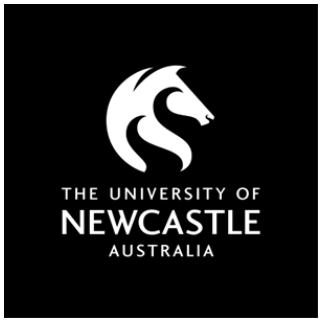
PERINATAL PROGRAMMING OF FEMALE SUBFERTILITY: THE IMPACT OF NEONATAL IMMUNE ACTIVATION ON BEHAVIOUR, OVARIAN DEVELOPMENT, AND THE BRAIN



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Declaration

I hereby certify that the work embodied in the thesis is my own original work, conducted under normal supervision. The thesis contains published scholarly work of which I am a co-author. For each such work a written statement, endorsed by the other authors, attesting to my contribution to the joint work, has been included. 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.

Signed:

Erin Alexandra Fuller,

December 14th, 2017

This thesis consist of an introduction comprised of a written literature review and a published review paper. Experimental chapters are presented as both published works and traditional chapters, with figures and tables embedded throughout.

Published Works Incorporated in this Thesis

1. Sominsky, L.., **Fuller, E.A.**, Hodgson, D.M. (2015). Factors in early-life programming of reproductive fitness. *Neuroendocrinology*, 102 (3): 216-225. DOI: 10.1159/000431378

2. **Fuller, E.A**., Sominsky, L., Sutherland, J.M., Redgrove, K.A., Harms, L., McLaughlin, E.A., Hodgson, D.M. (2017). Neonatal immune activation depletes the ovarian follicle reserve and alters ovarian acute inflammatory mediators in neonatal rats. *Biology of Reproduction.* Accepted 7th October, 2017. DOI--: 10.1093/biolre/iox123

3. Ong, L.K., **Fuller, E.A**., Sominsky, L., Hodgson, D.M., Dunkley, P.R., Dickson, P.W. (2017). Early life peripheral lipopolysaccharide challenge reprograms catecholaminergic neurons. *Scientific Reports* (7), DOI: 10.1038/srep40475.

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Thesis Abstract.

Perinatal programming of female subfertility:
Impact of neonatal immune activation on behaviour, ovarian development, and the brain.

The early life environment prescribes long-term health and disease outcomes. Accumulating evidence suggests that female reproductive health is shaped by perinatal factors, such as immune status. The fundamentals of female reproductive success and longevity are established in early life, where the dynamics of ovarian development are co-regulated via immune pathways to establish the ovarian reserve. Additionally, the immune system is known to be especially sensitive to perinatal stressors. This suggests that the early life environment plays an important role in sustained ovarian health and female fertility. Thus, inflammatory stressors during this critical period may permanently modify female ovarian development and immune-drive reproductive functioning, altering sexual behaviour and leading to a suboptimal female phenotype.

Using a rat model, we have previously demonstrated that neonatal immune activation (NIA) with bacterial mimetic lipopolysaccharide (LPS) is associated with; altered immune milieu, hypothalamic-pituitary-adrenal axis dysfunction, adult stress vulnerability, and an anxiety-like phenotype in males. The current thesis aimed to examine both the acute and long-term alterations in reproductive parameters in female rats exposed to an intraperitoneal injection of saline (control) or LPS (0.05mg/kg) to induce NIA on postnatal days 3 and 5.

Firstly, the behavioural phenotype of females in this model was examined in order to confirm and refine previous findings pertaining to female mating behaviour deficits, and establish if these alterations were driven by altered motivational states. The results of this study indicate that NIA leads to impairments in proceptive and receptive mating behaviours and an altered reproductive developmental trajectory. Secondly, the acute effects of NIA on

female rats was analysed, where by NIA treatment was demonstrated to significantly deplete early ovarian follicle populations and increase ovarian inflammation, suggesting that immune-mediated development of the ovary is perturbed by NIA in the female rat. Thirdly, the long term ramifications of neonatal bacterial exposure was examined in the adult female rat, demonstrating that NIA led to significantly advanced puberty onset, sustained ovarian reserve depletion, exaggerated peripheral inflammatory responses to stress, and increased ovarian inflammatory pathway gene expression. Lastly, the central gene expression of mediators associated with inflammation, stress regulation, and reproductive function were examined to elucidate on potential central mechanisms that may contribute to behavioural alterations and ovarian inflammation and reserve depletion. Furthermore, prospective mechanisms are suggested and data is presented demonstrating the potential of these for investigation in a female rat model of subfertility. The findings presented in this thesis suggest that NIA has the potential to perinatally program long-term central and ovarian immune functioning to a proinflammatory bias. This may detrimentally affect female reproductive fitness, fecundity, and stress responsivity, and as such, have implications for both physiological and psychological female health.

