UNIVERSITI PUTRA MALAYSIA

ADENOVIRUS-VECTORED IMMUNOCONTRACEPTION IN RAT MODEL

LO SEWN CEN

FPV 2012 31

ADENOVIRUS-VECTORED IMMUNOCONTRACEPTION IN RAT MODEL

By

LO SEWN CEN

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of
Master of Science**

April 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

ADENOVIRUS-VECTORED IMMUNOCONTRACEPTION IN RAT MODEL

By

LO SEWN CEN

April 2012

Chairman : Associate Professor Zeenathul Allaudin Nazariah, PhD

Faculty : Veterinary Medicine

The rat is an invasive threat to human health, agriculture and environment. Current control methods such as trapping, poisoning and by biological means are less than satisfactory to bring the growing population under control. As such, reproductive control by immunocontraceptive technique would be a more effective method for pest management. Immunocontraceptive vaccine prevents conception by stimulating the production of antibodies that bioneutralize gamete or hormone antigen and block to fertility. Conventional booster vaccine requires recapturing of the target animal and not practical in wild. Therefore, a virus-vectored immunocontraceptive vaccine is an alternative delivery system for wild pest population control. Virus-based immunocontraceptive vaccine offers several advantages that enable natural self

spreading through the pest population which is suitable for pest with high population densities and high reproductive potential. The critical involvement of the female gamete protein zona pellucida 3 (ZP3) in the fertilization process has been well documented. Therefore, an adenovirus (Ad) vector encoding rat ZP3 (rZP3) protein has been constructed. Briefly, rZP3 gene was first cloned into the shuttle vector, pSCMV (pSCMV-rZP3) followed by subsequent cloning into Ad vector (rec pAd-rZP3). Restriction enzyme analysis and PCR detection gave a distinct 1.3 kb rZP3 band. The rec pAd-rZP3 was then further transfected into the human embryonic kidney (HEK-293A) complementary cell to produce infectious replication-defective recombinant adenovirus-rZP3 (rec Ad-rZP3). Transfected HEK-293A cells showed cytopathic effect (CPE) at 12 days post infection (p.i). It was then followed by *in vitro* and *in vivo* protein expression assessment. The *in vitro* expression of rZP3 gene produced was screened for the presence of rZP3 gene and gene expression using PCR and reverse transcriptase PCR (RT-PCR) that gave a distinct 1.3 kbp band. The expression of rZP3 was further confirmed by SDS-PAGE and western blot analysis. High-leveled expression of rZP3 was achieved which gave an ~75kDa protein band. Furthermore, immunofluorescence staining of HEK-293A cells infected with rec Ad-rZP3 emitted strong fluorescence at the nucleus of the cells. This showed that the rZP3 gene is being expressed *in vitro*. The efficacy of the rec Ad-rZP3 construct was evaluated in rat model. Vaccination of laboratory rats with rec Ad-rZP3 resulted in reduction of fertility up to 30%, although statistically still below significant level ($\rho>0.05$). Histological examination of the treated rats demonstrated a normal follicular development. ELISA results showed that rats treated with rec Ad-rZP3 raised low anti-rZP3 antibody titers. Rat ZP3 protein was successfully expressed *in vitro* and *in vivo*. Immunization with rec Ad-rZP3 has shown

incomplete suppression of fertility in the target. This may due to the vector triggering strong innate immunity which leads to the elicitation of humoral and cellular immune response that results in suppression of rZP3 protein expression. Another reason may be due to the low antibody titers produced, as it has been demonstrated that high titer of antibody was directly associated with the ability to induce infertility. As infertility is associated with ZP3 antibodies, enhancing the antibody response of rec Ad-rZP3 construct would be beneficial. However, this study serves as an important platform to transfer the rZP3 gene into a host specific viral immunocontraceptive vaccine delivery vector such as rat cytomegalovirus (RCMV).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

VEKTOR ADENOVIRUS IMUNOKONTRASEPSI DALAM MODEL TIKUS

Oleh

LO SEWN CEN

April 2012

Pengerusi : Profesor Madya Zeenathul Allaudin Nazariah, PhD

Fakulti : Perubatan Veterinar

Tikus merupakan haiwan pengancam kesihatan manusia, pertanian dan alam sekitar. Kaedah pengawalan masa kini seperti memerangkap, keracunan dan cara biologi adalah kurang memuaskan untuk mengawal populasi yang semakin meningkat. Oleh itu, kawalan pembiakan menggunakan teknik imunokontraseptif akan menjadi satu kaedah yang lebih berkesan untuk pengurusan haiwan perosak. Vaksin imunokontraseptif merangsang penghasilan antibodi yang meneutralkan antigen gamet atau hormon bagi menghalang penghamilan. Vaksin berganda konvensional memerlukan penangkapan semula haiwan sasaran dan tidak praktikal bagi hidupan liar. Oleh yang demikian, virus vektor vaksin pencegah penghamilan merupakan sistem alternatif untuk mengawal populasi perosak liar. Vaksin imunokontraseptif berasaskan virus menawarkan beberapa

kelebihan yang membolehkan merebak secara semulajadi melalui populasi perosak yang sesuai untuk haiwan perosak dengan kepadatan populasi dan potensi pembiakan yang tinggi. Penglibatan kritikal gamet perempuan protein zona palusida 3 (*ZP3*) dalam proses persenyawaan telah didokumentasikan dengan luas. Oleh itu, adenovirus (*Ad*) vektor mengkodkan gen tikus *ZP3* (*rZP3*) telah dibangunkan. Secara ringkas, gen *rZP3* diklonkan ke dalam vektor shuttle, *pSCMV* (*pSCMV-rZP3*) diikuti oleh pengklonan berikutnya ke dalam vektor Ad (*rec pAd-rZP3*). Analisis sekatan enzim dan pengesanan *PCR* memberikan 1.3 kb jalur *rZP3* yang tersendiri. Rekombinan *pAd-rZP3* kemudiannya dijangkitkan kepada sel pelengkap buah pinggang embrio manusia (*HEK-293A*) untuk menghasilkan rekombinan adenovirus-*rZP3* (*rec Ad-rZP3*) yang berjangkit tidak mampu bereplikasi. Sel *HEK-293A* yang dijangkiti menunjukkan kesan sitopatik (*CPE*) pada 12 hari selepas jangkitan (p.i). Ia kemudian diikuti oleh penilaian ekspresi protein secara *in vitro* dan *in vivo*. Ekspresi gen *rZP3* yang dihasilkan secara *in vitro* telah disaring untuk kehadiran gen *rZP3* dan ekspresi gen menggunakan *PCR* dan *PCR* transkrip berbalik (*RT-PCR*) yang memberikan 1.3 kb jalur tersendiri. Ekspresi gen *rZP3* telah disahkan selanjutnya melalui *SDS-PAGE* dan *Western blot* analisis. Gen *rZP3* diekspres pada paras yang tinggi yang memberikan satu jalur protein bersaiz ~75kDa. Tambahan pula, sel-sel *HEK-293A* yang dijangkiti dengan *rec Ad-rZP3* melalui pewarnaan imunopendafluor memancarkan pendarfluor yang kuat di nukleus sel-sel. Ini menunjukkan bahawa gen *rZP3* telah diekspreskan secara *in vitro*. Keberkesanan *rec Ad-rZP3* telah dinilaikan pada model tikus. Vaksinasi dengan *rec Ad-rZP3* mampu menyebabkan pengurangan kesuburan sehingga 30%, walaupun statistik masih di bawah paras yang ketara ($\rho>0.05$). Pemeriksaan histologi tikus-tikus yang menerima rawatan *rec Ad-rZP3* menunjukkan perkembangan folikel yang normal.

Keputusan *ELISA* menunjukkan bahawa tikus yang dirawat dengan *rec Ad-rZP3* memperolehi titer antibodi anti-*rZP3* yang rendah. Protein *rZP3* telah berjaya diekspreskan secara *in vitro* dan *in vivo*. Imunosasi menggunakan *Rec Ad-rZP3* telah menunjukkan pengawalan kesuburan yang tidak lengkap pada sasaran. Ini mungkin disebabkan oleh vektor mencetuskan imuniti semula jadi yang kuat yang seterusnya merangsangkan imun humoral dan selular yang menyebabkan penekanan ekspresi protein *rZP3*. Sebab lain yang mungkin ialah titer antibodi yang rendah dihasilkan, dimana ia telah ditunjukkan bahawa titer antibodi yang tinggi adalah dikaitkan secara langsung dengan keupayaan untuk mendorong kemandulan. Sebagai kemandulan adalah dikaitkan dengan *ZP3* antibodi, meningkatkan tindak balas antibodi konstruk *rec Ad-rZP3* akan mendatangkan manfaat. Walau bagaimanapun, kajian ini berfungsi sebagai penanda aras yang penting untuk memindahkan gen *rZP3* ke perumah khusus imunokontraseptif virus vektor seperti virus sitomegalo tikus (*RCMV*).

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to my supervisor, Associate Professor Dr. Zeenathul Nazariah Allaudin, for her constant guidance, support and invaluable advice. Her encouragement was the motivation that helped me in completion of this study.

I would like to express my heartiest thanks and appreciation to Professor Dr. Mohd. Azmi Mohd. Lila and Professor Dato' Dr. Sheikh Omar Abdul Rahman, as members of the supervisory committee, for their helpful discussion, constructive comments and proper guidance during the course of this study.

I am grateful to all friends and staffs in LIVES laboratory, Virology Laboratory, Histology Laboratory and Pathology Laboratory for providing the research facilities, technical assistance and infinite support.

I am deeply grateful to my family for their encouragement, endless support and understanding throughout this long and demanding project. Last but not least, my gratitude goes to everybody who has helped or contributed in one way or another towards the completion of this project.

I certify that a Thesis Examination Committee has met on **6th April 2012** to conduct the final examination of Lo Sewn Cen on her thesis entitled "**Adenovirus-Vectored Immunocontraception in Rat Model**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Abdul Rani Bahaman, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Rozita Rosli, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Hassan Hj Mohd Daud, PhD
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Sharifah Binti Syed Hassan, PhD
Associate Professor
School of Medicine and Health Sciences
Monash University
Sunway Campus, Malaysia
(External Examiner)

_____
SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Zeenathul Nazariah bt Allaudin, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohd Azmi bin Mohd Lila, PhD, MBA, LLM
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Dato' Sheikh Omar Abdul Rahman, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

LO SEWN CEN

Date: 06 April 2012

TABLE OF CONTENTS

		Page
ABSTRACT		ii
ABSTRAK		v
ACKNOWLEDGEMENTS		viii
APPROVAL		ix
DECLARATION		xi
LIST OF TABLES		xv
LIST OF FIGURES		xvi
LIST OF ABBREVIATIONS		xviii
CHAPTER		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	
	Introduction to Adenovirus	8
	Adenovirus Biology	
	Pathogenesis	8
	Taxonomy	9
	Morphology	9
	Penetration	11
	Transcription	11
	Adenoviral Vector for Foreign Protein Expression	12
	Folliculogenesis and Ovulation	16
	Fertilization	19
	Development of Immunocontraceptive Vaccines	21
	The Zona Pellucida: Structure and Function	27
	Primary Sperm Receptor: Zona Pellucida 3	
	Structure of Zona Pellucida 3 Polypeptide Chain	30
	The Location of Sperm Combining Site	31
	Fertility Regulation	
	Zona Pellucida Glycoproteins Immunisation	32
	Recombinant Zona Pellucida 3 Immunisation	34
	Zona Pellucida 3 Peptides Immunisation	36
	Review on Cloning and Expression of Zona Pellucida 3 Genes	39
3	**DEVELOPMENT OF RECOMBINANT ADENOVIRUS PLASMID ENCODING RAT ZONA PELLUCIDA 3 GENE**	
	Introduction	44
	Materials and Methods	
	Amplification of Rat Zona Pellucida 3	47
	Cloning of Rat Zona Pellucida 3 Gene into pSCMV	48

Transformation into *E.coli* 48
Plasmid Isolation 49
Co-transformation of Recombinant pSCMV-rZP3 together with Supercoiled Adenovirus Vector into *E.coli* 50
Amplification of Recombinant pAd-rZP3 51
Computer-Aided Sequence Analysis 51
Results
Amplification of Rat Zona Pellucida 3 51
Recombinant pSCMV Encoding Rat Zona Pellucida 3 Gene Plasmid 52
Recombinant pAd-rZP3 Plasmid 53
Sequence Alignment Results 56
Discussion 59

4 *IN-VITRO* STUDIES OF RECOMBINANT ADENOVIRUS EXPRESSING RAT ZONA PELLUCIDA 3 PROTEIN
Introduction 62
Materials and Methods
Plasmid Preparation for Transfection 65
Propagation of HEK-293A Cell Line 66
Expression of Rat Zona Pellucida 3 Protein in HEK-293A Cell Line 66
Extraction of Recombinant Ad-rZP3 DNA 67
Polymerase Chain Reaction Screening of Recombinant Ad-rZP3 68
RNA Preparation from Infected Cell Cultures 68
Screening of Recombinant Ad-rZP3 by Reverse Transcriptase-PCR (RT-PCR) 69
Upscaling the Production of Viral Particles in HEK-293A Cells 69
Adenovirus Purification 71
Adenovirus Titration by Tissue Culture Infectious Dose 50 ($TCID_{50}$) 71
Expression of Rat Zona Pellucida 3 Fusion Protein 72
Purification of Rat Zona Pellucida 3 Fusion Protein 72
Production of Hyperimmune Serum Against Rat Zona Pellucida 3 (HIS-rZP3) in Mice 73
Concentration of Adenovirus 73
SDS-PAGE Analysis of Adenovirus 74
Electrophoretic Transfer 74
Western Blotting 75
Adenovirus Infectivity Test 75
Immunofluorescence Assays 76
Results
Transfection of Recombinant pAd-rZP3 Plasmid into HEK-293A Complementary Cells 77
Screening of Recombinant Ad-rZP3 by PCR 79

