The Role of Nuclear Progesterone Receptor (PGR) in Regulating Gene Expression, Morphology and Function in the Ovary and Oviduct during the Periovulatory Period

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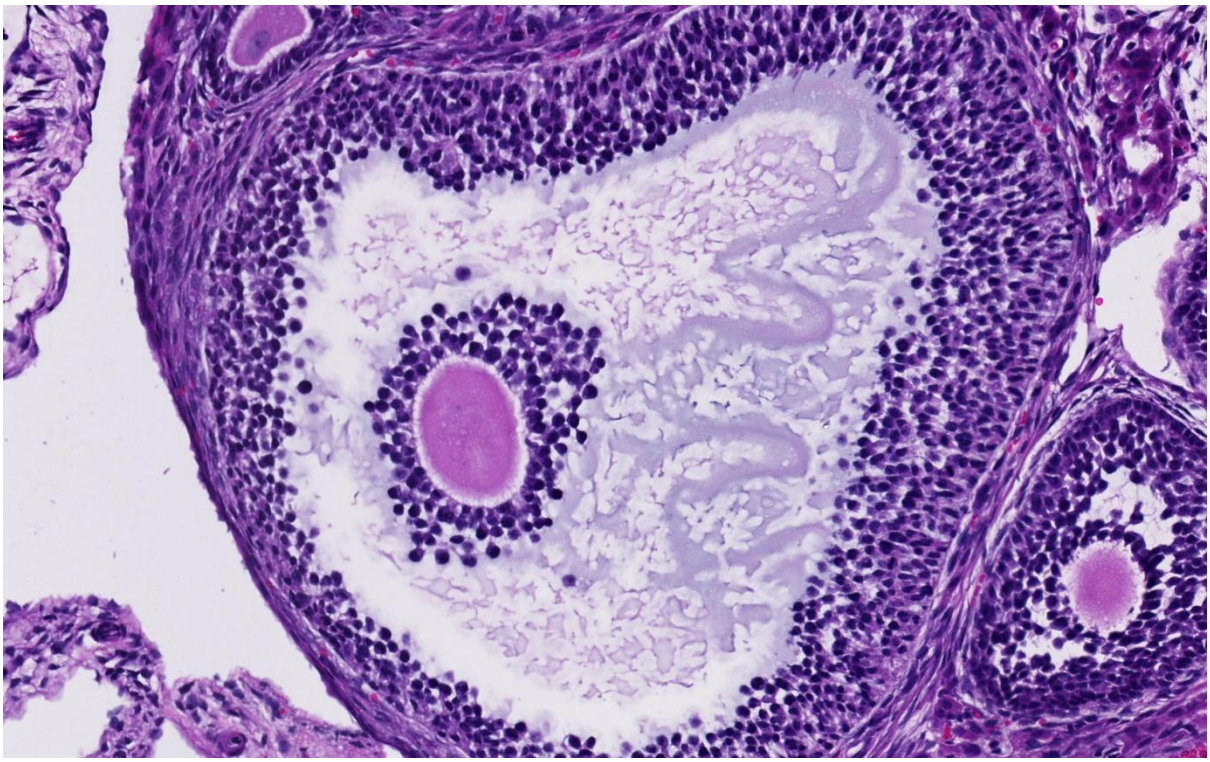

"The mystery of nature in the wonderful operation of animal reproduction has from the earliest period courted the attention of physiologists, and though experiment and imagination have toiled and fancied through all the ages, and opinions have been various and ingenious, the present day still finds the subject unconcluded."

Pulley, J. (1801). An essay on the proximate cause of animal impregnation; being the substance of a paper read and discussed in the medical society at Guy's Hospital, in October 1799. London

More than 200 years later...

"The overwhelming impression is that the molecular events of ovulation are far more complex...than originally imagined."

Espey, L. L. & Richards, J. S. (2002). Temporal and spatial patterns of ovarian gene transcription following an ovulatory dose of gonadotropin in the rat. *Biology of Reproduction* 67:1667.

