

**INVESTIGATION OF VERTICAL MANDIBULAR DISTRACTION
OSTEOGENESIS ON THE MASTICATORY MUSCLES IN A
'UNILATERAL HEMIFACIAL MICROSOMIA LIKE' DEFECT
IN THE SHEEP MODEL**

**RUMAIZI SHAARI
D.V.M (UPM), M.V.M (UPM)**



**Thesis submitted for the degree of
DOCTOR OF PHILOSOPHY (PhD)**

**Oral and Maxillofacial Surgery Unit
Dental School Faculty of Health Sciences
The University of Adelaide
Adelaide, South Australia, 5005**

**October, 2005
Revision September, 2008**

Table of Contents

TABLE OF CONTENTS	I
LIST OF TABLES	VII
LIST OF GRAPHS	X
LIST OF FIGURES	XII
STATEMENT	XIX
ACKNOWLEDGEMENTS	XXI
ABSTRACT	XXIV
PUBLICATIONS ARISING FROM THIS THESIS	XXVII
ABBREVIATIONS AND SYMBOLS	XXVIII
CHAPTER 1: GENERAL INTRODUCTION	1
CHAPTER 2: LITERATURE REVIEW	7
2.0 Distraction Osteogenesis	7
2.1 History of Distraction Osteogenesis	7
2.2 Biology of Distraction Osteogenesis	10
2.2.1 Osteotomy	11
2.2.2 Latency period	12
2.2.3 Distraction period	13
2.2.3.1 Rate and Frequency of distraction	17
2.2.4 Consolidation period	17
2.2.5 Remodelling period	18
2.3 Craniofacial Distraction Osteogenesis	19
2.3.1 Dentofacial traction	19
2.3.2 Craniofacial osteotomies	20
2.3.3 Mandibular distraction	20
2.3.4 Mechanical movement in mandibular distraction	24
2.4 Complication Related to Distraction Osteogenesis	26
2.4.1 Relapse	26

2.4.2 Muscle Responses	28
2.4.2.1 Level of Changes in the distracted muscle	30
2.4.2.2 Weight of the distracted muscle	30
2.4.2.3 Length of distracted muscle	31
2.4.2.4 Cross section and thickness of distracted muscle	32
2.5 Cellular Changes of Skeletal Muscle.	34
2.5.1 Normal histology of muscle	34
2.5.2 Muscle injury and healing	35
2.6 Muscle healing in distraction osteogenesis	38
2.6.1 Destruction phase	38
2.6.2 Necrosis of myofibres	38
2.6.3 Inflammation	39
2.6.4 Repair and Remodelling Phase	39
2.7 Histopathology of distracted muscle	41
2.7.1 Histopathology	42
2.7.2 Histomorphological evaluation	47
2.8 Lack of Distracted Muscle Adaptation	47
2.9 HEMIFACIAL MICROSOMIA	49
2.9.1 Aetiology of Hemifacial Microsomia	50
2.9.1.1 Branchial arches and facial development	50
2.10 Structural Deficits in Hemifacial Microsomia	57
2.10.1 Hard Tissues	57
2.10.2 Soft tissues	57
2.11 Development of the animal model for this study	62
2.11.1 Sheep as Animal Model	63
2.11.2 Biomechanics of the sheep masticatory system	66
2.11.3 Current research on mandibular distraction osteogenesis in sheep	67
2.12 OBJECTIVES AND HYPOTHESIS	70
CHAPTER 3: MATERIALS AND METHODS	72
3.0 Animal Model for “Hemifacial Microsomia Like Defect”	72
3.1 Animal	72
3.2 Surgical Protocol	72
3.3 Mandibular Distraction Osteogenesis Protocol	80
3.3.1 Age of Animal	80
3.3.2 Surgical Placement of Distractor	80
3.3.3 Latency Period	82
3.3.4 Distraction Period	82

3.3.5 Consolidation and Remodelling Periods	85
3.3.6 Removal of the distractor	87
3.4 Gross Assessment of Soft and Hard Tissues Pre and Post Mortem.	88
3.4.1 Ultrasonography (scanning) of masseter muscles	88
3.4.2 Euthanasia procedure	89
3.4.3 Anatomical Descriptions of Masseter and Pterygoid Muscles	89
3.4.5 Post mortem description	90
3.4.6 Length of Masseter Muscles	90
3.4.7 Weight of Masseter and Medial Pterygoid Muscles	91
3.5 Histological Examination	95
3.5.1 Muscle tissue sampling	95
3.5.2 Histomorphometry evaluation	96
3.6 Statistical analysis	101
3.6.1 Metric Data	102
3.6.2 Non-parametric Data	102
3.7 Mandibular Ramus (Hard tissues components)	102
3.7.1 Radiographic procedure	103
3.7.2: Tissue processing	106
3.7.3 Histological analysis	108
3.7.4 Histomorphometry	108
3.7.5 Quantimet analysis	108
CHAPTER 4: RESULTS	111
4.0 Animals	111
4.1 Anaesthesia	113
4.2 Post Mortem	113
4.3 'Unilateral Hemifacial Microsomia-like Defect' model	116
4.4 Mandibular Ramus (Bone) Measurements	116
4.4.1 Radiology	116
4.4.1.1 Distraction gap	116
4.4.1.2 Measurement of vertical and horizontal distance	116
4.4.1.3 Vertical height distance (AB)	118
4.4.1.4 Horizontal distance (BC)	119
4.4.1.5 Oblique distance (AC)	119
4.5 Direct Measurement	120
4.5.1 Vertical Distance (AB)	120
4.6 Bone Histology	120
4.6.1 Pre-distraction (Group1a)	120
4.6.2 Immediately post distraction (Group 1)	120

