic these physiological conditions in vitro using a carefully designed capacitation medium.



In the laboratory, sperm capacitation is induced in a chemically defined medium containing electrolytes, energy substrates, and a cholesterol acceptor. This controlled capacitation process ensures that sperm used in ART are functionally optimized,

Historical Development of Capacitation Research

Early 1950s

Austin and Chang, using rabbit as a model, independently described the changes required for sperm to fertilize oocytes in vivo. These changes were originally grouped under the name of "capacitation" (Austin, 1952).

1

1963

Yanagimachi and Chang used a medium with a defined chemical composition to capacitate hamster sperm and achieved the first successful IVF.

2

1971

IVF was performed in mice using epididymal sperm and a chemically defined medium (Toyoda et al., 1971).

3

1960s-1970s

First attempts to capacitate human sperm (Norman et al., 1960; Edwards et al., 1966, 1969), leading to the birth of Louise Brown by human IVF (Steptoe and Edwards, 1978).

4

The remarkable initial discoveries of the fertilization process in mammals were achieved in non-human species such as rabbit, rat, and hamster. The possibility to capacitate mammalian sperm in vitro and fertilize the eggs led to the first attempts to capacitate human sperm. Although little was known about the molecular aspects of human sperm capacitation, these were important steps for achieving the birth of Louise Brown by human IVF.

Mouse as a Model for Human Sperm Capacitation

From a molecular point of view, sperm capacitation has been well studied in vitro in several species such as bovine, humans, rats, and hamsters, but without any doubt the best characterized model is the mouse. Most of the remarkable discoveries have been generally achieved in mice and later explored in other species.
As a scientific tool, mice have helped to speed up the progress of research in all fields, and in sperm physiology, this is true due to several reasons:

- The possibility to use transgenic tools to create knockout (KO) or transgenic sperm
- It is easy to perform assisted reproductive techniques
- They are closely related to humans (~99% of mouse genes have an equivalent in humans)
- Their genome has been fully sequenced (published in 2002)

- Mice are small, have a short generation time, and have an accelerated lifespan
- Mice are cost effective because they are inexpensive and easy to look after
- Spermatogenesis in mice is comparable with humans

Key Differences Between Human and Mouse Sperm

Morphological Differences

Human sperm are highly pleomorphic in the sense that a large number of cells in the ejaculate display a great variety of morphological forms. In contrast, the proportion of mouse sperm with morphological variations is rather small.

Ejaculation Site

Humans deposit the ejaculate in the vagina, in contrast to mice that ejaculate in the uterus (Kawano et al., 2014).

Sperm Selection

Human sperm are selected in the cervix, where only morphologically normal or slightly abnormal sperm can migrate through this channel. A second round of selection occurs in the uterotubal junction (UTJ). In contrast, mouse sperm are only selected in the UTJ.

Experimental Conditions

The study of human sperm starts from a semen sample, whereas in mice, it starts from sperm recovered from the epididymis. In vitro incubation under capacitating conditions for human sperm ranges from 3 to 24 h, while most studies in mouse sperm use 1–1.5 h.

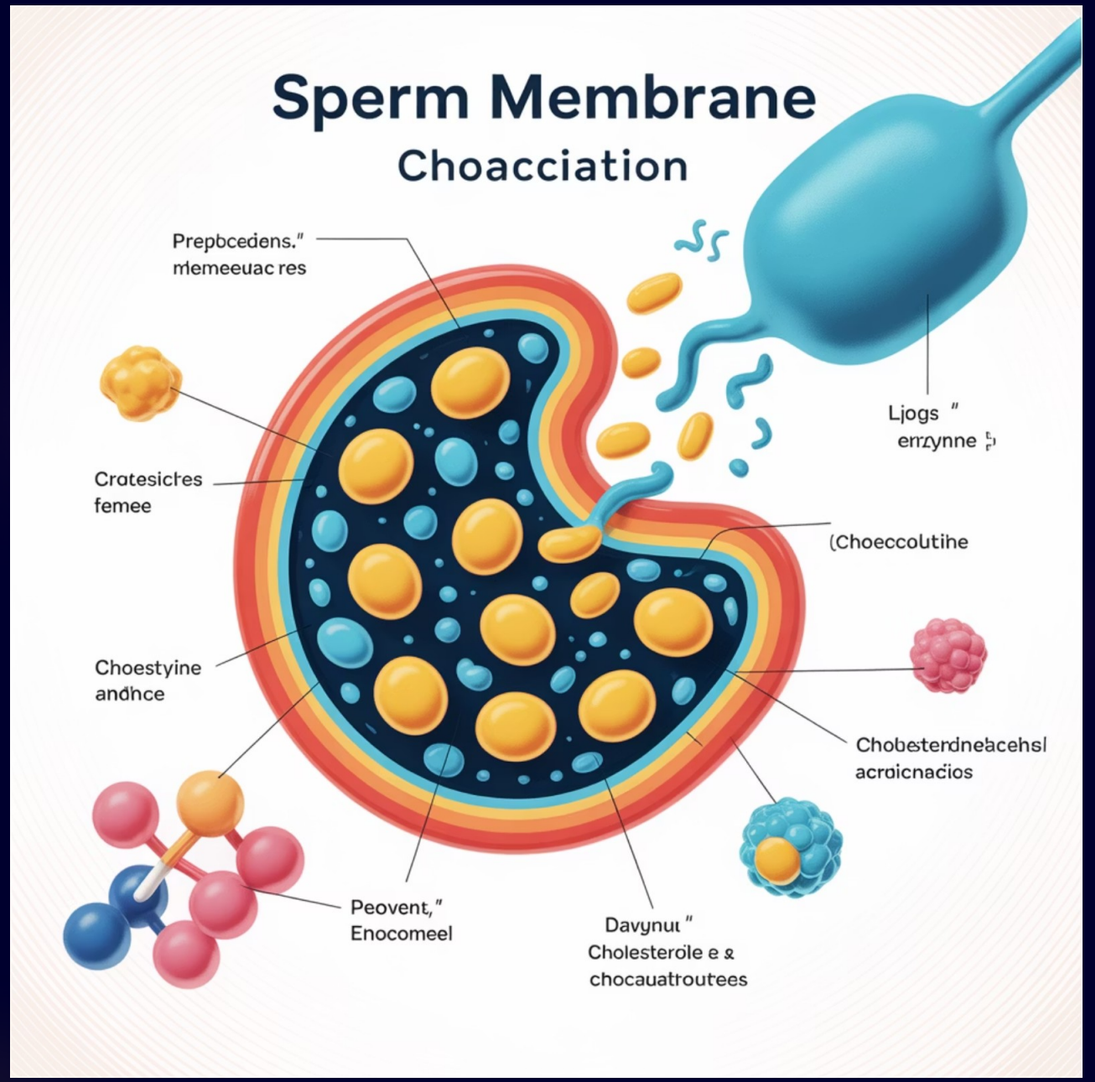
For all these reasons (and many others), caution while transferring molecular and cellular concepts between species was proposed recently. Alternatively, sperm from a given species should be studied using a vertical research strategy.

