THE INFLUENCE OF MENISCECTOMY AND OVARIECTOMY ON THE OVINE ANTERIOR CRUCIATE LIGAMENT

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

2013
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 degree at any tertiary educational institution.

Alison Daniel

30th May 2013
ABSTRACT

Current research supports a link between osteoarthritis (OA) and the decline in oestrogen levels at menopause. It is not known whether altered hormone levels exert their effect primarily on articular cartilage, or whether associated degeneration of other oestrogen-responsive joint tissues such as cruciate ligaments may significantly contribute. This study investigated the influence of ovariectomy and/or the presence of concurrent osteoarthritis (bilateral meniscectomy model) on the fibrillar structure of collagen within the anterior cruciate ligament (ACL).

Sheep (n=24) were treated experimentally with one or more of the following treatments: ovariectomy (OVX), meniscectomy (MENX) or non-operated control (NOC). ACLs were examined using transmission and scanning electron microscopy (TEM & SEM), gene expression, biochemical analysis and histology.

TEM studies showed OVX and MENX affect collagen fibril size and arrangement, while the combination of prior OVX and concurrent osteoarthritis (MENX) produced a different pattern of derangement to either treatment alone and may indicate a synergistic effect. Observed structural changes complemented molecular findings of altered mRNA expression, and changes in the collagen and sulphated glycosaminoglycan content of ACL tissue.

This study demonstrates that ovariectomy significantly affects ACL collagen fibril structure, and influences the response of the ACL to surgical OA (MENX). These results show the potential for changes in ACL structure post-menopause to influence joint integrity.
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ACKNOWLEDGEMENTS

I would like to thank the following people for their assistance with this study:

Professor Rick Read and Associate Professor Martin Cake (Murdoch University, Veterinary & Biomedical Sciences) for all their support and guidance, and for providing me with the opportunity to undertake this study.

Dr Margaret Smith of the Raymond Purves Bone and Joint Research Laboratories (Royal North Shore Hospital, Sydney) for her invaluable assistance with the RT-PCR protocols, and for kindly providing me with the ovine primer sequences used in this study.

Mr Peter Fallon (Murdoch University, Biological Sciences) for his instruction in the use of the transmission electron microscope and assistance in the cutting of the grids.

Ms Susan Smith of the Raymond Purves Bone and Joint Research Laboratories (Royal North Shore Hospital, Sydney) for providing me with the ligament tissue processing protocols for the histology sections and advice on special stains.

Mr Gerard Spoelstra and Mr Mike Slavin (Murdoch University, Veterinary & Biomedical Sciences) for use of their lab and assistance in cutting and staining of the histology sections.

Mr Richard Krummins and anatomy staff (Murdoch University, Veterinary & Biomedical Sciences) for their support and for kindly allowing me to use their dissection labs and equipment whenever necessary.

Associate Professor Phil Nicholls and the pathology department (Murdoch University, Veterinary & Biomedical Sciences) for use of their digital photographic microscope.

The staff of the Western Australian Institute for Medical Research (Perth) for allowing me to use their Agilent bioanalyser.

Mr Kim Thomas and the Murdoch University farm staff for their care of the animals.

Murdoch University for providing my scholarship.

Most of all thanks to my family for all their love, support and encouragement.
LIST OF ABBREVIATIONS