4.6.3 Consolidation for 2 months (Group 2a)	121
4.6.4 Consolidation 2 months and remodelling 1 month (Group 2)	121
4.6.5 Consolidation 3 months (Group 3)	122
4.6.6 Consolidation 3 months and remodelling 1 month (Group 4)	122
4.6.7 Consolidation 3 months and remodelling 2 month (Group 5)	122
4.6.8 Consolidation 4 months (Group 6)	123
4.7 Bone Structure	123
4.8 Gross Masticatory Muscle Changes	124
4.8.1 Weight of masticatory muscles	124
4.8.1.1 Weight of masseter muscles	124
4.8.1.2 Weight of Medial Pterygoid Muscle	128
4.8.2 Length of Four Planes on Masseter Muscles.	131
4.8.2.1 Length of Anterior Plane of Masseter Muscle (AB)	132
4.8.2.2 Length of middle plane of masseter muscle (CD)	134
4.8.2.3 Length of Posterior Plane of Masseter Muscle (EF)	137
4.8.2.4 Length of Oblique plane of Masseter Muscle (AD)	140
4.8.3 Ultrasonography	144
4.8.3.1 Cross Section (mm ²) of Masseter Muscle	144
4.8.3.1.1 Cross Section (mm ²) of proximal Masseter Muscle	145
4.8.3.1.2 Cross Section (mm ²) of Middle Masseter Muscle	149
4.8.3.1.3 Cross Section (mm ²) of Distal Masseter Muscle	152
4.8.3.2 Thickness (mm) of Masseter Muscle	156
4.8.3.2.1 Thickness (mm) of Proximal Masseter Muscle	157
4.8.3.2.2 Thickness (mm) of Middle Masseter Muscle	159
4.8.3.2.3 Thickness (mm) of Distal Masseter Muscle	162
4.9 Histopathology Quantitative Score	165
4.9.1 Immediately post distraction (Group 1)	167
4.9.2 Consolidation 2 months and remodelling 1 month (Group 2)	167
4.9.3 Consolidation 3 months (Group 3)	168
4.9.4 Consolidation 3 months and remodelling 1 month (Group 4)	168
4.9.5 Consolidation 3 months and remodelling 2 months (Group 5)	169
4.9.6 Consolidation 4 months (Group 6)	169
4.10 Other Findings	184
4.10.1 Sarcocystis Species	184
4.10.2 Prevalence of Sarcocystis species infection in sheep	186
CHAPTER 5: DISCUSSION	187
5.1 Selection of Sheep as Animal Model	187
5.2 Mortality and Morbidity	188

5.3 Mandibular Growth	190
5.4 Issues relating to the model	191
5.4.1 Biomechanics of masticatory forces, effect on position and fixation	191
5.4.2 Device design and stability	192
5.5 Vertical Mandibular Distraction Osteogenesis on ‘Unilateral Hemifacial Microsomia Like’ Defect.	193
5.6 Repeatability Coefficient	194
5.7 Distracted bone	195
5.7.1 Direct measurement	195
5.7.2 Radiological measurement	195
5.7.3 Bone histology	196
5.8 Effect of Mandibular Distraction Osteogenesis on Masticatory Muscles in sheep limb muscle distraction.	198
5.8.1 Weight of Masticatory Muscle	198
5.8.1.1 Weight of Masseter Muscle	198
5.8.1.2 Weight of Medial Pterygoid Muscle	200
5.8.2 Length of Landmarks of Masseter Muscles	201
5.8.2.1 Length of Anterior Plane of Masseter Muscle (AB)	201
5.8.2.2 Length of Middle Plane of Masseter Muscle (CD)	202
5.8.2.3 Length of Posterior Plane of Masseter Muscle (EF)	203
5.8.2.4 Length of Oblique Plane of Masseter Muscle (AD)	203
5.8.3 Ultrasonography	204
5.8.3.1 Cross Section (mm ²) of Masseter Muscle	204
5.8.3.1.1 Cross Section (mm ²) at the Proximal of the Masseter Muscle	204
5.8.3.1.2 Cross Section (mm ²) of Middle Masseter Muscle	205
5.8.3.1.3 Cross Section (mm ²) of Distal Masseter Muscle	206
5.8.3.2 Thickness (mm) of Masseter Muscle	207
5.8.3.2.1 Thickness (mm) of Proximal of Masseter Muscle	207
5.8.3.2.2 Thickness of Middle of Masseter Muscle	207
5.8.3.2.3 Thickness (mm) of Distal of Masseter muscle	207
5.9 Histopathological Evaluation	208
5.9.1 Immediately post distraction	208
5.9.2 Consolidation 2 months and remodelling 1 month	209
5.9.3 Consolidation 3 months	210
5.9.4 Consolidation 3 months and remodelling 1 month	210
5.9.5 Consolidation 3 months and remodelling 2 months	211
5.9.6 Consolidation 4 months	211
5.10 Comparison of the findings of this study to the literature	212