Sperm Plasma Membrane and Cholesterol

The sperm plasma membrane not only serves as the cell boundary but also presents a dynamic structure that has an impact on sperm capacitation and acrosomal exocytosis (AE). During capacitation, several changes in the sperm membrane have been described:

- Increase in membrane fluidity
- Lateral movement of cholesterol to the apical region of the sperm head
- Cholesterol efflux from the sperm plasma membrane to the extracellular environment

The approximate lipid content of mammalian sperm is composed of 70% phospholipids, 25% neutral lipids (cholesterol), and 5% glycoproteins, with cholesterol being the main sterol in the cellular plasma membrane (~90%).



The cholesterol/phospholipid (C/PL) ratio in sperm varies between species: 0.20 in boar sperm, 0.36 in stallion sperm, about 0.40 in bovine sperm, 0.43 in ram sperm, and 0.83 in human sperm. Davis reported a correlation between the C/PL ratio in sperm and the

Cholesterol Efflux During Capacitation

Initial State

Sperm cholesterol content is finely regulated within the male reproductive tract. Cholesterol is found in high abundance in seminal plasma, which has an inhibitory effect on capacitation.

Cholesterol Acceptors

Albumin is the most used cholesterol acceptor in in vitro experiments, and it has been described to be in high abundance in the oviduct. The lipid transfer protein-I (LTP-I) is present in the reproductive fluids and also serves as a cholesterol acceptor.

Membrane Reorganization

Sterol-rich microdomains, known as lipid rafts, are organization centers involved in membrane protein distribution. A capacitation-associated movement of GM1 has been observed during capacitation due to cholesterol efflux.

Signaling Activation

Phospholipid scrambling, one of the earliest capacitation events, is initiated by an increase in intracellular HCO_3^- followed by the activation of the cAMP/PKA pathway and may be essential to facilitate albumin-mediated cholesterol efflux.

It has been well demonstrated in vitro that capacitation is associated with removal of cholesterol from the plasma membrane. Sperm of patients with unexplained infertility showed a higher C/PL ratio due to lower phospholipid content, and normospermic patients who failed in IVF had either an atypically high content of cholesterol or a slow efflux of cholesterol during in vitro incubation.

Activation of cAMP-PKA Pathway

Human sperm capacitation can be mimicked in vitro in a chemically defined medium containing electrolytes (Na^+, K^+, Cl^-, HCO_3^-, Mg^{2+}, Ca^{2+}, and $PO_3^{-}{}_4$), energy substrates (glucose, pyruvate, and lactate), and a cholesterol acceptor (usually serum albumin).

The activation of intracellular signaling pathways is dependent on the presence of the chemicals in the capacitation medium. Once human sperm are exposed to seminal plasma or the female reproductive tract, they encounter higher concentration of HCO_3^-, which stimulates the soluble adenylyl cyclase ADCY10. Activation of ADCY10 primarily by HCO_3^-, but also by Ca^{2+} leads to an increase in cyclic adenosine monophosphate (cAMP) synthesis.

The initial HCO_3^- entrance in human sperm occurs through NBC cotransporters. In addition, inhibition of the cystic fibrosis transmembrane conductance regulator channel (CFTR) affects HCO_3^--entrance-dependent events, such as phosphorylation in substrates of protein kinase A (PKA) and tyrosine phosphorylation (pY).

