UNIVERSITI PUTRA MALAYSIA

DETECTION OF PHOSPHOLIPASE C ZETA (PLC-ζ) IN TESTIS OF Rattus argentiventer Robinson & Kloss (RICE-FIELD RAT) USING MOLECULAR TECHNIQUES

FADZLINA BINTI AMIR SHAPUDDIN

FPSK(M) 2017 19

DETECTION OF PHOSPHOLIPASE C ZETA (PLC-ζ) IN TESTIS OF *Rattus argentiventer* Robinson & Kloss (RICE-FIELD RAT*)* USING MOLECULAR TECHNIQUES

By

FADZLINA BINTI AMIR SHAPUDDIN

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of
Master of Science**

May 2013

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

DETECTION OF PHOSPHOLIPASE C ZETA (PLC- ζ) IN TESTIS OF *Rattus argentiventer* Robinson & Kloss (RICE-FIELD RAT) USING MOLECULAR TECHNIQUES

By

FADZLINA BINTI AMIR SHAPUDDIN

May 2013

Chair : Associate Professor Sabrina Sukardi, PhD
Faculty: Medicine and Health Sciences

Phospholipase C-zeta (PLC-ζ) is a specific enzyme found in sperm of mammals responsible for triggering calcium oscillations leading to oocyte activation during fertilization. The activation causes the fertilized oocyte to divide and develop into an embryo. *Rattus argentiventer* (Rice Field Rat) is often responsible for destruction of agricultural crops specifically paddy fields. The mammal is known for its rapid reproductive potential and as a carrier of pathogen. This rodent pest population is currently being controlled with baits, traps and biological control such as *Tyto alba*. However the current methods are quite expensive, needs long term monitoring and are hazardous to the environment. Thus the knowledge of fertility such as sperm factor of this rodent could be helpful in a way to prevent overpopulation of this rodent. Two detection methods to identify PLC-ζ gene fragments from the testis of *Rattus argentiventer* using conventional Polymerase Chain Reaction (Reverse Transcriptase RT-PCR) and Real Time Polymerase Chain Reaction (qRT-PCR) techniques were used *in* order to obtain the bands of PLC-ζ. Following that, sequencing of DNA structure and cloning of PLC were performed to amplify gene fragment of PLC-ζ. Approximately 420 bp nucleotides identified sequence was then keyed in into the Basic Local Alignment Search Tool (BLAST) portal in National Centre of Bioinformatics Information (NCBI) for comparison with standard nucleotide sequences from other species for sequence alignment. The specific target 420 bp nucleotides of PLC-ζ were cloned using a kit through ligation and transformation. The result showed that PLC-ζ enzyme was present in *Rattus argentiventer* using PCR technique. However, the cloning procedure carried out was not able to show the presence of target gene fragments of the enzyme. It is confirmed that PLC-ζ enzyme was present in *Rattus argentiventer* through the two detection

methods used. The study is a novel study to detect of PLC-ζ in paddy rats using molecular approaches. The result obtained with RT-PCR was validated with qRT-PCR and the sequence obtained showed excellent similarity homology with published sequence of PLC-ζ in *Rattus norvegicus*. As such this would give valuable baseline information for future researches to be carried out in the approach of controlling and preventing the continous growth of *Rattus argentiventer* population.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN PHOSPHOLIPASE C ZETA (PLC- ζ) DALAM TESTIS *Rattus argentiventer* Robinson & Kloss (TIKUS SAWAH) MENGGUNAKAN KONVENSIONAL REVERSE TRANCRIPTASE (RT-PCR) DAN *REAL TIME* POLYMERASE CHAIN REACTION (qRT-PCR)

Oleh

FADZLINA BINTI AMIR SHAPUDDIN

Mei 2013

Pengerusi : Profesor Madya Sabrina Sukardi, PhD
Fakulti: Perubatan dan Sains Kesihatan

Phospholipase C-zeta (PLC-ζ) adalah enzim spesifik yang ditemui di dalam sperma mamalia yang bertanggungjawab mencetuskan ayunan kalsium menyebabkan pengaktifan oosit semasa persenyawaan. Dengan pengaktifan ini oosit yang tersenyawa membahagi dan berkembang menjadi embrio. *Rattus argentiventer* (Tikus Sawah Padi) sering menyebabkan kemusnahan tanaman pertanian khususnya sawah padi. Mamalia spesis ini berpotensi membiak dengan cepat dan pembawa patogen. Tikus perosak ini biasanya dikawal dengan umpan, perangkap dan kawalan biologi contohnya dengan menggunakan perangkap *Tyto alba*. Walau bagaimanapun, kaedah semasa yang digunakan adalah agak mahal, memerlukan pemantauan jangka panjang dan berbahaya kepada alam sekitar. Oleh itu, pengetahuan mengenai faktor kesuburan seperti sperma tikus ini boleh membantu dalam cara untuk mengawal pembiakan yang terlalu tinggi. Dua kaedah pengesanan untuk mengenal pasti gen PLC-ζ dari testis *Rattus argentiventer* adalah menggunakan *Reverse Transcriptase* (RT-PCR) dan *Real Time Polymerase Chain Reaction* (qRT-PCR) dalam usaha untuk mendapat PLC-ζ nukleotida. Dengan itu, penjujukan struktur DNA dan pengklonan PLC-ζ telah dilakukan untuk mengenali struktur DNA gen PLC-ζ. Kira-kira 420 bp nukleotida yang dikenalpasti kemudiannya dimasukkan ke dalam portal BLAST di *National Centre of Bioinformatics Information* (NCBI) untuk perbandingan dengan spesis lain. Sasaran 420 bp PLC-ζ nukleotida diklon menggunakan kit melalui proses ligasi dan transformasi. Hasilnya menunjukkan bahawa enzim PLC-ζ hadir dalam *Rattus argentiventer* menggunakan kaedah PCR. Walau bagaimanapun, prosedur pengklonan tidak dapat menunjukkan kehadiran enzim PLC-ζ. Dua kaedah kajian pengesanan menggunakan pendekatan molekular mengesahkan bahawa enzim PLC-ζ hadir dalam testis *Rattus*

argentiventer. Hasil yang diperolehi dengan RT-PCR telah disahkan dengan qRT-PCR dan urutan yang diperolehi menunjukkan persamaan homologi tikus piawai PLC-ζ yang telah dikenalpasti. Maklumat yang diperolehi ini akan memberikan panduan asas yang sangat berharga untuk kajian akan datang dengan pendekatan mengawal dan mencegah populasi pembiakan tikus yang tinggi daripada spesis *Rattus argentiventer*.

ACKNOWLEDGEMENTS

Thank you to Allah. This research project would not have been possible without the support of many people. First of all I would like to express my gratitude to my supervisor, Associate Professor Dr. Sabrina Sukardi for her guidance, motivation, support and opportunity to do this study. Deepest gratitude to my co-supervisor, Associate Professor Dr. Cheah Yoke Kqueen for assisting me in many ways, without your knowledge and assistance, this study would not have been successful.