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS	216
6.1 Conclusions	216
General Objectives	216
Specific Objectives	219
6.2 Future Directions	222
CHAPTER 7: REFERENCES	226
CHAPTER 8: APPENDIXES	241

List of Tables

Table 2. 1: The stages of distraction osteogenesis, osteotomy, latency, distraction, consolidation and remodelling.	10
Table 2. 2: External ear classification system by Meurman and Marx.	58
Table 2. 3: Classification of hemifacial microsomia Pruzansky for Type I, II and III Kaban for Type II A and B.	59
Table 3. 1: Animal for pilot study, number of sheep per group (n) and the description of the groups.	85
Table 3. 2: Definition of the score for histopathological changes in masseter and medial pterygoid muscles.	97
Table 4. 1: Number of sheep with problems; general health and technical problems.	111
Table 4. 2: The distraction gaps in the different groups.	117
Table 4. 3: The vertical distance of AB changes at post distraction (Post-D) and sacrifice (Sac). Percentage (%) of the increase (↑); decrease (↓) and remain the same (↔).	118
Table 4. 4: Estimated reduction of the masseter muscle by superficial masseter myectomy.	124
Table 4. 5: Paired t-test for weight of masseter muscle between control and experimental sides for six groups.	125
Table 4. 6: The net change in weight (gm) of masseter muscles between the control and experimental sides in six groups.	127
Table 4. 7: Paired t-test for weight of medial pterygoid muscle between control and experimental sides in six groups.	128
Table 4. 8: The net change in weight (gm) of medial pterygoid muscles between the control and experimental sides in six groups.	129
Table 4. 9: Repeatability coefficient for four planes and groups.	131
Table 4. 10: Paired t-test for length AB (mm) (anterior plane) between control and experimental sides in six groups.	132
Table 4. 11: The net change in length of anterior plane (AB) (mm) of masseter muscle between the control and experiment sides in six groups.	133
Table 4. 12: Paired t-test for length CD (mm) (middle plane) between control and experimental sides in six groups.	135

Table 4. 13: The net change in length of middle plane (CD) (mm) of masseter muscles between the control and experimental sides in six groups.	136
Table 4. 14: Paired t-test for length EF (mm) (posterior plane) between control and experimental sides in six groups.	138
Table 4. 15: The net change in length of posterior plane (EF) (mm) of masseter muscles between the control and experimental sides in six groups.	139
Table 4. 16: Paired t-test for length AD (mm) (oblique plane) between control and experimental sides in six groups.	141
Table 4. 17: The net change in length of oblique plane (AD) (mm) of masseter muscles between the control and experimental sides in six groups.	142
Table 4. 18: Repeatability coefficient for cross section (mm ²) of image scans at three masseter levels for control and experimental sides in six groups.	144
Table 4. 19: Paired t-test for the cross sectional area (mm ²) of scanned images of the proximal masseter muscles between control and experimental sides in six groups.	146
Table 4. 20: The net change in cross section (mm ²) of the scan images from proximal masseter muscle between the control and experimental sides in six groups.	147
Table 4. 21: Paired t-test for the cross sectional area (mm ²) of scanned images of the middle masseter muscle between control and experimental sides in six groups.	149
Table 4. 22: The net change in cross section (mm ²) of the scan images from the middle masseter muscles between the control and experimental sides in six groups.	150
Table 4. 23: Paired t-test for cross sectional area (mm ²) of scanned images at distal masseter muscles between control and experimental sides in six groups.	153
Table 4. 24: The net change in cross section (mm ²) of the scan images from the distal masseter muscles between the control and experimental sides in six groups.	154
Table 4. 25: Repeatability coefficient for thickness (mm) of scan images at three levels of masseter muscle for control and experimental sides in six groups.	156
Table 4. 26: Paired t-test for thickness (mm) of scan images at the proximal level of masseter muscles between control and experimental sides in six groups.	157
Table 4. 27: The net change in thickness (mm) of the scan images from the proximal masseter muscles between the control and experimental sides in six groups.	158
Table 4. 28: Paired t-test for thickness (mm) of scan images for middle masseter muscles between control and experimental sides in six groups.	160
Table 4. 29: The net change in thickness (mm) of the scan images from the middle masseter muscles between the control and experimental sides in six groups.	161

Table 4.30: Paired t-test for thickness (mm) of scan images for distal masseter muscles between control and experimental sides in six groups.	163
Table 4. 31: The net change in thickness (mm) of the scan images from the distal masseter muscles between the control and experimental sides in six groups.	164
Table 4. 32: Double determination for the histological scoring.	165
Table 4. 33: Histopathological scores for Group 1, sacrifice immediately post distraction.	170
Table 4. 34: Histopathological scores for Group 2, consolidation 2 months and remodelling 1 month.	171
Table 4. 35: Histopathological scores for Group 3, consolidation period for 3 months.	172
Table 4. 36: Histopathological scores for Group 4, consolidation period for 3 months and remodelling 1 month.	173
Table 4. 37: Histopathological scores for Group 5, consolidation period 3 months and remodelling 2 months.	174
Table 4. 38: Histopathological scores for Group 6, consolidation period 4 months.	175

