

**Investigating porcine and feline zona pellucida as
immunocontraceptive antigens in the female domestic cat**

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This thesis is presented for the degree of Doctor of Philosophy at Murdoch University

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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ABSTRACT

Immunocontraception, or contraception mediated by the immune system, is being widely studied as an alternative, humane form of population control. The induction of an immune response against a specific component of the mammalian oocyte, termed zona pellucida (ZP) has been shown to be an effective immunocontraceptive in several species. Comparatively little work has been done investigating the use of ZP antigens in the domestic cat. This study aimed to investigate porcine ZP(B+C) and feline ZPA, B and C as immunogens in the domestic cat, and further to elucidate their effects on reproduction. Immunisation of female cats with porcine ZP(B+C) failed to elicit a detectable antibody response as assessed by ELISA, immunoblotting and immunohistochemistry. Additionally, there was no effect on the structure of the ovaries nor on breeding performance. Feline ZPA, B and C were cloned into the pkCMVint.polyli mammalian expression vector and used to immunise female cats. ELISA revealed that immunisation with either pkCMVint.fZP(B+C) or pkCMVint.fZPA DNA (Treated) resulted in a low-level circulating antibody response, which was apparently short-lived. Immunoblotting did not reveal any common pattern of recognition of antigenic polypeptides between responding animals. Ovaries from fZP Treated animals, however, showed antibody binding specifically on the ZP of follicles from late primary / early secondary, through to antral stages. Despite the antibody binding, the overall structure of the ovaries remained unaffected in all but two of the fZP Treated cats. Two fZPA immunised cats exhibited ovaries that had no recognisable follicular structures, however, the observed abnormalities could not be conclusively linked to fZPA immunisation. Overall, a significant lymphoproliferative response was shown in fZP Treated cats when compared with Controls. Fertility was not significantly affected in fZP Treated cats, although there was a tendency towards increased incidence of unsuccessful matings or pregnancies. The fZP DNA preparations studied here exhibit potential as an immunocontraceptive, with the ability

to generate a lymphoproliferative response against fZP and elicit antibodies specifically recognizing fZP *in situ*. Further studies should continue to investigate the immunogenicity of, and characterise the immune response against, such fZP DNA preparations.

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ABBREVIATIONS, ACRONYMS and UNITS

pZP	porcine zona pellucida
fZPA,B,C	feline zona pellucida A, B, C
mZP	mouse zona pellucida
rZP	rabbit zona pellucida
ZP	zona pellucida
FIV	feline immunodeficiency virus
LB	Luria Bertiani medium
kan	kanamycin
amp	ampicillin
TBroth	Terrific broth
EtOH	ethanol
MetOH	methanol
UP H ₂ O	Ultra pure water
dH ₂ O	distilled water
dIH ₂ O	deionised water
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
dNTP	dinucleotide triphosphate
AGE	agarose gel electrophoresis
MCT	microcentrifuge tube
ECL	enhanced chemiluminescence
PCR	polymerase chain reaction
AOD	autoimmune ovarian disease
APC	antigen presenting cell
CTL	cytolytic T-lymphocyte
CMI	cell-mediated immunity
DC	dendritic cell
IFN	interferon
Ig	immunoglobulin
IL	interleukin
MHC	major histocompatibility complex
T _h	T helper cell
T _{reg}	T regulatory cell
i.d.	intradermal
i.m.	intramuscular

RBC	red blood cell
WBC	white blood cell
<i>g</i>	gravity/ relative centrifugal force
rpm	revolutions per minute
min	minutes
h	hour
V	volts
RT	room temperature
L	litre
mL	millilitre
g	gram
μg	microgram
μL	microlitre
mol	mole
nmol	nanomole
fmol	femtomole
pmol	picomole

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“The scientist does not study nature because it is useful; he studies it because he delights in it, and he delights in it because it is beautiful. If nature were not beautiful, it would not be worth knowing, and if nature were not worth knowing, life would not be worth living.”

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Jules Henri Poincaré (1854-1912)

French mathematician.