Screening of Recombinant Ad-rZP3 by RT-PCR 80
Immunofluorescence detection of rZP3 protein expression 81
SDS-PAGE Analysis 83
Western Blotting Analysis of Rat Zona Pellucida 3 Protein Expression 84
Adenovirus Infectivity Test 85
Discussion 86

5 EFFICACY OF RECOMBINANT AD-RZP3 ON RAT FERTILITY
Introduction 91
Materials and Methods
Immunisation of Rats with Recombinant Ad-rZP3 93
Evaluation of Fertility
Fertility Trial Program 1 94
Fertility Trial Program 2 96
Histopathology Assessment of Ovaries 98
Yeast Expressing Rat Zona Pellucida 3 Fusion Protein, $(His)_6$-rZP3 99
Purification of $(His)_6$-rZP3 99
Detection of Anti Rat Zona Pellucida 3 Antibodies 100
Results
Effects of Recombinant Ad-rZP3 on Rat Fertility 100
Long-term Fertility Trial (Program1) 101
Short-term Fertility Trial (Program 2) 102
Histological Assessment of Ovarian Changes 105
Detection of Anti rZP3 Antibodies 115
Discussion 117

6 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION 128

BIBLIOGRAPHY 132
APPENDICES
A Plasmid Preparation 161
B Common Solutions 162
C Chemicals for Virus DNA Extraction 163
D Pichia Media Recipes 164
E Chemicals for SDS-PAGE Electrophoresis and Western Blotting 166
F Buffers and Solutions for ELISA 169
G AUP Approval Letter 170
BIODATA OF STUDENT 171
LIST OF PUBLICATIONS 172

LIST OF TABLES

Table		Page
2.1	ZP glycoprotein-based synthetic peptide immunogens	38
3.1	List of primers used in the present study	47
5.1	The effects of rec Ad-rZP3 vaccine on rat fertility	103

LIST OF FIGURES

Figure		Page
2.1	Structure of Ad particle	10
2.2	Adenoviral vector	15
2.3	Several steps involved in the initial stage of mammalian fertilization	20
2.4	Schematic representation of the overall architecture of mZP3	31
3.1	Restriction enzyme analysis of recombinant pSCMV-rZP3	52
3.2	PCR amplification of rZP3 gene from recombinant pSCMV-rZP3	53
3.3	Restriction enzyme analysis of rec pAd-rZP3 plasmid	54
3.4	PCR amplification of rZP3 gene from rec pAd-rZP3 plasmid	55
3.5	Sequence alignment of rec Ad-rZP3 with R. *rattus diarrdi* ZP3 from NCBI (AY 338396.1)	56
4.1	The formation of CPE by transfection of rec pAd-rZP3 into HEK-293A complementary cells results in the formation of recombinant Ad virus	78
4.2	PCR screening for rZP3 gene detection from rec Ad-rZP3 viral lysates	79
4.3	RT-PCR based detection of rZP3 gene expression from rec Ad-rZP3 mRNA	80
4.4	Immunofluorescence detection of rZP3 proteins expression on rec Ad-rZP3 infected HEK-293A complementary cells	82
4.5	Expression analysis of rec Ad-rZP3 grown in HEK-293A cells	83
4.6	Western blot analysis for rZP3 protein expression study of rec Ad-rZP3	84
4.7	Infectivity test on REF cells	85
5.1	Fertility trial program 1	95
5.2	Fertility trial program 2	97
5.3	Percentage of fertility reduction rate of fertility trial program 1 and 2	104
5.4	Ovarian section of rat 2 weeks after receiving 1st injection for fertility trial program 1 stained with hematoxylin-eosin	106

5.5 Ovarian section of rat 1 month after mating trial 1 for fertility trial program 1 stained with hematoxylin-eosin 107

5.6 Ovarian section of rat 2 weeks after receiving first booster for fertility trial program 1 stained with hematoxylin-eosin 108

5.7 Ovarian section of rat 2 weeks after receiving second booster for fertility trial program 1 stained with hematoxylin-eosin 109

5.8 Ovarian section of rat 1 month after mating trial 2 for fertility trial program 1 stained with hematoxylin-eosin 110

5.9 Ovarian section of rat 2 weeks after receiving first injection for fertility trial program 2 stained with hematoxylin-eosin 111

5.10 Ovarian section of rat 2 weeks after receiving first booster for fertility trial program 2 stained with hematoxylin-eosin 112

5.11 Ovarian section of rat 2 weeks after receiving second booster for fertility trial program 2 stained with hematoxylin-eosin 113

5.12 Ovarian section of rat 1 month after mating trial for fertility trial program 2 stained with hematoxylin-eosin 114

5.13 Anti-rZP3 antibodies titer raised by rec Ad-rZP3 immunization for program 1 and 2 116

5.14 Activation of the host's immune system upon Ad administration 125

LIST OF ABBREVIATIONS

$(His)_6$-rZP3	Yeast-rZP3 Fusion Protein
Ad	Adenovirus
Ad2	Adenovirus Serotype 2
Ad5	Adenovirus Serotype 5
Ad5E3$^-$	E3-deleted Ad5
AMH	Anti-Mullerian Hormone
APCs	Antigen Presenting Cells
BMGY	Buffered Glycerol-complex Medium
BMMY	Buffered Methanol-complex Medium
BMP15	Bone Morphogenetic Factor 15
bmZP1	Bonnet Monkey Zona Pelllucida 1
bmZP3	Bonnet Monkey Zona Pellucida 3
BSA	Bovine Serum Albumin
BVDV	Bovine Viral Diarrhea Virus
CAR	Coxsackievirus and Adenovirus Receptor
cDNA	Complementary Deoxyribonucleic Acid
CFA	Complete Freund's Adjuvant
CFCS	Consensus Furin Cleavage -site
CHO	Chinese Hamster Ovary Cells
CHV	Canine Herpesvirus
CHV-BAC	Canine Herpesvirus-Bacterial Artificial Chromosome
CPE	Cytopathic Effect
CTL	Cytotoxic T-lymphocytes
C*x*	Connexins
DAB	3'-3' Diaminobenzidine

DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
DT	Diphtheria Toxoid
dZP3	Dog Zona Pellucida 3
E1	Early Region 1
E2	Early Region 2
E3	Early Region 3
E4	Early Region 4
EDTA	Ethylene Diamine Tetra-Acetate
ELISA	Enzyme Linked Immunosorbent Assay
FA-1	Fertilisation Antigen-1
FBS	Foetal Bovine Serum
FIG-α	Factor In the Germline, Alpha
FSH	Follicle Stimulating Hormone
fZP3	Fox Zona Pellucida 3
fZPC	Fox Zona Pellucida C
GDF9	Growth Differentiation Factor 9
GFP	Green Fluorescence Protein
GnRH	Gonadotrophin Releasing Hormone
GonaCon-B™	GnRH-Blue Protein Hemocyanin
GonaCon™	GnRH Peptide-KLH
GPI	Glycosyl Phosphatidylinositol
H&E	Hematoxylin & Eosin
hAd	Human Adenovirus
hCG	Human Chorionic Gonadotropin
HCMV	Human Cytomegalovirus

HEK-293A	Human Embryonic Kidney Cells
HIS-rZP3	Hyperimmune Serum against rZP3
HIV	Human Immunodeficiency Virus
hZP2	Human Zona Pellucida 2
hZP3	Human Zona Pellucida 3
i.m	Intramuscular
i.p	Intraperitoneal
IFA	Incomplete Freud's Adjuvant
ITR	Inverted Terminal Repeats
IVF	*In Vitro* Fertilization
KLH	Keyhole Limpet Hemacyanin
L1-5	Late Region 1 to 5
LB	Luria Bertani
LDH-C_4	Lactate Dehydrogenase C_4
LF2000	Lipofectamine 2000
LH	Luteinizing Hormone
m.u.	Map Unit
mAb	Monoclonal Antibodies
MCMV	Murine Cytomegalovirus
MHCI	Major Histocompatibility Complex I
MHCII	Major Histocompatibility Complex II
MII	Metaphase II
mRNA	Messenger Ribonucleic Acid
mstZP3	Marmoset Zona Pellucida 3
MV-ZPB	Myxoma Virus Encoding Rabbit Zona Pellucida B
mZP3	Mouse Zona Pellucida 3
NP	Nucleoprotein

OTC	Ornithine Transcarbamylase
p.i.	Post Infection
P1	Program 1
P2	Program 2
PBS	Phosphate Buffer Saline
PBST	Phosphate Buffer Saline Tween
PEG8000	Polyethylene Glycol 8000
Pfu	Plaque Forming Unit
PH-20	Sperm Surface Protein
pSCMV	Shuttle Vector
pSCMV-rZP3	Recombinant Shuttle Plasmid
PVDF	Polyvinylidene Difluoride
pZP	Porcine Zona Pellucida
pZP3	Porcine Zona Pellucida 3
pZPC	Porcine Zona Pellucida C
RCMV	Rat Cytomegalovirus
rec Ad-rZP3	Recombinant Adenovirus rZP3
rec pAd-rZP3	Recombinant rZP3 Adenovirus Plasmid
REF	Rat Embryonic Fibroblast Cells
RNA	Ribonucleic Acid
RNase	Ribonuclease
RT-PCR	Reversed-Transcriptase Polymerase Chain Reaction
rZP3	Rat Zona Pellucida 3
SARS-CoV	Severe Acute Respiratory Syndrome Corona Virus
SDS-PAGE	Sodium Dodecyl Sulphate-polyacrylamide Gel Electrophoresis
SIV	Simian Immunodeficiency Virus
SOC	Super Optimal Broth with Catabolite Repression

SP10	Sperm Protein 10
TA	Tibialis Anterior
$TCID_{50}$	Tissue Culture Infectious Dose 50
TE	Tris-EDTA
TGF-β	Transforming Growth Factor β
tk	Thymidine Kinase
TNF	Tumor Necrosis Factor
TP	Terminal Protein
TPL	Tripartite Leader
TT	Tetanus Toxoid
VSV	Vesicular Stomatitis Virus
wtAd5	Wild Type Ad5
YNB	Yeast Nitrogen Base
YPD	Yeast Extract Peptone Dextrose Medium
ZP	Zona Pellucida
ZP1	Zona Pellucida 1
ZP2	Zona Pellucida 2
ZP3	Zona Pellucida 3
α^{v}-integrins	Alpha-v-Integrins

CHAPTER 1

INTRODUCTION

Rats have become world major pests with huge economic impact. They are important reservoirs of numerous diseases such as plaque, leptospirosis, salmonellosis (food poisoning), murine typhus and rat bite fever. They consume stored food and grain and damage the storage structures and containers. Spillage, spoilage or contamination due to rat gnawing caused condemnation or rejection of crop shipments. In Indonesia, they annually consume enough rice to feed 20 million people sufficiently (Ylönen, 2001). Similar threats from this pest species occur in many Southeast Asian and African countries that also have severe problems in producing sufficient food. In close association with people in dense settlements, they cause problems by gnawing on electrical wiring resulting in fires and wooden structures such as doors, ledges and in wall materials. They cause a devastating impact on many native ecosystems; for example by preying on the eggs or young wild birds thus become important concerns in the recovery of endangered species especially in island environments (Dunlevy *et al.*, 2000).

Current control methods for wild rats population involve trapping, poisoning and biological means. Trapping generally is not practical for managing large infestations or removing entire populations over extensive areas. It is very labor intensive and requires skill to be used effectively. Most measures to control rats depend on the application of poison. Although rodenticides have the potential for rapid knock down of the pest population but success often declines with repeated applications because animals that

survive quickly learn to avoid the bait. The naturally cautious behavior of rats helps them to survive poisoning campaigns. This method of population control can cause negative side effects through primary and secondary poisoning of non-target species.

Biological control or the introduction of exotic vertebrate predators into new areas for pest control purposes has never been successfully demonstrated and, in some cases, has resulted in unanticipated, calamitous ecological effects. During the late 1800s, the small Indian mongoose (*Herpestes javanicus*) was introduced into both the West Indies and Hawaii to control rats populations in sugarcane fields. Although this predator survives in some areas on a diet composed mainly of rats, the introductions failed to achieve the desired result of reducing rats populations in sugarcane fields (Baldwin *et al.*, 1952; Kami, 1964). In both the West Indies and Hawaii, mongooses have severely impacted ground-nesting bird species by preying on their eggs and young (Ebenhard, 1988). In some areas in the Caribbean, the species has become a reservoir for rabies. The predators exert a controlling influence on their prey only under rare circumstances, such as when prey populations are already at low densities and alternative prey are scarce. More commonly, the presence of high rodent or other prey populations attracts and sustains predators which relocate when prey animals become more difficult to find and capture. Thus, except under rare conditions, predators do not hunt their prey to the low levels required for effective management of rodent damage. There are also dangers of the introduced predators becoming pests themselves.

Biological rodenticide using the *Sarcocystis singaporensis*, has been reported (Jäkel *et al.*, 1999; Jäkel *et al.*, 2006; Wood, 1985; Wood and Fee, 2003). This protozoan reproduces in the intestine of reticulated pythons and is transmitted via faeces to rats. Rats eat coprophagic invertebrates which carry sporocysts or drink water from streams in which reticulated pythons preferably defecate. It causes a debilitating muscle infection and a fatal pneumonia to rats (Wood, 1985; Zaman and Colley, 1975). However, the use of disease and parasites has only temporary effects on the population and rats can easily adopt and become resistant.