Special thanks to Ms Norfaezah Mohd Noordin and En Nasyriq Anuar for ups and downs throughout the study. To all heads of Department Psychiatry Associate Professor Dr Brian Ho Kong Wai, Dr Bharathi Vengadasalam and Dr Hamidin Awang for giving me permission to further my studies and not forgetting all the staff of Department of Psychiatry especially Dr Normala Ibrahim who was very helpful, Pn Nahariah Mat Lia, En Roslan Yusoff and En Asnoor Abdul Jalil for their understanding. To Mr Lee Learn Han, Dr Crystale Lim Siew Ying, Ms Rosfayati Othman, Ms Nor Azimah Norbidin, Ms Norhayati Sajali and Ms Norhayati Shamsul, thanks for sharing the literature and invaluable assistance and the good times we shared. Also to our laboratory officers Ms Normayati Sulaiman, Ms Hasnijah Yaakob, Mr Nasrul Ridzal Zainal Abidin and Mr Kufli for helping me through the study. To En Salleh from MARDI, Bumbung Lima, for supplying the rats, Dr Anita Abdul Rahman, Dr Zubaidah Jamil@Osman, Ms Armania Nurdin, Mohd Rohaizad Md Roduan and Ms Nurshahira Sulaiman for their moral support to continue this study.

Finally, an honourable mention goes to my family especially to my husband Mr Adli bin Bahari and two beloved daughters, Nurhananni Farzana and Nurhadirah Azmina, thank you for giving me the courage, support and stay beside me through all the study. To my mother, Sadariah binti Hamzah and late father, Amir Shapuddin bin Abdullah Sani, my siblings Fairuz Atida and Amirul Farid for their love and patience. My sincere appreciation and apologies to all colleagues their names are not mentioned. Thank you all.

APPROVAL

I certify that an Examination Committee has met on 15 May 2013 to conduct the final examination of Fadzlina Amir Shapuddin on her Master of Science thesis entitled "Detection of Phospholipase C Zeta (PLC- ζ) In Testis Of *Rattus argentiventer* Robinson & Kloss (Rice-Field Rat*)* Using Molecular Techniques" in accordance with Universities and Universitiy College Act 1971 and Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Member of the Thesis Examination Committee were as follows:

Mohd Nasir Desa, PhD
Senior lecturer
Faculty of Medicine and Health Sciences
University Putra Malaysia
(Chairman)

Huzwah Khaza'ai, PhD
Senior lecturer
Faculty of Medicine and Health Sciences
University Putra Malaysia
(Internal Examiner)

Roslida Abd Hamid @ Abd Razak, PhD
Associate Professor
Faculty of Medicine and Health Sciences
University Putra Malaysia
(Internal Examiner)

Dr Mahanem Bt. Mat Noor, PhD
Associate Professor
UniversitiKebangsaan Malaysia (External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date : 22 March 2017

This thesis was submitted to Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science**.** The members of the Supervisory Committee were as follows:

Sabrina Binti Sukardi, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Cheah Yoke Kqueen, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

A	Adenine
A260	Absorbance at 260 nm
A280	Absorbance at 280 nm
Amp	Ampicillin
APC	Anaphase promoting complex
BAPTA	*N,N*-(1,2-ethanediylbis(oxy-2'l-phenelyne)bisN(carboxymathyl))-glysine
BLAST	Basic Local Alignment Search Tool
bp	Base pair
C	Cytosine
Ca^{2+}	Calcium
CaMKII	Calmodulin-dependent protein kinase
CAMP	Cyclic adenosine monophosphate
cDNA	Complemetary deoxyribonucleic acid
Ct	Cycle threshold
DAG	Diacylglycerol
DdNTPs	Dideoxynucleotide triphosphates
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
EST	Express sequence taq
EtOH	Ethanol
FA	Formaldehyde Agarose
Fg	force exerted by gravity
FSH	Follicle Stmulating Hormone
G	Guanine
g	Gram
GPI	Glycosylphosphatidyl-inositol
ICSI	Intracytoplasmic Sperm Injection
IP3	Inositol triphosphate
IPTG	Isoprapnol β-D-galactopyranoside
IVF	In vitro fertilization
L	Litre
LB	Luria-Bertani
Mg	Miligram
MIQE	Minimum Information for Publication of Quantitative Real Time PCR Experiments
Mins	Minute
ml	Millilitre
mM	Millimolar
MOPS	3-(N-morpholino) propanesulfonic acid
MW	Molecular weight
NA	Nutrient Agar
N	Nucleus

NaCl	Sodium chloride
NCBI	National Centre of Bioinformatics Information
Nm	Nanometer
ºC	Degree celcius
OD	Optical density
PAGE	Polyacrymide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PD	Plasmid DNA
PIP2	Phosphotidylinositol 4,5-bisphosphate
PKC	Protein kinase C
PLC- ζ	Phospholipase C zeta
PN	Pronucleus
qPCR	Relative quantitative real-time polymerase chain reaction
RA	*Rattus argentiventer*
RNA	Ribonucleic acid
rpm	Revolution per minute
RT-PCR	Reverse trancriptase polymerase chain reaction
S	Svedberg
SF	Sperm Factor
SYBR	Sybergreen
T	Thymine
TBE	Tris Borate
Tm	Melting temperature
V	Volt
X-gal	5-bromo-4chloro-3-indolyl-β-D-galactopyranoside
β-ME	β-mercaptoethanol

CHAPTER 1

INTRODUCTION

1.1 Research Background

Phospholipase C zeta (PLCζ) is an enzyme which induces a surge of calcium in the fertilized ovum causing it to divide and develop into an embryo. This enzyme is found in the sperm which triggers calcium oscillations leading to oocyte activation during fertilization. Oocyte activation is an important biological process that allows the release of mammalian oocytes which are arrested at the metaphase of the second meiotic division (MII). Saunders et al., (2002) uses mouse express sequence tag databases and discovered a novel, and importantly testis-specific, Phospholipase C (PLC), termed PLC zeta (PLCζ), which is a protein of ~74 kDa in mice and ~70 kDa in humans. This is the smallest PLC isoform and is found to play an important part in mammalian oocyte stimulation and demonstrate that immunodepletion of PLCζ from sperm extracts suppresses Ca^{2+} releasing ability whereas sperm studies have indicated that the presence of PLCζ in sperm correlates with the sperm's ability to induce Ca^{2+} oscillations in the oocyte (Fujimoto et al., 2004; Kurokawa et al., 2005). Jones et al., (2000) and Jones, (2005) demonstrated that PLCζ has been involved in the release of this arrest as a physiological agent of oocyte activation. Therefore it is found that PLCζ is the physiological agent of mammalian oocyte stimulation (Swann et al., 2006; Whitaker, 2006; Parrington et al., 2007; Kashir et al., 2010). PLCζ mRNA was found to exist as early as day 17 (Young et al., 2009) in nothern blot analyses of testes from postnatal hamsters. It is predominantly localized to post-acrosomal parts of the sperm head in mice, a pattern preserved before the acrosome reaction in immunofluorescence studies (Fujimoto et al., 2004; Yoon & Fissore, 2007; Young et al., 2009). It has been shown to be a potential target in manipulating fertility of male mammals. One of the molecular approaches that can be used is silencing the sperm factor known as Phospholipase C-zeta (PLC-ζ), an enzyme that is involved in fertilization (Swann et al., 2004). In this study Conventional Reverse Tranciptase PCR (RT-PCR) Polymerase Chain Reaction is the molecular approach chosen to detect sperm-specific factor PLC-ζ in rodents pest namely *Rattus argentiventer*. These rodent pests are a rapid breeder and a carrier of pathogens. The method is based on the study conducted by Fujimoto in 2004 who designed the primary sequence for PLCζ in rodents. Real Time PCR (qRT-PCR) was used to detect and validate the result obtained from RT-PCR using the same primers.