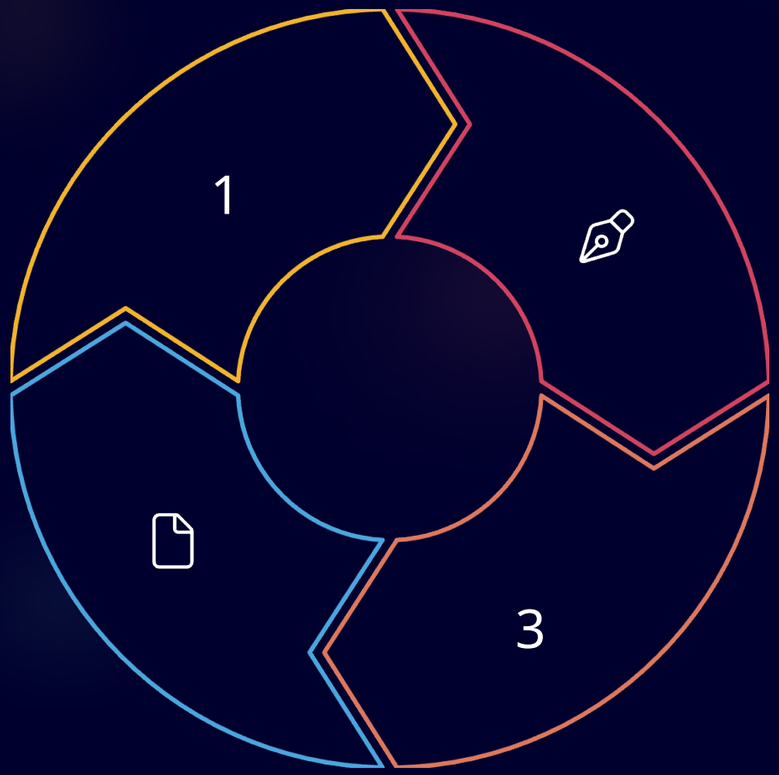
cAMP Signaling in Sperm Function

cAMP Synthesis

In sperm, intracellular cAMP levels are highly dynamic. Its concentration relies on the simultaneous action of both synthesis by ADCY10 and degradation by phosphodiesterases (PDE).

Tyrosine Phosphorylation

One of the best characterized events in sperm capacitation is the time-dependent increase in tyrosine phosphorylation (pY). The increase in sperm pY is downstream of a cAMP/PKA-dependent pathway.



PKA Activation

One of the main targets of cAMP is PKA, which is essential in sperm biology. PKA is an heterotetramer composed of two catalytic subunits (C) and two regulatory subunits (R).

AKAP Localization

A-kinase-anchoring proteins (AKAPs) anchor the R subunit of PKA, restricting its activity to discrete locations within the sperm. Several AKAPs such as AKAP3 and AKAP4 are present in human sperm.

In human sperm, the mechanism by which PKA activates pY was reported to be mediated by proline-rich tyrosine kinase 2 (PYK2). In summary, the cAMP/PKA signaling pathway is essential for human sperm capacitation and is activated by HCO_3^- and Ca^{2+} influx during the sperm transit from the epididymis to the oviduct.

pH Regulation in Sperm Capacitation

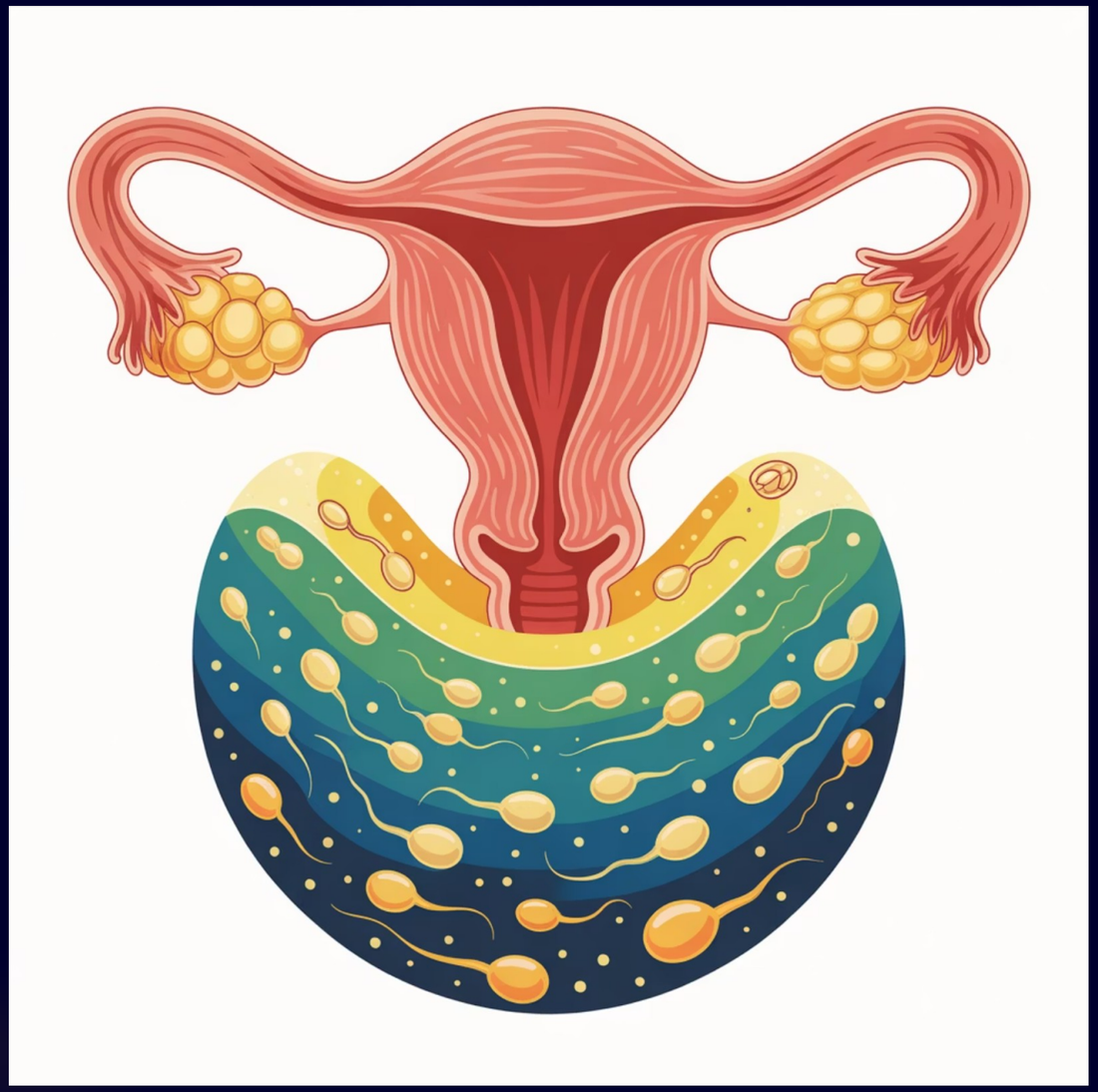
pH Changes During Sperm Transit

Sperm encounter a variety of dramatic changes in H^+ extracellular concentration during their transit from the epididymis to the site of fertilization in the female tract:

- Extracellular pH (pHe) from epididymis is acidic (approx. 6.8)
- In humans, the pH of semen is approximately 7.2–8.4
- In the human female reproductive tract, pH is graduated, with lowest pH in the vagina (approx. pH 4.4), increasing toward the endocervix and uterus (approx. pH 7)

In addition to different H^+ concentrations, sperm encounter a variety of different ionic compositions such as HCO_3^-, which varies from approximately 2–4 mM in the epididymis to approximately 25 mM in seminal plasma, and approximately 20–60 mM in the female tract.

