

## Index

List of figures .....	VII
List of abbreviations .....	VII
1 Introduction.....	- 1 -
1.1 Fertilization in mammals .....	- 1 -
1.2 Zona pellucida glycoproteins.....	- 4 -
1.3 Ion channels and transporters required for sperm fertility .....	- 8 -
<b>1.3.1 CatSper, the principal sperm Ca<sup>2+</sup> channel</b> .....	- 8 -
<b>1.3.2 Slo3 potassium channel</b> .....	- 10 -
<b>1.3.3 The proton channel Hv1</b> .....	- 10 -
<b>1.3.4 Sodium-proton exchanger family</b> .....	- 11 -
1.4 ZP signaling .....	- 12 -
1.5 Aim of this PhD thesis.....	- 14 -
2 Materials and methods .....	- 15 -
2.1 Materials.....	- 15 -
<b>2.1.1 Chemicals</b> .....	- 15 -
<b>2.1.2 Antibodies</b> .....	- 15 -
2.2 Escherichia coli culture .....	- 17 -
<b>2.2.1 Bacterial strains and vectors</b> .....	- 17 -
<b>2.2.2 Composition and preparation of E. coli culture media</b> .....	- 17 -
<b>2.2.3 Amplification of E. coli cultures for plasmid preparation</b> .....	- 17 -
<b>2.2.4 Generation of competent E. coli cells</b> .....	- 18 -
2.3 Preparation of nucleic acids .....	- 18 -
<b>2.3.1 Mini-preparation of plasmid DNA via alkaline lysis</b> .....	- 18 -
<b>2.3.2 Midi- and maxi-preparation of plasmid DNA</b> .....	- 18 -
<b>2.3.3 Preparation of genomic DNA from mouse tissue</b> .....	- 19 -
2.4 Separation, purification and quantification of plasmid DNA and DNA fragments ....	- 19 -
<b>2.4.1 Agarose-gel electrophoresis</b> .....	- 19 -
<b>2.4.2 Elution of DNA from agarose gels</b> .....	- 20 -
<b>2.4.3 Ethanol precipitation</b> .....	- 20 -

2.4.4	<b>Purification with SureClean</b> .....	- 20 -
2.4.5	<b>Photometric quantification of nucleic acid concentration</b> .....	- 21 -
2.4.6	<b>Quantification of nucleic acid concentration by agarose-gel electrophoresis.</b> -	21 -
2.5	Modification of nucleic acids.....	- 21 -
2.5.1	<b>Restriction digest of plasmid DNA</b> .....	- 21 -
2.5.2	<b>Ligation of DNA fragments</b> .....	- 21 -
2.5.3	<b>Transformation</b> .....	- 21 -
2.6	Polymerase chain-reaction (PCR) .....	- 22 -
2.6.1	<b>Primer synthesis</b> .....	- 22 -
2.6.2	<b>PCR conditions</b> .....	- 22 -
2.7	Mammalian cell culture.....	- 23 -
2.7.1	<b>Sterile work</b> .....	- 23 -
2.7.2	<b>Cell lines</b> .....	- 23 -
2.7.3	<b>Continuous culture of HEK293, HEK293T cells, and hybridoma cells</b> .....	- 24 -
2.7.4	<b>Cryopreservation of mammalian cell lines</b> .....	- 24 -
2.7.5	<b>Transient transfection</b> .....	- 24 -
2.7.6	<b>Stem cell culture</b> .....	- 25 -
2.8	Immunofluorescence.....	- 25 -
2.8.1	<b>Immunocytochemistry</b> .....	- 25 -
2.8.2	<b>Sectioning of frozen tissue</b> .....	- 25 -
2.8.3	<b><math>\beta</math>-galactosidase staining of testis sections</b> .....	- 26 -
2.9	Protein preparation .....	- 26 -
2.9.1	<b>Protein preparation from mammalian cells</b> .....	- 26 -
2.9.2	<b>Protein preparation from mouse tissue</b> .....	- 26 -
2.9.3	<b>Protein quantification with bicinchoninic acid</b> .....	- 27 -
2.10	Purification of proteins from cell supernatant .....	- 27 -
2.10.1	<b>Batch purification via Ni-NTA agarose</b> .....	- 27 -
2.10.2	<b>Large-scale protein purification using the ÄKTA system</b> .....	- 28 -
2.10.3	<b>Buffer exchange</b> .....	- 28 -
2.10.4	<b>Protein quantification</b> .....	- 28 -
2.10.5	<b>PNGase digestion</b> .....	- 28 -