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Abstract

Ovulation requires sequential molecular events and structural remodelling in the ovarian follicle for the successful release of a mature oocyte into the oviduct. Critical to this process is progesterone receptor (PGR), a transcription factor highly yet transiently expressed in granulosa cells (GC) of preovulatory follicles and abundant in the oviduct. Progesterone receptor knockout (PRKO) mice are anovulatory, with a specific and complete defect in follicle rupture. Therefore, this model was used to examine the critical molecular and biochemical mechanisms necessary for successful ovulation. Progesterone is known to affect oviductal cells in vitro, but how PGR regulates oviductal structure and function is poorly understood. A systematic evaluation of ovarian and oviductal morphology during the periovulatory period revealed no structural defects in PRKO mice relative to heterozygous (PR+/-) littermates. However, in response to the LH surge/hCG treatment, ovulation only occurred in PR+/- ovaries, with numerous corpora lutea observed and cumulus oocyte complexes (COCs) in oviducts, while PRKO ovaries did not ovulate and showed entrapped COCs within large, luteinising follicles. Transplantation of PRKO ovaries into wild-type mice (PRWT) did not rescue the infertility phenotype. Therefore, although PGR is expressed in other tissues, ovarian PGR is essential for ovulation.

Further experiments identified PGR-regulated processes at multiple levels. In whole ovaries 10 h post-hCG, inflammatory genes were disrupted in PRKO mice, including cytokines, endothelial adhesion factors, vasoconstrictors, T-cell antigens, and the prostaglandin synthase, Ptgs2. In GCs and COCs isolated 8 h post-hCG, microarray analyses identified 296 and 44 differentially expressed genes respectively between PRKO and PR+/- samples. Gene ontology analysis revealed associations with the processes of proteolysis, vascular remodelling/angiogenesis, inflammatory responses, cell adhesion, migration and invasion. The latter three processes were characterised in periovulatory COCs using in vitro assays and were shown to be transiently activated, peaking at ovulation then declining dramatically in COCs collected from the oviduct immediately post-ovulation. However, periovulatory PRKO and PR+/- COCs showed similar rates of adhesion, migration and invasion in the presence of collagen I. Upregulation of the chemokine receptor, Cxcr4, by LH/hCG via PGR in both GCs and COCs was validated by RT-PCR and immunohistochemistry. Mitochondrial membrane potential was altered in PRKO oocytes compared to PR+/- and therefore their developmental potential may be reduced. Further, a bioassay measuring retention of prostaglandin (PGE_2) within the matrix of expanded COCs suggested that the matrix integrity of PRKO COCs may be compromised. Therefore, PGR in granulosa cells appears to have down-stream impacts on COCs.

In oviducts, microarray analysis comparing gene expression in PRKO and PR+/- mice 8 h post-hCG, when P4 levels are high, identified 1003 PGR-regulated genes. Gene ontology analysis identified

significant associations with the functions of cell adhesion, migration, invasion, chemotaxis, muscle contraction and vasoconstriction. Several genes were confirmed to be PGR-regulated by RT-PCR (Adamts1, Itga8 and Edn3) and were induced by LH/hCG.

Therefore, the identification of novel gene targets for PGR regulation in the ovary and oviduct exposes several new, down-stream influences of PGR on inflammation, the COC and oviductal function, highlighting the essential role of PGR as master regulator in the ovary and oviduct during the periovulatory period.

Thesis declaration:

I certify that this work contains no material which has been accepted 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Robker, R.L., **Akison, L.K.** & Russell, D.L. (2009). Control of oocyte release by Progesterone Receptor-regulated gene expression. *Nuclear Receptor Signaling* 7, 1-12.

Akison, L.K.*, Alvino, E.*, Dunning, K.R. Robker, R.L. & Russell, D.L. (2012). Transient invasive migration in mouse cumulus oocyte complexes induced at ovulation by luteinizing hormone. *Biology of Reproduction* 86 (4), 125, 1-8. * Both authors contributed equally to this work.

Akison, L.K. & Robker, R.L. (2012). The critical roles of progesterone receptor (PGR) in ovulation, oocyte developmental competence and oviductal transport in mammalian reproduction. *Reproduction in Domestic Animals* 47 (Supplement 4), 288-296.