Reproductive control would seem to be a more effective method for pest animal management. The rapid reproductive potential of rats often enables them to rapidly overcome other population reduction measures. Three basic available techniques include surgical sterilization, hormonal contraception and immunocontraception (Artois, 1997; Cowan and Tyndale-Biscoe, 1997; Sinclair, 1997). However, it is costly and impractical to conduct surgical sterilization for free ranging wild pest population. Hormonal contraception using highly conserved reproductive steroid sex hormones such as gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) to disrupt the estrus cycle is not species-specific. It often leads to undesirable side effects that raise ethical issues, such as premature termination of pregnancies or damage to non-reproductive tissues (Delves, 2004; Ferro and Mordini, 2004; Talwar, 1997), alterations in sexual and social behavior in the target species (Tuyttens and Macdonald, 1998; Tyndale-Biscoe, 1994). Therefore, immunocontraception remains an attractive alternative for fertility control. It aims to trick the host's immune system into generating an immune reaction against cells or

molecules that are essential for reproduction, such as hormones (e.g. FSH and LH) or against the gamete themselves. If successful, this approach will effectively reduce the rat population density. It is a non-lethal and more humane alternative with the potential to provide long-lasting control.

The first step in the development of a contraceptive vaccine is selecting a potential agent to deliver the vaccine and the second step to indentify the antigen to use in the vaccine. Conventional vaccination of anaesthetized individuals which require frequent repetitive doses works well for large vertebrates. For pest with high population densities and high reproductive potential, the most effective distribution of the immunocontraceptive agent is via a virus or some other contagious agent that spreads naturally through the pest population. In a previous study, RCMV was identified as a suitable vector to carry the vaccine (Lai *et al.*, 1998). RCMV is a promising vector for fertility associated gene due to its narrow host range and the capability of establishing latent and persistent infections. However, RCMV vector construction is still ongoing by our group.

Currently, recombinant Ad vectors have become one of the most useful gene-delivery vehicles in the study of gene transfer *in vitro*, vaccination *in vivo*, and gene therapy (Brun *et al.*, 2008; Vanniasinkam and Ertl, 2005; Verma and Weitzman, 2005; Xing *et al.*, 2005). Several reasons have made Ad vectors an attractive candidate for gene expression such as well characterized viral genome and high titers virus can be produced. They are stable and can be lyophilized and administered through various routes. They express transgenes to high levels, activate the innate immune system, and stimulate dendritic cell maturation (Morelli *et al.*, 2000; Rea *et al.*, 1999; Tan *et al.*,

2005) leading to elicitation of strong immune responses. Therefore, Ad vector was choosen to serve as a model laboratory system for examining the potential of viral-based immunocontraception for managing wild rat populations before engineering the RCMV-vectored immunocontraceptive vaccine.

Target for immunocontraception can be grouped into three main categories, targeting: a) gamete production (GnRH); b) targeting gamete function (sperm or oocyte); c) gamete outcome (hCG). Targeting gamete function was adopted because gamete proteins are tissue-specific and non-circulatory therefore complications arising from an immune complex formation should not happen. It was reported that 70% of vasectomized men produce anti sperm antibodies and up to 30% of cases of human infertility is associated with the production of anti sperm antibodies in an infertile couple (Govind *et al.*, 2001; Lo *et al.*, 2011; Scarselli *et al.*, 1973; Witkin and David, 1986). Autoantibodies against the egg protein, zona pellucida (ZP) have also been reported in infertile patients that otherwise appear healthy (Buckshee and Mhaskar, 1985; Kamada *et al.*, 1992; Nishimoto *et al.*, 1980). This indicates that an immunological block to fertility is prevalent in human situation without side effects. Egg proteins is preference for this study because the ova surfaces consist of only three proteins and their individual functions are well understood compared to sperm antigens.

ZP is an extracellular glycoprotein matrix that surrounds the oocyte. It comprises three sulfated glycoproteins, zona pellucida 1 (ZP1), zona pellucida 2 (ZP2) and zona pellucida (ZP3) (Dunbar *et al.*, 1994; McLeskey *et al.*, 1997; Prasad *et al.*, 2000), which are expressed by the developing oocyte and are unique to the ovary (Epifano *et al.*,

1995; Philpott *et al.*, 1987). ZP plays a critical role during mammalian fertilization, which includes species-specific recognition and binding of spermatozoa to the oocyte, induction of acrosome-reaction, prevention of polyspermy and providing protection to the pre-implanted blastocyst. The crucial role of tissue specific ZP in mammalian fertilization has made it an ideal target antigen for the development of an immunocontraceptive vaccine.

The use of ZP in the development of immunocontraceptive vaccine has been widely documented. Porcine zona pellucida (pZP) has been extensively used in these studies due to its easy accessibility at slaughterhouses. Immunization with pZP has resulted in significant zona antibody titers and inhibition of fertility in a number of species including rabbits (Skinner *et al.*, 1984; Wood *et al.*, 1981), primates (Bagavant *et al.*, 1994; Paterson *et al.*, 1992), horses (Kirkpatrick and Turner, 2008; Turner Jr *et al.*, 2002), hamsters (Hasegawa *et al.*, 1992), elephant (Perdock *et al.*, 2008) and deer (Curtis *et al.*, 2007). Unfortunately, rats and mice inoculated with whole pZP remained fertile (Sacco *et al.*, 1981; Wood *et al.*, 1981). Hence, 'self' ZP is required to suppress fertility in these animals.

As such, hypothetically, a recombinant adenovirus encoding rZP3 gene express rZP3 protein *in vitro* and *in vivo*. The infected rat will raise antibodies against the self ZP3 protein of oocytes, thus providing an immunocontraceptive barrier to fertility.

The objectives of this study are:

1) to construct and characterize a recombinant adenovirus immunocontraceptive vaccine expressing rat zona pellucida 3 protein.
2) to evaluate the *in vitro* expression of the recombinant adenovirus expressing rat zona pellucida 3 (rec Ad-rZP3) protein.
3) to evaluate the immunocontraceptive efficacy of the recombinant adenovirus expressing rat zona pellucida 3 protein in rat model.

BIBLIOGRAPHY

Ackert, C.L., J.E.I. Gittens, M.J. O'Brien, J.J. Eppig, and G.M. Kidder. 2001. Intercellular communication via connexin43 gap junctions is required for ovarian folliculogenesis in the mouse. *Developmental Biology*. 233:258-270.

Aitken, R.J. 2002. Immunocontraceptive vaccines for human use. *Journal of Reproductive Immunology*. 57:273-287.

Aitken, R.J., E.A. Rudak, D.W. Richardson, J. Dor, O. Djahanbahkch, and A.A. Templeton. 1981. The influence of anti-zona and anti-sperm antibodies on sperm-egg interactions. *Journal of Reproduction and Fertility*. 62:597-606.

Araki, Y., M.C. Orgebin-Crist, and D.R. Tulsiani. 1992. Qualitative characterization of oligosaccharide chains present on the rat zona pellucida glycoconjugates. *Biology of Reproduction*. 46:912-919.

Artois, M. 1997. Managing problem wildlife in the'Old World': a veterinary perspective. *Reproduction, Fertility, and Development*. 9:17-25.

Bagavant, H., F.M. Fusi, J. Baisch, B. Kurth, C.S. David, and K.S. Tung. 1997. Immunogenicity and contraceptive potential of a human zona pellucida 3 peptide vaccine. *Biology of Reproduction*. 56:764-770.

Bagavant, H., P. Thillai-Koothan, M.G. Sharma, G.P. Talwar, and S.K. Gupta. 1994. Antifertility effects of porcine zona pellucida-3 immunization using permissible adjuvants in female bonnet monkeys (*Macaca radiata*): reversibility, effect on follicular development and hormonal profiles. *Journal of Reproduction and Fertility*. 102:17-25.

Baldwin, P.H., C.W. Schwartz, and E.R. Schwartz. 1952. Life history and economic status of the mongoose in Hawaii. *Journal of Mammalogy*. 33:335-356.

Bangari, D.S., and S.K. Mittal. 2006. Development of nonhuman adenoviruses as vaccine vectors. *Vaccine*. 24:849-862.

Barber, M.R., and R.A. Fayrer-Hosken. 2000. Possible mechanisms of mammalian immunocontraception. *Journal of Reproductive Immunology*. 46:103-124.

Barouch, D.H., P.F. McKay, S.M. Sumida, S. Santra, S.S. Jackson, D.A. Gorgone, M.A. Lifton, B.K. Chakrabarti, L. Xu, and G.J. Nabel. 2003. Plasmid chemokines and colony-stimulating factors enhance the immunogenicity of DNA priming-viral vector boosting human immunodeficiency virus type 1 vaccines. *Journal of Virology*. 77(16):8729-8735.

Beaton, S., J. Have, A. Cleary, and M.P. Bradley. 1995. Cloning and partial characterization of the cDNA encoding the fox sperm protein FSA-Acr. 1 with

similarities to the SP-10 antigen. *Molecular Reproduction and Development*. 40:242-252.

Beebe, S.J., L. Leyton, D. Burks, M. Ishikawa, T. Fuerst, J. Dean, and P. Saling. 1992. Recombinant mouse ZP3 inhibits sperm binding and induces the acrosome reaction. *Developmental Biology*. 151:48-54.

Bennett, E.M., J.R. Bennink, J.W. Yewdell, and F.M. Brodsky. 1999. Cutting edge: adenovirus E19 has two mechanisms for affecting class I MHC expression1. *The Journal of Immunology*. 162:5049-5052.

Bergelson, J.M., J.A. Cunningham, G. Droguett, E.A. Kurt-Jones, A. Krithivas, J.S. Hong, M.S. Horwitz, R.L. Crowell, and R.W. Finberg. 1997. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science*. 275:1320-1323.

Berkner, K.L. 1998. Development of adenovirus vectors for the expression of heterologous genes. *Biotechniques*. 6:616-629.

Berkner, K.L., and P.A. Sharp. 1983. Generation of adenovirus by transfection of plasmids. *Nucleic Acids Research*. 11:6003-6020.

Bett, A.J., W. Haddara, L. Prevec, and F.L. Graham. 1994. An efficient and flexible system for construction of adenovirus vectors with insertions or deletions in early regions 1 and 3. *Proceedings of the National Academy of Sciences*. 91:8802-8806.

Boone, S.A., and C.P. Gerba. 2007. Significance of fomites in the spread of respiratory and enteric viral disease. *Applied and Environmental Microbiology*. 73:1687-1696.

Bradley, M.P., L.A. Hinds, and P.H. Bird. 1997. A bait-delivered immunocontraceptive vaccine for the European red fox (*Vulpes vulpes*) by the year 2002? *Reproduction, Fertility, and Development*. 9:111-116.

Bristol, J.A., P. Shirley, N. Idamakanti, M. Kaleko, and S. Connelly. 2000. *In vivo* dose threshold effect of adenovirus-mediated factor VIII gene therapy in hemophiliac mice. *Molecular Therapy*. 2:223-232.

Bronson, R.A., G.W. Cooper, E.J. Margalioth, R.K. Naz, and M.S. Hamilton. 1989. The detection in human sera of antisperm antibodies reactive with FA-1, an evolutionarily conserved antigen, and with murine spermatozoa. *Fertility and Sterility*. 52:457-462.

Brun, A., E. Albina, T. Barret, D.A.G. Chapman, M. Czub, L.K. Dixon, G.M. Keil, B. Klonjkowski, M.F. Le Potier, and G. Libeau. 2008. Antigen delivery systems for veterinary vaccine development: viral-vector based delivery systems. *Vaccine*. 26:6508-6528.

Buckshee, K., and A. Mhaskar. 1985. Status of autoantibodies to zona pellucida in human reproduction. *International Journal of Fertility*. 30:13-17.

Burgert, H.G., J.L. Maryanski, and S. Kvist. 1987. " E3/19K" protein of adenovirus type 2 inhibits lysis of cytolytic T lymphocytes by blocking cell-surface expression of histocompatibility class I antigens. *Proceedings of the National Academy of Sciences*. 84:1356-1360.

Campbell, M., S. Qu, S. Wells, H. Sugandha, and R.A. Jensen. 2003. An adenoviral vector containing an arg-gly-asp (RGD) motif in the fiber knob enhances protein product levels from transgenes refractory to expression. *Cancer Gene Therapy*. 10:559-570.

Cao, H., D.R. Koehler, and J. Hu. 2004. Adenoviral vectors for gene replacement therapy. *Viral Immunology*. 17:327-333.

Capone, S., A. Meola, B.B. Ercole, A. Vitelli, M. Pezzanera, L. Ruggeri, M.E. Davies, R. Tafi, C. Santini, and A. Luzzago. 2006. A novel adenovirus type 6 (Ad6)-based hepatitis C virus vector that overcomes preexisting anti-Ad5 immunity and induces potent and broad cellular immune responses in rhesus macaques. *Journal of Virology*. 80:1688-1699.

Casimiro, D.R., L. Chen, T.M. Fu, R.K. Evans, M.J. Caulfield, M.E. Davies, A. Tang, M. Chen, L. Huang, and V. Harris. 2003. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. *Journal of Virology*. 77(11):6305-6313.

Catanzaro, A.T., R.A. Koup, M. Roederer, R.T. Bailer, M.E. Enama, Z. Moodie, L. Gu, J.E. Martin, L. Novik, and B.K. Chakrabarti. 2006. Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 candidate vaccine delivered by a replication-defective recombinant adenovirus vector. *Journal of Infectious Diseases*. 194:1638-1649.

Cecconi, S., C. Ciccarelli, M. Barberi, G. Macchiarelli, and R. Canipari. 2004. Granulosa cell-oocyte interactions. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 115:S19-S22.

Chapman, N., E. Kessopoulou, P. Andrews, D. Hornby, and C.R. Barratt. 1998. The polypeptide backbone of recombinant human zona pellucida glycoprotein-3 initiates acrosomal exocytosis in human spermatozoa *in vitro*. *Biochemical Journal*. 330:839-845.

Chartier, C., E. Degryse, M. Gantzer, A. Dieterle, A. Pavirani, and M. Mehtali. 1996. Efficient generation of recombinant adenovirus vectors by homologous recombination in *Escherichia coli*. *Journal of Virology*. 70(7):4805-4810.

