Functional Characterization of Dynamin in Spermatozoa Epididymal Maturation and Acrosomal Exocytosis

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Master Degree of Science

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Declaration

Statement of Originality

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision.

The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

Thesis by publication

I hereby certify that this thesis is in the form of a series of papers. I have included as part of the thesis a written declaration from each co-author, endorsed in writing by the Faculty Assistant Dean (Research Training), attesting to my contribution to any jointly authored papers.

Signed: **.....**

Wei Zhou

Acknowledgments

I still could not believe that three and a half years come to an end. As an international student, it is not an easy decision for me to come over to another country to conquer a PhD degree. However, this decision turns out to be the most right one in my life and now I feel Newcastle is my second hometown and it is really hard to say goodbye. To me, it has been a wonderful journey and I would like to express my sincere gratitude to all that have helped me during my PhD study.

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Publications and awards arising from work in this thesis

1. Publications

Chapter 1: Introduction and literature review

Zhou W, De Iuliis GN, Dun MD, Nixon B (2018). Characteristics of the Epididymal Luminal Environment Responsible for Sperm Maturation and Storage. Front Endocrinol; 9: 59. DOI: 10.3389/fendo.2018.00059.

Invited Review, Published | Frontiers in Endocrinology

Chapter 2:

Zhou W, Sipila P, De Iuliis GN, Dun MD, Nixon B (2018). Analysis of Epididymal Protein Synthesis and Secretion. J Vis Exp; 138: e58308. DOI: 10.3791/58308.

Published | Journal of Visualized Experiments

Chapter 3:

Zhou W, De Iuliis GN, Turner AP, Reid AT, Anderson AL, McCluskey A, McLaughlin EA, Nixon B (2016). Developmental expression of the dynamin family of mechanoenzymes in the mouse epididymis. Biol Reprod; 96: 159-173. DOI: 10.1095/biolreprod.116.145433.

Published | Biology of Reproduction

Chapter 4:

Zhou W, Stanger SJ, Anderson AL, De Iuliis GN, McCluskey A, McLaughlin EA, Dun MD, Nixon B (2018). Mechanistic insights into mouse epididymosome-sperm interactions.

Submitted | BMC Biology

Chapter 5:

Zhou W, Anderson AL, Turner AP, De Iuliis GN, McCluskey A, McLaughlin EA, Nixon B (2017). Characterization of a novel role for the dynamin mechanoenzymes in the regulation of human sperm acrosomal exocytosis. Mol Hum Reprod; 23(10): 657-673. DOI: 10.1093/molehr/gax044.

Published | Molecular Human Reproduction

2. Statements of Contribution

I attest that the Research Higher Degree candidate Wei Zhou has contributed upward of 50% towards data collection/analysis and manuscript preparation for all the publications included in this thesis for which I am a co-author.

…….
Prof. Adam McCluskey
Date:05/12/2018

…….
Dr. Adrian P Turner
Date:04/12/2018

…….
Amanda L Anderson
Date:30/11/2018

…….
Dr. Andrew T Reid
Date:04/12/2018

…….
Prof. Brett Nixon
Date:30/11/2018

…….
Prof. Eileen A Mclaughlin
Date:06/12/2018

…….
Dr. Geoffry De Iuliis
Date:03/12/2018

…….
Dr. Matthew D Dun
Date:30/11/2018

…….
A/Prof. Petra Sipilä
Date:07/12/2018

…….
Simone J Stanger
Date:04/12/2018

…….
Prof. Frances Martin
Assistant Dean of Research Training
Date:11/12/2018

3. Conference proceedings relevant to this thesis

Zhou W, Stanger SJ, Anderson AL, De Iuliis GN, McCluskey A, McLaughlin EA, Dun MD, Nixon B.
Mechanistic insights into epididymosome-sperm interactions. 7th International Conference on the Epididymis. Montreal, Canada. September 2018. *Poster presentation* **|** Travel award for the excellent quality of the abstract.