LIST OF GRAPHS

Graph 4. 1: The weight gain of sheep (kg) of sheep at different stages throughout experimental period.	112
Graph 4. 2: The weight (gm) of masseter muscles of the experimental and control sides in six groups.	126
Graph 4. 3: The net change in weight (gm) of masseter muscles between the control and experimental sides in six groups.	127
Graph 4. 4: The weight (gm) of medial pterygoid muscles of the experimental and control sides in six groups.	129
Graph 4. 5: The net change in weight (gm) of medial pterygoid muscles between the control and experimental sides in six groups.	130
Graph 4. 6: The length of anterior plane (AB) (mm) of masseter muscles for the experimental and control sides in six groups.	133
Graph 4. 7: The net change in length of anterior plane (AB) (mm) of masseter muscle between the control and experimental sides in six groups.	134
Graph 4. 8: The length of middle plane (CD) (mm) of masseter muscle for the experimental and control sides in six groups.	135
Graph 4. 9: The net change in length of middle plane (CD) (mm) of masseter muscles between the control and experimental sides in six groups.	136
Graph 4. 10: The length of posterior plane (EF) (mm) of masseter muscles for the experimental and control sides in six groups.	138
Graph 4. 11: The net change in length of posterior plane (EF) (mm) of masseter muscles between the control and experimental sides in six groups.	140
Graph 4. 12: The length of oblique plane (AD) (mm) of masseter muscles for the experimental and control sides in six groups.	141
Graph 4. 13: The net change in length of oblique plane (AD) (mm) of masseter muscles between the control and experimental sides in six groups.	142
Graph 4. 14: The cross section (mm ²) of the scan images from the proximal masseter muscles on the experimental and control sides in six groups.	146
Graph 4. 15: The net change in cross section (mm ²) of the scan images from proximal masseter muscle between the control and experimental sides in six groups.	147
Graph 4. 16: The cross section (mm ²) of the scan images of the middle masseter muscles on the experimental and control sides in six groups.	150

Graph 4. 17: The net change in cross section (mm ²) of the scan images from the middle masseter muscles between the control and experimental sides in six groups.	151
Graph 4. 18: The cross section (mm ²) of the scan images from the distal masseter muscles on the experimental and control sides in six groups.	153
Graph 4. 19: The net change in cross section (mm ²) of the scan images from the distal masseter muscles between the control and experimental sides in six groups.	154
Graph 4. 20: The thickness (mm) of the scanned images from the proximal masseter muscles on experimental and control sides in six groups.	158
Graph 4. 21: The net change in thickness (mm) of the scan images from the proximal masseter muscles between the control and experimental sides in six groups.	159
Graph 4. 22: The thickness (mm) of the scan images from the middle masseter muscle on the experimental and control sides in six groups.	161
Graph 4. 23: The net change in thickness (mm) of the scan images from the middle masseter muscles between the control and experimental sides in six groups.	162
Graph 4. 24: The thickness (mm) of the scan images from the distal masseter muscles on the experimental and control sides in six groups.	163
Graph 4. 25: The net change in the thickness (mm) of the scan images from the distal masseter muscles between the control and experimental sides in six groups.	164
Graph 4. 26: Histopathological activity at the proximal, middle and distal levels of the masseter muscles on the experimental side in six groups.	166
Graph 4. 27: Histopathological activity at proximal, middle and distal levels of the medial pterygoid muscles on the experimental side in six groups.	166

List of Figures

- Figure 2. 1: The combination of external frame and plaster casting for limbs lengthening, design by Codivilla. 9
- Figure 2. 2: The external ring to perform lower limbs lengthening, design by Ilizarov. One pair of crossed un-tensioned wires (A), one pair of crossed tensioned wires (B) and two pairs of crossed tensioned wires (C). 9
- Figure 2. 3: Radiograph (left) and schematic drawing (right) of a goat tibia demonstrating five zonal structures of distraction regeneration. FZ fibrous interzone (radiolucent), MZ: mineralization zones (radiodense), RZ: remodelling zone (radiolucent) and RHBS: residual bone host segments. 15
- Figure 2. 4: Radiograph (left) and schematic drawing (right) of a goat tibia demonstrating structure of distraction regenerate during the consolidation periods. Note two radiolucent zones of remodelling (RZ) adjacent to the residual host bone segment (RHBS) and divided by the mineralization zones (MZ). 16
- Figure 2. 5: Photomicrographs of goat tibial distraction regenerate demonstrating the three types of bone maturation during the consolidation period. 16
- Figure 2. 6: Angell's palatal expansion appliance on the maxillary arch. 19
- Figure 2. 7: Mandibular distraction osteogenesis (unidirectional), placement of the device to hold two fragments and arrow showing the vector of distraction. 23
- Figure 2. 8: Mandibular distraction osteogenesis with bi-directional design by Molina. Vertical vector (thin arrows) and horizontal vector (solid arrows). 24
- Figure 2. 9: Mandibular distraction osteogenesis on canine mandible, (an oblique osteotomy was performed). The masseter muscle illustrated in this figure was smaller than normal. 29
- Figure 2. 10: Mandibular distraction osteogenesis on porcine mandible. An angle osteotomy on mandible. The muscles were perpendicular to the distraction vector. The movement of the bone fragments (arrows). 29
- Figure 2. 11: A drawn illustration of the small fascicle of the myofibre and structures. Perimysium (1), endomysium (2), individual myofibre (3), elongated nuclei (4), contractive myofibres (5), small myosatellite cells (6) and capillaries (7). 36
- Figure 2. 12: Normal muscle fibres (M) and muscle spindle (S), presence of several muscle fascicles and perimysium (P) x 350; H&E. 37
- Figure 2. 13: The contractile component of the skeletal muscle (sarcomere). The micro filament known as actine (A) and myosin (M). 37
- Figure 2. 14: Mechanism of skeletal muscle adapted in distraction process. Myofibril proliferation and regeneration to bridge the damage muscle. 40