1.2 Problem Statements

Rice is a staple food for the Asean region. Rat infestation can cause paddy yield to decrease. *Rattus argentiventer* known as Rice Field Rat (Robinson and Kloss, 1918) was normally cought at paddy fields. These rats are serious pest of paddy and plantation such as oil palm and cocoa (Lam, 1982). The rice field rat is one of the rodent species that is responsible for destructions of all stages of paddy growth in South East Asia such as Malaysia (Wood & Fee, 2003). Precautions need to be implemented at each stage of the paddy growth in order to avoid destruction by these pest which are time consuming and costly. This rodent breeds during the reproductive phase of the rice crop (Lam, 1983). They breed rapidly and could reach high population density in a very short time. Every single breeding pair is able to produce 600 offspring in three months (Vail, 2008). A few studies in Malaysia showed that *Rattus argentiventer* is a carrier of pathogens that could be harm to humans even though the percentage carried is lower compared to other pests studied in Malaysia. These parasite includes intracellular parasites such as ectoparsite, nematodes and protozoa (Shafiyyah et al., 2012). Currently, rodent pest populations are controlled by rodenticides such as warfarin (Buckle et al., 1984) baits, traps (an upgrade of traps combination with physical barrier known as –TBS) and biological methods using owls such as Tyto alba. The excessive use of anticoagulant rodenticides such as vitamin K antagonist that disrupt normal blood clothing mechanism causing lethal haemorrhage resulted in the animals developing resistance to the lethal effects. In addition, improper use of baits could be lethal to non-target animals. Residues of rodenticides found in dead or dying rodents could also be toxic to scavengers and predators (Hoare & Hare, 2006). A recent approach controlling rice field rat populations based on fertility control was by using chemical sterilant that accelerates the natural reproductive ageing process in the rat resulting in sterility or reproductive 'senescence' known as 'contrapest' while removing the adverse effect of poisons on non-target species (Vail, 2008).

As PLCζ has been shown to be a potential target in manipulating the fertility of male mammals (Swan et al., 2004), therefore the aim of this study was to isolate and identify the presence of PLCζ in *Rattus argentiventer* testis. It is hope with the discovery of PLCζ from this rodent could further propagate more research in this area for future potential in developing new strategies in pest control.

1.3 Objective

1.3.1 General objective

To identify Phospholipase C-zeta (PLC-ζ) in *Rattus argentiventer* (Rice Field Rat).

1.3.2 Specific Objectives:

1. To isolate and amplify PLC-ζ gene fragment from testis of *Rattus argentiventer*.
2. To validate PLC gene fragment obtained from conventional PCR with qRTPCR.
3. To determine the sequence of PLC-ζ *Rattus argentiventer* and compare the sequence of PLC-ζ *Rattus argentiventer* with published PLC-ζ sequences.
4. To clone the PLC-ζ sequence.

1.4 Research Hypothesis

PLC-ζ enzyme is present in testis of *Rattus argentiventer* using conventional Reverse Transcriptase (RT-PCR) and Real Time (qRT-PCR) techniques.

REFERENCES

Aghajanpour, S., Ghaedi, K., Salamian, A., Deemeh, M., Tavalaee, M., Moshtaghian, J., NasrEsfahani, M. (2011). Quantitative expression of phospholipase C zeta, as an index to assess fertilization potential of a semen sample. *Human Reproduction*, 26(11), 2950 - 2956.

Bedford-Guaus, S. J., McPartlin, L. A., Xie, J., Westmiller, S. L., Buffone, M. G., & Roberson, M. S. (2011). Molecular Cloning and Characterization of Phospholipase C Zeta in Equine Sperm and Testis Reveals Species-Specific Differences in Expression of Catalytically Active Protein. *Biology of Reproduction*, 85(1), 78-88.

Berridge, M. J., Bootman, M. D., & Roderick, H. L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Natural Revision Molecular Cell Biology*, 4(7), 517-529.

Bleil, J. D., & Wassarman, P. M. (1980). Structure and function of the zona pellucida: Identification and characterization of the proteins of the mouse oocyte's zona pellucida. *Developmental Biology*, 76(1), 185-202.

Boerke, A., Tsai, P. S., Garcia-Gil, N., Brewis, I. A., & Gadella, B. M. (2008). Capacitation-dependent reorganization of microdomains in the apical sperm head plasma membrane: Functional relationship with zona binding and the zona-induced acrosome reaction. *Theriogenology*, 70(8), 1188-1196.

Borg, C. L., Wolski, K. M., Gibbs, G. M., & O'Bryan, M. K. (2010). Phenotyping male infertility in the mouse: how to get the most out of a 'non-performer'. *Human Reproduction Update*, 16(2), 205-224.

Buckle, A. P., Rowe, F. P., & Husin, A. R. (1984). Field trials of warfarin and brodifacoum wax block baits for the control of the rice field rat, *Rattus argentiventer* in peninsular Malaysia. *Tropical Pest Management*, 30(1), 51-58.

Busa, W. B., & Nuccitelli, R. (1985). An elevated free cytosolic Ca^{2+} wave follows fertilization in eggs of the frog, Xenopus laevis. *The Journal of Cell Biology*, 100(4), 1325-1329.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Wittwer, C. T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry*, 55(4), 611-622.

Carmosino, M., Mazzone, A., Laforenza, U., Gastaldi, G., Svelto, M., & Valenti, G. (2005). Altered expression of aquaporin 4 and H+/K+-ATPase in the stomachs of peptide YY (PYY) transgenic mice. *Biology of the Cell*, 97(9),

735-742.