Zhou W, Stanger SJ, Anderson AL, De Iuliis GN, McCluskey A, McLaughlin EA, Dun MD, Nixon B.
Mechanistic insights into epididymosome-sperm interactions. 49th Annual Conference of the Society for Reproductive Biology. Adelaide, Australia. August 2018. *Oral presentation*.

Zhou W, Stanger SJ, Anderson AL, De Iuliis GN, McCluskey A, McLaughlin EA, Dun MD, Nixon B.
Elucidating the role of dynamin in epididymosome mediated transfer of fertility-modulating proteins to maturing spermatozoa. Australian Society for Medical Research Scientific Meeting. Newcastle, Australia. December 2017. *Oral presentation*.

Zhou W, Stanger SJ, Anderson AL, De Iuliis GN, McCluskey A, McLaughlin EA, Dun MD, Nixon B.
Elucidating the role of dynamin in epididymosome mediated transfer of fertility-modulating proteins to maturing spermatozoa. 22nd annual biology RHD conference. Newcastle, Australia. November 2017. *Oral presentation* **|** winner of best 3rd-year student presentation.

Zhou W, Anderson AL, Turner AP, De Iuliis GN, McCluskey A, McLaughlin EA, Nixon B.
Characterization of a novel role for the dynamin mechanoenzymes in the regulation of human sperm acrosomal exocytosis. 48th Annual Conference of the Society for Reproductive Biology. Perth, Australia. August 2017. *Oral presentation* **|** Finalist in Oozoa award section.

Zhou W, Reid AT, Anderson AL, De Iuliis GN, McCluskey A, McLaughlin EA, Nixon B.
Developmental expression of the dynamin family of mechanoenzymes in the mouse epididymis. 47th Annual Conference of the Society for Reproductive Biology. Gold Coast, Australia. August 2016. *Poster presentation*.

Zhou W, Reid AT, Anderson AL, De Iuliis GN, McCluskey A, McLaughlin EA, Nixon B.
Developmental expression of the dynamin family of mechanoenzymes in the mouse epididymis. 20th Annual biology RHD conference. Newcastle, Australia. November 2015. *Oral presentation*.

4. Additional publications

Nixon B, De Iuliis GN, Hart H, **Zhou W**, Mathe A, Bernstein I, Anderson A, Larsen MR, Dun MD (2018). Proteomic profiling of mouse epididymosomes reveals their contributions to post-testicular sperm maturation. Mol Cell Proteomics; pii: mcp.RA118.000946. DOI: 10.1074/mcp.RA118.000946.

Published | Molecular & Cellular Proteomics

5. Awards

Best HDR Publication Award "Highly Commended" **|** University of Newcastle **|** 2018

Travel award for the excellent quality of the abstract **|** 7th International Conference on the Epididymis **|** 2018

HDR International Conference Scholarship **|** University of Newcastle **|** 2018

