SPERM ACTIVATION IN NILE TILAPIA *OREOCHROMIS NILOTICUS* AND THE EFFECTS OF ENVIRONMENTALLY RELEVANT POLLUTANTS ON SPERM FITNESS

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DOCTOR OF PHILOSOPHY
UNIVERSITY OF STIRLING
2010

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by

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A thesis submitted to the University of
Stirling for the degree of
Doctor of Philosophy

INSTITUTE OF AQUACULTURE

UNIVERSITY OF
STIRLING

MAY 2010

Dedicated to my late father who passed away on December 18, 2006.

Declaration

I declare that this thesis has been compiled by myself and is the result of my own scientific investigations. It has not been submitted for any other degree and all sources of information have been duly acknowledged.

Signature _____

Signature of supervisor _____

Date _____

Acknowledgements

I would like to give my gratitude to Prof. Dr Stephen G. George, Dr. Krishen J. Rana and Dr Michael J. Leaver for their expert guidance and who have made a special effort to make this study possible; your generosity on time, help, critism and ideas are truly appreciated.

Thanks are also due to Mr Keith Ranson for providing help on collection of the samples. I would also like to acknowledge my appreciation to Billy (WP), Linton Brown and Dr James Bron of Institute of Aquaculture, your help on technical skill and suggestion are gratefully acknowledged. I am also extremely grateful for the assistance and guidance from numerous staff members at the Institute of Aquaculture and staff at Institute of Zoology (Zoological Society of London), especially to Dr Katrien Van Look and Prof. William Holt, without their training, perseverance, and patience I would never have learned the techniques required to finish this project. I am also especially grateful to Dr Elsa Cabrita of Portugal for her valuable guidance throughout my period of study.

Lastly but not least I would like to thank the Director of Aquaculture himself and Vice Director of the Institute for giving me the opportunity to study in the Institute of Aquaculture, University of Stirling.

I am also grateful to the Public Service Department and Government of Malaysia for financial assistance for the study. And not forgotten Universiti Malaysia Terengganu, especially to the head department of Fisheries Science and Aquaculture and Dean of the Faculty of Agriculture and Food Science for their support during my study.

Finally, my warmest gratitude goes to both my parents, my elder sister (Yong), my husband and my two daughters for their endless love and continuous support throughout the study period.

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Abstract

In externally fertilizing fishes, multiple factors of the spawning environment may affect the sperm viability, and thus the fertilization rate. In this thesis, the sperm activation effect of osmolality of non-electrolytes and electrolytes activation media, pH and ion channel inhibitors on Nile tilapia, *Oreochromis niloticus,* and the effect of environmentally relevant pollutants (cadmium, malathion and rotenone) on sperm fitness (motility and morphology) were investigated.

Seminal fluid samples collected from male fishes (200-250g) were subjected to activation treatments, then analyzed for sperm motility using motility score, and motility variables using Hobson sperm tracker for straight line velocity (VSL), beat cross frequency (BCF) and percentage of motile cells (MOT). For the ion channel inhibitors and pollutants, the effect on sperm motility variables of VSL, VCL (curvilinear velocity) and LIN (linearity) were determined. Multivariate analysis was also carried out to determine the effects of ion channel inhibitors and pollutants on sperm subpopulations. The effects of pollutants on sperm morphology were observed using microscopy techniques, namely, scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Sperm motility was initiated when the sperm were exposed to hypoosmotic electrolytes and non-electrolytes solution. We also found that sperm show optimal activity at pH range of 6-8 which depicts that the effect of pH on sperm motility is negligible. Lanthanum (calcium channel blocker) and flunarizine (sodium-calcium exchanger pump blocker) were found to inhibit sperm motility at 25 and 5 μM, respectively, suggesting that both ion channels play a significant role in sperm activation in *O. niloticus.* In contrast amiloride, ouabain and quinine showed no effects on activation, indicating that epithelial sodium channels, sodium-potassium ATPase and voltage gated potassium channels respectively are unlikely to have major roles in sperm activation or motility. The spermatozoa of *Oreochromis niloticus* were uniflagellate with clearly differentiated oval-shaped head, midpiece and flagellum. Sperm exposed to hypoosmotic shock showed swelling of the midpiece and sleeve structure.

The pollutants showed dose- and time-dependent effect on sperm motility of the fast linear sperm subpopulation. Sperm morphology was not affected. Sperm motility was

inhibited at 0.44, 0.03 and 0.063 μM, cadmium, malathion and rotenone respectively. Both cadmium and malathion exerted effects very quickly after exposure. The effect of cadmium, which can exert toxicity by calcium antagonism, is consistent with the effects of calcium channel blockes and further supports an important role for calcium in sperm activation and motility. Malathion had effects at relatively low, environmentally relevant concentrations, suggesting the presence of functionally important acetylcholinesterase activity in sperm, and also the presence of activation cytochrome P450 activity. Rotenone, a well known mitochondrial poison, affected motility only after 15 min of pretreatment. The alteration of sperm trajectories in fast linear spermatozoa subpopulation by pollutants at submicromolar concentrations as demonstrated in our study implies potentially serious consequences for fish populations in polluted environments. Furthermore the results indicate that fish sperm motility as assessed by CASA could be an ecologically relevant, sensitive, and ethically acceptable method for toxicity testing in environmental risk assessment.