Chen, J., E.S. Litscher, and P.M. Wassarman. 1998. Inactivation of the mouse sperm receptor, mZP3, by site-directed mutagenesis of individual serine residues located at the combining site for sperm. *Proceedings of the National Academy of Sciences*. 95:6193-6197.

Chen, Y., D. Zhang, N. Xin, Y.Z. Xiong, P. Chen, B. Li, X.D. Tu, and F.H. Lan. 2008. Construction of sperm-specific lactate dehydrogenase DNA vaccine and experimental study of its immunocontraceptive effect on mice. *Science in China Series C: Life Sciences*. 51(4):308-316.

Chuah, M.K.L., G. Schiedner, L. Thorrez, B. Brown, M. Johnston, V. Gillijns, S. Hertel, N. Van Rooijen, D. Lillicrap, and D. Collen. 2003. Therapeutic factor VIII levels and negligible toxicity in mouse and dog models of hemophilia A following gene therapy with high-capacity adenoviral vectors. *Blood*. 101:1734-1743.

Cohen, C.J., J.T.C. Shieh, R.J. Pickles, T. Okegawa, J.T. Hsieh, and J.M. Bergelson. 2001. The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. *Proceedings of the National Academy of Sciences*. 98:15191-15196.

Cohen, P. 2006. Immunity's yin and yang. A successful vaccine must first avoid being eliminated by pre-existing immunity before it can promote a protective immune response. *International AIDS Vaccine Initiative Report*. 10(1):1-5.

Cowan, P.E., and C.H. Tyndale-Biscoe. 1997. Australian and New Zealand mammal species considered to be pests or problems. *Reproduction, Fertility, and Development*. 9:27-36.

Cui, C., V.C. Stevens, and S.P. Schwendeman. 2007. Injectable polymer microspheres enhance immunogenicity of a contraceptive peptide vaccine. *Vaccine*. 25(3):500-509.

Curtis, P.D., M.E. Richmond, L.A. Miller, and F.W. Quimby. 2007. Pathophysiology of white-tailed deer vaccinated with porcine zona pellucida immunocontraceptive. *Vaccine*. 25:4623-4630.

D'Occhio, M.J. 1993. Immunological suppression of reproductive functions in male and female mammals. *Animal Reproduction Science*. 33:345-372.

Danthinne, X., and M.J. Imperiale. 2000. Production of first generation adenovirus vectors: a review. *Gene Therapy*. 7:1707-1714.

Dean, J. 2002. Oocyte-specific genes regulate follicle formation, fertility and early mouse development. *Journal of Reproductive Immunology*. 53:171-180.

Delsink, A.K., J.J. Van Altena, D. Grobler, H.J. Bertschinger, J.F. Kirkpatrick, and R. Slotow. 2007. Implementing immunocontraception in free-ranging African

elephants at Makalali Conservancy. *Journal of the South African Veterinary Association*. 78:25-30.

Delves, P.J. 2004. How far from a hormone-based contraceptive vaccine? *Journal of Reproductive Immunology*. 62:69-78.

Delves, P.J., and I.M. Roitt. 2005. Vaccines for the control of reproduction--status in mammals, and aspects of comparative interest. *Developments in Biologicals*. 121:265-273.

Doolittle, R.F. 1986. Of URFs and ORFs: a primer on how to analyze derived amino acid sequences. Univ Science Books, Mill Valley, C.A.

Dormond, E., M. Perrier, and A. Kamen. 2009. From the first to the third generation adenoviral vector: What parameters are governing the production yield? *Biotechnology Advances*. 27(2):133-144.

Douglas, J.T., M. Kim, L.A. Sumerel, D.E. Carey, and D.T. Curiel. 2001. Efficient oncolysis by a replicating adenovirus (ad) *in vivo* is critically dependent on tumor expression of primary ad receptors. *Cancer Research*. 61:813-817.

Drell, D.W., D.M. Wood, D. Bundman, and B.S. Dunbar. 1984. Immunological comparison of antibodies to porcine zonae pellucidae in rats and rabbits. *Biology of Reproduction*. 30:435-444.

Dudek, T., and D.M. Knipe. 2006. Replication-defective viruses as vaccines and vaccine vectors. *Virology*. 344:230-239.

Dunbar, B.S., S. Avery, V. Lee, S. Prasad, D. Schwahn, E. Schwoebel, S. Skinner, and B. Wilkins. 1994. The mammalian zona pellucida: its biochemistry, immunochemistry, molecular biology, and developmental expression. *Reproduction, Fertility and Development*. 6:331-347.

Dunbar, B.S., C. Lo, and B. Stevens. 1989. Effect of immunization with purified porcine zona pellucida proteins on ovarian function in baboons. *Fertility and Sterility*. 52(2):311-318.

Dunbar, B.S., and G.O. Michael. 1991. *A comparative overview of mammalian fertilization*. Plenum Press, New York.

Dunlevy, P.A., E.W. CampbellIii, and G.D. Lindsey. 2000. Broadcast application of a placebo rodenticide bait in a native Hawaiian forest. *International Biodeterioration & Biodegradation*. 45:199-208.

Durlinger, A.L.L., M.J.G. Gruijters, P. Kramer, B. Karels, H.A. Ingraham, M.W. Nachtigal, J.T.J. Uilenbroek, J.A. Grootegoed, and A.P.N. Themmen. 2002. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. *Endocrinology*. 143:1076-1084.

Ebenhard, T. 1988. Introduced birds and mammals and their ecological effects. *Swedish Wildlife Research*. 13:1-107.

Epifano, O., and J. Dean. 1994. Biology and structure of the zona pellucida: a target for immunocontraception. *Reproduction, Fertility and Development*. 6:319-330.

Epifano, O., and J. Dean. 2002. Genetic control of early folliculogenesis in mice. *Trends in Endocrinology and Metabolism*. 13:169-173.

Epifano, O., L.F. Liang, M. Familari, M.C. Moos, and J. Dean. 1995. Coordinate expression of the three zona pellucida genes during mouse oogenesis. *Development*. 121:1947-1956.

Eppig, J.J. 1991. Intercommunication between mammalian oocytes and companion somatic cells. *Bioessays*. 13:569-574.

Erickson, G.F. 1996. Physiologic basis of ovulation induction. *Seminars in Reproductive Endocrinology*. 14:287-298.

Fair, T. 2003. Follicular oocyte growth and acquisition of developmental competence. *Animal Reproduction Science*. 78:203-216.

Fechner, H., A. Haack, H. Wang, X. Wang, K. Eizema, M. Pauschinger, R. Schoemaker, R. Veghel, A. Houtsmuller, and H.P. Schultheiss. 1999. Expression of coxsackie adenovirus receptor and alphav-integrin does not correlate with adenovector targeting *in vivo* indicating anatomical vector barriers. *Gene Therapy*. 6(9):1520.

Fenner, F., and F. Ratcliffe. 1965. *Myxomatosis*. Cambridge University Press, Cambridge.

Ferreira, T., P. Alves, J. Aunins, and M. Carrondo. 2005. Use of adenoviral vectors as veterinary vaccines. *Gene Therapy*. 12:S73-S83.

Ferro, V.A., and E. Mordini. 2004. Peptide vaccines in immunocontraception. *Current Opinion in Molecular Therapeutics*. 6:83-89.

Fisher, K., Y. Stallwood, K. Ulbrich, V. Mautner, and L. Seymour. 2000. Protection and retargeting of adenovirus using a multifunctional hydrophilic polymer. *Molecular Therapy1*. 57.

Fitchen, J., R.N. Beachy, and M.B. Hein. 1995. Plant virus expressing hybrid coat protein with added murine epitope elicits autoantibody response. *Vaccine*. 13:1051-1057.

Fitzgerald, J.C., G.P. Gao, A. Reyes-Sandoval, G.N. Pavlakis, Z.Q. Xiang, A.P. Wlazlo, W. Giles-Davis, J.M. Wilson, and H.C.J. Ertl. 2003. A simian replication-defective adenoviral recombinant vaccine to HIV-1 gag. *The Journal of Immunology*. 170:1416-1422.

Florman, H.M., and P.M. Wassarman. 1985. O-linked oligosaccharides of mouse egg ZP3 account for its sperm receptor activity. *Cell*. 41:313-324.

Gadkari, R., R. Deshpande, and R.R. Dighe. 2003. Hyperexpression and purification of biologically active human luteinizing hormone and human chorionic gonadotropin using the methylotropic yeast, Pichia pastoris. *Protein expression and purification*. 32:175-184.

Galloway, S.M., K.P. McNatty, L.M. Cambridge, M.P.E. Laitinen, J.L. Juengel, T.S. Jokiranta, R.J. McLaren, K. Luiro, K.G. Dodds, and G.W. Montgomery. 2000. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature Genetics*. 25:279-283.

Gao, W., A.C. Soloff, X. Lu, A. Montecalvo, D.C. Nguyen, Y. Matsuoka, P.D. Robbins, D.E. Swayne, R.O. Donis, and J.M. Katz. 2006. Protection of mice and poultry from lethal H5N1 avian influenza virus through adenovirus-based immunization. *Journal of Virology*. 80:1959-1964.

Garrott, R.A., J.G. Cook, M.M. Bernoco, J.F. Kirkpatrick, L.L. Cadwell, S. Cherry, and B. Tiller. 1998. Antibody response of elk immunized with porcine zona pellucida. *Journal of Wildlife Diseases*. 34(3):539-546.

Gilardi, P., M. Courtney, A. Pavirani, and M. Perricaudet. 1990. Expression of human [alpha] 1-antitrypsin using a recombinant adenovirus vector. *FEBS letters*. 267:60-62.

Gilden, R.V., J. Kern, Y. Ki Lee, F. Rapp, J.L. Melnick, J.L. Riggs, E.H. Lennette, B. Zbar, H.J. Rapp, and H.C. Turner. 1970. Serologic surveys of human cancer patients for antibody to adenovirus T antigens. *American Journal of Epidemiology*. 91:500-509.

Ginsberg, H.S., U. Lundholm-Beauchamp, R.L. Horswood, B. Pernis, W.S. Wold, R.M. Chanock, and G.A. Prince. 1989. Role of early region 3 (E3) in pathogenesis of adenovirus disease. *Proceedings of the National Academy of Sciences*. 86:3823-3827.

Goldberg, E. 1990. Lactate dehydrogenase Cd as an immunocontraceptive model. *In Gamete Interaction: Prospects for Immunocontraception*. Wiley-Liss, New York. 63-73.

Goldberg, E., T.E. Wheat, J.E. Powell, and V.C. Stevens. 1981. Reduction of fertility in female baboons immunized with lactate dehydrogenase C4. *Fertility and Sterility*. 35:214-217.

Goncalves, M.A.F.V., and A.A.F. de Vries. 2006. Adenovirus: from foe to friend. *Reviews in Medical Virology*. 16:167-186.

Gonin, P., W. Oualikene, A. Fournier, and M. Eloit. 1996. Comparison of the efficacy of replication-defective adenovirus and Nyvac poxvirus as vaccine vectors in mice. *Vaccine*. 14:1083-1087.

Gooding, L.R., and W.S. Wold. 1990. Molecular mechanisms by which adenoviruses counteract antiviral immune defenses. *Critical Reviews in Immunology*. 10:53-71.

Gorman, S., M.L. Lloyd, L.M. Smith, A.R. McWhorter, M.A. Lawson, A.J. Redwood, and G.R. Shellam. 2008. Prior infection with murine cytomegalovirus (MCMV) limits the immunocontraceptive effects of an MCMV vector expressing the mouse zona-pellucida-3 protein. *Vaccine*. 26:3860-3869.

Govind, C.K., G.K. Gahlay, S. Choudhury, and S.K. Gupta. 2001. Purified and refolded recombinant bonnet monkey (Macaca radiata) zona pellucida glycoprotein-B expressed in Escherichia coli binds to spermatozoa. *Biology of reproduction*. 64:1147-1152.

Govind, C.K., A. Hasegawa, K. Koyama, and S.K. Gupta. 2000. Delineation of a conserved B cell epitope on bonnet monkey (*Macaca radiata*) and human zona pellucida glycoprotein-B by monoclonal antibodies demonstrating inhibition of sperm-egg binding. *Biology of Reproduction*. 62:67-75.

Graham, F.L., J. Smiley, W.C. Russell, and R. Nairn. 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *Journal of General Virology*. 36:59-72.

Gu, W., M. Holland, P. Janssens, R. Seamark, and P. Kerr. 2004. Immune response in rabbit ovaries following infection of a recombinant myxoma virus expressing rabbit zona pellucida protein B. *Virology*. 318(2):516-523.

Gupta, S.K., and P. Bansal. 2010. Vaccines for immunological control of fertility. *Reproductive Medicine and Biology*. 9(2):61-71.

Gupta, S.K., N. Gupta, P. Suman, S. Choudhury, K. Prakash, T. Gupta, R. Sriraman, S.B. Nagendrakumar, and V.A. Srinivasan. 2011. Zona pellucida-based contraceptive vaccines for human and animal utility. *Journal of Reproductive Immunology*. 88:240-246.

Gupta, S.K., N. Srivastava, S. Choudhury, A. Rath, N. Sivapurapu, G.K. Gahlay, and D. Batra. 2004. Update on zona pellucida glycoproteins based contraceptive vaccine. *Journal of Reproductive Immunology*. 62:79-89.

Gwatkin, R.B., D.T. Williams, and D.J. Carlo. 1977. Immunization of mice with heat-solubilized hamster zonae: production of anti-zona antibody and inhibition of fertility. *Fertility and Sterility*. 28:871-877.

Halpin, D.M.G., A. Jones, G. Fink, and H.M. Charlton. 1986. Postnatal ovarian follicle development in hypogonadal (hpg) and normal mice and associated changes in

the hypothalamic-pituitary ovarian axis. *Journal of Reproduction and Fertility*. 77:287-296.

