

The Molecular Basis of Sperm – Oocyte Interactions

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Declaration

I hereby certify that this thesis is submitted in the form of a series of published papers of which I am the lead author. I have included as part of the thesis a written statement from each co-author; and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

(Signed).....

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“Success is the ability to go from one failure to another with no loss of enthusiasm”

“Out of intense complexities intense simplicities emerge”

Winston Churchill, 1874 – 1965

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LIST OF PUBLICATIONS INCLUDED AS PART OF THE THESIS

1. **Dun MD**, Mitchell LA, Aitken RJ, Nixon B. 2010. Sperm-zona pellucida interaction: molecular mechanisms and the potential for contraceptive intervention. *Handb Exp Pharmacol*(198):139-178. **Published.**

This project was led by Matthew, 70% contribution. Matthew conducted the literature search, compiled all data for tables, and contributed to the figures. He also took the lead role in manuscript preparation. Other authors contributed as follows: B. Nixon (20%), R.J. Aitken (5%) and L.A. Mitchel (5%).

2. **Dun MD**, Smith ND, Baker MA, Lin M, Aitken RJ, Nixon B. 2011. The chaperonin containing TCP1 complex (CCT/TRiC) is involved in mediating sperm-oocyte interaction. *J Biol Chem.* **Published.**

This project was led by Matthew, 75% contribution. Matthew led the study design collected all of the data and prepared the manuscript. Other authors contributed as follows: B. Nixon (10%), R.J. Aitken (5%), Nathan Smith (5%) and Baker and Lin contributed the remainder.

3. **Dun MD**, Anderson AL., Bromfield EG, Asquith KL, McLaughlin EA, Aitken RJ, Nixon B. 2011. Investigation of the expression and functional significance of the novel mouse sperm protein, a disintegrin and metalloprotease with thrombospondin type 1 motifs number 10 (ADAMTS10). *Int J Androl.* **Accepted.**

This project was led by Matthew, 60% contribution. Matthew completed all data analyses took the lead role in manuscript preparation and collected most of the data that were analysed. Other authors contributed as follows: B. Nixon (10%), Amanda Anderson (10%), Kelly Asquith (10%), and McLaughlin, Aitken and Bromfield contributed the remainder.

4. **Dun MD**, Aitken RJ, Nixon B. 2011. The role of molecular chaperones in spermatogenesis and the post-testicular maturation of mammalian spermatozoa. *Hum Reprod Update.* **Accepted.**

This project was led by Matthew, 90% contribution. Matthew conducted the literature search, compiled all data and tables, designed and produced all figures and also took

the lead role in manuscript preparation. Other authors contributed as follows: B. Nixon (5%) and R.J. Aitken (5%).

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ADDITIONAL PUBLICATION

Redgrove KA, Anderson AL, **Dun MD**, McLaughlin EA, O'Bryan MK, Aitken RJ, Nixon B. 2011. Involvement of multimeric protein complexes in mediating the capacitation-dependent binding of human spermatozoa to homologous zonae pellucidae. *Dev Biol* 356(2):460-474. ***Published.***

As a result of the significant findings described in publication 1 and 2, these techniques have been employed in the above mentioned publication (Redgrove et al., 2011). Matthew contributed to the experimental design as well as performed a key figure of this manuscript.

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1

LIST OF ABBREVIATIONS

| | |
|-------------------|--|
| 1-D | one-dimensional |
| 1-D BN-PAGE | one-dimensional blue native polyacrylamide gel electrophoresis |
| 2-D | two-dimensional |
| ADAM | a disintegrin and a metalloprotease domain |
| ADAMTS10 | a disintegrin and a metalloprotease domain with thrombospondin10 |
| ADAMTS10-A | ADAMTS10 active enzyme |
| ADAMTS10-Z | ADAMTS10 zymogen |
| Ahal | Activator of HSP90 ATPase |
| AKAP | A kinase anchoring protein |
| AMP | ampicillin |
| ANOVA | analysis of variance |
| apY | anti-phosphotyrosine |
| AMP | adenosine monophosphate |
| ATP | adenosine triphosphate |
| bp | base pairs |
| BN-PAGE | blue native polyacrylamide gel electrophoresis |
| BSA | bovine serum albumin |
| BWW | Biggers, Whitten and Whittingham medium |
| CABYR | calcium-binding tyrosine phosphorylation-regulating protein |
| cAMP | cyclic adenosine monophosphate |
| cDNA | complementary DNA |
| cGMP | complementary guanosine monophosphate |
| CHAPS | 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate |
| CHIP | carboxyl terminus of HSC-70 interaction protein (CHIP) |
| CCT α /1 | chaperonin alpha/1 |
| CCT β /2 | chaperonin beta/2 |
| CCT γ /3 | chaperonin gamma/3 |
| CCT δ /4 | chaperonin delta/4 |
| CCT ϵ /5 | chaperonin epsilon |
| CCT ζ /6A | chaperonin zeta |
| CCT η /7 | chaperonin eta |
| CCT θ /8 | chaperonin theta |
| Cpn10 | chaperonin10 |
| CRISP | cysteine-rich secretory protein |
| DABCO | 1,4-diazobicyclo-[2.2.2]-octane |
| dATP | deoxyadenosine triphosphate |
| db | dense bodies |
| dbcAMP | dibutyl cyclic adenosine monophosphate |
| dCTP | deoxycytidine triphosphate |
| DF | decapacitation factor |
| dGTP | deoxyguanosine triphosphate |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| dNTP | deoxyribonucleotide |

