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THE EFFECTS AND MECHANISMS OF PAEONIFLORIN ON MURINE OVARIAN CELLS FOR THE TREATMENT OF POLYCYSTIC OVARIAN SYNDROME

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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This research is supported by an Australian Government Research Training Program Scholarship.

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Abstract

Polycystic Ovarian Syndrome (PCOS) is a complex disorder associated with various reproductive, metabolic and cardiovascular abnormalities and is present in approximately 15% of women of reproductive age. The hallmarks include androgen excess, ovulatory dysfunction and insulin resistance which is believed to play a role in the pathogenesis of the disorder. Although the exact mechanisms of PCOS are unknown, intrinsic dysfunction of ovarian theca and granulosa cells are also thought to contribute to altered steroid production and follicle development which may explain the clinical features of the syndrome.

Metformin, an insulin sensitising agent may improve both metabolic and reproductive aspects of the disorder, however, the development of new therapeutic agents for PCOS is still required. Women with PCOS are inclined to seek complementary and alternative treatment options such as Chinese Herbal Medicine, warranting further investigation into the efficacy of the herbs commonly used. Paeoniflorin, the major compound of the herb, Radix Paeoniae Albus has demonstrated the ability to ameliorate insulin resistance in animal models, however the effects and mechanisms of paeoniflorin for the treatment of PCOS has yet to be elucidated.

This study therefore used a dexamethasone-induced *in vitro* model of PCOS in murine theca and granulosa cells to determine the effects of paeoniflorin on secretion of key hormones testosterone, progesterone and oestradiol, cell

proliferation as well as the molecular mechanisms in which paeoniflorin may regulate steroid production. Dexamethasone (10 μM) increased theca cell androgen production and adversely affected oestradiol: progesterone ratios in granulosa cells. Meanwhile, paeoniflorin (100 μg/mL) decreased androgen production in dexamethasone-induced theca cells and maintained normal oestradiol: progesterone ratios in granulosa cells. In theca cells, this was shown to be through downregulation of cholesterol side-chain cleavage enzyme and 17,20-lyase protein expression. Paeoniflorin also increased mRNA gene expression of *CYP11A1* which may indicate influence over transcription factors or post-translation modifiers, particularly in relation to cell differentiation.

Together, these results suggest that firstly, dexamethasone can be considered a useful *in vitro* model of PCOS in murine ovarian cells. Secondly, paeoniflorin may be a novel agent for the treatment of PCOS by ameliorating hyperandrogenism and improving ovarian function. Further research into the effect of paeoniflorin in differentiation of theca cells as well as the molecular mechanisms in which paeoniflorin attenuates hormones in granulosa cells is needed. Finally, this research can potentially support future animal or clinical studies to further improve the treatment options and quality of life for women with PCOS.

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Publications and Communications

Publications

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Abbreviations

AMH	Anti-mullerian hormone
AR	Androgen receptor
BPA	Bisphenol A
BSA	Bovine serum albumin
CC	Clomiphene citrate
CHM	Chinese herbal medicine
CM	Complete medium
COC	Combined oral contraceptive pill
CRP	C-reactive protein
CVD	Cardiovascular disease
CYP11	Cytochrome p450scc
CYP17	Cytochrome P45017
DEX	Dexamethasone
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
E2	Oestradiol
ECL	Enhanced chemiluminescent
ER	Endoplasmic reticulum
FBS	Fetal bovine serum
FSH	Follicle stimulating hormone
G	Gravity
GDF-9	Growth differential factor 9
GLUT4	Glucose transporter 4
GnRH	Gonadotropin-releasing hormone
HPLC	High performance liquid chromatography
IL-6	Interleukin 6
IR	Insulin resistance
IRS-1	Insulin receptor substrate 1

IVF	In vitro fertilisation
LH	Luteinising hormone
LHR	Luteinising hormone receptor
MAPK	Mitogen-activated protein kinases
NAFLD	Non-alcoholic fatty liver disease
OCP	Oral contraceptive pill
P	Progesterone
PCOM	Polycystic ovarian morphology
PCOS	Polycystic ovarian syndrome
PCR	Polymerase chain reaction
PFE	Paeoniflorin extract
PI3K	Phosphoinositide 3 kinase
PR	Progeterone receptor
qRT-PCR	Quantitative reverse-transcriptase polymerase chain reaction
RIPA	Radioimmunoprecipitation assay buffer
RNA	Ribonucleic acid
RT	Reverse transcriptase
SHBG	Sex hormone binding globulin
T	Testosterone
T2DM	Type 2 diabetes mellitus
TNFα	Tumour necrosis factor alpha

Contents