Choi, D., Lee, E., Hwang, S., Jun, K., Kim, D., Yoon, B.-K., Lee, J.-H. (2001). Animal Experimentation: The Biological Significance of Phospholipase C β1 Gene Mutation in Mouse Sperm in the Acrosome Reaction, Fertilization, and Embryo Development. *Journal of Assisted Reproduction and Genetics*, 18(5), 305-310.

Cipriani, G., Lot, G., Huang, W. G., Marrazzo, M. T., Peterlunger, E., & Testolin, R. (1999). AC/GT and AG/CT microsatellite repeats in peach [Prunus persica (L) Batsch]: isolation, characterisation and cross-species amplification in Prunus. *Theoretical and Applied Genetics*, 99(1-2), 65-72.

Cooper, T. W. (1998). Interactions between epididymal secreations and spermatozoa. *Reproduction Fertility Supplementary*, 53, 119-136.

Coward, K., Ponting, C. P., Chang, H.-Y., Hibbitt, O., Savolainen, P., Jones, K. T., & Parrington, J. (2005). Phospholipase Cζ, the trigger of egg activation in mammals, is present in a non-mammalian species. *Reproduction*, 130(2), 157-163.

Cox, L., Larman, M., Saunders, C., Hashimoto, K., Swann, K., & Lai, F. (2002). Sperm phospholipase Czeta from humans and cynomolgus monkeys triggers Ca^{2+} oscillations, activation and development of mouse oocytes. *Reproduction*, 124(5), 611-623.

Cran, D. G., Moor, R. M., & Irvine, R. F. (1988). Initiation of the cortical reaction in hamster and sheep oocytes in response to inositol trisphosphate. *Journal of Cell Science*, 91(1), 139- 144.

Cuasnicu, P., Cohen, P., Ellerman, D., Busso D., Daros, V., & Morganfeld M. (2002). Changes in sperm protein during epidydermal maturation. In Robaire, B. Hinton, B.T, (Ed.), *The epididymis: From Molecules to Clinical Practice* (pp. 389-404). New York: Plenum Press.

Dacheux, J. L. C., S. J.L; Dacheux F. (2005). Epididymal cell secretory activities and he role of protein in boar sperm matration. *Theriogenology*, 63, 319-341.

Deng, M. Y., Wang, H., Ward, G. B., Beckham, T. R., & McKenna, T. S. (2005). Comparison of six RNA extraction methods for the detection of classical swine fever virus by real-time and conventional reverse Transcription–PCR. *Journal of Veterinary Diagnostic Investigation*, 17(6), 574.

Dias Neto, E., Harrop, R., Correa-Oliveira, R., Wilson, R. A., Pena, S. D. J., & Simpson, A. J. G. (1997). Minilibraries constructed from cDNA generated by arbitrarily primed RT-PCR: An alternative to normalized libraries for the generation of ESTs from nanogram quantities of mRNA. *Genes*, 186(1), 135-142.

Ducibella, T., Huneau, D., Angelichio, E., Xu, Z., Schultz, R., Kopf, G., Ozil, J.-P.

(2002). Eggto-embryo transition is driven by differential responses to Ca^{2+} oscillation number. *Developmental Biology,* 250(2), 280 - 291.

Dupont, G., McGuinness, O M, Johnson, M H, Berridge, M J, Borgese, F. (1996). Phospholipase C in mouse oocytes: characterization of beta and gamma isoforms and their possible involvement in sperm-induced Ca^{2+} spiking. *Biochemistry Journal,*(316), 583-591.

Durden, L. A., & Page, B. F. (1991). Ectoparasites of commensal rodents in Sulawesi Utara, Indonesia, with notes on species of medical importance. *Medical and Veterinary Entomology,* 5(1), 1-7.

Eddy, E. M., Toshimori, K., & O'Brien, D. A. (2003). Fibrous sheath of mammalian spermatozoa. *Microscopic Research and Technique,* 61(1), 103-115.

Eickhoff, R., Baldauf, C., Koyro, H.W., Wennemuth, G., Suga, Y., Seitz, J., Henkel, R., & Meinhardt, A. (2004). Influence of microphage migration inhibitory factor (MIF) on the zinc contain and redox state of potein bound sulfydryl groups in rat sperm; indications foe a new role of MIF I sperm maturation. *Molecular Human Reproduction,* 10,605-611.

Eisen, A., & Reynolds, G. T. (1984). Calcium transients during early development in single starfish (Asterias forbesi) oocytes. *The Journal of Cell Biology,* 99(5), 1878-1882.

Essen L.O, P. O., Cheung R, Katan M, Williams, R. L. (1996). Crystal structure of a mammalian phosphoinositide-specific phospholipase C delta. *Nature* 380(6575), 595-602.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature,* 391(6669), 806.

Frenette, G., Legare, C., Saez, F., & Sullivan, R. (2005). Macrophage Migration inhibitory factor in the human epididymis and semen. *Molecular Human Reproduction,* 11, 575-582.

Fujimoto, S., Yoshida, N., Fukui, T., Amanai, M., Isobe, T., Itagaki, C., Perry, A. C. F. (2004). Mammalian phospholipase Cζ induces oocyte activation from the sperm perinuclear matrix. *Developmental Biology,* 274(2), 370-383.

Fukami, K., Inanobe, S., Kanemaru, K., & Nakamura, Y. (2010). Phospholipase C is a key enzyme regulating intracellular calcium and modulating the phosphoinositide balance. *Progress in Lipid Research,* 49(4), 429-437.

Gerard, G., Fox, D., Nathan, M., & D'Alessio, J. (1997). Reverse Transcriptase. *Molecular Biotechnology,* 8(1), 61-77.

Gradil, C., Yoon, S.Y., Brown, J., He, C., Visconti, P & Fissore, R. (2006). PLC ζ: a marker of fertility for stallions? *Animal Reproduction Science* 94, 23-25.

Goni, R., Gracia, P & Foissac, S., (2009). The qRT-PCR data statistical analysis. *Integromics White Paper* (pp. 1-9). Integromics SL, Madrid Science Park, 2 Santiago Grisolía, 28760 Tres Cantos, Spain.

Hannon, G. J., Rossi, John J. (2004). Unlocking the potential of the human genome with RNA interference. *Nature,* 431(7006), 371.

Hermo, L.. Oko, R., Morales & C.R. (1994). Secreation and endocytsis in the male reproductive tract: a role in sperm maturation. *International Reproduction Cytology* 154, 106-189.

Heytens, E., Parrington, J., Coward, K., Young, C., Lambrecht, S., Yoon, S.-Y., De Sutter, P. (2009). Reduced amounts and abnormal forms of phospholipase C zeta (PLCzeta) in spermatozoa from infertile men. *Human Reproduction,* 24(10), 2417 - 2428.

Hoare, J. M., & Hare, K. M. (2006). The impact of brodifacoum on non-target wildlife: Gaps in knowledge. *New Zealand Journal of Ecology,* 30(2), 157-167.

