
# The impact of environmental insult on mouse epididymal spermatozoa

**Natalie A. Trigg**

B. Biotechnology (Honours Class I)

University of Newcastle

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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. 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.

Signed:……
Natalie A. Trigg

## ACKNOWLEDGMENT OF AUTHORSHIP

I hereby certify that the work embodied in this thesis contains published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis a written declaration endorsed in writing by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.

By signing below, I confirm that Natalie Trigg contributed upward of 50% towards data collection/analysis and manuscript preparation for all the papers / publications included in this thesis

Signed:… ……
Brett Nixon

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*This thesis was written and researched on Awabakal Lands.*

*Wherever we walk in Australia, we walk on Aboriginal land.*

# PUBLICATIONS AND AWARDS ARISING FROM THIS THESIS:

## Publications:

Chapter 1: Introduction and Literature Review

1. **Trigg, N.A**., Eamens, A.L., & Nixon, B. (2019). The contribution of epididymosomes to the sperm small RNA profile, Reproduction, 157, 6: R209-R223. DOI: 10.1530/REP-18-0480

**Invited Review. Published | *Reproduction***

Chapter 2: Original Research Article

2. **Trigg. N.A.,** Skerrett-Byrne, D.A., Xavier, M.J., Zhou. W., Anderson. A.L., Stanger. S.J., De Iuliis. G.N., Dun, M.D., Roman, S.D., Eamens. A.L., & Nixon, B. The reproductive toxicant acrylamide modulates the mouse epididymal proteome to drive alterations to the sperm epigenome and dysregulate embryo development

**Submitted | *Nature Communications***

Chapter 3: Original Research Article

3. **Trigg. N.A.,** Stanger. S.J., Zhou. W., Skerrett-Byrne, D.A., Sipilä, P., Dun, M.D., Eamens. A.L., De Iuliis, G.N., Bromfield, E.G., Roman, S.D., & Nixon, B. A novel role for milk fat globule-EGF factor 8 protein (MFGE8) in the mediation of sperm-extracellular vesicle interactions

**Accepted with Minor Revisions | *Proteomics***

STATEMENT OF CONTRIBUTION

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

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**Brett Nixon**

Date: 07/12/2020

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**Andrew Eamens**

Date: 02/12/2020

……

**Shaun Roman**

Date: 07/12/2020

……

**Geoffry De Iuliis**

Date: 03/12/2020

……

**David Skerrett-Byrne**

Date: 04/12/2020

……

**Miguel Xavier**

Date: 02/12/2020

……

**Elizabeth Bromfield**

Date: 04/12/2020

……

**Matthew Dun**

Date: 02/12/2020

……

**Wei Zhou**

Date: 03/12/2020

……

**Amanda Anderson**

Date: 29/11/2020

……

**Simone Stanger**

Date: 01/12/2020

……

**Petra Sipilä**

Date: 04/12/2020

# CONFERENCE PROCEEDINGS RELEVANT TO THESIS:

**2020:**

1. **Trigg, N.A**., Skerrett-Byrne, D.A., Xavier, M.J., Zhou, W., Anderson, A.L., Stanger, S.J., De Iuliis, G.N., Roman, S.D., Eamens, A.L., Nixon, B (2020) Acrylamide exposure drives alterations to the small RNA landscape of mature spermatozoa and influences early embryo gene expression. Society for Reproductive Biology Virtual Awards. *Oral Presentation*

**2019:**

2. **Trigg, N.A**., Roman, S.D., Eamens, A.L., Xavier, M.J., Nixon, B (2019) The impact of acute acrylamide exposure on the small RNA profile of spermatozoa. 24th Annual Newcastle University Higher Degree Research Conference, Newcastle, Australia. *Oral Presentation*

3. **Trigg, N.A**., Roman, S.D., Eamens, A.L., Xavier, M.J., Nixon, B (2019) The impact of acute acrylamide exposure on the small RNA profile of spermatozoa. Australian Reproduction Update, Melbourne, Australia. *Oral presentation*

4. **Trigg, N.A**., Roman, S.D., Eamens, A.L., Xavier, M.J., Nixon, B (2019) The impact of acute acrylamide exposure on the small RNA profile of spermatozoa. 50th Annual Scientific Meeting of the Society for Reproductive Biology, Sydney, Australia. *Oral Presentation*

**2018:**

5. **Trigg, N.A**., Roman, S.D., Eamens, A.L., Xavier, M.J., Nixon, B. (2018) The impact of acute acrylamide exposure on the small RNA profile of spermatozoa. 23rd Annual Newcastle University Higher Degree Research Conference. Newcastle, Australia. *Poster Presentation*