Alkalinization During Capacitation



During their transit through the female reproductive tract, sperm encounter an alkaline pH, higher HCO_3^- concentration, and albumin. All these factors contribute to the cytoplasmic alkalinization that

Mechanisms of pH Regulation in Human Sperm

1

Voltage-gated H^+ channels (Hv1)

Hv1 is the dominant proton conductance in human sperm, located in the principal piece of the flagellum. It is an H^+-selective channel whose activity is potentiated by capacitation, anandamide, membrane depolarization, and alkaline extracellular pH. Interestingly, this channel is inhibited by Zn^{2+}, which is present in high concentration in seminal plasma.

2

Na^+/H^+ Exchangers (NHE)

The expression of three NHEs has been identified in human sperm: NHE1, NHE5, and NHE10. In addition, a sperm-specific NHE (sNHE) is expressed, whose localization is restricted to the principal piece. In human sperm, sNHE is mainly localized in the principal piece and its expression is downregulated in sperm from asthenozoospermic patients.

3

HCO_3^- transporters

HCO_3^- transporters include the SLC26 and SLC4 families and the CFTR. In human sperm, NBC2, NDCBE, and NBCn2 were detected in testis. It has recently been shown that NBC is involved in the initial HCO_3^- uptake in humans. CFTR is a selective ion channel to Cl^- that also transports other anions including HCO_3^-.

4

Carbonic anhydrases (CAs)

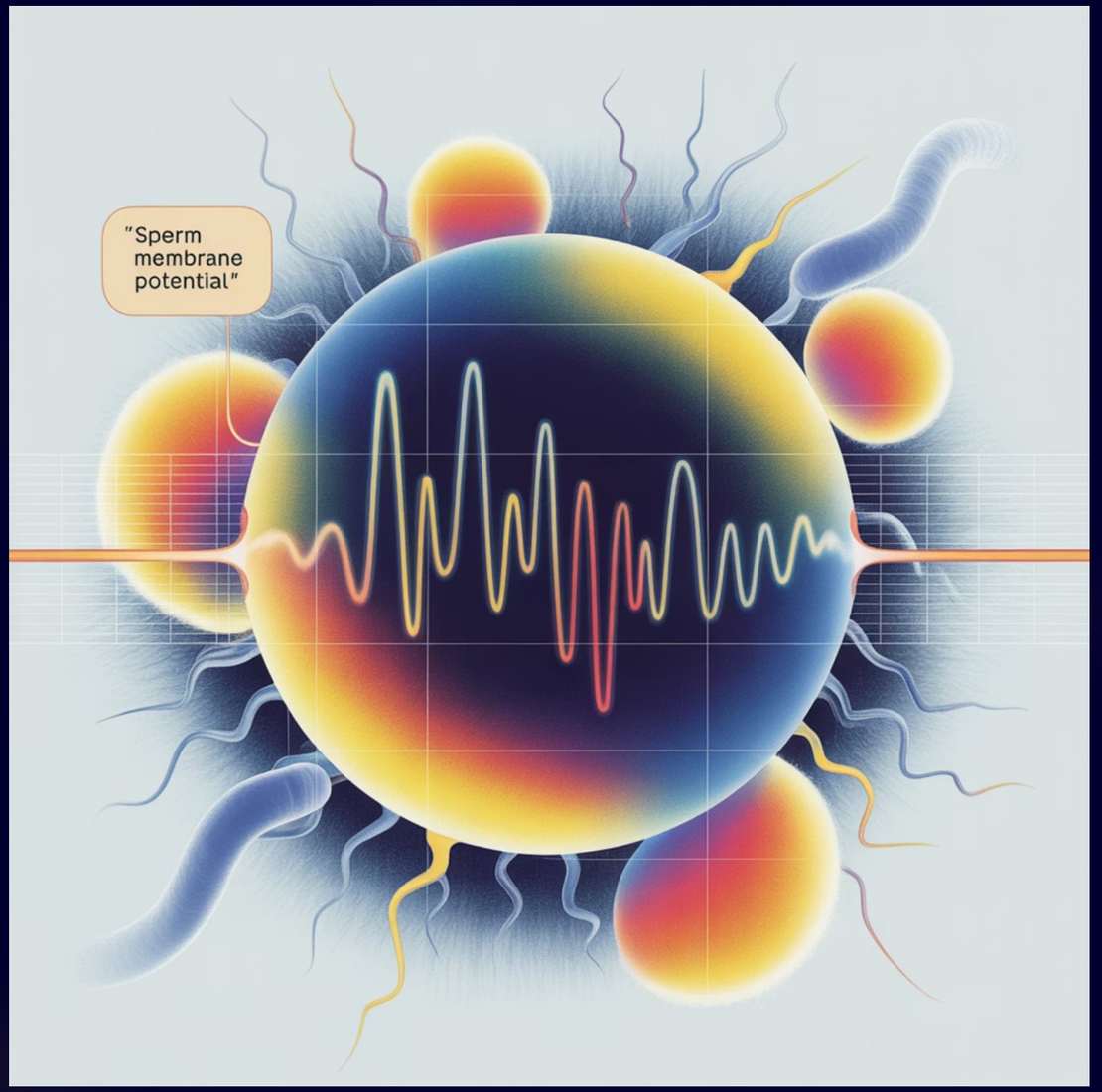
Carbonic anhydrases (CAs) catalyze the reversible hydration of carbon dioxide to HCO_3^-. The expression of some CAs has been reported in human sperm, including CAI, CAII, and CAXIII. The function of CAs is not yet fully understood, but the use of general blockers against these enzymes affects motility and increases the acrosome reaction in capacitated human sperm.