Abbreviations

5-HT	Serotonin
AA	Arachidonic acid
ABS	Australian Bureau of Statistics
ACEC	Animal Ethics Committee
ACTH	Adrenocorticotropic hormone
ADHD	Attention deficit/hyperactivity disorder
AG	Anogenital
ANOVA	Analysis of variance
aNS	Adulthood no stress
ANS	Autonomic nervous system
APAF	Australian Proteome Analysis Facility
APC	Antigen presenting cells
ARC	Animal Resources Centre
ARTs	Assisted reproductive therapies
aST	Adulthood restraint stress
BBB	Blood-brain barrier
CASP3	Caspase 3
cDNA	Complementary deoxyribonucleic acid
CORT	Corticosterone
COX	Cyclooxygenase
CRH	Corticotropin releasing hormone
CRHR1/2	Corticotropin releasing hormone receptor 1/2
C-RP	C-reactive protein
CSF	Colony stimulating factor
CTCF	Corrected total cell florescence
DA	Dopamine
DAB	3, 3-diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
DEHP	Di(2-ethylhexyl)phthalate
DEPc	Diethyl pyrocarbonate

DES	Diethylstilboestrol
DEX	Dexamethasone
DG	Dentate gyrus
DNA	Deoxyribonucleic acid
DOHaD	Developmental Origins and Health and Disease
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, version 5
DVO	Day of vaginal opening
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPI	Epinephrine
EPM	Elevated plus maze
ER	Oestrogen receptor
Foxo3	Forkhead box O3
FSH	Follicle stimulating hormone
FWD	Forward
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GD	Gestation day
Gdf	Growth differentiation factor
GFAP	Glial fibrillary acidic protein
GnRH	Gonadotropin releasing hormone
GnRHR	Gonadotropin releasing hormone receptor
GR	Glucocorticoid receptor
H&E	Hematoxylin and eosin
HC	Hippocampus
HD	Habituation day
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadal
HTH	Hypothalamus
Iba-1	Ionized calcium-binding adapter molecule 1
IDA	Information dependent acquisition
IDO	Indolamine-2,3-Dioxygenase

IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
ip	Intraperitoneal
IPA	Ingenuity Pathway Analysis
IRAK	Interleukin-1 receptor-associated kinase 1
IUGR	Intrauterine growth restriction
IVF	In vitro fertilisation
JAK/STAT	Janus kinase/signal transducers and activators of transcription
KP	Kynurenine pathway
Kyn	Kynurenine
LBP	LPS-binding protein
LC3	Light chain 3
LC	Locus coeruleus
L-Dopa	3,4-dihydroxy-l-phenylalanine
LH	Luteinizing hormone
LPS	Lipopolysaccharide
LSV	Lateral saphenous vein
LXR/RXR	Liver X receptor/retinoic X receptor
MANOVA	Multivariate analysis of variance
MAPK	Map kinase
Mapk8/Jnk1	Mitogen activated protein kinase 8/Jun N-terminal kinase
MD	Myeloid differentiation protein
MDD	Major Depressive Disorder
MHC	Major histocompatibility complex
MIA	Maternal immune activation
mPOA	Medial pre optic area
MR	Mineralocorticoid
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
mTOR	Mechanistic target of rapamycin

MyD88	Myeloid differentiation primary response 88
NCRIS	National Collaborative Research Infrastructure Strategy
NE	Norepinephrine
NF	Nuclear factor
NHMRC	National Health and Medical Research Council of Australia
NIA	Neonatal immune activation
NK	Natural killer
nLPS	Neonatal lipopolysaccharide
NMDA	N-methyl-D-aspartate
Nos1	Nitric oxide synthase-1
NSAI	Nonsteroidal anti-inflammatory pharmaceutical
nSAL	Neonatal saline
PAH	Polycyclic aromatic hydrocarbon
PAMP	Pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PCB	Polychlorinated biphenyl
PCOS	Polycystic ovarian syndrome
PFC	Prefrontal cortex
PG	Prostaglandin
PMT	Paced-mating test
PND	Post-natal day
PNS	Parasympathetic nervous system
POF	Premature ovarian failure
POI	Primary ovarian insufficiency
Poly I:C	Polyinosinic:polycytidylic acid
Prkc	Protein kinase C
PRR	Pattern-recognition receptor
PTSD	Post-traumatic stress disorder
PVC	Polyvinyl chloride
PVN	Paraventricular nucleus
qRT-PCR	Quantitative reverse transcription polymerase chain reaction

REV	Reverse
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulfate
SIT	Social interaction test
SN	Substantia nigra
SNS	Sympathetic nervous system
SPSS	Statistical Package for the Social Sciences
SPT	Sucrose preference test
STI	Sexually transmitted infection
TB	Time bin
TBS	Tris-buffered saline
T_C	T cytotoxic
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TDO	Tryptophan-2,3-Dioxygenase
TGF	Transforming growth factor
T_H	T helper
TH	Tyrosine hydroxylase
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TOFMS	Time of flight mass spectrometry
TRAF	Tumour necrosis factor receptor associated factor
Trp	Tryptophan
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labelling
TX	Thromboxane
VTA	Ventral tegmental area

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