Htwe, N. M., Singleton, G. R., Hinds, L. A., Propper, C. R., & Sluydts, V. (2012). Breeding ecology of rice field rats, Rattus argentiventer and R. tanezumi in lowland irrigated rice systems in the Philippines. *Agriculture, Ecosystems and Environment,* 161, 39-45.

Hyslop, L. A., Nixon, V. L., Levasseur, M., Chapman, F., Chiba, K., McDougall, A., Jones, K. T. (2004). Ca^{2+}-promoted cyclin B1 degradation in mouse oocytes requires the establishment of a metaphase arrest. *Developmental Biology,* 269(1), 206-219.

Ito, M., Shikano, T., Oda, S., Horiguchi, T., Tanimoto, S., Awaji, T., Miyazaki, S. (2008). Difference in Ca^{2+} Oscillation-Inducing Activity and Nuclear Translocation Ability of PLCZ1, an Egg-Activating Sperm Factor Candidate, Between Mouse, Rat, Human, and Medaka Fish. *Biology Reproduction,* 78(6), 1081 - 1090.

Jacob, J., Herawati, N. A., Davis, S. A., & Singleton, G. R. (2004). The Impact of Sterilized Females on Enclosed Populations of Rice Field Rat. *The Journal of Wildlife Management,* 68(4), 1130-1137.

Jones, K. T., Matsuda, M, Parrington, J, Katan, M, Swann, K. (2000). Different Ca^{2+}-releasing abilities of sperm extracts compared with tissue extracts and phospholipase C isoforms in sea urchin egg homogenate and mouse eggs. *Biochemistry Journal,* 346(3), 743-749.

Jones, K. T. (2005). Mammalian egg activation: from Ca^{2+} spiking to cell cycle

progression. *Reproduction,* 130(6), 813-823.

Kashir, J., Heindryckx, B., Jones, C., De Sutter, P., Parrington, J., & Coward, K. (2010). Oocyte activation, phospholipase C zeta and human infertility. *Human Reproduction Update,* 16(6), 690 - 703.

Kashir, J., Jones, C., Lee, H., Rietdorf, K., Nikiforaki, D., Durrans, C., Coward, K. (2011). Loss of activity mutations in phospholipase C zeta (PLCzeta) abolishes calcium oscillatory ability of human recombinant protein in mouse oocytes. *Human Reproduction,* 26(12), 3372 - 3387.

Kashir, J., Jones, C., & Coward, K. (2012). Calcium Oscillations, Oocyte Activation, and Phospholipase C zeta. In M. S. Islam (Ed.), *Calcium Signaling,* 740, 1095-1121.

Katan, M. (1998). Families of phosphoinositide-specific phospholipase C: structure and function. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids,* 1436(1–2), 5-17.

Katz, Z. B., Wells, A. L., Park, H. Y., Wu, B., Shenoy, S. M., & Singer, R. H. (2012). β-Actin mRNA compartmentalization enhances focal adhesion stability and directs cell migration. *Genes and Development,* 26(17), 1885-1890.

Kelleher, K. L., Leck, K.-J., Hendry, I. A., & Matthaei, K. I. (2001). A one-step quantitative reverse transcription polymerase chain reaction procedure. *Brain Research Protocols,* 6(3), 100-107.

Kelley, G., Grant, G., Sarah, E., Ondrako, M., Joanne, S., Alan, V., (2001). Phospholipase C[epsi]: A novel Ras effector. *Embriology Journal,* 20(4), 743- 754.

Kline, D., & Kline, J. (1992). Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg. *Developmental Biology,* 149(1), 80 - 89.

Knott, J. G., Kurokawa, M., & Fissore, R. A. (2003). Release of the Ca^{2+} oscillation-inducing sperm factor during mouse fertilization. *Developmental Biology,* 260(2), 536-547.

Kouchi, Z., Fukami, K., Shikano, T., Oda, S., Nakamura, Y., Takenawa, T., & Miyazaki, S. (2004). Recombinant phospholipase Czeta has high Ca^{2+} sensitivity and induces Ca^{2+} oscillations in mouse eggs. *Journal of Biology Chemistry,* 279(11), 10408 - 10412.

Kupker, W., Diedrich, K., & Edwards, R.G. (1988). Principles of mammalian fertilization. *Human Reproduction,* 13, 20-32.

Kurokawa, M., Sato, K.-i., Wu, H., He, C., Malcuit, C., Black, S., Fissore, R. (2005).

Functional, biochemical, and chromatographic characterization of the complete [Ca^{2+}]i oscillation-inducing activity of porcine sperm. *Developmental Biology*, 285(2), 376 - 392.

Kurokawa, M., Yoon, S., Alfandari, D., Fukami, K., Sato, K.-i., & Fissore, R. (2007). Proteolytic processing of phospholipase Czeta and [Ca2+]i oscillations during mammalian fertilization. *Developmental Biology*, 312(1), 407 - 418.

Lalancette, C., Miller, D., Li, Y., & Krawetz, S. A. (2008). Paternal contributions: New functional insights for spermatozoal RNA. *Journal of Cellular Biochemistry*, 104(5), 1570-1579.

Lam, Y. M. (1982). Rats as rice field pests-their importance and control. In K. et al (Ed.), *Rodent pests of agricultural crops in Malaysia* (pp. 9-17). Kuala Lumpur: Malaysian Plant Protection Society.

Lam, Y. M. (1983). Reproduction in the rice field rat, Rattus argentiventer. *Malayan Nature Journal*, 36(4), 249-282.

Larman, M., Saunders, C., Carroll, J., Lai, F., & Swann, K. (2004). Cell cycle-dependent Ca^{2+} oscillations in mouse embryos are regulated by nuclear targeting of PLCzeta. *Journal of Cell Science*, 117(12), 2513 - 2521.

Lawrence, Y., Whitaker, M., & Swann, K. (1997). Sperm-egg fusion is the prelude to the initial Ca^{2+} increase at fertilization in the mouse. *Development*, 124(1), 233 - 241.

Lee, W. K., Kim, J. K., Seo, M.-S., Cha, J.-H., Lee, K. J., Rha, H. K., Lee, K.-H. (1999). Molecular Cloning and Expression Analysis of a Mouse Phospholipase C-δ1. *Biochemical and Biophysical Research Communications*, 261(2), 393-399.

Legare, C., Berube, B., Bone, F., Lefevre L., Morales, C.R., ; El- Alfy, M., & Sullivan R. (1999). Hamster sperm antigen P26h is a phosphatidyllinositol-anchored protein. *Molecular Reproduction Development*, 52, 225-233.

Leung, L. K. P., Singleton, G., Sudarmaji, Rahmini, (1999). Ecologically based population management of the rice-field rat in Indonesia. In Singleton, G.R., Hinds, L.A., Leir, H., Zhang, Z., (Ed.). *Ecologically based Management of Rodent Pest* (pp. 305-318). ACIAR, Canberra.