Hardy, C., and A. Braid. 2007. Vaccines for immunological control of fertility in animals. *Revue Scientifique et Technique (International Office of Epizootics)*. 26:461-470.

Hardy, C.M., G. Clydesdale, and K.J. Mobbs. 2004a. Development of mouse-specific contraceptive vaccines: infertility in mice immunized with peptide and polyepitope antigens. *Reproduction*. 128:395-407.

Hardy, C.M., G. Clydesdale, K.J. Mobbs, J. Pekin, M.L. Lloyd, C. Sweet, G.R. Shellam, and M.A. Lawson. 2004b. Assessment of contraceptive vaccines based on recombinant mouse sperm protein PH20. *Reproduction*. 127(3):325-334.

Hardy, C.M., L.A. Hinds, P.J. Kerr, M.L. Lloyd, A.J. Redwood, G.R. Shellam, and T. Strive. 2006. Biological control of vertebrate pests using virally vectored immunocontraception. *Journal of Reproductive Immunology*. 71:102-111.

Hardy, C.M., J.F. Ten Have, J. Pekin, S. Beaton, R.J. Jackson, and G. Clydesdale. 2003. Contraceptive responses of mice immunized with purified recombinant mouse zona pellucida subunit 3 (mZP3) proteins. *Reproduction*. 126:49-59.

Harrod, K.S., T.W. Hermiston, B.C. Trapnell, W.S.M. Wold, and J.A. Whitsett. 1998. Lung-specific expression of adenovirus E3-14.7 K in transgenic mice attenuates adenoviral vector-mediated lung inflammation and enhances transgene expression. *Human Gene Therapy*. 9:1885-1898.

Hasegawa, A., K. Koyama, M. Inoue, T. Takemura, and S. Isojima. 1992. Antifertility effect of active immunization with ZP4 glycoprotein family of porcine zona pellucida in hamsters. *Journal of Reproductive Immunology*. 22:197-210.

Hashimoto, M., J.L. Boyer, N.R. Hackett, J.M. Wilson, and R.G. Crystal. 2005. Induction of protective immunity to anthrax lethal toxin with a nonhuman primate adenovirus-based vaccine in the presence of preexisting anti-human adenovirus immunity. *Infection and Immunity*. 73:6885-6891.

He, T.C., S. Zhou, L.T. Da Costa, J. Yu, K.W. Kinzler, and B. Vogelstein. 1998. A simplified system for generating recombinant adenoviruses. *Proceedings of the National Academy of Sciences*. 95:2509-2514.

Hemmi, S., R. Geertsen, A. Mezzacasa, I. Peter, and R. Dummer. 1998. The presence of human coxsackievirus and adenovirus receptor is associated with efficient adenovirus-mediated transgene expression in human melanoma cell cultures. *Human Gene Therapy*. 9:2363-2373.

Henderson, C.J., M.J. Hulme, and R.J. Aitken. 1987. Analysis of the biological properties of antibodies raised against intact and deglycosylated porcine zonae pellucidae. *Gamete Research*. 16:323-341.

Herr, J.C., C.J. Flickinger, M. Homyk, K. Klotz, and E. John. 1990. Biochemical and morphological characterization of the intra-acrosomal antigen SP-10 from human sperm. *Biology of Reproduction*. 42:181-193.

Hodges, B.L., D. Serra, H. Hu, C.A. Begy, J.S. Chamberlain, and A. Amalfitano. 2000. Multiply deleted [E1, polymerase-, and pTP-] adenovirus vector persists despite deletion of the preterminal protein. *The Journal of Gene Medicine*. 2:250-259.

Holland, M.K., J. Andrews, H. Clarke, C. Walton, and L.A. Hinds. 1997. Selection of antigens for use in a virus-vectored immunocontraceptive vaccine: PH-20 as a case study. *Reproduction, Fertility, and Development*. 9:117-124.

Holman, D.H., D. Wang, K. Raviprakash, N.U. Raja, M. Luo, J. Zhang, K.R. Porter, and J.Y. Dong. 2007. Two complex, adenovirus-based vaccines that together induce immune responses to all four dengue virus serotypes. *Clinical and Vaccine Immunology*. 14(2):182-189.

Holman, D.H., D. Wang, J. Woraratanadharm, and J.Y. Dong. 2009. Viral vectors. *In Vaccines for Biodefense and Emerging and Neglected Diseases*. Vol. 7. Academic Press. 77-91.

Huebner, R.J., W.P. Rowe, and W.T. Lane. 1962. Oncogenic effects in hamsters of human adenovirus types 12 and 18. *Proceedings of the National Academy of Sciences of the United States of America*. 48(12):2051-2058.

Ilan, Y., G. Droguett, N.R. Chowdhury, Y. Li, K. Sengupta, N.R. Thummala, A. Davidson, J.R. Chowdhury, and M.S. Horwitz. 1997. Insertion of the adenoviral E3 region into a recombinant viral vector prevents antiviral humoral and cellular immune responses and permits long-term gene expression. *Proceedings of the National Academy of Sciences*. 94:2587-2592.

Imler, J.L. 1995. Adenovirus vectors as recombinant viral vaccines. *Vaccine*. 13:1143-1151.

Jackson, R.J., D.J. Maguire, L.A. Hinds, and I.A. Ramshaw. 1998. Infertility in mice induced by a recombinant ectromelia virus expressing mouse zona pellucida glycoprotein 3. *Biology of Reproduction*. 58(1):152-159.

Jäkel, T., Y. Khoprasert, S. Endepols, C. Archer-Baumann, K. Suasa-Ard, P. Promkerd, D. Kliemt, P. Boonsong, and S. Hongnark. 1999. Biological control of rodents using *Sarcocystis singaporensis*. *International Journal for Parasitology*. 29:1321-1330.

Jäkel, T., Y. Khoprasert, P. Promkerd, and S. Hongnark. 2006. An experimental field study to assess the effectiveness of bait containing the parasitic protozoan *Sarcocystis singaporensis* for protecting rice crops against rodent damage. *Crop Protection*. 25:773-780.

Janett, F., R. Stump, D. Burger, and R. Thun. 2009. Suppression of testicular function and sexual behavior by vaccination against GnRH (Equity (TM)) in the adult stallion. *Animal Reproduction Science*. 115:88-102.

Johnston, D.S., W.W. Wright, J.H. Shaper, C.H. Hokke, D.H. Van den Eijnden, and D.H. Joziasse. 1998. Murine sperm-zona binding, a fucosyl residue is required for a high affinity sperm-binding ligand. *Journal of Biological Chemistry*. 273:1888-1895.

Jones, G.R., A.G. Sacco, M.G. Subramanian, M. Kruger, S. Zhang, E.C. Yurewicz, and K.S. Moghissi. 1992. Histology of ovaries of female rabbits immunized with deglycosylated zona pellucida macromolecules of pigs. *Journal of Reproduction and Fertility*. 95:513-525.

Jooss, K., H.C.J. Ertl, and J.M. Wilson. 1998. Cytotoxic T-lymphocyte target proteins and their major histocompatibility complex class I restriction in response to adenovirus vectors delivered to mouse liver. *Journal of Virology*. 72:2945-2954.

Jovine, L., E.S. Litscher, and P.M. Wassarman. 2002a. Egg zona pellucida, egg vitelline envelope, and related extracellular glycoproteins. *Advances in Developmental Biology and Biochemistry*. 12:31-54.

Jovine, L., H. Qi, Z. Williams, E. Litscher, and P.M. Wassarman. 2002b. The ZP domain is a conserved module for polymerization of extracellular proteins. *Nature Cell Biology*. 4:457-461.

Juillard, V., P. Villefroy, D. Godfrin, A. Pavirani, A. Venet, and J.G. Guillet. 1995. Long-term humoral and cellular immunity induced by a single immunization with replication-defective adenovirus recombinant vector. *European Journal of Immunology*. 25:3467-3473.

Kamada, M., T. Daitoh, K. Mori, N. Maeda, K. Hirano, M. Irahara, T. Aono, and T. Mori. 1992. Etiological implication of autoantibodies to zona pellucida in human female infertility. *American Journal of Reproductive Immunology* 28:104-109.

Kami, H.T. 1964. Foods of the mongoose in the Hamakua District, Hawaii. *Zoonoses Research*. 3:165-170.

Keenan, J.A., A.G. Sacco, M.G. Subramanian, M. Kruger, E.C. Yurewicz, and K.S. Moghissi. 1991. Endocrine response in rabbits immunized with native versus deglycosylated porcine zona pellucida antigens. *Biology of Reproduction*. 44:150-156.

Kerr, P.J., R.J. Jackson, A.J. Robinson, J. Swan, L. Silvers, N. French, H. Clarke, D.F. Hall, and M.K. Holland. 1999. Infertility in female rabbits (*Oryctolagus cuniculus*) alloimmunized with the rabbit zona pellucida protein ZPB either as a purified recombinant protein or expressed by recombinant myxoma virus. *Biology of Reproduction*. 61(3):606-613.

Khan, M.A.H., V.A. Ferro, S. Koyama, Y. Kinugasa, M. Song, K. Ogita, T. Tsutsui, Y. Murata, and T. Kimura. 2007. Immunisation of male mice with a plasmid DNA vaccine encoding gonadotrophin releasing hormone (GnRH-I) and T-helper epitopes suppresses fertility *in vivo*. *Vaccine*. 25(18):3544-3553.

Killian, G., T.J. Kreeger, J. Rhyan, K. Fagerstone, and L. Miller. 2009. Observations on the use of GonaCon in captive female elk (*Cervus elaphus*). *Journal of Wildlife Diseases*. 45(1):184-188.

Kirkpatrick, J.F., A. Rowan, N. Lamberski, R. Wallace, K. Frank, and R. Lyda. 2009. The practical side of immunocontraception: zona proteins and wildlife. *Journal of Reproductive Immunology*. 83:151-157.

Kirkpatrick, J.F., and A. Turner. 2008. Achieving population goals in a long-lived wildlife species (*Equus caballus*) with contraception. *Wildlife Research*. 35:513-519.

Kit, S., M. Kit, and E.C. Pirtle. 1985. Attenuated properties of thymidine kinase-negative deletion mutant of pseudorabies virus. *American Journal of Veterinary Research*. 46(6):1359.

Kitchener, A.L., L.M. Edds, F.C. Molinia, and D.J. Kay. 2002. Porcine zonae pellucidae immunisation of tammar wallabies (*Macropus eugenii*): fertility and immune responses. *Reproduction, Fertility and Development*. 14:215-223.

Klinger, F.G., and M. De Felici. 2002. *In vitro* development of growing oocytes from fetal mouse oocytes: stage-specific regulation by stem cell factor and granulosa cells. *Developmental Biology*. 244:85-95.

Kobinger, G.P., H. Feldmann, Y. Zhi, G. Schumer, G. Gao, F. Feldmann, S. Jones, and J.M. Wilson. 2006. Chimpanzee adenovirus vaccine protects against Zaire Ebola virus. *Virology*. 346(2):394-401.

Kong, W., L. Xu, K. Stadler, J.B. Ulmer, S. Abrignani, R. Rappuoli, and G.J. Nabel. 2005. Modulation of the immune response to the severe acute respiratory syndrome spike glycoprotein by gene-based and inactivated virus immunization. *Journal of Virology*. 79:13915-13923.

Kurth, B.E., L. Digilio, P. Snow, L.A. Bush, M. Wolkowicz, J. Shetty, A. Mandal, Z. Hao, P.P. Reddi, and C.J. Flickinger. 2008. Immunogenicity of a multi-component recombinant human acrosomal protein vaccine in female *Macaca fascicularis*. *Journal of Reproductive Immunology*. 77(2):126-141.

Kutzler, M., and A. Wood. 2006. Non-surgical methods of contraception and sterilization. *Theriogenology*. 66:514-525.

Kuzmin, A.I., M.J. Finegold, and R.C. Eisensmith. 1997. Macrophage depletion increases the safety, efficacy and persistence of adenovirus-mediated gene transfer *in vivo*. *Gene Therapy*. 4:309-316.

Kvirkvelia, N., J.D. McBride, N. Porakishvili, T. Lund, I.M. Roitt, and P.J. Delves. 2003. The use of Hepatitis B core antigen particles as a presentation scaffold for a peptide-based contraceptive vaccine. *Journal of Reproductive Immunology*. 58:138-147.

Ladd, A. 1993. Progress in the development of anti-LHRH vaccine. *American Journal of Reproductive Immunology* 29:189-194.

Lai, K.Y. 2004. Development of an immunocontraceptive vaccine for biocontrol of rats. . *PhD. Thesis, Universiti Putra Malaysia*.

Lai, K.Y., M.L. Mohd Azmi, and A.R. Sheikh-Omar. 1998. Identification of new strains of rat cytomegalovirus by western blot. *In Proceedings of 10th National Biotechnology Seminar*, Selangor, Malaysia. 264-267.

Lane, V.M., I.K. Liu, K. Casey, E.M. vanLeeuwen, D.R. Flanagan, K. Murata, and C. Munro. 2007. Inoculation of female American black bears (*Ursus americanus*) with partially purified porcine zona pellucidae limits cub production. *Reproduction, Fertility and Development*. 19:617-625.

Lea, I.A., E.E. Widgren, and M.G. O'Rand. 2002. Analysis of recombinant mouse zona pellucida protein 2 (ZP2) constructs for immunocontraception. *Vaccine*. 20:1515-1523.

Lee, M.G., M.A. Abina, H. Haddada, and M. Perricaudet. 1995. The constitutive expression of the immunomodulatory gp19k protein in E1-, E3-adenoviral vectors strongly reduces the host cytotoxic T cell response against the vector *Gene Therapy*. 2:256-262.

Levy, J.K., M. Mansour, P.C. Crawford, B. Pohajdak, and R.G. Brown. 2005. Survey of zona pellucida antigens for immunocontraception of cats. *Theriogenology*. 63(5):1334-1341.