Membrane Potential in Human Sperm

In any given cell, the metabolic state and specific ion channels and transporters determine the internal and the external ion concentration and the plasma membrane permeability that defines the membrane potential (Em). Sperm encounter different concentrations of extracellular K^+, Na^+, Cl^-, and HCO_3^- throughout their journey from the testis to the site of fertilization in the female tract.

In human sperm, it was demonstrated that the regulation of Em is related to male fertility due to the modulation of ion channels and transporters such as CatSper (sperm-specific Ca^{2+} channel) and Hv1. It was reported that idiopathic and asthenozoospermic infertile men have more depolarized Em than fertile men, and that depolarization of Em is associated with low IVF success rate in subfertile men.

Hyperpolarization During Capacitation



Hyperpolarization of the Em occurs when there is an increase in the concentration of net negative charges in the intracellular compartment. Membrane hyperpolarization during capacitation has been demonstrated in murine, bovine, equine, and human sperm.

Regulation of Membrane Potential

K^+ channels SLO1 and SLO3

In mammalian sperm, hyperpolarization associated with capacitation is inhibited using blockers such as Ba^{2+} and sulfonylureas. Two members of the Slo family of K^+ channels were proposed to have a role in this phenomenon: Slo1 and sperm-specific Slo3.

In human sperm, the participation of K^+ channels is not as well established as it is in mouse sperm. Human sperm K^+ current (KSper) is less sensitive to pH and more sensitive to $[Ca^{2+}]i$, and is inhibited by progesterone.

Na^+ transport

Na^+ participates in establishing the resting Em in sperm. The Na^+ epithelial channels (ENaC) is an heteromultimeric channel composed of the combination of α, β, γ, or δ subunits. The activity of ENaC channels is closely associated with CFTR, as this channel negatively regulates ENaC.

In human sperm, the presence of the ENaC-δ subunit in the testis, ENaC-α in the mid-piece of the sperm flagellum, and the expression of ENaC-β have been demonstrated.

Na^+/K^+ ATPase

The Na^+/K^+ pump is an electrogenic transmembrane ATPase that catalyzes Na^+ and K^+ transport by using the energy derived from ATP hydrolysis. The α4-subunit is specifically expressed in germ cells of rat, mouse, and human mature sperm.

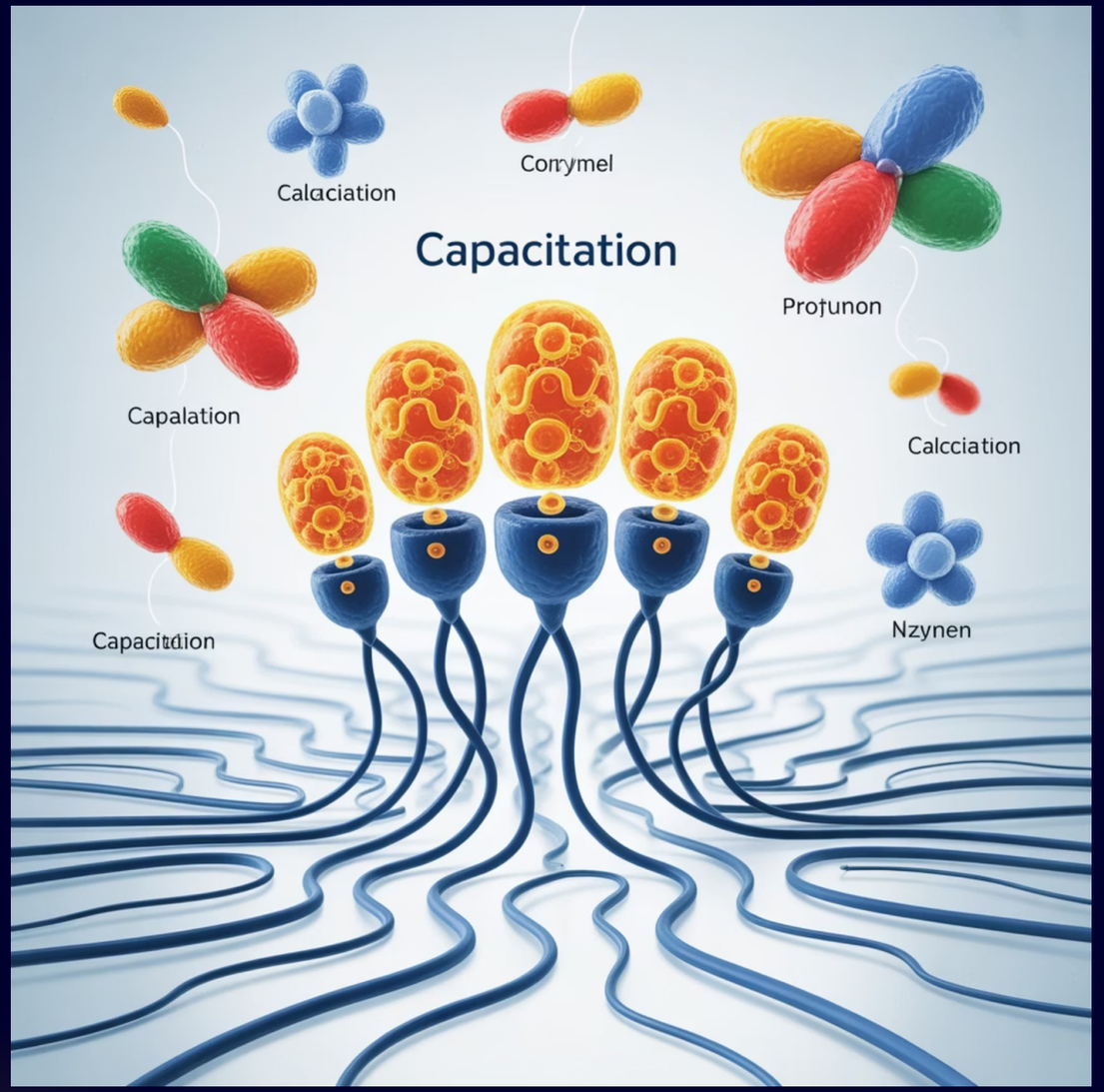
Human sperm treated with ouabain showed an $[Na^+]i$ increase at concentrations that inhibit Na^+/K^+ ATPase α1 and α4 and a decrease in sperm motility at concentrations that selectively inhibited Na^+/K^+ ATPase α4.

