Summary

The non-genomic actions of steroids are rapid in nature and are relevant for fast changes in the membrane and their effects on ion channels. Various reports suggested that these fast actions of steroids are responsible for various important physiological functions such as sperm hypermotility at the time of fertilization and others. Reports indicate that CatSper, a Ca^{2+} ion channel present in mature sperm, is modulated by Progesterone (P4), a steroid molecule. To further enhance the understanding of P4 and ion channel interaction, the phylogeny of this channel was analysed in this study and it was observed that the CatSper channel represents a channel with gnomically and sequence-wise diversity. The synteny analysis of this channel indicates the "gene loss" event in various taxa especially in amphibians. The amino acid sequence at critical regions, such as in the Lipid Water Interface (LWI) region and possible TM region, loop-region, and other interacting motifs remain highly diverse. Such diversity may be relevant for the diverse environment in which sperm cells from different species must survive to complete fertilization. The data also indicates the possibility of the presence of ion channels other than CatSper that can recognize P4.

To further analyze the effect of Progesterone on this ion channel, the function of sperm collected was studied from bull and mouse, two closely related mammals. It was observed that the various parameters, such as sperm motility, cytosolic Ca^{2+}, mitochondrial Ca^{2+}, ratio of cytosolic vs mitochondrial Ca^{2+}, are modulated differently. Such results suggest possibilities of the presence of alternate ion channels which can also be the target for P4 and can alter the functionality of mature sperm cells. It was explored if TRPV4 (which is known to be present in sperm endogenously) can function as another non-selective Ca^{2+} channel and can get modulated by Progesterone. Experimental data suggests that TRPV4 can modulate Progesterone-mediated downstream signaling events in sperm cells, suggesting that TRPV4 can also act as a P4-sensitive ion channel. Further, the possible Progesterone and TRPV4 ion

channel interaction was analysed, and docking studies were performed. It was observed that there are different binding pockets present in the TRPV4 where P4 can interact. Further, the molecular simulation experiments suggest that Progesterone can form hydrogen bonds at certain residues and increase the pore radius. Collectively the *in silico* and biochemical experiments suggest that the TM4-loop-TM5 region of TRPV4 serves as a potential region where Progesterone can bind and modulate the channel.

To further analyze the possibilities of Progesterone-mediated TRPV4 channel opening, F11 cell was chosen as a neuronal and heterologous system as F11 cells do not have endogenous expression of TRPV4. Wild-type TRPV4 ion channel was expressed along with their disease-causing mutations, namely L596P, R616Q and L618P to explore the effect of P4 on Ca^{2+}-influx. It was observed that P4 can induce Ca^{2+}-influx in cells expressing TRPV4-Wt but not with its mutations. TRPV4 seems to alter the effect of P4 both in short-term and in long-term. Further, the effect of Progesterone on the bone lineage cell was analysed, since Progesterone has long-term effect on osteoblast regulation and is relevant for osteoporosis. Thus bio-mineralization as well as other functional parameters performed by MSCs isolated from male and female BALB/c mice in presence of different modulators of TRPV4 and or Progesterone were compared. Differences in the cytosolic Ca^{2+}, mitochondrial potential, cytosolic ROS and mitochondrial ROS in cells isolated from both males and females were observed, both after 1-day as well as after 12-days. Data also suggests that TRPV4 modulation can differentially alter the correlation between cytosolic Ca^{2+} and mitochondrial potential, mostly in a context-dependent manner.

The major findings of this thesis work can be summarized as, TRPV4 as an alternate ion channel where P4 may act and have broad implications in health, especially in several pathophysiologies. In particular, such understandings may have relevance in reproduction, neuropathic pain, and bone-disorders.