Figure 2. 15: The traction process caused the contractile unit to increase in number as adaptation to the new length. Circle shows the new sarcomere.	40
Figure 2. 16: Mechanism of the adaptation of skeletal muscle, which involved the connective tissues. Healing by fibrous tissues formation (sclerosis).	41
Figure 2. 17: Degeneration of the muscle fibres and sclerosis, which appeared as light pink within muscle fibres (arrows).	45
Figure 2. 18: Increased fibrous tissues between the perimysium (arrows) and endomysium (yellow arrow heads). (H&E; X80).	46
Figure 2. 19: Regeneration occurred at the musculotendinous junction (arrows), tendon (T) and Muscle (M).	46
Figure 2. 20: Unilateral hemifacial microsomia patient (above) and the same patient with the device in place on the right mandible that was treated with distraction osteogenesis.	49
Figure 2. 21: Sagittal view of the brachial region at 4 weeks. Observe the blood vessels that arise from the heart below and passes through each branchial arch.	52
Figure 2. 22: Development of masticatory muscles. Establishment of masseter, temporalis and medial and lateral pterygoid muscles at 10 weeks.	52
Figure 2. 23: Facial muscles overlying skull and positioned almost at final location at 10 weeks	53
Figure 2. 24: Facial blood supply of internal carotid artery by the stapelial artery, which is related to the common and external carotid arteries at 7 weeks.	53
Figure 2. 25: <i>Hfm</i> transgenic mouse. Small ear pinna (arrow) (A), Embryo with smaller pinna (arrow) (B), Midline shift to the right side (C), and underdevelopment of mandibular arch (D).	56
Figure 2. 26: Right middle masseter muscle (dot arrow), which is almost parallel to the vertical outline of the mandibular ramus (solid arrow).	65
Figure 2. 27: Right medial pterygoid muscle (dot arrow), which is positioned parallel to the vertical orientation of the mandibular ramus (solid arrow).	66
Figure 2. 28: Graphic presentation of the stress distribution on sheep mandible when the masticatory muscle is contracted at the maximum level, focus mainly at the insertion of muscles.	69
Figure 2. 29: The anticipated movement (arrow) of the proximal and distal fragments when the device is activated. The masseter muscle is expected to experience a direct effect from the distraction process as the outline is parallel to the distraction vector.	69

- Figure 3. 1: Preauricular skin incision to approach to the right condyle (arrow head) and submandibular incision to expose the superficial masseter muscle (small arrow) in the first surgery. 74
- Figure 3. 2: Exposure of the right condyle (arrow) (A) and the right condyle after removal (B). 74
- Figure 3. 3: The right parotid duct (arrow), which has been identified before completion of the right superficial masseter myectomy. 77
- Figure 3. 4: The right superficial masseter muscle (arrow) (A), the blunt dissection process to separate the superficial layer from the middle masseter (B), the right superficial masseter (arrow) after removal and exposed fascia of the middle masseter muscle. 78
- Figure 3. 5: The right midline shift resulted from condylectomy and superficial masseter myectomy. 79
- Figure 3. 6: The mandibular osteotomy and the placement of the bone-borne Mathys mandibular distractor (Mathys Australia Pty. Ltd, AUS- Kensington , N.S.W). 79
- Figure 3. 7: The lateral cephalogram before (A) and after distraction (B). The right midline shift was corrected when 10 mm gap showed on radiograph. 83
- Figure 3. 8: The midline shifting toward the right side resulted from condylectomy and superficial myectomy (A), the distraction process corrected the midline (B). 84
- Figure 3. 9: The experimental design for the pilot study, which involved one animal each for eight groups. 86
- Figure 3. 10: Placement of marker screws A, B and C on the mandible. 90
- Figure 3. 11: The placement of transducer to scan 3 levels of the masseter muscle; proximal, middle and distal. 92
- Figure 3. 12: The ultrasound machine, Aloka (Echo camera), model SSD 650 cl. with transducer 7.5 MHz. 93
- Figure 3. 13: The six anatomical points to measure four landmarks on the masseter muscle. 94
- Figure 3. 14: The three levels of sampling at proximal, middle and distal levels of the masseter muscle. 97
- Figure 3. 15: Normal architecture of masseter muscle. Normal muscle fibres (M), perimysium (P), collagen deposition (C), artery (A) and vein (V). H&E (100x). 98
- Figure 3. 16: Muscle dystrophy-presence of various sizes of muscle fibres within one fascicle. Normal muscle fibres (M), atrophic muscle (m), perimysium (P) and fat tissue (F). H&E (100x) 98
- Figure 3. 17: Muscle atrophy (a), generalize uniform reduction in fibre size with increased endomysium (E), perimysium (P) spaces and collagen deposition (C). H&E (100x) 99

