DECLARATION

I, Kathrine Elizabeth Theron, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

……..

……day of……., 2010

Dedicated to:

My husband Nicky and my unborn son Pieter,
for giving me the strength to persevere and the
courage to face each new day.
My family, for always believing in me.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Publications:

Scholtz K. E., Penny C. B. and Hosie M. J. (2008) A High Resolution SEM Study on the Effects of RU486, Used as a Postcoital Contraceptive, on the Rat Uterus During Early Pregnancy. *Cell Biology International*, 32, 436-446.

Conference presentations:

Health Science Faculty Research Day, University of the Witwatersrand, RSA (20th August 2008) – Oral presentation in the subgroup Molecular and Comparative Biosciences.
A High Resolution SEM Study on the Effects of RU486, Used as a Postcoital Contraceptive, on the Rat Uterus during Early Pregnancy.

Molecular and Cell Biology Group (MCBG) of South Africa Symposium, University of Pretoria, South Africa (17th October 2007) – poster presentation.
A High Resolution SEM Study on the Effects of RU486, Used as a Postcoital Contraceptive, on the Rat Uterus during Early Pregnancy.

46th Annual Meeting of the American Society of Cell Biology (ASCB), San Diego, USA (9-14th December 2006) – Poster presentation
An SEM Study on the Effects of RU486, Used as a Postcoital Contraceptive, on the Rat Uterus during Early Pregnancy.

ABSTRACT

Tissue specific regulation of the progesterone receptor is central to female health. The synthetic steroid, RU486 is a partial progesterone and oestrogen receptor antagonist, functioning to actively silence progesterone receptor gene associated transcription. For this reason, it has been used both as a contraceptive and quite controversially as an abortive agent.

In this study, both cellular and gene specific effects of RU486 were investigated in a rat model of early pregnancy, this including the key phases of the plasma membrane transformation, the window of receptivity and early implantation. As all of these stages are hormonally regulated by progesterone and oestrogen, the focus here was to elucidate the mechanisms of action of a single dose of RU486, used as a postcoital contraceptive, at day 3.0 of pregnancy, to successfully prevent implantation of a viable blastocyst and subsequent pregnancy.

In association with the cellular preparation of uterine epithelial cells for implantation, selected molecular targets and events were investigated at a protein and gene expression level, both prior to and after RU486 treatment, to assess the effects of either a deficit or excessive expression of these gene products on uterine preparation and eventual implantation. Factors here included the progesterone receptor, markers of apoptosis (Bax and Bcl2), mediators of angiogenesis (VEGF, bFGF and PDGF) and biomarkers of endometrial implantation (LIF, Calcitonin and Muc-1).

Together, an ultrastructural and light microscopy analysis showed RU486 to morphologically alter the uterine endometrial cells and to disrupt the plasma membrane transformation of early pregnancy, predisposing these cells towards apoptosis. In association with this, progesterone receptor gene and protein expression was ubiquitously decreased throughout pregnancy.

With regards to the implantation process of early pregnancy, the luminal epithelial cells undergo apoptosis to allow the hatching blastocyst to penetrate and implant within the uterine wall. This is partially mediated by the ratio of the expression of the apoptotic factors Bax and Bcl-2. Surprisingly here, RU486 caused an overall anti-apoptotic environment, despite previously observed high levels of apoptotic activity. This indicates that factors other than Bax and Bcl-2 influence the RU486-induced apoptosis.

A crucial event of early pregnancy is the establishment of an adequate blood supply to sustain and nourish the implanting blastocyst. There was a decided reduction in the angiogenic response of early pregnancy, as a direct consequence of RU486 treatment; the normally high levels of VEGF and bFGF during early pregnancy, were markedly decreased at all three days of pregnancy. This was reflected in the lack of increased vascularisation as normally signalled by the indicator dye, Pontamine Sky blue. In contrast to the overall increase in VEGF and bFGF at the time of blastocyst implantation during early pregnancy, increased PDGF expression was localised to the implantation sites, strongly suggesting a role for this angiogenic factor in endothelial cell proliferation.

The endometrial biomarkers are indicative of implantation, their expression patterns varying around the phase of implantation. These markers are essential to implantation, as when LIF and Calcitonin are deregulated and Muc-1 persists on the apical surface of the endometrium, implantation fails. These events are precisely what occur following RU486 treatment.

In summary, the overall effects of RU486 in the rat model of early pregnancy, when used as a postcoital contraceptive, indicate highly effective inhibition of progesterone and oestrogen effects on the endometrium, mediated by their receptors. More specifically, the structural and molecular events mirror those described in ovariectomised animal models, suggesting a hormonally under-stimulated endometrium. Clearly from the present study, the precise priming of the endometrium in preparation for blastocyst implantation is severely impaired by RU486 through a number of signalling pathways, thus predisposing the uterus to pregnancy failure.

ACKNOWLEDGEMENTS

I wish to thank my supervisors, Associate Professor Margot Hosie and Doctor Clement Penny for their invaluable support, guidance and assistance throughout my PhD.

I wish to thank the Head of the School of Anatomical Sciences. I value my time spent in the school and all the knowledge and lessons I take with me.

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

To the University of the Witwatersrand Financial Aid and Scholarships for financial assistance during the course of this degree.

To all the technical staff in the School of Anatomical Sciences for all their hard work and patience in assisting me with my techniques.