Levy, M., & Miller, S. L. (1988). The stability of the RNA bases: Implications for the origin of life. *Proceedings of the National Academy of Sciences*, 95(14).

Liat, L. B., Fong, Y. L., & Krishnasamy, M. (1977). Capillaria hepatica infection of wild rodents in Peninsular Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 8(3), 354-358.

Liat, L. B., Wah, L. T., Cheah, W., & Fong, Y. L. (1976). Angiostrongylus malaysiensis from Tuaran Sabah, with reference to the distribution of the parasite in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 7(3), 384-389.

Lopez, I., Mak, E. C., Ding, J., Hamm, H. E., & Lomasney, J. W. (2001). A Novel Bifunctional Phospholipase C That Is Regulated by Gct12 and Stimulates the Ras/Mitogen-activated Protein Kinase Pathway. *Journal of Biological Chemistry*, 276(4), 2758-2765.

Lwanga, S, K, & Lemeshow, S. (1991). *Sample size determination in health studies. A practical manual*. WHO.

Malcuit, C., Kurokawa, M., & Fissore, R. (2006). Calcium oscillations and mammalian egg activation. *Journal of Cell Physiology*, 206(3), 565 - 573.

Markoulaki, S., Matson, S., & Ducibella, T. (2004). Fertilization stimulates long-lasting oscillations of CaMKII activity in mouse eggs. *Developmental Biology*, 272(1), 15-25.

Maxam, A. M., & Gilbert, W. (1977). A new method for sequencing DNA. *Proceedings of the National Academy of Sciences*, 74(2), 560-564.

McLachlan, R. I., O'Donnell, L., Meachem, S. J., Stanton, P. G., de, K., Pratis, K., & Robertson, D. M. (2002). Hormonal regulation of spermatogenesis in primates and man: insights for development of the male hormonal contraceptive. *Journal of Andrology*, 23(2), 149-162.

Mehlmann, L. M., Chattopadhyay, A., Carpenter, G., & Jaffe, L. A. (2001). Evidence That Phospholipase C from the Sperm Is Not Responsible for Initiating Ca^{2+} Release at Fertilization in Mouse Eggs. *Developmental Biology*, 236(2), 492-501.

Miyazaki, S., & Ito, M. (2006). Calcium signals for egg activation in mammals. *Journal of Pharmacology Science*, 100(5), 545 - 552.

Miyazaki, S., Shirakawa, H., Nakada, K., & Honda, Y. (1993). Essential role of the inositol 1,4,5-trisphosphate receptor/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. *Developmental Biology*, 158(1), 62 - 78.

Mizushima, S., Takagi, S., Ono, T., Atsumi, Y., Tsukada, A., Saito, N., & Shimada, K. (2009). Phospholipase Czeta mRNA expression and its potency during spermatogenesis for activation of quail oocyte as a sperm factor. *Molecular Reproduction Development*, 76(12), 1200 - 1207.

Moore, A., Penfold, L., Iatchman, D., & Moore, H. (1990). A human cDNA clone encodes a protein which blocks sperm-zona binding in vitro. *Cell Biology*

International, 14(69).

Musser, G. G. & Carleton M. D. C. (1993). Family Muridae. In Wilson, D.E. & Reeder, D.M. (Ed.), *Mammal Species of the World* (2nd ed.) (pp. 501-755). Washington D.C., U.S.A. and London, U.K.: Smithsonian Institution Press.

Muul, I., Lim, B. L., & Walker, J. S. (1977). Scrub typhus infection in rats in four habitats in Peninsular Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 71(6), 493-497.

Muul, I., & Chai, K. S. (1978). Distribution of rats infected with Rickettsia tsutsugamushi (Scrub typhus) in an edge habitat. *Southeast Asian Journal of Tropical Medicine and Public Health*, 9(4), 489-493.

Naz, R. K. R. C. (2004). Passive immunization for immunocontraceptive : lessons learned from infectious diseases. *Reproduction Biology and Endocrinology*, 2(75-87).

Nomikos, M., Blayney, L., Larman, M., Campbell, K., Rossbach, A., Saunders, C., Lai, K. (2005). Role of phospholipase C-zeta domains in Ca^{2+}-dependent phosphatidylinositol 4,5-bisphosphate hydrolysis and cytoplasmic Ca^{2+} oscillations. *Journal of Biology Chemsitry*, 280(35), 31011 - 31018.

Nomikos, M., Mulgrew-Nesbitt, A., Pallavi, P., Mihalyne, G., Zaitseva, I., Swann, K., McLaughlin, S. (2007). Binding of phosphoinositide-specific phospholipase C-zeta (PLC-zeta) to phospholipid membranes. *Journal of Biology Chemistry*, 282(22), 16644 - 16653.

Nomikos, M., Elgmati, K., Theodoridou, M., Calver, B., Nounesis, G., Swann, K., & Lai, F. (2011a). Phospholipase Czeta binding to PtdIns(4,5)P2 requires the XY-linker region. *Journal of Cell Science*, 124(15), 2582 - 2590.

Nomikos, M., Elgmati, K., Theodoridou, M., Georgilis, A., Gonzalez-Garcia, J., Nounesis, G., Lai, F. (2011b). Novel regulation of PLCzeta activity via its XY-linker. *Biochemistry Journal*, 438(3), 427 - 432.

Non-chemical Control of Rodents in Lowland Irrigated Rice Crops. Canberra: Australian Centre for International Agricultural Research (ACIAR), 2002.

Nowak, R. (1991). *Walker's mammals of the worlds* (5th ed.). Baltimore: The Johns Hopkins University Press.

Oda, S. (2006). Mammalian sperm factor and phospholipase C zeta. *Journal of Mammalian Ovarian Respiration*, 23(1), 2-9.

Palermo, G. D., Avrech, O. M., Colombero, L. T., Wu, H., Wolny, Y. M., Fissore, R. A., & Rosenwaks, Z. (1997). Human sperm cytosolic factor triggers Ca^{2+} oscillations and overcomes activation failure of mammalian oocytes. *Molecular Human Reproduction*, 3(4), 367-374.

Parrington, J., Brind, S., De Smedt, H., Gangeswaran, R., Anthony Lai, F., Wojcikiewicz, R., & Carroll, J. (1998). Expression of Inositol 1,4,5-Trisphosphate Receptors in Mouse Oocytes and Early Embryos: The Type I Isoform Is Upregulated in Oocytes and Downregulated after Fertilization. *Developmental Biology*, 203(2), 451-461.

Parrington, J., Jones, M., Tunwell, R., Devader, C., Katan, M., & Swann, K. (2002). Phospholipase C isoforms in mammalian spermatozoa: potential components of the sperm factor that causes Ca^{2+} release in eggs. *Reproduction*, 123(1), 31-39.