Figure 3. 18: Muscle necrosis, presence of inflammatory cells and phagocytic process. Normal muscle fibres (M), necrotic fibres (N), fat tissue (F) and regeneration (R). H&E (100x).	99
Figure 3. 19: Proliferation of fibroblasts (arrow) increase in number of fibroblast nuclei (fb) between connective tissues at perimysium (P) and endomysium (E). Normal muscle fibres (M). H&E (400x).	100
Figure 3. 20: Sclerotic muscle, increased inter fascicle space and muscle fibre with connective tissues (collagen). Normal muscle fibres (M) and perimysium (P). H&E (100x)	100
Figure 3. 21: Regeneration of muscle (R), presence of new muscle cells (arrow) within damaged muscles. Normal muscle fibres (M). H&E (400x)	101
Figure 3. 22: Lateral cephalometry using cephalostat head frame.	104
Figure 3. 23: The sheep positioned in cephalostat head frame (A) and the result of lateral cephalometry X-ray (B). The white tape in (A) is holding the ruler in place. The marker screws, distance and the distracted gap can be visualized on radiograph.	105
Figure 3. 24: The marker screws A, B and C in circles. Distances between screws AB, BC and AC were measured directly on the specimen.	107
Figure 3. 25: Bone blocks sectioned coronally, most anterior on the right.	107
Figure 3. 26: Bone specimen from an experimental side to demonstrate the distracted gap, which is the zone of interest for the histomorphometric study. Van Gieson actual size.	110
Figure 4. 1: The post mortem finding, the intestinal perforation at duodenum, which contaminated abdominal cavity.	114
Figure 4. 2: Presence of yellow necrotic tissues and pus surrounding the distracted gap at post mortem.	115
Figure 4. 3: Presence of whitish fibrous tissues on the right distal surface of masseter muscle. The edge of the superficial masseter stump (point of forceps).	115
Figure 4. 4: The scan images of the proximal masseter muscle. Control side (A) and experimental side (B).	148
Figure 4. 5: The scan images of the middle masseter muscles and example of tracing (white tracing). Control side (A) and experimental side (B).	152
Figure 4. 6: The scan images of the distal masseter muscles. Control side (A) and experimental side (B).	155
Figure 4. 7: Normal muscle (control side). Normal muscle fibres (M), perimysium (P), artery (A), vein (V) and collagen (C). H&E (100x).	176

Figure 4. 8: Normal muscle (control side). Normal muscle fibres (M), muscle spindle (S), perimysium (P), vein (V) and artery (A). H&E (100x).	176
Figure 4. 9: Musculotendinous junction (MTJ) normal muscle (control side). Normal muscle fibres (M), tendon (T) and perimysium (P). H&E (100x).	177
Figure 4. 10: Dystrophy in several muscle fascicles. Normal muscle fibres (M), dystrophic muscles (Ds), perimysium (P) and fat tissues (F). H&E (40x).	177
Figure 4. 11: Muscular dystrophy. Dystrophic muscle (Ds), normal muscle (M), perimysium (P) and fat tissue (F). H&E (100x).	178
Figure 4. 12: Generalised muscle atrophy and increase in perimysial (P) and endomysial (e) spaces. Normal muscle fibre size (M). (Group 1). H&E (100x).	178
Figure 4. 13: Generalised muscle atrophy (m), increased perimysial (P) and endomysial (E) spaces. Normal muscle fibres (M) and fat tissues (F). (Group 1) H&E (40x).	179
Figure 4. 14: Generalised muscle atrophy. Atrophic muscle (m), increased perimysial spaces. Artery (A). (Group 2). H&E (100x).	179
Figure 4. 15: Necrosis of muscle fibres. Necrotic muscle (N), normal muscle (M), regeneration (R) and fat tissue (F). H&E (400x).	180
Figure 4. 16: Necrosis of muscle fibres. Necrotic muscle (N), normal muscle (M), regeneration (R) and fat tissue (F). H&E (400x).	180
Figure 4. 17: Necrosis of muscle fibre (N). Normal muscle (M), musculotendinous junction (MTJ) and tendon (T). H&E (400x).	181
Figure 4. 18: Increased connective tissues (green) in the perimysium (P) and endomysium (E). Red striping (arrows) on the collagen. Normal muscle (M) and fat tissues (F). One step Gomori's trichrome (100x).	181
Figure 4. 19: Presence of the red striping amongst the collagen (green). One step Gomori's trichrome (400x).	182
Figure 4. 20: Increase of the connective tissues at perimysium and proliferation of fibroblasts (Fb), collagen (C), normal muscle (M) and perimysium (P). H&E (400x).	182
Figure 4. 21: Presence of new muscle cells within the damaged muscles. Regeneration (R), necrotic muscle (N), normal muscle (M) and fat tissues (F). H&E (250x).	183
Figure 4. 22: Presence of regeneration (R) within damage muscle (N). Normal muscle (M). H&E (400x).	183
Figure 4. 23: The cross section of the <i>Sarcocystis sp</i> (arrow), within the muscle fibre. Normal muscle (M), nucleus of muscle (n) and endomysium (E). H&E (400x).	185