It was reported that hyperpolarization of the plasma membrane occurs downstream of cAMP elevation in mouse and human sperm. In human sperm, the PKA-dependent activation of CFTR also contributes to the regulation of Em. It is postulated that hyperpolarization may occur as a result of either the increase of K^+ permeability and/or the reduction of Na^+ permeability.

Calcium Requirements During Capacitation

Sperm functional changes that take place during capacitation depend on a combination of sequential and concomitant signaling processes, which includes complex signaling cascades where intracellular Ca^{2+} plays a central role. There are some reports where Ca^{2+} levels were measured and showed an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) during mammalian sperm capacitation.

It has been described that Ca^{2+} can directly bind to membrane phospholipids and to numerous enzymes, modifying the membrane properties and enzymatic activity. This ion may also bind to calmodulin (CaM), and CaM antagonists have been shown to inhibit certain aspects of sperm function, as hyperactivated motility.



Ca^{2+} binding to CaM causes conformational changes, and this complex modulates the activity of adenylyl cyclases, phosphatases, phosphodiesterases, and protein kinases. Interestingly, testis specific ADCY10 is Ca^{2+}-dependent but CaM-independent, suggesting that Ca^{2+} regulates capacitation through multiple pathways.

Calcium Transport Systems in Sperm

1

Calcium Efflux Systems

The plasma membrane Ca^{2+} ATPase (PMCA) and the Na^{+}/Ca^{2+}-exchanger (NCX) pump Ca^{2+} out of the cell or into intracellular Ca^{2+} stores. PMCA is localized in the principal piece of the flagellum and is relevant for sperm function. NCX is present in the plasma membrane of mammalian sperm and is thought to be of great importance for the regulation of Ca^{2+} homeostasis.

2

Calcium Influx Systems

Ca^{2+} influx involves mainly the sperm-specific Ca^{2+} channel CatSper. Other Ca^{2+} plasma membrane channels have also been identified in spermatogenic and sperm cells, including voltage-gated Ca^{2+} (Cav) channel subunits, cyclic nucleotide-gated channels (CNG), and some members of the transient receptor potential channel (TRPC) family.

3

Intracellular Calcium Stores

Sperm intracellular Ca^{2+} can be exchanged to or from internal stores localized in the acrosome, as well as in the neck (redundant nuclear envelope, RNE) by inositol triphosphate and ryanodine receptors (IP3R and RyR, respectively). In human sperm, the RyR was located mainly in the neck region, whereas the IP3 receptors were found in the neck region and in the acrosome.

4

Calcium Pumps

Two Ca^{2+} pumps were identified and located in human sperm: sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase (SERCA) and secretory pathway Ca^{2+} ATPases (SPCA). SERCA 2 has been localized in the acrosome and mid-piece regions and it has been suggested to participate in Ca^{2+} sequestration in internal stores during sperm capacitation.