Parrington, J., Davis, L., Galione, A., & Wessel, G. (2007). Flipping the switch: How a sperm activates the egg at fertilization. *Developmental Dynamic*, 236(8), 2027 - 2038.

Perry, A. C. F., Wakayama, T., Cooke, I. M., & Yanagimachi, R. (2000). Mammalian Oocyte Activation by the Synergistic Action of Discrete Sperm Head Components: Induction of Calcium Transients and Involvement of Proteolysis. *Developmental Biology*, 217(2), 386- 393.

Peter, R. B., Nguyen, P. T., Grant, R. S., Dao, T. H., Phung, T. H., Phi, T. H., Nguyen, V. T. (2005). Population dynamics of rattus argentiventer, rattus losea, and rattus rattus inhabiting a mixed-farminsg system in the red river delta, Vietnam. *Population Ecology*, 47, 247-256.

Phillips, S., Yu, Y., Rossbach, A., Nomikos, M., Vassilakopoulou, V., Livaniou, E., Swann, K. (2011). Divergent effect of mammalian PLCzeta in generating Ca^{2+} oscillations in somatic cells compared with eggs. *Biochemistry Journal*, 438(3), 545 - 553.

Phung, N. T. M., Brown, P. R., & Leung, L. K. P. (2012). Changes in population abundance, reproduction and habitat use of the rice-field rat, Rattus argentiventer, in relation to rice-crop growth stage in a lowland rice agroecosystem in Vietnam. *Wildlife Research*, 39(3), 250-257.

Primakoff, P., & Myles, D. G. (2007). Cell–cell membrane fusion during mammalian fertilization. *FEBS Letters*, 581(11), 2174-2180.

Qiagen PCR Cloning Handbook. (2001). (Vol. 04). Germany: QIAGEN.

Ramadan, W., Kashir, J., Jones, C., & Coward, K. (2012). Oocyte activation and phospholipase C zeta (PLCzeta): diagnostic and therapeutic implications for assisted reproductive technology. *Cell Communication and Signaling*, 10(1), 12.

Rappolee, D. A., Mark, D., Banda, M.J & Werb, Z. (1988). Wound macrophages express TGFalpha and other growth factors in vivo: analysis by mRNA phenotyping (transforming growth factor). *Science*, 241, 708-712.

Real Time PCR Application Guide. (2006). Retrieved from http://www.genequantification.de/real-time-pcr-guide-bio-rad.pdf

Rhee, S. G. (2001). Regulation of phosphoinositide-specific phospholipase C. *Annual Review Biochemistry* 70, 281-312.

Rice, A., Parrington, J., Jones, K. T., & Swann, K. (2000). Mammalian Sperm Contain a Ca2+- Sensitive Phospholipase C Activity That Can Generate InsP3 from PIP2 Associated with Intracellular Organelles. *Developmental Biology,* 228(1), 125-135.

Robinson and Kloss, Species authority Robinson, H.C and Kloss, CB. (1918). Mammals of Korinchi. Journal of the Federated Malay States Museum 8: 1-72.

Ridgway, E. B., Gilkey, J. C., & Jaffe, L. F. (1977). Free calcium increases explosively in activating medaka eggs. *Proceedings of the National Academy of Sciences,* 74(2), 623- 627.

Robaire, B & Hermo, L. (1994). Efferent ducts, epididymis and vas deferens. In Knobil E, Niel J, (Ed,), *Physiology of Reproduction* (pp. 999-1080). New York: Raven Press.

Rogers, N., Hobson, E., Pickering, S., Lai, F., Braude, P., & Swann, K. (2004). Phospholipase Czeta causes Ca^{2+} oscillations and parthenogenetic activation of human oocytes. *Reproduction,* 128(6), 697 - 702.

Roux, K. H. (2009). Optimization and Troubleshooting in PCR. *Cold Spring Harbor Protocols,* 2009(4).

Runft, L. L., Jaffe, L. A., & Mehlmann, L. M. (2002). Egg Activation at Fertilization: Where It All Begins. *Developmental Biology,* 245(2), 237-254.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences,* 74(12), 5463-5467.

Saunders, C., Larman, M., Parrington, J., Cox, L., Royse, J., Blayney, L., Lai, F. (2002). PLCzeta: a sperm-specific trigger of Ci^{+} oscillations in eggs and embryo development. *Development,* 129(15), 3533 - 3544.

Saunders, C., Swann, K., & Lai, F. (2007). PLCzeta, a sperm-specific PLC and its potential role in fertilization. *Biochemistry Social Symposium,* 74, 23 - 36.

Sherwood, L. (2004). The reproductive system. In Thomson, Brooks & Cole (Ed.), *Human physiology: from cells to systems* (pp. 731-748). USA, Yolanda Cassio.

Shimizu, S., Tsuji, M., & Dean, J. (1983). In vitro biosynthesis of three sulfated glycoproteins of murine zonae pellucidae by oocytes grown in follicle culture. *Journal of Biological Chemistry*, 258(9), 5858-5863.

Sinniah, B. (1979). Parasites of some rodents in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 10(1), 115-121.

Siti Shafiyyah, C. O., Jamaiah, I., Rohela, M., Lau, Y. L., & Siti Aminah, F. (2012). Prevalence of intestinal and blood parasites among wild rats in Kuala Lumpur, Malaysia. *Tropical Biomedicine*, 29(4), 544-550.

Skarnes, W.C. Barry, R., Anthony P.W., Manousos, K., Wendy, B., Vivek, L., Alejandro O.M., Mark, T., Jennifer, H., Tony, C., David, J., Jessica, S., Patrick, B., Jun, F., Michael, N., Peter, J., Francis, A., & Allan, (2011). A conditional knockout resource for the genomewide study of mouse gene function. *Nature*, 474(7351), 337-342.

Sone, Y., Ito, M., Shirakawa, H., Shikano, T., Takeuchi, H., Kinoshita, K., & Miyazaki, S. (2005). Nuclear translocation of phospholipase C-zeta, an egg-activating factor, during early embryonic development. *Biochemistry Biophysiology Respiration Communication*, 330(3), 690 - 694.

Song, C., Hu, C.-D., Masago, M., Kariya, K.-i., Yamawaki-Kataoka, Y., Shibatohge, M., Kataoka, T. (2001). Regulation of a Novel Human Phospholipase C, PLCε, through Membrane Targeting by Ras. *Journal of Biological Chemistry*, 276(4), 2752-2757.

Steinhardt, R. A., Epel, D., Carroll, E.J., & Yanagimachi, R. (1974). Is calcium ionophore a universal activator for unfertilised eggs? *Nature*, 252(5478), 41-43.

Stith, B. J., Espinoza, R., Roberts, D., & Smart, T. (1994). Sperm Increase Inositol 1,4,5- Trisphosphate Mass in Xenopus laevis Eggs Preinjected with Calcium Buffers or Heparin. *Developmental Biology*, 165(1), 206-215.