Figure 4. 24: The longitudinal section of *Sarcocystis sp* (arrow) within the muscle fibre of the sheep. Normal muscle (M), nucleus of muscle (n) and endomysium (E). H&E (400x). 185

Figure 4. 25: Life cycle of the genus *Sarcocystis* 186

This thesis is dedicated to my wife Halilah Wan Muhammad and our four children Alya Fatini, Asna Qistina, Ahnaf Imran and Aniq Hafry. Not forgotten to my father Haji Shaari Haji Abdullah, my late mother Allahyarhamah Hajjah Halimah Ahmad, My in laws Haji Wan Muhammad Wan Jaffar and Hajjah Wan Hasnah Wan Yussof.

STATEMENT

This study is part of a much larger study into distraction osteogenesis in the sheep model, which is being directed by Professor Alastair Goss. Various defined aspects of the study have involved other postgraduate students.

Specifically they are:

All of the animal surgery was performed jointly by myself and Dr Sharifah A.J. Syed Zainal. Her thesis specifically focuses on the bone aspect of this research project. As alteration in the soft tissue directly relates to changes in the bone, I have acknowledged her study in the body of this thesis.

Dr Con Vanco, in association with Dr. Sharifah, performed the initial pilot study. He looked at the bone morphogenic protein by using immunohistochemistry techniques. Dr Neena Durairaj, studied the histology of the condyle and the regenerated condyle from the condylectomy.

Both Dr. Sharifah A.J. Syed Zainal and Dr. Con Vanco have received the Doctorate of Clinical Dentistry (Oral Maxillofacial Surgery and Orthodontics and Dr. Neena Durairaj is in the process of getting her Honours Degree.

Unless otherwise stated as above, and acknowledged in the body of the thesis, this work is original and contains no material which has been accepted for the award of any other degree or diploma in any University or any other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where the due reference has been made in the text.

I give consent for this copy of my thesis, when deposited in the University library, to be available for loan and photocopying.

Signed _____

Rumaizi Shaari

Date: _____

ACKNOWLEDGEMENTS

I was greatly encouraged by my supervisors, Professor Alastair Goss and Dr. Timothy Kuchel who gave me the opportunity to conduct this study. I would like to thank both of them for their endless support, patience and guidance throughout this programme.

Acknowledgements also go to my research colleague, Dr S.A.J Syed Zainal, an Oral and Maxillofacial Surgery Registrar who always shared her experience in surgical skill and knowledge in the clinical application which was helpful to understand and relate to the study. Not forgotten was Dr. J. Varughese for sharing his knowledge and experience in the distraction osteogenesis in sheep.

Thanks are also dedicated to people who give me the knowledge and shared their experience of laboratory animal experiment and management. These include Glenda Summerside (Supervisor of laboratory animal theatre of IMVS), Brian Lewis (supervisor of laboratory animal husbandry) and other staff of Institute of Medical and Veterinary Sciences (IMVS) Veterinary Division. Special thanks also go to the Animal Ethics Committee of the IMVS for allowing me to attend some of the meetings and sessions to give me an idea of how to evaluate the animal research applications.

My thanks also go to Visiting Researcher in the Australian/Japanese Jaw Joint Project, Dr. Mikio Shimitzu for sharing his experience in animal surgery and tissue processing. I also would like to express my appreciation to Sandy Hughes for her help in coordinating the training and organisation to complete my histological experiments. I would also extend my thanks to Dr. John Finnie for assisting me with the histopathology of masticatory muscles, Dr. Richard

Logan, Oral Pathology Unit, the University of Adelaide and Mrs. Marjorie Quin, for her experience in histological technique and for helping me to improve my skill and knowledge in laboratory work.

Special thanks also to Mathys Australia and the Australian New Zealand Association of Oral & Maxillofacial Surgeons, who sponsored this research. Mr. Rocco Fazzalari and Mrs Juliet Forgan of Mathys Australia, who constantly assisted us when needed.

Special thanks also to Dr. Janet Fuss, for arranging me to attend the radiological course, sit for an examination and obtain a licence. Not forgotten thanks to Mr Steve Johnson from Radiation Protection Branch, Environment Protection Authority for giving me guidance and teaching me the way to operate the X-ray machine and processing of materials.

I would also like to thank Miss Emmae Ramsay and Kristyn Willson (statistician) from Department of Public Health, General Practice, and the University of Adelaide for assisting me in statistical analysis. Special thanks also to my friend, Dr. Mohd Fadhli Khamis for helping me with data management and some statistical analyses.

I am also would like to thank the Department of General Services of Malaysia and the Science University of Malaysian for granting me a four year scholarship to pursue my PhD programme at the University of Adelaide. A special thanks also to Alison Hambour and Juliet Hugo for their help with the English, as English is not my first language.