Li, H.P., X.J. He, C. Tang, X.Y. Yao, and D.J. Li. 2008. Fusion of hC3d3 to hCG [beta] enhances responsiveness *in vitro* of human peripheral immunocompetent cells upon the antigen primary challenge. *Journal of Reproductive Immunology*. 78(2):115-124.

Li, S., E. Locke, J. Bruder, D. Clarke, D.L. Doolan, M.J.E. Havenga, A.V.S. Hill, P. Liljestrom, T.P. Monath, and H.Y. Naim. 2007. Viral vectors for malaria vaccine development. *Vaccine*. 25(14):2567-2574.

Litscher, E.S., K. Juntunen, A. Seppo, L. Penttila, R. Niemela, O. Renkonen, and P.M. Wassarman. 1995. Oligosaccharide constructs with defined structures that inhibit binding of mouse sperm to unfertilized eggs *in vitro*. *Biochemistry*. 34:4662-4669.

Liu, I.K.M., M. Bernoco, and M. Feldman. 1989. Contraception in mares heteroimmunized with pig zonae pellucidae. *Journal of Reproduction and Fertility*. 85(1):19-29.

Lloyd, M.L., G.R. Shellam, J.M. Papadimitriou, and M.A. Lawson. 2003. Immunocontraception is induced in BALB/c mice inoculated with murine cytomegalovirus expressing mouse zona pellucida 3. *Biology of Reproduction*. 68:2024-2032.

Lo, S., N. Zeenathul, A. Sheikh Omar, and M. Mohd Azmi. 2011. Current ZP3-based immunocontraceptive vaccine for free ranging wild pest. *Pertanika Journal of Tropical Agricultural Science*. 34:1-16.

Locke, S.L., M.W. Cook, L.A. Harveson, D.S. Davis, R.R. Lopez, N.J. Silvy, and M.A. Fraker. 2007. Effectiveness of Spayvac® for reducing white-tailed deer fertility. *Journal of Wildlife Diseases*. 43:726-730.

Lou, Y., J. Ang, H. Thai, F. McElveen, and K.S. Tung. 1995. A zona pellucida 3 peptide vaccine induces antibodies and reversible infertility without ovarian pathology. *The Journal of Immunology*. 155:2715-2720.

Lou, Y.H., K.K. Park, S. Agersborg, P. Alard, and K.S.K. Tung. 2000. Retargeting T cell-mediated inflammation: a new perspective on autoantibody action. *The Journal of Immunology*. 164:5251-5257.

Ma, C., K. Yao, F. Zhou, and M. Zhu. 2006. Comparative immunization in BALB/c mice with recombinant replication-defective adenovirus vector and DNA plasmid expressing a SARS-CoV nucleocapsid protein gene. *Cellular and Molecular Immunology*. 3:459-465.

Mackenzie, S.M., E.A. McLaughlin, H.D. Perkins, N. French, T. Sutherland, R.J. Jackson, B. Inglis, W.J. Müller, B.H. van Leeuwen, and A.J. Robinson. 2006. Immunocontraceptive effects on female rabbits infected with recombinant myxoma virus expressing rabbit ZP2 or ZP3. *Biology of Reproduction*. 74:511-521.

Mahi-Brown, C.A. 1996. Primate response to immunization with a homologous zona pellucida peptide. *Journal of Reproduction and Fertility. Supplement*. 50:165-174.

Mahi-Brown, C.A., R. Yanagimachi, J.C. Hoffman, and T.T. Huang. 1985. Fertility control in the bitch by active immunization with porcine zonae pellucidae: use of

different adjuvants and patterns of estradiol and progesterone levels in estrous cycles. *Biology of Reproduction*. 32:761-772.

Majhen, D., and A. Ambriović-Ristov. 2006. Adenoviral vectors—How to use them in cancer gene therapy? *Virus Research*. 119:121-133.

Massie, B., F. Couture, L. Lamoureux, D.D. Mosser, C. Guilbault, P. Jolicoeur, F. Belanger, and Y. Langelier. 1998. Inducible overexpression of a toxic protein by an adenovirus vector with a tetracycline-regulatable expression cassette. *Journal of Virology*. 72:2289-2296.

McConnell, M.J., P.C. Hanna, and M.J. Imperiale. 2007. Adenovirus-based prime-boost immunization for rapid vaccination against anthrax. *Molecular Therapy*. 15:203-210.

McLaughlin, E., and R. Aitken. 2011. Is there a role for immunocontraception? *Molecular and Cellular Endocrinology*. 335:78-88.

McLaughlin, E.A., and R.J. Aitken. 2010. Is there a role for immunocontraception? *Molecular and Cellular Endocrinology*. 335:78-88.

McLeskey, S.B., C. Dowds, R. Carballada, R.R. White, and P.M. Saling. 1997. Molecules involved in mammalian sperm-egg interaction. *International Review of Cytology*. 177:57-113.

McNees, A.L., C.T. Garnett, and L.R. Gooding. 2002. The adenovirus E3 RID complex protects some cultured human T and B lymphocytes from Fas-induced apoptosis. *Journal of Virology*. 76:9716-9723.

Millar, J., D. Dissanayake, T.C. Yang, N. Grinshtein, C. Evelegh, Y. Wan, and J. Bramson. 2007. The magnitude of the CD8+ T cell response produced by recombinant virus vectors is a function of both the antigen and the vector. *Cellular Immunology*. 250:55-67.

Millar, S.E., S.M. Chamow, A.W. Baur, C. Oliver, F. Robey, and J. Dean. 1989. Vaccination with a synthetic zona pellucida peptide produces long-term contraception in female mice. *Science*. 246:935-938.

Miller, L.A., J.P. Gionfriddo, K.A. Fagerstone, J.C. Rhyan, and G.J. Killian. 2008. The Single-shot GnRH immunocontraceptive Vaccine (GonaCon^{TM}) in white-tailed deer: comparison of several GnRH preparations. *American Journal of Reproductive Immunology*. 60(3):214-223.

Miller, L.A., B.E. Johns, D.J. Elias, and K.A. Crane. 1997. Comparative efficacy of two immunocontraceptive vaccines. *Vaccine*. 15:1858-1862.

Miller, L.A., and G.J. Killian. 2002. In search of the active PZP epitope in white-tailed deer immunocontraception. *Vaccine*. 20:2735-2742.

Miyake, S., M. Makimura, Y. Kanegae, S. Harada, Y. Sato, K. Takamori, C. Tokuda, and I. Saito. 1996. Efficient generation of recombinant adenoviruses using adenovirus DNA-terminal protein complex and a cosmid bearing the full-length virus genome. *Proceedings of the National Academy of Sciences*. 93:1320-1324.

Mizuguchi, H., and M.A. Kay. 1998. Efficient construction of a recombinant adenovirus vector by an improved *in vitro* ligation method. *Human Gene Therapy*. 9:2577-2583.

Morelli, A.E., A.T. Larregina, R.W. Ganster, A.F. Zahorchak, J.M. Plowey, T. Takayama, A.J. Logar, P.D. Robbins, L.D. Falo, and A.W. Thomson. 2000. Recombinant adenovirus induces maturation of dendritic cells via an NF-kappa B-dependent pathway. *Journal of Virology*. 74:9617-9628.

Morin, J.E., M.D. Lubeck, J.E. Barton, A.J. Conley, A.R. Davis, and P.P. Hung. 1987. Recombinant adenovirus induces antibody response to hepatitis B virus surface antigen in hamsters. *Proceedings of the National Academy of Sciences*. 84:4626-4630.

Myers, L.E., L.J. McQuay, and F.B. Hollinger. 1994. Dilution assay statistics. *Journal of Clinical Microbiology*. 32:732-739.

Naugle, R.E., A.T. Rutberg, H.B. Underwood, J.W. Turner Jr, and I.K. Liu. 2002. Field testing of immunocontraception on white-tailed deer (*Odocoileus virginianus*) on Fire Island National Seashore, New York, USA. *Reproduction Supplement*. 60:143-153.

Naz, R., and A. Aleem. 2007. Effect of immunization with six sperm peptide vaccines on fertility of female mice. *Society of Reproduction and Fertility Supplement*. 63:455-464.

Naz, R.K. 1987. The fertilization antigen (FA-1) causes a reduction of fertility in actively immunized female rabbits. *Journal of Reproductive Immunology*. 11:117-133.

Naz, R.K. 2000b. Fertilization-Related Sperm Antigens and their Immunocontraceptive Potentials. *American Journal of Reproductive Immunology*. 44(1):41-46.

Naz, R.K., and K.K. Bhargava. 1990. Antibodies to sperm surface fertilization antigen (FA-1): Their specificities and site of interaction with sperm in male genital tract. *Molecular Reproduction and Development*. 26:175-183.

Naz, R.K., and S.C. Chauhan. 2002. Human sperm-specific peptide vaccine that causes long-term reversible contraception. *Biology of Reproduction*. 67:674-680.

Naz, R.K., S.K. Gupta, J.C. Gupta, H.K. Vyas, and G.P. Talwar. 2005. Recent advances in contraceptive vaccine development: a mini-review. *Human Reproduction*. 20:3271-3283.

Naz, R.K., A.G. Sacco, and E.C. Yurewicz. 1991. Human spermatozoal FA-1 binds with ZP3 of porcine zona pellucida. *Journal of Reproductive Immunology*. 20:43-58.

Naz, R.K., X. Zhu, and A.L. Kadam. 2000a. Identification of human sperm peptide sequence involved in egg binding for immunocontraception. *Biology of Reproduction*. 62(2):318-324.

Nemerow, G.R. 2000. Cell receptors involved in adenovirus entry. *Virology*. 274:1-4.

Nemerow, G.R., L. Pache, V. Reddy, and P.L. Stewart. 2009. Insights into adenovirus host cell interactions from structural studies. *Virology*. 384:380-388.

Nishimoto, T., T. Mori, I. Yamada, and T. Nishimura. 1980. Autoantibodies to zona pellucida in infertile and aged women. *Fertility and Sterility*. 34:552-556.

Nunes, F.A., E.E. Furth, J.M. Wilson, and S.E. Raper. 1999. Gene transfer into the liver of nonhuman primates with E1-deleted recombinant adenoviral vectors: safety of readministration. *Human Gene Therapy*. 10:2515-2526.

O'Hern, P.A., C.S. Bambra, M. Isahakia, and E. Goldberg. 1995. Reversible contraception in female baboons immunized with a synthetic epitope of sperm-specific lactate dehydrogenase. *Biology of Reproduction*. 52:331-339.

O'Leary, S., M.L. Lloyd, G.R. Shellam, and S.A. Robertson. 2008. Immunization with recombinant murine cytomegalovirus expressing murine zona pellucida 3 causes permanent infertility in BALB/c mice due to follicle depletion and ovulation failure. *Biology of Reproduction*. 79:849-860.

O'Riordan, C.R., A. Lachapelle, C. Delgado, V. Parkes, S.C. Wadsworth, A.E. Smith, and G.E. Francis. 1999. PEGylation of adenovirus with retention of infectivity and protection from neutralizing antibody *in vitro* and *in vivo*. *Human Gene Therapy*. 10:1349-1358.

Papp, Z., L.A. Babiuk, and M.E. Baca-Estrada. 1999. The effect of pre-existing adenovirus-specific immunity on immune responses induced by recombinant adenovirus expressing glycoprotein D of bovine herpesvirus type 1. *Vaccine*. 17:933-943.

Paterson, M., Z.A. Jennings, M. van Duin, and R.J. Aitken. 2000. Immunocontraception with zona pellucida proteins. *Cells Tissues Organs*. 166:228-232.

Paterson, M., Z.A. Jennings, M.R. Wilson, and R.J. Aitken. 2002. The contraceptive potential of ZP3 and ZP3 peptides in a primate model. *Journal of Reproductive Immunology*. 53:99-107.

Paterson, M., P.T. Koothan, K.D. Morris, K.T. O'Byrne, P. Braude, A. Williams, and R.J. Aitken. 1992. Analysis of the contraceptive potential of antibodies against

native and deglycosylated porcine ZP3 *in vivo* and *in vitro*. *Biology of Reproduction*. 46(4):523-534.

Paterson, M., M.R. Wilson, Z.A. Jennings, M. Van Duin, and R.J. Aitken. 1999. Design and evaluation of a ZP3 peptide vaccine in a homologous primate model. *Molecular Human Reproduction*. 5:342-352.

Peeters, M.J.T.F.D.V., G.A. Patijn, A. Lieber, L. Meuse, and M.A. Kay. 1996. Adenovirus-mediated hepatic gene transfer in mice: comparison of intravascular and biliary administration. *Human Gene Therapy*. 7:1693-1699.

Perdock, A.A., W.F. de Boer, and T.A.E. Stout. 2008. Prospects for managing African elephant population growth by immunocontraception: a review. *Pachyderm*. 42:97-107.

Philipson, L., and R.F. Pettersson. 2001. The coxsackie-adenovirus receptor- a new receptor in the immunoglobulin family involved in cell adhesion. *In Adenoviruses: Model and Vectors in Virus-Host Interactions*. Vol. 137. Springer-Verlag, Berlin. 87-111.

Philpott, C.C., M.J. Ringuette, and J. Dean. 1987. Oocyte-specific expression and developmental regulation of ZP3, the sperm receptor of the mouse zona pellucida. *Developmental Biology*. 121:568-575.

Pickles, R.J., D. McCarty, H. Matsui, P.J. Hart, S.H. Randell, and R.C. Boucher. 1998. Limited entry of adenovirus vectors into well-differentiated airway epithelium is responsible for inefficient gene transfer. *Journal of Virology*. 72:6014-6023.

Prasad, S.V., S.M. Skinner, C. Carino, N. Wang, J. Cartwright, and B.S. Dunbar. 2000. Structure and function of the proteins of the mammalian zona pellucida. *Cells Tissues Organs*. 166:148-164.