CatSper Channel: Structure and Regulation

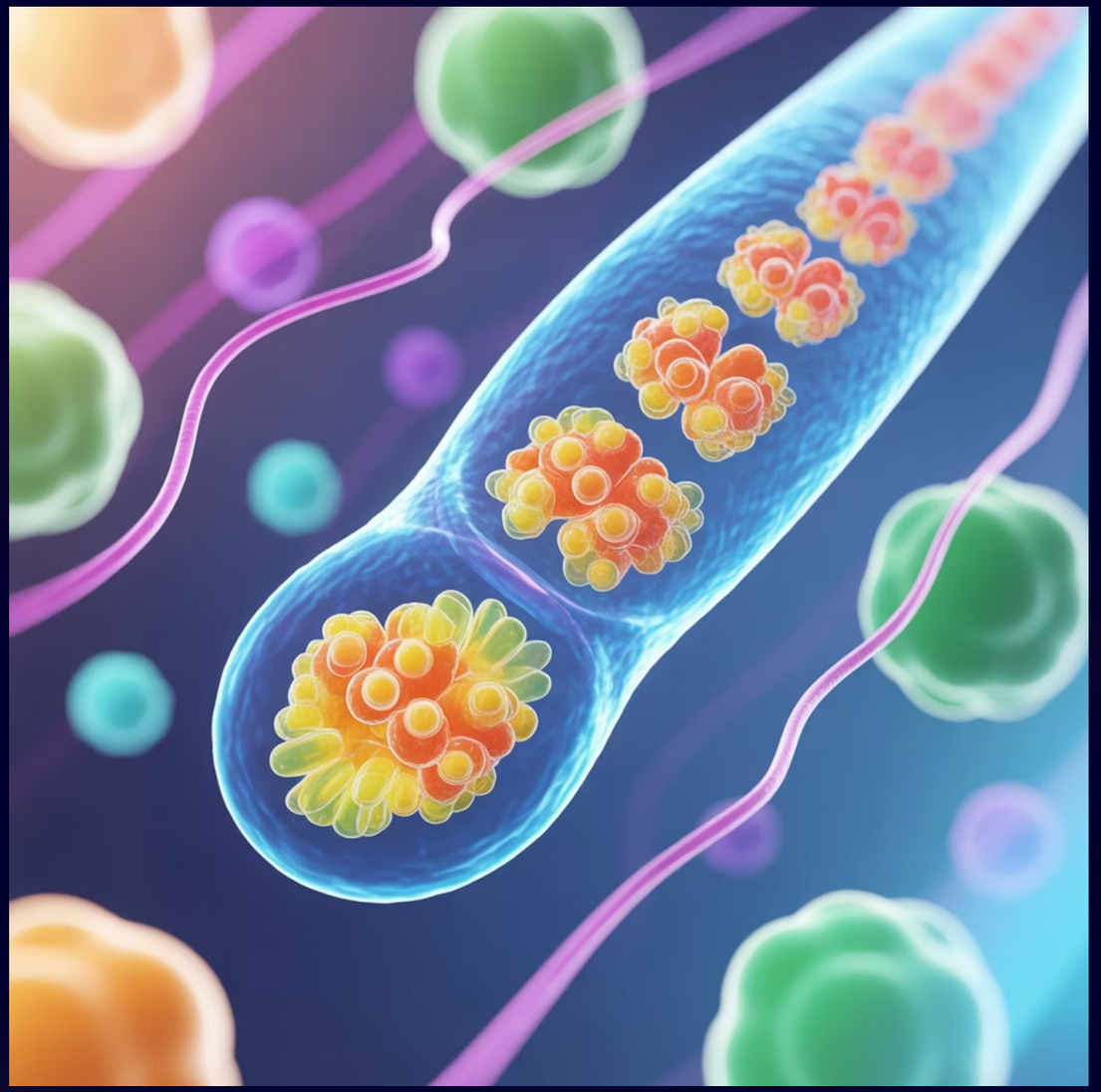
Despite a large body of evidence indicating the presence of multiple Ca^{2+} channels in human sperm, their activity has not been totally elucidated. The advent of sperm electrophysiology allowed the characterization of Ca^{2+} currents through CatSper channels.
This channel complex is localized in the sperm flagellum and comprises four homologous α subunits (CatSper 1–4) and auxiliary subunits: CatSper β, CatSper ɣ, CatSper δ, CatSper ξ and CatSper ζ. Deficiency of any subunit affects the expression of all the other subunits and is detrimental to male fertility.

Evidence from KO mice has shown that CatSper is essential for hyperactivation and fertilization. CatSper-KO sperm are unable to migrate efficiently in vivo and penetrate the egg cumulus and the zona pellucida. In humans, point mutations within the CatSper1 gene as well as deletion of the CatSper2 gene are related to male infertility.

CatSper Channel Distribution and Function

Recent groundbreaking work using super-resolution microscopy (STORM) in the mouse model showed that CatSper distributes longitudinally following four backbone lines, which are localized in the plasma membrane of the principal piece, close to the fibrous sheath. Similarly, it has been reported that human CatSper ξ is arranged in four domains along the flagellum.

Together with CatSper, other signaling molecules display a similar spatial distribution along the principal piece, which reveals a complex organization of signaling pathways in the sperm flagellum that focuses tyrosine phosphorylation in time and space.



It has been reported that approximately 30% of sperm presented a quadrilateral CatSper1 domain organization and they were able to display hyperactivated motility and tyrosine phosphorylation. This is consistent with the observations that only a subpopulation of sperm achieved hyperactivation upon capacitation.

Regulation of CatSper Channel Activity

Voltage and pH Sensitivity

The human CatSper channel is slightly more voltage dependent in comparison to the mice one. Although intracellular alkalinization allows the opening of mice CatSper channels, this is not sufficient for human sperm. The highly enriched histidine composition of the N-termini of both CatSper1 proteins is thought to be involved in the pH sensitivity of the channel.

Steroid Regulation

In humans but not mice, progesterone activates CatSper via binding to the serine hydrolase ABHD2 (α/β hydrolase domain–containing protein 2). It has been shown that at rest the human CatSper channel is inhibited by the endocannabinoid 2-arachidonoylglycerol (2-AG); after progesterone binding, ABHD2 degrades 2-AG, relieving CatSper inhibition.

Other Physiological Regulators

Prostaglandins also activate the human CatSper channel, but independent of the ABHD2 mechanism. The prostaglandins-induced Ca^{2+} influx evokes acrosomal exocytosis and increases motility. Both progesterone and prostaglandin modulation is suggested to be restricted to human and primate sperm and do not involve classical nuclear receptors or G protein-coupled receptors.

Interaction with Other Signaling Pathways

Evidence in the mouse sperm suggests that SLO3 K^+ channels control Ca^{2+} entry through CatSper. High concentrations of HCO_3^- trigger an initial change in the pHi, which activates SLO3 channels; the resulting membrane hyperpolarization raises pHi even more, probably through an NHE mechanism. This intracellular alkalization activates the CatSper channel, which results in a very rapid $[Ca^{2+}]i$ increase.