I wish to thank CSIR, Pretoria, South Africa for the use of their scanning electron microscopy and Mrs Loukie Adlem for her guidance in the use of the microscope.

I wish to thank the EM unit at Medunsa University, South Africa for the use of their transmission electron microscope and Dr Chantelle Baker for her assistance and guidance in the use of the microscope.

I would also like to thank my friend and colleague Dr Victoria Gibbon for the years of friendship, coffee breaks, sound advice and assistance with the RT-qPCR analysis.

To all my friends who have given me love, support and encouragement throughout the years, thank you. To Natalie, Louis and Tacara, thank you for putting up with me in the last few weeks and for giving me the strength and encouragement to see it through to the end.

Finally, my grateful thanks to my family; my Dad, Mom and Stepmom for giving me constant encouragement and support, and to my sister for her love, and faith in me and my abilities throughout the years. And most importantly I'd like to thank my loving and ever patient husband, Nicky. Thank you for always believing in me and for being proud of me no matter what. I owe you big time baby!!

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NOMENCLATURE

α	alpha
β	beta
ΔCT	change in threshold cycle
1° Ab	primary antibody
2° AB	secondary antibody
°C	degrees Celsius
%	percent
(-/-)	wild type
A_{230}	absorbance at 230nm
A_{260}	absorbance at 260nm
A_{280}	absorbance at 280nm
A	adenine
Ab	antibody
ACE	Associated Chemical Enterprises
ANOVA	one-way analysis of variance
AR	androgen receptor
bFGF	basic fibroblast growth factor
bps	base pairs
BSA	bovine serum albumin
BV	blood vessels
C	cytosine
cAMP	cyclic adenosine monophosphate
cDNA	complementary deoxyribonucleic acid
CO_2	carbon dioxide
CT	threshold cycle
DBD	DNA binding domain
DF	degrees of freedom
dH_2O	distilled water
DNA	deoxyribonucleic acid
E	endogenous control
EC	emergency contraception
ECM	extracellular matrix
EDTA	ethylenediamine tetraacetate
EM	electron microscopy
ER	oestrogen receptor
ERα	oestrogen receptor alpha

ERβ	oestrogen receptor beta
ERKO	oestrogen receptor knockout
FasL	Fas ligand
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
Flt-1	c-fms-like tyrosine kinase
Flk-1	kinase domain insert-containing receptor
FN	fibronectin
FRET	fluorescent resonance energy transfer
G	guanine
gp130	glycoprotein 130
GR	glucocorticoid receptor
HeLa	uterine cervical adenocarcinoma cell line
HP	Hewlett Packard
hsp90	90kDa heat shock protein
IF	immunofluorescence
IFN-γ	interferon γ
IFP	intermediate filament protein
IgG	immunoglobulin-G
IP	intraperitoneal
IS	implantation sites
kDa	kilo Daltons
KDR	kinase domain insert-containing receptor
kV	kilo volts
LBD	ligand binding domain
LIF	leukaemia inhibitory factor
LIFR	leukaemia inhibitory factor receptor
mA	milliamps
mg	milligram
MGB	minor groove binder
ml	millilitre
mm	millimetre
mM	millimolar
mRNA	messenger ribonucleic acid
Muc-1	mucin 1
n	sample size (number of observations)
NaOH	sodium hydroxide

NCBI	National Centre for Biotechnology Information
NcoR	nuclear receptor co-repressor
NEM	*N*-ethylmaleimide
NFQ	nonfluorescent quencher
N.H.	Null Hypothesis
NIS	non-implantation sites
NK	natural killer cells
nM	nanomolar
nm	nanometre
NRP-1	neuropilin-1
NTC	no template control
ODN	oligodeoxynucleotide
OPN	osteopontin
P	progesterone
$p<0.05$	significantly different at the 5% level of significance
$p>0.05$	not significantly different at the 5% level of significance
PA	progesterone receptor antagonist
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
pH	potential hydrogen
PMT	plasma membrane transformation
PO_4	phosphate buffer (pH 7.2)
PPI	pixels per inch
PR	progesterone receptor
PR-A	progesterone receptor isoform A
PR-B	progesterone receptor isoform B
PRAKO	progesterone receptor-A knockout
PRBKO	progesterone receptor-B knockout
PRE	progesterone response element
PRM	progesterone receptor modulator
PRKO	progesterone receptor knockout
RNA	ribonucleic acid
ROI	region of interest
RQ	relative quantification
rRNA	ribosomal ribonucleic acid

RT	room temperature
RT	reverse transcriptase/transcription
-RT	minus reverse transcriptase control
RT-qPCR	quantitative reverse transcription polymerase chain reaction
SEM	scanning electron microscopy
SEM	standard error of the mean
SMRT	silencing mediator of retinoic acid and thyroid hormone receptor
SRC	steroid receptor co-activator
T	target
T	thymine
T	treated (RU486-treated animals)
TBE	tris borate EDTA
TEM	transmission electron microscopy
TNF-α	tumour necrosis factor α
TNFR	tumour necrosis factor receptor
µg	microgram
UGKO	uterine gland knockout
µl	microlitre
µm	micrometer
USA	United States of America
UT	untreated (control pregnant animals)
UTMP	uterine milk protein
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VNTR	variable number of tandem repeats
VPF	vascular permeability factor
v/v	volume to volume
W	watt
WHO	World Health Organisation
w/v	weight to volume