Stricker, S. (1999). Comparative biology of calcium signaling during fertilization and egg activation in animals. *Developmental Biology*, 211(2), 157 - 176.

Sullivan, R., Saez, F., Girouard, J., & Frenette, G. (2005). Role of exosomes in sperm maturation during the transit along the male reproductive tract. *Blood Cells, Molecules, and Diseases*, 35(1), 1-10.

Sutovsky, P., Manandhar, G., Wu, A., & Oko, R. (2003). Interactions of sperm perinuclear theca with the oocyte: Implications for oocyte activation, anti-polyspermy defense, and assisted reproduction. *Microscope Respiration Technology*, 61(4), 362 - 378.

Swann, K., & Ozil, J.-P. (1994). Dynamics of the calcium signal that triggers mammalian egg activation. *International Revision Cytology,* 152, 183 - 222.

Swann, K., Larman, M. G., Saunders, C. M., & Lai, F. A. (2004). The cytosolic sperm factor that triggers Ca2+ oscillations and egg activation in mammals is a novel phospholipase C: PLCζ. *Reproduction,* 127(4), 431-439.

Swann, K., Saunders, C., Rogers, N., & Lai, F. (2006). PLCzeta (zeta): A sperm protein that triggers Ca^{2+} oscillations and egg activation in mammals. *Seminar Cell Developmental Biology,* 17(2), 264 - 273.

Swann, K., & Yu, Y. (2008). The dynamics of calcium oscillations that activate mammalian eggs. *International Journal Developmental Biology,* 52(5-6), 585 - 594.

Tebes, S. J., & Kruk, P. A. (2005). The genesis of RNA interference, its potential clinical applications, and implications in gynecologic cancer. *Gynecologic Oncology,* 99(3), 736- 741.

Tesarik, J., & Testart, J. (1994). Treatment of sperm-injected human oocytes with Ca^{2+} ionophore supports the development of Ca^{2+} oscillations. *Biology Reproduction,* 51(3), 385 - 391.

Torres, J. M., & Ortega, E. (2004). Quantitation of mRNA levels of steroid 5a-reductase isozymes: A method that combines one-step reverse transcription-polymerase chain reaction and separation by capillary electrophoresis. *Electrophoresis,* 25(3), 415-420.

Tortora, G. J., & Grabowski, S. R. (2002). The reproductive system. In B. Roesch (Ed.), *Principles of Anatomy and Physiology* (pp 1014-1025). New York: Von Hoffman Press.

Tuschl, T. (2001). RNA Interference and Small Interfering RNAs. *Biochemistry,* 2(4), 239-245.

Vail, T. L. (2008). A 21st century approach to rice field rat control. *International Pest Control,* 50(4), 190.

Van Peenen, P. (1969). *Preliminary Identification Manual for Mammals of South Vietnam*. United States National Museum Smithsonian Institution, Washington.

Visel, A., Thaller, C., & Eichele, G. (2004). GenePaint.org: an atlas of gene expression patterns in the mouse embryo. *Nucleic Acids Research, 32*(suppl 1), D552-D556.

Walensky, L. D., & Snyder, S. H. (1995). Inositol 1,4,5-trisphosphate receptors selectively localized to the acrosomes of mammalian sperm. *The Journal of*

Cell Biology, 130(4), 857-869.

Whitaker, M. (2006). Calcium at fertilization and in early development. *Physiology Revision*, 86(1), 25 - 88.

Wood, B. J., & Fee, C. G. (2003). A critical review of the development of rat control in Malaysian agriculture since the 1960s. *Crop Protection*, 22(3), 445-461.

Wu, H., He, C., & Fissore, R. (1997). Injection of a porcine sperm factor triggers calcium oscillations in mouse oocytes and bovine eggs. *Molcular Reproductive Development*, 46(2), 176 - 189.

Wu, H., Smyth, J., Luzzi, V., Fukami, K., Takenawa, T., Black, S. L., Fissore, R. A. (2001). Sperm Factor Induces Intracellular Free Calcium Oscillations by Stimulating the Phosphoinositide Pathway. *Biology of Reproduction*, 64(5), 338-1349.

Yamamoto, T. M., Iwabuchi, M., Ohsumi, K., & Kishimoto, T. (2005). APC/C–Cdc20-mediated degradation of cyclin B participates in CSF arrest in unfertilized Xenopus eggs. *Developmental Biology*, 279(2), 345-355.

Yanagimachi, R. (2011). Mammalian Sperm Acrosome Reaction: Where Does It Begin efore Fertilization? *Biology of Reproduction*, 85(1), 4-5.

Yasukawa, K., Nemoto, D., & Inouye, K. (2008). Comparison of the Thermal Stabilities of Reverse Transcriptases from Avian Myeloblastosis Virus and Moloney Murine Leukaemia Virus. *Journal of Biochemistry*, 143(2), 261-268.

Yoda, A., Oda, S., Shikano, T., Kouchi, Z., Awaji, T., Shirakawa, H., Miyazaki, S. (2004). Ca^{2+} oscillation-inducing phospholipase C zeta expressed in mouse eggs is accumulated to the pronucleus during egg activation. *Developmental Biology*, 268(2), 245 - 257.

Yoneda, A., Kashima, M., Yoshida, S., Terada, K., Nakagawa, S., Sakamoto, A., Watanabe, T. (2006). Molecular cloning, testicular postnatal expression, and oocyte-activating potential of porcine phospholipase Czeta. *Reproduction*, 132(3), 393 - 401.

Yoon, S.Y., & Fissore, R. (2007). Release of phospholipase C zeta and [Ca^{2}**i**i oscillation- inducing activity during mammalian fertilization. *Reproduction*, 134(5), 695 - 704.

Yoon, S.-Y., Jellerette, T., Salicioni, A., Lee, H., Yoo, M.-s., Coward, K., Fissore, R. (2008). Human sperm devoid of PLC, zeta 1 fail to induce Ca^{2+} release and are unable to initiate the first step of embryo development. *Journal of Clinical Investigation*, 118(11), 3671 - 3681.

Young, C., Grasa, P., Coward, K., Davis, C., & Parrington, J. (2009). Phospholipase C zeta undergoes dynamic changes in its pattern of localization in sperm during capacitation and the acrosome reaction. *Fertilization Sterility*, 91(5), 2230 - 2242.

Yu, Y., Nomikos, M., Theodoridou, M., Nounesis, G., Lai, F., & Swann, K. (2011). PLC(zeta)zeta causes Ca^{2+} oscillations in mouse eggs by targeting intracellular and not plasma membrane PI(4,5)P2. *Molecular Biology Cell*, 23(2), 371 - 380.

Zhang, H. M.-D., P.A. (2003). Mouse epididymal Spam 1 (pH-20) is released in luminal fluid with its lipid anchor. *Journal of Andrology*, *24*, 267-275.