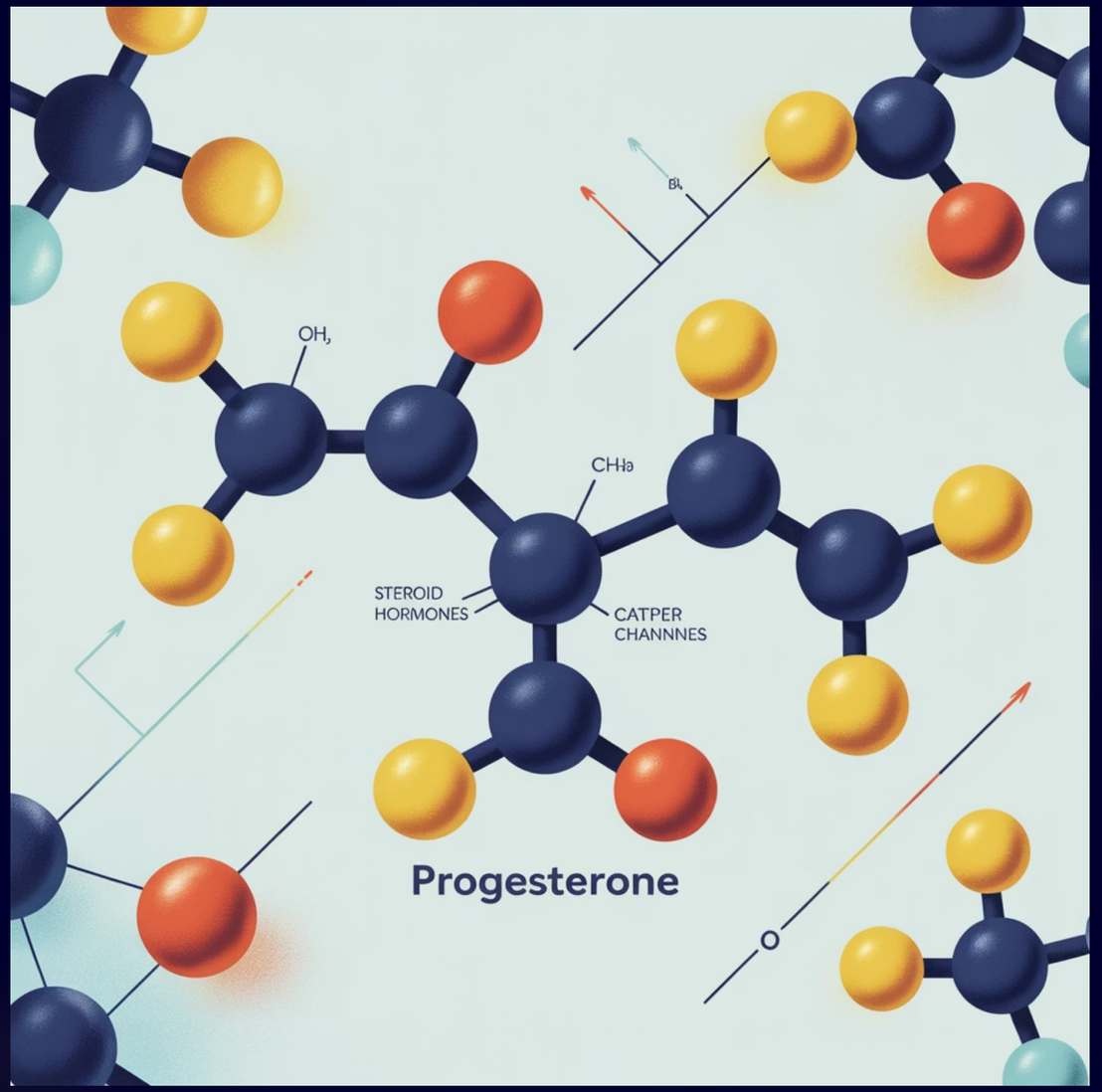
Additional Regulators of CatSper Activity

Neurosteroids and CatSper

Patch-clamp recordings from human sperm revealed that the neurosteroid pregnenolone sulfate exerted similar effects as progesterone on CatSper currents. CatSper-deficient patients were described as infertile, and their sperm did not produce any progesterone nor pregnenolone sulfate-activated currents.

There are controversies about the effects of testosterone, estrogen, and hydrocortisone on CatSper currents. Some results revealed that they abolish CatSper activation by progesterone but these steroids do not activate CatSper themselves. On the other hand, other evidence determined that testosterone, hydrocortisone, and estradiol are agonists that activate CatSper.

Other Modulators



Human ß-defensin 1, a small secretory peptide with antimicrobial activities, interacts with the sperm chemokine receptor type 6 (CCR6), triggering Ca^{2+} mobilization. CCR6 colocalizes and interacts with CatSper in human sperm, and both are required for the Ca^{2+} entry/current induced by physiological

Summary of Molecular Mechanisms in Human Sperm Capacitation

Sperm are exposed to higher HCO_3^- concentration at the time of ejaculation and during their transit through the female reproductive tract. In addition, removal of sperm cholesterol from the plasma membrane to acceptors present in the uterus and fallopian tubes, such as albumin, results in biophysical modification of the plasma membrane.

The initial HCO_3^- transport through NBC cotransporters activates ADCY10 and that in turn produce an increase in cAMP concentration, leading to the activation of PKA. Phosphorylation by PKA is essential for CFTR activity, and together with other Cl^-/HCO_3^- cotransporters, it produces a sustained increase in HCO_3^-.

Activation of PKA led to protein tyrosine phosphorylation by mechanisms that are not completely elucidated, which involved the kinases PYK2/FERT. At the same time, upon contact with HCO_3^-, there is an increase in sperm intracellular pH. Human sperm alkalinization is also favored by the efflux of proton through Hv1 channels. Alkalinization and certain steroids present in the female reproductive tract such as progesterone activate CatSper channels and produce a sustained increase in $[Ca^{2+}]i$. Activation of cAMP/PKA pathways also leads to hyperpolarization of the plasma membrane through the opening of K^+ channels and the closure of Na^+ channels.