| | |
|---------------|---|
| DSS | disuccinimidyl suberate |
| DTT | dithiothreitol |
| dTTP | deoxythymidine triphosphate |
| ECL | enhanced chemiluminescence |
| ECM | extracellular matrix |
| EMMPRIN | extracellular matrix metalloprotease inducer |
| ER | endoplasmic reticulum |
| ERK | extracellular-signal regulated kinase |
| ERP99 | endoplasmin |
| FA-1 | fertilisation antigen-1 |
| FITC | fluorescein isothiocyanate |
| GalTase | galactosyltransferase |
| GCNA | germ cell nuclear antigen |
| GPI | glycosylphosphatidylinositol |
| GRP78 | glucose regulated protein 78 |
| GRP94 | glucose regulated protein 94 (endoplasmin) |
| h | hours |
| HBSS | Hanks buffered salt solution |
| hCG | human chorionic gonadotropin |
| HDL | high density lipoprotein |
| HIP | HSP70-interacting protein |
| His | Histidine |
| HK-1 | hexokinase type 1 |
| HOP | HSP-organising protein |
| HOS | hyperosmotic swelling |
| HRP | horseradish peroxidase |
| HSP | heat shock protein |
| HSP10 | heat shock protein 10 |
| HSP60 | heat shock protein 60 |
| HSP70 | heat shock protein 70 |
| HSP90 | heat shock protein 90 |
| i.d. | inner diameter |
| IEF | isoelectric focussing |
| IgG | immunoglobulin G |
| IPTG | isopropylthio- β -D-galactoside |
| IU | international units |
| KAN | kanamycin |
| kDA | kilodalton |
| LB | Luria broth |
| M | molar |
| MALDI-TOF | matrix-assisted laser desorption ionisation – time of flight |
| MALDI-TOF/TOF | matrix-assisted laser desorption ionisation – time of flight/time of flight |
| MAPK | mitogen-activated protein kinase |
| Min | minutes |
| MMP | matrix metalloprotease |
| mRNA | messenger ribonucleic acid |
| MS/MS | tandem mass spectrometry |
| MW | molecular weight |
| NCBI | National Center for Biotechnology Information (USA) |
| o.d. | outer diameter |
| OD | optical density |
| ODF | outer dense fiber |
| OSP | oviductal secretory protein |
| PBS | phosphate buffered saline |
| PCR | polymerase chain reaction |

| | |
|----------|---|
| PDE | phosphodiesterase |
| PDI | protein disulphide isomerases |
| PI | propidium iodide |
| PKA | protein kinase A |
| PKC | protein kinase C |
| PLAC | protease and lacunin |
| PTB | phosphotyrosine binding |
| PTP | protein tyrosine phosphatase |
| ptx | pentoxifylline |
| PVA | polyvinylalcohol |
| pY | phosphotyrosine |
| RNA | ribonucleic acid |
| RT | reverse transcriptase |
| RT-PCR | reverse transcriptase polymerase chain reaction |
| rZP | recombinant zona pellucida |
| rZP3 | recombinant zona pellucida protein 3 |
| sAC | soluble adenylyl cyclase |
| SDS | sodium dodecyl sulfate |
| SDS-PAGE | sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| sec | seconds |
| ser/thr | serine/threonine |
| SGG | sulfogalactosylglycerolipid |
| sp56 | sperm protein 56 |
| TAE | tris/acetate/EDTA |
| TBS | tris buffered saline |
| TBS-T | tris buffered saline with Tween-20 |
| TCP-1 | T-complex polypeptide 1 |
| TE | tris/EDTA |
| TEMED | N,N,N',N'-tetramethylethylenediamine |
| tmAC | transmembrane adenylyl cyclase |
| Tween-20 | polyoxyethylenesorbitan monolaurate |
| U | units |
| v/v | volume per volume |
| w/v | weight per volume |
| X-gal | 5-bromo-4-chloro-3-indolyl- β -galactoside |
| ZP | zona pellucida |
| ZP3 | zona pellucida protein 3 |
| ZBP1 | zona pellucida binding protein 1 |
| ZBP2 | zona pellucida binding protein 2 |
| ZRC | zona receptor complex |
| ZRK | zona receptor kinase |