Prasad, S.V., B. Wilkins, S.M. Skinner, and B.S. Dunbar. 1996. Evaluating zona pellucida structure and function using antibodies to rabbit 55 kDa ZP protein expressed in baculovirus expression system. *Molecular Reproduction and Development*. 43:519-529.

Primakoff, P., W. Lathrop, L. Woolman, A. Cowan, and D. Myles. 1988. Fully effective contraception in male and female guinea pigs immunized with the sperm protein PH-20. *Nature*. 335:543-546.

Primakoff, P., L. Woolman-Gamer, K.S. Tung, and D.G. Myles. 1997. Reversible contraceptive effect of PH-20 immunization in male guinea pigs. *Biology of Reproduction*. 56:1142-1146.

Qin, M., S. Chen, T. Yu, B. Escuadro, S. Sharma, and R.K. Batra. 2003. Coxsackievirus adenovirus receptor expression predicts the efficiency of adenoviral gene transfer

into non-small cell lung cancer xenografts. *Clinical Cancer Research*. 9:4992-4999.

Raghupathy, R., and G.P. Talwar. 1992. Vaccines against fertility. *Current Opinion in Immunology*. 4:597-602.

Randrianarison-Jewtoukoff, V., and M. Perricaudet. 1995. Recombinant adenoviruses as vaccines. *Biologicals*. 23:145-157.

Rankin, T., S. Soyal, and J. Dean. 2000. The mouse zona pellucida: folliculogenesis, fertility and pre-implantation development. *Molecular and Cellular Endocrinology*. 163:21-25.

Rankin, T., P. Talbot, E. Lee, and J. Dean. 1999. Abnormal zonae pellucidae in mice lacking ZP1 result in early embryonic loss. *Development*. 126:3847-3855.

Rankin, T.L., M. O'Brien, E. Lee, K. Wigglesworth, J. Eppig, and J. Dean. 2001. Defective zonae pellucidae in Zp2-null mice disrupt folliculogenesis, fertility and development. *Development*. 128:1119-1126.

Raper, S.E., M. Yudkoff, N. Chirmule, G.P. Gao, F. Nunes, Z.J. Haskal, E.E. Furth, K.J. Propert, M.B. Robinson, and S. Magosin. 2002. A pilot study of *in vivo* liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. *Human Gene Therapy*. 13:163-175.

Raschperger, E., J. Thyberg, S. Pettersson, L. Philipson, J. Fuxe, and R.F. Pettersson. 2006. The coxsackie-and adenovirus receptor (CAR) is an *in vivo* marker for epithelial tight junctions, with a potential role in regulating permeability and tissue homeostasis. *Experimental Cell Research*. 312(9):1566-1580.

Rath, A., D. Batra, R. Kaur, S. Vrati, and S.K. Gupta. 2003. Characterization of immune response in mice to plasmid DNA encoding dog zona pellucida glycoprotein-3. *Vaccine*. 21:1913-1923.

Rea, D., F.H.E. Schagen, R.C. Hoeben, M. Mehtali, M.J.E. Havenga, R.E.M. Toes, C.J.M. Melief, and R. Offringa. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *Journal of Virology*. 73:10245-10253.

Redwood, A.J., N.L. Harvey, M. Lloyd, M.A. Lawson, C.M. Hardy, and G.R. Shellam. 2007. Viral vectored immunocontraception: Screening of multiple fertility antigens using murine cytomegalovirus as a vaccine vector. *Vaccine*. 25(4):698-708.

Redwood, A.J., M. Messerle, N.L. Harvey, C.M. Hardy, U.H. Koszinowski, M.A. Lawson, and G.R. Shellam. 2005. Use of a murine cytomegalovirus K181-derived bacterial artificial chromosome as a vaccine vector for immunocontraception. *Journal of Virology*. 79:2998-3008.

Reed, L.J., and H. Muench. 1938. A simple method of estimating fifty percent endpoints. *The American Journal of Hygiene*. 27:493-497.

Reubel, G.H., S. Beaton, D. Venables, J. Pekin, J. Wright, N. French, and C.M. Hardy. 2005. Experimental inoculation of European red foxes with recombinant vaccinia virus expressing zona pellucida C proteins. *Vaccine*. 23:4417-4426.

Robert-Guroff, M. 2007. Replicating and non-replicating viral vectors for vaccine development. *Current Opinion in Biotechnology*. 18:546-556.

Rosenfeld, M.A., W. Siegfried, K. Yoshimura, K. Yoneyama, M. Fukayama, L.E. Stier, P.K. Paakko, P. Gilardi, L.D. Stratford-Perricaudet, and M. Perricaudet. 1991. Adenovirus-mediated transfer of a recombinant alpha 1-antitrypsin gene to the lung epithelium *in vivo*. *Science*. 252:431-434.

Rosenthal, K.L., K.F.T. Copeland, and W.S. Gallichan. 1996. Recombinant adenoviruses as vectors for mucosal immunity. *Mucosal Vaccines*. 11:147-158.

Rosiere, T.K., and P.M. Wassarman. 1992. Identification of a region of mouse zona pellucida glycoprotein mZP3 that possesses sperm receptor activity. *Developmental Biology*. 154:309-317.

Rowe, W.P., R.J. Huebner, L.K. Gillmore, R.H. Parrott, and T.G. Ward. 1953. Isolation of a cytogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. *Proceedings of the Society for Experimental Biology and Medicine*. 84(3):570-579.

Russell, W.C. 2000. Update on adenovirus and its vectors. *Journal of General Virology*. 81:2573-2604.

Rutberg, A.T., and R.E. Naugle. 2008. Population-level effects of immunocontraception in white-tailed deer (*Odocoileus virginianus*). *Wildlife Research*. 35:494-501.

Sabeur, K., G.N. Cherr, A.I. Yudin, P. Primakoff, M.W. Li, and J.W. Overstreet. 1997. The PH-20 protein in human spermatozoa. *Journal of Andrology*. 18:151-158.

Sacco, A.G., E.C. Yurewicz, M.C. Subramanian, and F.J. DeMayo. 1981. Zona pellucida composition: species cross reactivity and contraceptive potential of antiserum to a purified pig zona antigen (PPZA). *Biology of Reproduction*. 25:997-1008.

Sacco, A.G., E.C. Yurewicz, and M.G. Subramanian. 1986. Carbohydrate influences the immunogenic and antigenic characteristics of the ZP3 macromolecule (Mr 55 000) of the pig zona pellucida. *Journal of Reproduction and Fertility*. 76:575-586.

Sacco, A.G., E.C. Yurewicz, M.G. Subramanian, Y. Lian, and W.R. Dukelow. 1991. Immunological response and ovarian histology of squirrel monkeys (*Saimiri*

sciureus) immunized with porcine zona pellucida ZP3 (Mr= 55,000) macromolecules. *American Journal of Primatology*. 24:15-28.

Sailaja, G., H. HogenEsch, A. North, J. Hays, and S.K. Mittal. 2002. Encapsulation of recombinant adenovirus into alginate microspheres circumvents vector specific immune response. *Gene Therapy*. 9:1722-1729.

Sambrook, J., and D.W. Russell. 2001. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory press, New York.

Sarukhan, A., S. Camugli, B. Gjata, H. Von Boehmer, O. Danos, and K. Jooss. 2001. Successful interference with cellular immune responses to immunogenic proteins encoded by recombinant viral vectors. *Journal of Virology*. 75:269-277.

Saxena, B.B., A. Clavio, M. Singh, P. Rathnam, E.Y. Bukharovich, T.J. Reimers Jr, A. Saxena, and S. Perkins. 2003. Effect of immunization with bovine luteinizing hormone receptor on ovarian function in cats. *American Journal of Veterinary Research*. 64:292-298.

Scarselli, G., A. Succi, L. Berti, A.M. Pala, P. Zati, L. Coronella, and F. Branconi. 1973. Immunological factors in sterility: the appearance of antibodies against spermatozoa in the female. *Acta Europaea Fertilitatis*. 4:37-40.

Schagen, F.H.E., M. Ossevoort, R.E.M. Toes, and R.C. Hoeben. 2004. Immune responses against adenoviral vectors and their transgene products: a review of strategies for evasion. *Critical Reviews in Oncology/Hematology*. 50:51-70.

Schepp-Berglind, J., M. Luo, D. Wang, J.A. Wicker, N.U. Raja, B.D. Hoel, D.H. Holman, A.D.T. Barrett, and J.Y. Dong. 2007. Complex adenovirus-mediated expression of West Nile Virus C, preM, E, and NS1 proteins induces both humoral and cellular immune responses. *Clinical and Vaccine Immunology*. 14:1117-1126.

Scobie, G.A., L.E. Kerr, P. Macduff, and R.J. Aitken. 1999. Cloning, sequencing and site of origin of the rat sperm receptor protein, ZP3. *Zygote*. 7:27-35.

Shaha, C., A. Suri, and G.P. Talwar. 1990. Induction of infertility in female rats after active immunization with 24 kD antigens from rat testes. *International Journal of Andrology*. 13:17-25.

Shanley, J.D., and C.A. Wu. 2003. Mucosal immunization with a replication-deficient adenovirus vector expressing murine cytomegalovirus glycoprotein B induces mucosal and systemic immunity. *Vaccine*. 21:2632-2642.

Sharma, A., X. Li, D.S. Bangari, and S.K. Mittal. 2009. Adenovirus receptors and their implications in gene delivery. *Virus Research*. 143(2):184-194.

Shenk, T. 2001. Adenoviridae: the viruses and their replication *In Fields Virology*. Vol. 2. Lippincott Williams & Wilkins, Philadelphia. 2265-2300.

Shiver, J.W., T.M. Fu, L. Chen, D.R. Casimiro, M.E. Davies, R.K. Evans, Z.Q. Zhang, A.J. Simon, W.L. Trigona, and S.A. Dubey. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature*. 415:331-335.

Shu, Y., S. Winfrey, Z. Yang, L. Xu, S.S. Rao, I. Srivastava, S.W. Barnett, G.J. Nabel, and J.R. Mascola. 2007. Efficient protein boosting after plasmid DNA or recombinant adenovirus immunization with HIV-1 vaccine constructs. *Vaccine*. 25:1398-1408.

Simon, A.M., D.A. Goodenough, E. Li, and D.L. Paul. 1997. Female infertility in mice lacking connexin 37. *Nature*. 385:525-529.

Sinclair, A.R. 1997. Fertility control of mammal pests and the conservation of endangered marsupials. *Reproduction, Fertility, and Development*. 9:1-16.

Sivapurapu, N., A. Hasegawa, G.K. Gahlay, K. Koyama, and S.K. Gupta. 2005. Efficacy of antibodies against a chimeric synthetic peptide encompassing epitopes of bonnet monkey (*Macaca radiata*) zona pellucida-1 and zona pellucida-3 glycoproteins to inhibit *in vitro* human sperm-egg binding. *Molecular Reproduction and Development*. 70:247-254.

Sivapurapu, N., A. Upadhyay, A. Hasegawa, K. Koyama, and S.K. Gupta. 2002. Native zona pellucida reactivity and *in-vitro* effect on human sperm-egg binding with antisera against bonnet monkey ZP1 and ZP3 synthetic peptides. *Journal of Reproductive Immunology*. 56:77-91.

Sivapurapu, N., A. Upadhyay, A. Hasegawa, K. Koyama, and S.K. Gupta. 2003. Efficacy of antibodies against *Escherichia coli* expressed chimeric recombinant protein encompassing multiple epitopes of zona pellucida glycoproteins to inhibit *in vitro* human sperm-egg binding. *Molecular Reproduction and Development*. 65:309-317.

Skinner, S., T. Timmons, E. Schwoebel, and B. Dunbar. 1990. Zona pellucida antibodies. *Immunology and Allergy Clinics of North America*. 10:185-197.

Skinner, S.M., T. Mills, H.J. Kirchick, and B.S. Dunbar. 1984. Immunization with zona pellucida proteins results in abnormal ovarian follicular differentiation and inhibition of gonadotropin-induced steroid secretion. *Endocrinology*. 115:2418-2432.

Skinner, S.M., E.M. Niu, D.S. Bundman, C. Lo, and B.S. Dunbar. 1987. Use of immunoaffinity purified antibodies to zona pellucida to compare alloimmunization of male and female rabbits. *Journal of Reproductive Immunology*. 12:81-92.

Srivastava, N., R. Santhanam, P. Sheela, S. Mukund, S.S. Thakral, B.S. Malik, and S.K. Gupta. 2002. Evaluation of the immunocontraceptive potential of *Escherichia coli*- expressed recombinant dog ZP2 and ZP3 in a homologous animal model. *Reproduction*. 123:847-857.

Strive, T., C.M. Hardy, N. French, J.D. Wright, N. Nagaraja, and G.H. Reubel. 2006. Development of canine herpesvirus based antifertility vaccines for foxes using bacterial artificial chromosomes. *Vaccine*. 24:980-988.

Strive, T., C.M. Hardy, J. Wright, and G.H. Reubel. 2007. A virus vector based on Canine Herpesvirus for vaccine applications in canids. *Veterinary Microbiology*. 119:173-183.

Sullivan, N.J., A. Sanchez, P.E. Rollin, Z. Yang, and G.J. Nabel. 2000. Development of a preventive vaccine for Ebola virus infection in primates. *Nature*. 408:605-609.

Sun, Q.Y. 2003. Cellular and molecular mechanisms leading to cortical reaction and polyspermy block in mammalian eggs. *Microscopy Research and Technique*. 61:342-348.

Suri, A., G.P. Talwar, and C. Shaha. 1993. Oral immunization with sperm antigens. *In Local Immunity in Reproductive Tract Tissues* Oxford University Press, Oxford. 427-440.

Takasaki, S., E. Mori, and T. Mori. 1999. Structures of sugar chains included in mammalian zona pellucida glycoproteins and their potential roles in sperm-egg interaction. *Biochimica et Biophysica Acta*. 1473:206-215.