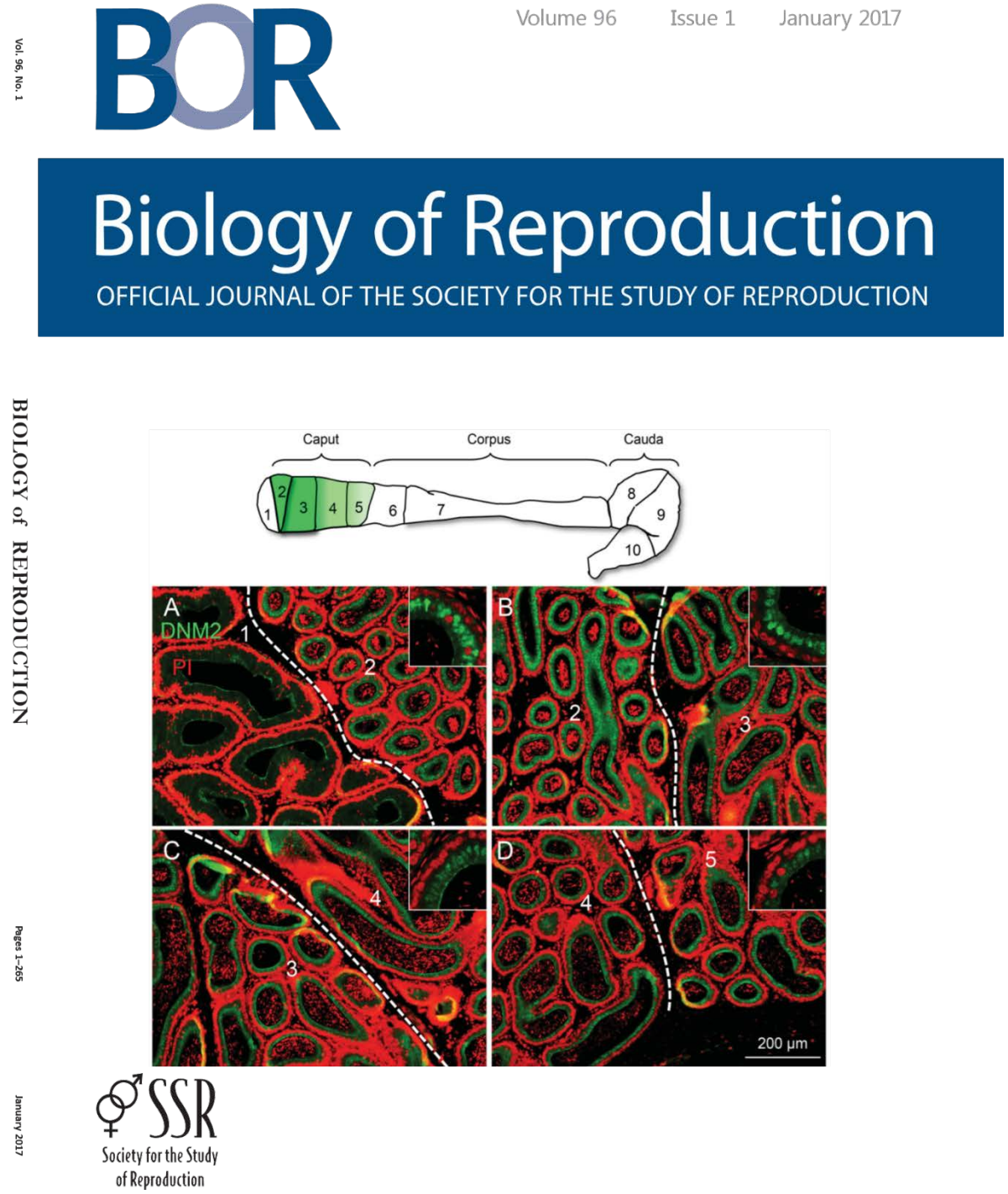
Best HDR Publication Award **|** University of Newcastle **|** 2017

Best 3rd year Oral Presentation **|** 22nd Annual Biology RHD Conference **|** University of Newcastle **|** 2017

Finalist for Oozoa Award for best student presentation **|** Society for Reproductive Biology **|** 2017

6. Journal cover image

Biology of Reproduction, Volume 96, Issue 1 (January 2017).



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Abstract

Human infertility is now a major clinical problem affecting approximately one in six couples; with a male factor contributing to nearly 50% of these cases. Clinical analysis has shown that a majority of male infertile patients are still able to produce enough spermatozoa to achieve fertilization. However, for reasons that remain poorly defined, the functionality of these cells has become compromised. Improved understanding of how sperm acquire functional maturity would not only beneficial in terms of uncovering the causative basis of male gamete dysfunction, but also for the provision of urgently needed biomarkers of sperm quality to reliably predict the outcome of assisted reproductive technology treatments. In this context, it is generally accepted that spermatozoa released from the testes require additional phases of post-testicular development that occur during their transit through the epididymis and female reproductive tract before acquiring functional competence. Both biophysical and biochemical changes occur along this journey, eventually culminating in the ability of sperm to undergo an acrosome reaction and recognize the oocyte. Notably, due to spermatozoa being both transcriptionally and translationally silent, the acquisition of functional maturity is reliant on communication between the spermatozoa and the extrinsic factors that they encounter within the male and female reproductive tracts.