ACL       anterior cruciate ligament
AM        anteromedial
Basic FGF basic fibroblast growth factor
BMD       bone mineral density
CaCL      caudal cruciate ligament
CCL       cranial cruciate ligament
cDNA      complimentary deoxyribonucleic acid
COX       cyclooxygenase
CS        chondroitin sulfate
CTGF      connective tissue growth factor
DC        dendritic cells
DH        Dunkin Hartley
DMAB      dimethyaminobenzaldehyde
DMMB      dimethylmethylene blue
DNA       deoxyribonucleic acid
DNase     deoxyribonuclease
E1        oestrone
E2        oestadiol
E3        oestriol
ECM       extracellular matrix
EDTA      ethylenediaminetetraacetic acid
ER        endoplasmic reticulum
EtOH      ethanol
FSH       follicle stimulating hormone
G1        amino-terminal globular domain
G2        additional globular domain
G3        carboxy-terminal domain
GAGs      glycosaminoglycans
GAPDH     glyceraldehyde 3-phosphate dehydrogenase
H&E       haematoxylin and eosin
HCl       hydrochloride
HRT       hormone replacement therapy
IDO       indoleamine 2-3 dioxygenase
IFN-      interferon
IgG       serum immunoglobulin G
IL-       interleukin
IL-ß      interleukin beta
IVD       intervertebral disc
KS        keratan sulfate
LAPs      large aggregating proteoglycans
LCL       lateral collateral ligament
LH        luteinising hormone
M         molar
MCL       medial collateral ligament
MENX      meniscectomised
mg        milligram
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>MGA</td>
<td>middle genicular artery</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>MMPs</td>
<td>matrix metalloproteinases</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>NaCl</td>
<td>sodium chloride</td>
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<td>NOC</td>
<td>non-operated control</td>
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<tr>
<td>NTC</td>
<td>non-template control</td>
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<tr>
<td>O+M</td>
<td>ovariectomised and meniscectomised</td>
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<td>osteoarthritis</td>
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<td>hydroxyproline</td>
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<tr>
<td>OVX</td>
<td>ovariectomised</td>
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<td>PCL</td>
<td>posterior cruciate ligament</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PDGF</td>
<td>platelet derived growth factor</td>
</tr>
<tr>
<td>PL</td>
<td>posterolateral</td>
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<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNase</td>
<td>ribonuclease</td>
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<tr>
<td>SABC</td>
<td>state agricultural biotechnology centre</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>S-GAG</td>
<td>sulfated glycosaminoglycan</td>
</tr>
<tr>
<td>SLRPs</td>
<td>small leucine rich proteoglycans</td>
</tr>
<tr>
<td>SYSADOA</td>
<td>symptomatic slow-acting drugs in OA</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TGF-β</td>
<td>transforming growth factor beta</td>
</tr>
<tr>
<td>TIMP</td>
<td>tissue inhibitor of matrix metalloproteinases</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumour necrosis factor alpha</td>
</tr>
<tr>
<td>Tol Blue</td>
<td>toluidine blue</td>
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<tr>
<td>TPA</td>
<td>tibial plateau angle</td>
</tr>
<tr>
<td>ul</td>
<td>microlitre</td>
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<tr>
<td>um</td>
<td>micrometers</td>
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<tr>
<td>β-actin</td>
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PREFACE

This study was conducted to investigate the influence of knee osteoarthritis on other important joint structures, specifically the anterior cruciate ligament, and determine what effect the decline in ovarian hormones associated with menopause has on ligament tissue. It was conducted concurrently with another study investigating cartilage changes in ovariectomised and meniscectomised sheep. The author was involved both studies, including all surgical procedures and tissue harvesting, and is personally responsible for all ligament investigations presented in this thesis. Prof Rick Read and his research team, in co-operation with the Raymond Purves Bone and Joint Research Laboratories (Royal North Shore Hospital, Sydney) generated the cartilage results, some of which have been included to confirm the occurrence of osteoarthritis as induced by the meniscectomy model.

For efficiency, and to minimise the number of animal subjects used, several hypotheses were tested within the design of a single large trial. The reader is initially provided with a general introduction relevant to all subsequent chapters (Chapter 1) followed by details of the methods and materials used in the study (Chapter 2). The thesis then discusses the effect of each treatment individually by chapter, first investigating the effect of osteoarthritis alone (Chapter 3), and then the effect of ovariectomy with or without concurrent osteoarthritis (Chapter 4). Chapters 3-4 are presented each with their own specific introductory information, results and discussion, while the combined results, providing the opportunity to compare and contrast the results from all test groups, are available to view in the appendices. For each method of investigation, all samples from the four groups were processed as a single batch to avoid variation between groups and therefore allow them to be directly compared. Finally, Chapter 5 presents a review of all hypotheses and potential areas for future research are discussed.