Takehashi, M., M. Kanatsu-Shinohara, K. Inoue, N. Ogonuki, H. Miki, S. Toyokuni, A. Ogura, and T. Shinohara. 2007. Adenovirus-mediated gene delivery into mouse spermatogonial stem cells. *Proceedings of the National Academy of Sciences*. 104:2596-2061.

Talwar, G.P. 1997. Vaccines for control of fertility and hormone-dependent cancers. *Immunology and Cell Biology*. 75:184-189.

Talwar, G.P., H.K. Vyas, S. Purswani, and J.C. Gupta. 2009. Gonadotropin-releasing hormone/human chorionic gonadotropin [beta] based recombinant antibodies and vaccines. *Journal of Reproductive Immunology*. 83:158-163.

Tan, P.H., S.C. Beutelspacher, S.A. Xue, Y.H. Wang, P. Mitchell, J.C. McAlister, D.F.P. Larkin, M.O. McClure, H.J. Stauss, and M.A. Ritter. 2005. Modulation of human dendritic-cell function following transduction with viral vectors: implications for gene therapy. *Blood*. 105:3824-3832.

Tatsis, N., and H.C.J. Ertl. 2004. Adenoviruses as vaccine vectors. *Molecular Therapy*. 10:616-629.

Tollefson, A.E., T.W. Hermiston, D.L. Lichtenstein, C.F. Colle, R.A. Tripp, T. Dimitrov, K. Toth, C.E. Wells, P.C. Doherty, and W.S.M. Wold. 1998. Forced degradation of Fas inhibits apoptosis in adenovirus-infected cells. *Nature*. 392:726-730.

Tollefson, A.E., A. Scaria, T.W. Hermiston, J.S. Ryerse, L.J. Wold, and W.S. Wold. 1996. The adenovirus death protein (E3-11.6 K) is required at very late stages of infection for efficient cell lysis and release of adenovirus from infected cells. *Journal of Virology*. 70:2296-2306.

Tollner, T.L., J.W. Overstreet, D. Branciforte, and P.D. Primakoff. 2002. Immunization of female cynomolgus macaques with a synthetic epitope of sperm-specific lactate dehydrogenase results in high antibody titers but does not reduce fertility. *Molecular Reproduction and Development*. 62(2):257-264.

Tsaadon, A., E. Eliyahu, N. Shtraizent, and R. Shalgi. 2006. When a sperm meets an egg: block to polyspermy. *Molecular and Cellular Endocrinology*. 252:107-114.

Turner, A., and J.F. Kirkpatrick. 2002. Effects of immunocontraception on population, longevity and body condition in wild mares (*Equus caballus*). *Reproduction Supplement*. 60:187-195.

Turner Jr, J.W., I.K. Liu, D.R. Flanagan, K.S. Bynum, and A.T. Rutberg. 2002. Porcine zona pellucida (PZP) immunocontraception of wild horses (*Equus caballus*) in Nevada: a 10 year study. *Reproduction Supplement*. 60:177-186.

Tuyttens, F.A.M., and D.W. Macdonald. 1998. Fertility control: an option for non-lethal control of wild carnivores? *Animal Welfare*. 7:339-364.

Tyndale-Biscoe, C.H. 1994. Virus-vectored immunocontraception of feral mammals. *Reproduction, Fertility and Development*. 6:281-287.

Van Duin, M., J.E. Polman, I.T. De Breet, K. Van Ginneken, H. Bunschoten, A. Grootenhuis, J. Brindle, and R.J. Aitken. 1994. Recombinant human zona pellucida protein ZP3 produced by chinese hamster ovary cells induces the human sperm acrosome reaction and promotes sperm-egg fusion. *Biology of Reproduction*. 51:607-617.

VandeVoort, C.A., E.D. Schwoebel, and B.S. Dunbar. 1995. Immunization of monkeys with recombinant complimentary deoxyribonucleic acid expressed zona pellucida proteins. *Fertility and Sterility*. 64:838-847.

Vanniasinkam, T., and H.C.J. Ertl. 2005. Adenoviral gene delivery for HIV-1 vaccination. *Current Gene Therapy*. 5:203-212.

Verma, I.M., and M.D. Weitzman. 2005. Gene therapy: twenty-first century medicine. *Annual Review of Biochemistry*. 74:711-738.

Vorburger, S.A., and K.K. Hunt. 2002. Adenoviral gene therapy. *The Oncologist*. 7:46-59.

Walters, R.W., P. Freimuth, T.O. Moninger, I. Ganske, J. Zabner, and M.J. Welsh. 2002. Adenovirus fiber disrupts CAR-mediated intercellular adhesion allowing virus escape. *Cell*. 110:789-799.

Walters, R.W., W. Van't Hof, S.M.P. Yi, M.K. Schroth, J. Zabner, R.G. Crystal, and M.J. Welsh. 2001. Apical localization of the coxsackie-adenovirus receptor by glycosyl-phosphatidylinositol modification is sufficient for adenovirus-mediated gene transfer through the apical surface of human airway epithelia. *Journal of Virology*. 75:7703-7711.

Wang, D., M. Hevey, L.Y. Juompan, C.M. Trubey, N.U. Raja, S.B. Deitz, J. Woraratanadharm, M. Luo, H. Yu, and B.M. Swain. 2006a. Complex adenovirus-vectored vaccine protects guinea pigs from three strains of Marburg virus challenges. *Virology*. 353:324-332.

Wang, D., N.U. Raja, C.M. Trubey, L.Y. Juompan, M. Luo, J. Woraratanadharm, S.B. Deitz, H. Yu, B.M. Swain, and K.M. Moore. 2006b. Development of a cAdVax-based bivalent ebola virus vaccine that induces immune responses against both the Sudan and Zaire species of Ebola virus. *Journal of Virology*. 80:2738-2746.

Wang, Y., and S. Huang. 2000. Adenovirus technology for gene manipulation and functional studies. *Drug Discovery Today*. 5:10-16.

Wang, Y., S. Wang, Y. Bao, C. Ni, N. Guan, J. Zhao, L.G. Salford, B. Widegren, and X. Fan. 2006c. Coxsackievirus and adenovirus receptor expression in non-malignant lung tissues and clinical lung cancers. *Journal of Molecular Histology*. 37:153-160.

Wassarman, P.M. 1999. Mammalian Fertilization: Review Molecular Aspects of Gamete Adhesion, Exocytosis, and Fusion. *Cell*. 96:175-183.

Wassarman, P.M., H.M. Florman, and J.M. Greve. 1985. Receptor-mediated sperm-egg interactions in mammals. *In Biology of Fertilization*. Vol. 2. Academic Press, New York. 341-360.

Wassarman, P.M., L. Jovine, and E.S. Litscher. 2001. A profile of fertilization in mammals. *Nature Cell Biology*. 3:E59-E64.

Wassarman, P.M., L. Jovine, E.S. Litscher, H. Qi, and Z. Williams. 2004. Egg-sperm interactions at fertilization in mammals. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 115:S57-S60.

Wheir, W.H., B. Dunbar, and S. Prasad. 2005. Immuno-sterilization in dogs using zona pellucida (Zp)-based vaccine. *In Humane Wildlife Solutions: The Role of Immunocontraception*. Humane Society Press, USA. 77-94.

Witkin, S.S., and S.S. David. 1986. Abnormal reactivity of spermatozoa with immunoglobulin: case report of an infertile couple. *Fertility and Sterility*. 45:138-140.

Wold, W.S.M. 1993. Adenovirus genes that modulate the sensitivity of virus-infected cells to lysis by TNF. *Journal of Cellular Biochemistry*. 53:329-335.

Wold, W.S.M., and A.E. Tollefson. 2007. *Adenovirus methods and protocols: adenoviruses, ad vectors, quantitation, and animal models*. Humana Press, New Jersey.

Wolff, G., S. Worgall, N. van Rooijen, W.R. Song, B.G. Harvey, and R.G. Crystal. 1997. Enhancement of *in vivo* adenovirus-mediated gene transfer and expression by prior depletion of tissue macrophages in the target organ. *Journal of Virology*. 71:624-629.

Wood, B.J. 1985. Biological control of vertebrates- a review, and an assessment of prospects for Malaysia. *Journal of Plant Protection in the Tropics*. 2(2):67-79.

Wood, B.J., and C.G. Fee. 2003. A critical review of the development of rat control in Malaysian agriculture since the 1960s. *Crop Protection*. 22:445-461.

Wood, D.M., C. Liu, and B.S. Dunbar. 1981. Effect of alloimmunization and heteroimmunization with zonae pellucidae on fertility in rabbits. *Biology of Reproduction*. 25:439-450.

Worgall, S., P.L. Leopold, G. Wolff, B. Ferris, N. van Roijen, and R.G. Crystal. 1997a. Role of alveolar macrophages in rapid elimination of adenovirus vectors administered to the epithelial surface of the respiratory tract. *Human Gene Therapy*. 8:1675-1684.

Worgall, S., G. Wolff, E. Falck-Pedersen, and R.G. Crystal. 1997b. Innate immune mechanisms dominate elimination of adenoviral vectors following *in vivo* administration. *Human Gene Therapy*. 8:37-44.

Wu, C., V.R. Nerurkar, R. Yanagihara, and Y. Lu. 2008. Effective modifications for improved homologous recombination and high-efficiency generation of recombinant adenovirus-based vectors. *Journal of Virological Methods*. 153:120-128.

Wu, J.Q.H., N.D. Barabé, Y.M. Huang, G.A. Rayner, M.E. Christopher, and F.L. Schmaltz. 2007. Pre-and post-exposure protection against Western equine encephalitis virus after single inoculation with adenovirus vector expressing interferon alpha. *Virology*. 369:206-213.

Wu, L., W. Kong, and G.J. Nabel. 2005. Enhanced breadth of CD4 T-cell immunity by DNA prime and adenovirus boost immunization to human immunodeficiency virus Env and Gag immunogens. *Journal of Virology*. 79(13):8024-8031.

Xiang, R.L., F. Zhou, Y. Yang, and J.P. Peng. 2003a. Construction of the Plasmid pCMV4-rZPC_ DNA Vaccine and Analysis of Its Contraceptive Potential1. *Biology of Reproduction*. 68:1518-1524.

Xiang, Z.Q., G.P. Gao, A. Reyes-Sandoval, Y. Li, J.M. Wilson, and H.C.J. Ertl. 2003b. Oral vaccination of mice with adenoviral vectors is not impaired by preexisting immunity to the vaccine carrier. *Journal of Virology*. 77:10780-10789.

Xiang, Z.Q., S. Pasquini, and H.C.J. Ertl. 1999. Induction of genital immunity by DNA priming and intranasal booster immunization with a replication-defective adenoviral recombinant. *The Journal of Immunology*. 162:6716-6723.

Xing, Z., M. Santosuosso, S. McCormick, T.C. Yang, J. Millar, M. Hitt, Y. Wan, J. Bramson, and H.M. Vordermeier. 2005. Recent advances in the development of adenovirus-and poxvirus-vectored tuberculosis vaccines. *Current Gene Therapy*. 5:485-492.

Xuan, X., K. Tuchiya, I. Sato, Y. Nishikawa, Y. Onoderaz, Y. Takashima, A. Yamamoto, A. Katsumata, A. Iwata, and S. Ueda. 1998. Biological and immunogenic properties of rabies virus glycoprotein expressed by canine herpesvirus vector. *Vaccine*. 16:969-976.

Yanagimachi, R. 1994. Mammalian fertilization. . *In The Physiology of Reproduction* Raven Press, New York. 189-317.

Yang, Y., Q. Li, H.C. Ertl, and J.M. Wilson. 1995. Cellular and humoral immune responses to viral antigens create barriers to lung-directed gene therapy with recombinant adenoviruses. *Journal of Virology*. 69:2004-2015.

Yang, Y., and J.M. Wilson. 1996. CD40 ligand-dependent T cell activation: requirement of B7-CD28 signaling through CD40. *Science*. 273:1862-1864.

Yang, Z., L.S. Wyatt, W. Kong, Z. Moodie, B. Moss, and G.J. Nabel. 2003. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *Journal of Virology*. 77(1):799-803.

Ylönen, H. 2001. Rodent plagues, immunocontraception and the mousepox virus. *Trends in Ecology & Evolution*. 16:418-420.

Zaman, V., and F.C. Colley. 1975. Light and electron microscopic observations of the life cycle of Sarcocystis orientalis sp. n. in the rat (*Rattus norvegicus*) and the Malaysian reticulated python (*Python reticulatus*). *Parasitology Research*. 47:169-185.

Zanin, M.P., D.E. Webster, and S.L. Wesselingh. 2007. A DNA prime, orally delivered protein boost vaccination strategy against viral encephalitis. *Journal of Neurovirology*. 13:284-289.

Zhang, X., and W. Godbey. 2006. Viral vectors for gene delivery in tissue engineering. *Advanced Drug Delivery Reviews*. 58:515-534.

Zhang, X., Y.H. Lou, M. Koopman, T. Doggett, K.S. Tung, and R. Curtiss. 1997. Antibody responses and infertility in mice following oral immunization with attenuated *Salmonella typhimurium* expressing recombinant murine ZP3. *Biology of Reproduction*. 56(1):33-41.

Zhang, Y., N. Chirmule, G. Gao, R. Qian, M. Croyle, B. Joshi, J. Tazelaar, and J.M. Wilson. 2001. Acute cytokine response to systemic adenoviral vectors in mice is mediated by dendritic cells and macrophages. *Molecular Therapy*. 3:697-707.

Zhao, H., F. Granberg, and U. Pettersson. 2007. How adenovirus strives to control cellular gene expression. *Virology*. 363:357-375.

Zhu, X., and R.K. Naz. 1999. Comparison of ZP3 protein sequences among vertebrate species: to obtain a consensus sequence for immunocontraception. *Frontiers in Bioscience*. 4:212-225.