Our recent work has shown that the dynamin family of enzymes may regulate several key steps in these communication pathways. The dynamin family comprises a group of large GTPases responsible for the regulation of membrane trafficking events such as endocytosis, exocytosis and intracellular trafficking. Such diverse functionality relies on the ability of dynamin to polymerize into a helix structure around the template lipid membrane, whereupon GTP hydrolysis drives the lengthwise constriction of the helix structure and leads to scission of the connection between the two membrane templates. Dynamin has three canonical isoforms, namely dynamin 1, dynamin 2 and dynamin 3. Although sharing over 80% sequence homology, recent studies have shown that each isoform may play distinct roles in regulating membrane trafficking events, depending on their localization and ability to interact with other protein targets. This is especially the case in male reproduction with our previously published studies having shown that dynamin 1 and 2 putatively regulate acrosomal exocytosis whilst dynamin 2 plays an essential role in regulating spermatogenesis in the mouse model.

Herein, we have provided further evidence that dynamin is involved in sperm maturation through the regulation of epididymal epithelium secretion. Our detailed

characterization of the three canonical dynamin isoforms have revealed that each are highly expressed during the early development phases of epididymal differentiation. Interestingly however, the widespread localization of these isoforms in the juvenile epididymis is replaced by segment and cell specific patterns coinciding with the arrival of testicular sperm into the tract. Notably, the expression of dynamin 2 in the Golgi apparatus of caput epithelial cells ideally positions the enzyme to regulate the classical merocrine pathway of protein secretion. This hypothesis was tested through the use of an *in vitro* caput epithelial cell line; i.e. mECap18 cells. Accordingly, pharmacological inhibition of dynamin selectively inhibited the secretion of a subset of proteins, such as CCT3 and CCT8, from the mECap18 cells.

Having demonstrated that dynamin influences the secretion of epididymal proteins, we elected to explore if members of this family also participate in downstream communication between epididymal soma and sperm via the control of extracellular vesicle uptake. For this purpose, we elected to focus on epididymosomes, small membrane encapsulated vesicles that have been implicated in establishing the sperm proteomic and epigenetic landscape. Through the establishment of an *in vitro* co-culture model, we have documented the kinetics of epididymosome-mediated transfer of proteins to spermatozoa and identified the post-acrosomal sheath as the domain responsible for initial epididymosome – sperm adhesion. Such adhesion appears to be followed by the uptake of epididymosome cargo into the cell, a process that is reliant on both dynamin 1 and lipid rafts.

In continuing our investigation of dynamin, we also elected to study the role of this family of mechanoenzymes in regulating the acrosome reaction in human spermatozoa. Based on previous data generated in a mouse model, we hypothesized that dynamin 1 and 2 play a conserved role in facilitating acrosomal exocytosis in human spermatozoa, and that this activity is linked to the phosphorylation status of the dynamin proteins. Consistent with this hypothesis, dynamin 1 and 2 were localized to the acrosomal domain of human spermatozoa and their pharmacological inhibition significantly compromised the ability of human spermatozoa to complete an acrosome reaction. This activity appears to be tied to the phosphorylation of dynamin, with our data identifying CDK1 as an important targeting kinase for dynamin 2. Accordingly, we recorded a significant loss of dynamin 2 expression in the acrosomal domain of poor quality human spermatozoa; a loss that was accompanied by a significant reduction in the ability of these cells to complete an acrosome reaction. Collectively these data support a conserved role for dynamin in regulating the acrosome reaction in both mouse and human spermatozoa.

In summary, the data summarized in this thesis implicates dynamin as a key regulatory enzyme in both epididymal sperm maturation and downstream acrosomal exocytosis. In contrast to the overlapping role of the dynamin family members in somatic cells, our findings raise the prospect that different dynamin members fulfill distinct functions in the male reproductive system. Such distinctions raise the intriguing possibility of being able to target specific dynamin members for the purpose of male fertility regulation. Moreover, the functional conservation we observed between human and mouse models supports the utility of conditional knockout mouse models as an important tool with which to further dissect the role of dynamin in human male (in)fertility.