2.11	Separation and detection of specific proteins.....	- 29 -
2.11.1	<b>Reducing SDS-polyacrylamide gel electrophoresis</b> .....	- 29 -
2.11.2	<b>Mass spectrometry</b> .....	- 30 -
2.11.3	<b>Transfer and immobilization of proteins by Western blotting</b> .....	- 30 -
2.11.4	<b>Immunostaining of immobilized proteins</b> .....	- 31 -
2.12	Laboratory animals .....	- 32 -
2.12.1	<b>Captive care and breeding</b> .....	- 32 -
2.12.2	<b>Isolation of native mouse zona pellucida</b> .....	- 33 -
2.13	Mouse and human sperm experiments.....	- 33 -
2.13.1	<b>Mouse sperm preparation</b> .....	- 33 -
2.13.2	<b>In-vitro fertilization</b> .....	- 34 -
2.13.3	<b>Human sperm preparation</b> .....	- 35 -
2.13.4	<b>Sperm membrane protein preparation</b> .....	- 36 -
2.13.5	<b>Acrosome reaction assay</b> .....	- 36 -
2.13.6	<b>Antigen retrieval for ICC on sperm</b> .....	- 37 -
2.13.7	<b>STORM analysis of sperm flagellar proteins</b> .....	- 37 -
2.13.8	<b>Flagellar beat analysis</b> .....	- 38 -
2.13.9	<b>Electrophysiological recordings from human sperm</b> .....	- 38 -
2.14	Fluorometric measurements in sperm .....	- 39 -
2.14.1	<b>Fluorescent Ca<sup>2+</sup> indicator CAL520</b> .....	- 39 -
2.14.2	<b>Fluorescent pH<sub>i</sub> indicator BCECF</b> .....	- 41 -
2.14.3	<b>Ca<sup>2+</sup> and pH<sub>i</sub> fluorimetry in multi-well plates</b> .....	- 43 -
2.14.4	<b>Stopped-flow device</b> .....	- 43 -
2.14.5	<b>Ca<sup>2+</sup> and pH<sub>i</sub> fluorimetry in the stopped-flow device</b> .....	- 44 -
2.14.6	<b>Calculation of EC<sub>50</sub> values from dose-response curves</b> .....	- 46 -
3	Results .....	- 47 -
3.1	The action of zona pellucida glycoproteins in mouse sperm .....	- 47 -
3.1.1	<b>Isolation and functional characterization of native mouse zona pellucida glycoproteins</b> .....	- 47 -
3.1.2	<b>ZP-evoked [Ca<sup>2+</sup>]<sub>i</sub> responses</b> .....	- 49 -
3.1.3	<b>ZP-evoked pH<sub>i</sub> responses</b> .....	- 51 -

3.1.4	<b>Molecular mechanism underlying the ZP-evoked <math>pH_i</math> response</b> .....	- 56 -
3.1.5	<b>The ZP-evoked <math>pH_i</math> response in mouse involves the NHA1 <math>Na^+/H^+</math> exchanger</b> - 60 -	- 60 -
3.1.6	<b>The ZP-induced acrosome reaction in mouse sperm requires a polarized membrane potential and alkalization</b> .....	- 65 -
3.1.7	<b>Summary</b> .....	- 66 -
3.1.8	<b>Heterologous expression and characterization of mouse zona pellucida glycoproteins</b> .....	- 67 -
3.2	<b>The action of human zona pellucida glycoproteins in human sperm</b> .....	- 72 -
3.2.1	<b>Heterologous expression and characterization of human zona pellucida glycoproteins</b> .....	- 72 -
3.2.2	<b>ZP-evoked <math>[Ca^{2+}]_i</math> responses</b> .....	- 73 -
3.2.3	<b>Species-specificity of heterologous mouse and human ZP glycoproteins</b> .....	- 77 -
3.2.4	<b>ZP-evoked <math>pH_i</math> responses</b> .....	- 78 -
3.2.5	<b>Molecular mechanism underlying the ZP glycoprotein-evoked <math>pH_i</math> response</b> - 80 -	- 80 -
3.2.6	<b>ZP glycoproteins directly activate human CatSper</b> .....	- 85 -
4	<b>Discussion</b> .....	- 88 -
4.1	<b>ZP signaling in mouse and human sperm</b> .....	- 89 -
4.2	<b>Regulation of ZP-evoked acrosome reaction</b> .....	- 91 -
4.3	<b>Which roles play different ZP glycoproteins in ZP signaling?</b> .....	- 92 -
4.4	<b>Outlook</b> .....	- 93 -
5	<b>References</b> .....	- 96 -
6	<b>Appendix</b> .....	- 113 -
7	<b>Acknowledgement</b> .....	- 115 -