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.

.................................................................

Joyce A. Eade
ABSTRACT

Immuonocontraception, 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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<tr>
<td>pZP</td>
<td>porcine zona pellucida</td>
</tr>
<tr>
<td>fZPA,B,C</td>
<td>feline zona pellucida A, B, C</td>
</tr>
<tr>
<td>mZP</td>
<td>mouse zona pellucida</td>
</tr>
<tr>
<td>rZP</td>
<td>rabbit zona pellucida</td>
</tr>
<tr>
<td>ZP</td>
<td>zona pellucida</td>
</tr>
<tr>
<td>FIV</td>
<td>feline immunodeficiency virus</td>
</tr>
<tr>
<td>LB</td>
<td>Luria Bertiani medium</td>
</tr>
<tr>
<td>kan</td>
<td>kanamycin</td>
</tr>
<tr>
<td>amp</td>
<td>ampicillin</td>
</tr>
<tr>
<td>TBROth</td>
<td>Terrific broth</td>
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<tr>
<td>EtOH</td>
<td>ethanol</td>
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<tr>
<td>MetOH</td>
<td>methanol</td>
</tr>
<tr>
<td>UP H$_2$O</td>
<td>Ultra pure water</td>
</tr>
<tr>
<td>dH$_2$O</td>
<td>distilled water</td>
</tr>
<tr>
<td>dIH$_2$O</td>
<td>deionised water</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>dinucleotide triphosphate</td>
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<tr>
<td>AGE</td>
<td>agarose gel electrophoresis</td>
</tr>
<tr>
<td>MCT</td>
<td>microcentrifuge tube</td>
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<tr>
<td>ECL</td>
<td>enhanced chemiluminescence</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>AOD</td>
<td>autoimmune ovarian disease</td>
</tr>
<tr>
<td>APC</td>
<td>antigen presenting cell</td>
</tr>
<tr>
<td>CTL</td>
<td>cytolytic T-lymphocyte</td>
</tr>
<tr>
<td>CMI</td>
<td>cell-mediated immunity</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
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<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>$T_h$</td>
<td>T helper cell</td>
</tr>
<tr>
<td>$T_{reg}$</td>
<td>T regulatory cell</td>
</tr>
<tr>
<td>i.d.</td>
<td>intradermal</td>
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<tr>
<td>i.m.</td>
<td>intramuscular</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<tr>
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<tr>
<td>RBC</td>
<td>red blood cell</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cell</td>
</tr>
<tr>
<td>g</td>
<td>gravity/ relative centrifugal force</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
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<tr>
<td>min</td>
<td>minutes</td>
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<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>V</td>
<td>volts</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
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<td>gram</td>
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<tr>
<td>μg</td>
<td>microgram</td>
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<td>μL</td>
<td>microlitre</td>
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<tr>
<td>nmol</td>
<td>nanomole</td>
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<tr>
<td>fmol</td>
<td>femptomole</td>
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<tr>
<td>pmol</td>
<td>picomole</td>
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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.”

Jules Henri Poincaré (1854-1912)
French mathematician.