6. **Trigg, N.A**., Roman, S.D., Eamens, A.L., Xavier, M.J., Nixon, B. (2018) The impact of acute acrylamide exposure on the small RNA profile of spermatozoa. Australian Reproduction Update Melbourne, Australia. *Oral Presentation*

7. **Trigg, N.A**., Eamens, A.L., De Iuliis, G.N., Bromfield, E.G., Nixon, B. (2018) The impact of environmental insult on the small RNA profile of spermatozoa. 49th Annual Scientific Meeting of the Society for Reproductive Biology, Adelaide, Australia. *Poster Presentation*

8. **Trigg, N.A**., De Iuliis, G.N., Bromfield, E.G., Eamens, A.L., Nixon, B. (2018). The impact of environmental insults on the small non-protein-coding RNA profile of spermatozoa. Australian Society for Medical Research (ASMR) Satellite Scientific Meeting. Newcastle, Australia. *Oral Presentation*

**2017:**

9. **Trigg, N.A**., De Iuliis, G.N., Bromfield, E.G., Eamens, A.L., Nixon, B. (2017) Regulation of the sperm epigenome by epididymosomes: A new paradigm. 22nd Annual Newcastle University Higher Degree Research Conference. Newcastle, Australia. *Oral Presentation*

## ADDITIONAL PUBLICATIONS:

### 2020

1. Fraser, B.A., Miller, K., **Trigg, N.A.**, Smith, N.D., Western, P.S., Nixon, B., and Aitken, J.R. (2020) A novel approach to non-surgical sterilization; application of menadione-modified gonocyte-targeting M13 bacteriophage for germ cell ablation in utero. **Pharmacology Research & Perspectives** https://doi.org/10.1002/prp2.654

2. Tamessar. C.T., **Trigg. N.A**., Sharkey. D.J., Robertson S.A., Bromfield. E.G., Nixon. B., and Schjenken. J.E. (2020) Roles of male reproductive tract extracellular vesicles in reproduction. **American Journal of Reproductive Immunology**, https://doi.org/10.1111/aji.13338

### 2019

1. Nixon, B., Bernstein, I.R., Cafe, S.L., Delehedde, M., Sergeant, N., Anderson, A.L., **Trigg, N.A.,** Eamens, A.L., Dun, M.D., De Iuliis, G.N., and Bromfield, E.G. (2019). A Kinase Anchor Protein 4 Is Vulnerable to Oxidative Adduction in Male Germ Cells. **Frontiers in Cell and Development Biology** https://doi.org/10.3389/fcell.2019.00319

2. Nixon, B., De Iuliis, G. N., Dun, M. D., Zhou, W., **Trigg, N. A.,** and Eamens, A. L. (2019), Profiling of epididymal small non-protein-coding RNAs. **Andrology**. https://doi:10.1111/andr.12640

### 2018

1. Houston, B.J., Nixon, B., Martin, J.H., De Iuliis, G.N., **Trigg, N.A**., Bromfield, E.G., McEwan, K.E., and Aitken, R.J. (2018) Heat exposure induces oxidative stress and DNA damage in the male germ line. **Biology of Reproduction** https://doi.org/10.1093/biolre/ioy009

## AWARDS:

**Finalist for David Healy New Investigator Award |** Society for Reproductive Biology Virtual Awards (2020)

**Faculty 1000 recommendation** for review entitled 'The contribution of epididymosomes to the sperm small RNA profile' (2019)

**Best PhD Student Poster Award** | Newcastle University, School of Environmental and Life Sciences 23rd annual HDR conference | Awarded by the University of Newcastle (2018)

Australian Government Research Training Scheme **PhD Scholarship** (2017)

TABLE OF CONTENTS:

# ABBREVIATIONS:

AA-E – acrylamide exposure timed to collect mature cauda spermatozoa exposed as epididymal sperm

AA-S – acrylamide exposure timed to collect mature cauda spermatozoa exposed as spermatocytes

ART – assisted reproductive technologies

EV – extracellular vesicles

ES – epididymosomes (also referred to as epididymal extracellular vesicles)