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Lisa Akison 2012

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Abbreviations

ΔΨm: mitochondrial membrane potential

AC: adenylate cyclise

ACTB: actin, beta

ACTG2: actin, gamma 2, smooth muscle, enteric

ADAMTS1: ADAM metallopeptidase with thrombospondin type 1 motif, 1

AGTII: angiotension II

AGTR: angiotensin II receptor

AMH: anti-Mullerian hormone

αMEM: minimum essential medium, alpha

ANGPT: angiopoietin

ANOVA: analysis of variance

AREG: amphiregulin

BAX: BCL2-associated X protein

bp: base pairs

BSA: bovine serum albumin

C3, C7: complement 3 and 7

cAMP: cyclic adenosine monophosphate

CBF: cilia beat frequency

CC: cumulus cells

CCR4: chemokine (C-C motif) receptor 4

cDNA: complementary DNA

C/EBPβ: CCAAT enhancer binding protein β

CL: corpus luteum

COC: cumulus oocyte complex

ColI: collagen

CREB: cAMP response element binding protein

CT: cycle threshold

CXCL12: chemokine (C-X-C motif) ligand 12 (previously known as SDF1)

CXCR4: chemokine (C-X-C motif) receptor 4

DES: desmin

DNA: deoxyribonucleic acid

dNTP: deoxyribonucleotide

E2: estradiol

ECE1: endothelin converting enzyme 1

eCG: equine chorionic gonadotrophin

ECM: extracellular matrix

EDN: endothelin

EFNB2: ephrin B2

EGF: epidermal growth factor

EGF-L: epidermal growth factor-like

EPAS1: endothelial PAS domain protein 1 (previously known as HIF2α)

EREG: epiregulin

ERK: extracellular signal-regulated kinase

EtOH: ethanol

F1: first filial

FCS: fetal calf serum

FSH: follicle stimulating hormone

FN: fibronectin

GC: granulosa cell

GVBD: germinal vesicle breakdown

GM-CSF: colony stimulating factor 2 (granulocyte-macrophage)

h: hour

HA: hyaluronan

hCG: human chorionic gonadotropin

H & E: haematoxylin and eosin

HIF: hypoxia inducible factor

HMOX1: heme oxygenase (decycling) 1

IL: interleukin

ILCL: interstitial Cajal-like cells

IFN: interferon

i.p.: intraperitoneal

IPA: Ingenuity Pathway Analysis

ITG: integrin

IU: international units

IVF: in vitro fertilisation

IVM: in vitro maturation

KO: knockout

Lam: laminin

LH: luteinising hormone

LHCGR: luteinising hormone/choriogonadotrophin receptor

MAPK: mitogen-activated protein kinase

min: minute

MMP: matrix metalloproteinase

mPR: membrane progestin receptor

mRNA: messenger ribonucleic acid

MYOCD: myocardin

NFKB2: nuclear factor of kappa light polypeptide gene enhancer in B-cells 2

NO: nitric oxide

OSF: oocyte secreted factor

OVGP1: oviductal glycoprotein 1 (previously known as MUC9)

P4: progesterone

PA: plasminogen activator

PAF: platelet activating factor

PAPPA: pregnancy-associated plasma protein A

PBS: phosphate buffered saline

PBST: phosphate buffered saline + Tween-20

PCA: principal component analysis

PCR: polymerase chain reaction

PG: prostaglandin

PKA: protein kinase A

PPARγ: peroxisome proliferator-activated receptor gamma

PGR: progesterone receptor

PGRMC1: progesterone receptor membrane component 1

PRE: progesterone response element

PRKO: progesterone receptor knockout

PRLR: prolactin receptor

PRWT: progesterone receptor wild-type

PTGS2: prostaglandin-endoperoxide synthase 2 (previously known as COX2)

RGMB: RGM domain family, member B

RNA: ribonucleic acid

RIN: RNA integrity number

RPL19: ribosomal protein L19

RTCA: real time cell analysis

RT-PCR: reverse transcription polymerase chain reaction

RUNX1/2: runt related transcription factor 1 & 2

SE: standard error

SELE: selectin E

SFM: serum free medium

SMC: smooth muscle cell

SOCS: suppressor of cytokine signaling

SP1/3: specificity protein 1/3

SPHK1: sphingosine kinase 1

STAT: signal transducer and activator of transcription

TBE: tris borate EDTA

TGF: transforming growth factor

TN: tenascin

VCAN: versican

VEGF: vascular endothelial growth factor

VN: vitronectin

ZBTB16: zinc finger and BTB domain containing 16 (previously known as PLZF)