ICSI – intracytoplasmic sperm injection

IPA® – ingenuity pathway analysis

IVF – *in vitro* fertilisation

mECap18 – SV40-immortalised mouse caput epididymal epithelial cell line 18

miRNA – microRNA

piRNA – piwi-interacting RNA

sRNA / sncRNA – small non-protein coding RNA

TF – transcription factor

tRF – transfer RNA derived RNA fragments

# ABSTRACT:

The possibility of inheritance that deviates from canonical Mendelian rules (epigenetic / non-genetic inheritance) has been considered since the beginning of genetics research, however, only in recent years has experimental work provided the evidence needed to substantiate epigenetic inheritance. Indeed, models of epigenetic inheritance have now been established in species ranging from worms to mice. The putative 'information carriers' responsible for epigenetic inheritance, include DNA and histone modifications, chromatin modifications and, small non-coding RNAs (sncRNAs) and the modifications they harbour. Recently, several lines of evidence have supported sperm-borne sncRNA as an integral intergenerational signalling molecule following their delivery to the oocyte at the moment of fertilisation. It is also now apparent that the sperm sncRNA profile is dynamic and susceptible to modification during sperm maturation and in response to paternal environmental and lifestyle factors; each of which have potentially significant post-fertilisation consequences. While, the importance of the sperm sncRNA profile emerges, what remains less clear are the molecular mechanism(s) underpinning the response to environmental insult that leads to an altered sperm sncRNA profile. The studies in this thesis were designed to begin to bridge this important knowledge gap. Specifically, we aimed to investigate how paternal exposure to environmental factors influence the sperm sncRNA profile and the consequences of the delivery of an altered sncRNA profile to the oocyte (Chapter 2).

The sncRNA profile of spermatozoa undergoes major remodelling during the sperm cells passage of the epididymis. Moreover, the balance of evidence has implicated the epididymis as a vulnerable site in which sperm acquire environmental signals, such as an altered sncRNA profile. Owing to the transcriptionally and translationally quiescent state of epididymal spermatozoa, modification to the sncRNA profile is facilitated by the complex luminal microenvironment. A key component of which are small membrane bound vesicles, termed epididymosomes. These vesicles are produced by the epididymal epithelial cells and deliver a diverse cargo of macromolecules, including sncRNA, to spermatozoa. Additionally, epididymosomes have recently been implicated in delivery of an altered sncRNA profile to sperm under paternal stress conditions. Hence, in examining the mechanism(s) driving sperm sncRNA changes, we focused on epididymosomes as mediators of this dialogue and more specifically, on the cognate receptor-ligand(s) that underpin epididymosome-sperm adhesion (Chapter 3).

The data presented within this thesis confirms the acute sensitivity of the sperm sncRNA payload to the environmental toxicant, acrylamide, encountered during their post testicular development. We have traced the differential accumulation of miRNAs to coincide with sperm transit of the proximal (caput) epididymal segment. Indeed, we identified alterations in the epididymosomes secreted by the caput epididymis following environmental insult. In expanding this mechanistic investigation, we profiled the proteome of the caput epididymal epithelium and

revealed that acrylamide exposure alters the expression of a subset of proteins. In resolving a causal link, we identified the increased expression of seven transcription factors in the caput epithelium of acrylamide exposed mice, each of which have been implicated in the regulation of acrylamide-sensitive miRNAs. In identifying the consequences of an altered sperm sncRNA profile following acrylamide exposure our analysis revealed a subset of dysregulated genes in embryos fertilised by exposed sperm. This gene dysregulation was demonstrated to be in part driven by the sncRNA changes in the sperm and thus substantiate sperm-borne sncRNAs as important epigenetic messengers.

Having confirmed an integral role for epididymosome mediated communication in facilitating a response to paternal environmental exposure (acrylamide), we next sought to further our understanding of the mechanistic basis underlying the interaction between epididymosomes and recipient sperm. In exploring the proteomic composition of epididymosomes, previous work from our laboratory identified a putative role for an epididymosome resident ligand, milk fat globule-EGF factor 8 (MFGE8), in the efficient transfer of cargo from epididymosomes to spermatozoa. MFGE8 has been implicated in the adhesion of extracellular vesicles (EVs) isolated from non-reproductive tissues to recipient cells and this finding indicated a conserved function for MFGE8 in EVs isolated from the epididymis. Therefore, the remaining studies herein focused on the role of MFGE8 as a key molecular ligand in this intercellular form of communication in the epididymis (Chapter 3). We utilised an immortalised caput epididymal (mECap18) cell line as a model and first confirmed its suitability with which to study the mechanism(s) of sperm-epididymosome interaction. Through additional inhibition studies and the ablation of MFGE8 functional domains, we identified MFGE8 as being of fundamental importance for efficient sperm-EV interaction. However, the failure to completely block sperm-EV interaction indicates redundancy in the EV tethering mechanisms and highlights the need for further research to gain a complete understanding of the cognate receptors and ligands that mediate this interaction.

Taken together, the findings of this thesis contribute to our understanding of the sncRNA profile of mature mouse spermatozoa and its dynamic response to environmental insult. Importantly, these studies have advanced our knowledge of the molecular basis of epididymosome-sperm interactions, the importance of this intercellular communication in directing sperm sncRNA changes following stress, the mechanistic understanding of how paternal exposures affect remodelling of the sperm small RNA profile and the consequences of such changes for embryonic development.