Special additional acknowledgements:

This thesis has received a substantial number of suggestions and recommendations from the examiners for further improvement. Both of my supervisors had reviewed the examination outcomes and contributed greatly in finalising this doctoral thesis. I would like to take this opportunity to thank Dr. Mohd Ayub Sadiq, a lecturer at the School of Dental Sciences, USM for clarifying all issues on statistical analysis throughout this thesis. My special thanks and appreciation goes to Dr. Saidi Jaafar, lecturer at School of Dental Sciences, USM for helping me rewrite this thesis with careful consideration of all the issues indicated by the examiners. Lastly, I would like to extend my gratitude to Madam Noraini @ Norizan binti Abdul Rahman, English teacher at Kubang Kerian Secondary School who has 15 years of experience for going through the language of this thesis.

ABSTRACT

Distraction osteogenesis is a recently developed option for surgical correction of the craniofacial discrepancy but there are few studies that look at the long-term effect of the relapse phenomenon. This study was conducted to look at the bone and muscle response at different consolidation and remodelling periods.

Thirty ten-week-old Merino lambs were subjected to this current study. The surgically created defect was performed on the experimental side (right) by superficial masseter myectomy and a condylectomy. The lambs showed a midline shift to the affected side three months later. Marker screws were placed on both sides of the mandible to examine the adjustment of the vertical and horizontal dimension of the mandible. In this present study, the left side was used as the control.

The affected mandible was surgically corrected using a vertical ramus distraction osteogenesis protocol. The latency period was 7 days where the device was inactive. Distraction was then performed at 1 mm per day until the distracted gap was 10 mm on a radiograph. Initially, the sheep were divided into 8 groups but later the groups were refined into 6 groups for statistical analysis;

Group 1: Immediately post distraction

Group 2: Consolidation 2 months and remodelling 1 month

Group 3: Consolidation 3 months

Group 4: Consolidation 3 months and remodelling 1 month

Group 5: Consolidation 3 months and remodelling 2 months

Group 6: Consolidation 4 months

A 'hemifacial microsomia like' defect was successfully created and it was then corrected using vertical ramus distraction. Cephalometric examination showed that the vertical height was significantly increased after the distraction was completed.

Examination of the bone and muscle was performed to look at the adjustment of bone structure in relation to relapse. The bone investigation was conducted using radiological analysis, histological analysis and direct measurement of the vertical screws at pre-distraction and at sacrifice as well as histomorphometric analysis. The radiological examination was conducted using cephalometric analysis of the distance between marker screws on both experimental and control sides. Bone histology was investigated on the middle and posterior position of the experimental side. The histomorphometric analysis was conducted using Quantiment analysis software.

The muscle responses and adaptation were investigated by measuring: the weight of the masseter and medial pterygoid muscles; length between 6 different points (4 landmarks); cross section and thickness of masseter muscles by ultrasound and by histopathological examination of both masseter and medial pterygoid muscles on the experimental and the control sides.

The distracted bone showed a completion of maturation after a three month consolidation period. Bone formation was shown to continue after longer consolidation periods. A two-month consolidation period was insufficient time for the bone to consolidate and stable, as there was evidenced of relapse during this period.

There was no increased in muscle mass after distraction. The distraction altered the length of anterior and posterior planes and the size of cross sectional area and thickness of origin and middle level of masseter muscles. There were no changes in the length of the middle and oblique planes and the cross sectional area of the insertion of the masseter muscle. The distracted muscle adapted well after a longer consolidation and remodelling period but was sensitive to any surgical procedure such as device removal. The adjustment was observed to continue within the first month after surgical removal of device. The weight, length of planes, cross section and thickness was temporarily reduced on the experimental side but continued to improve and stabled during the second month after removal of the device. The first month after device removal also showed that histopathological activity was increased after both 2 and 3 month consolidation periods. Importantly, muscle histopathology was back to almost normal activity after the second month of device removal, following a 3 month consolidation period.

This study showed that it was possible to create a 'hemifacial microsomia like' defect in very young lambs. The defect was then successfully corrected by a vertical distraction osteogenesis procedure. It was also shown that the sufficient time for the device to be fixed in place (consolidation period) was 3 months or longer as the bone and muscle was stable after that period of time. Results from this investigation have important implications to the management of similar conditions in humans.

PUBLICATIONS ARISING FROM THIS THESIS

ABSTRACTS

1. Shaari, R, Syed Zainal, S, Kuchel ,T and Goss, AN.

The effect of vertical mandibular distraction osteogenesis on masticatory muscles in sheep model. CACDRC (Colgate Australian Clinical Dental Research Centre Research Day): 22 August 2003. Adelaide, South Australia.

2. Shaari, R, Syed Zainal, S, Kuchel ,T and Goss, AN,

The effect of distraction on the masticatory muscles. 20th Biennial Conference ANZAOMS (Australian and New Zealand Association of Oral and Maxillofacial Surgeons). 3-6 September 2003. Adelaide, South Australia.

3. Shaari, R, Syed Zainal, S, Kuchel ,T and Goss, AN.

The effect of vertical mandibular distraction osteogenesis on masticatory muscles in sheep model. IADR (International Australian Dental Research). 29 September 2003. Melbourne, Victoria.

ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variances
°C	Degree Celsius
C.T	Computerised tomography
CCD	Charge-coupled device
gm	Gram
mg	Milligram
kg	Kilogram
µm	Micrometre
mm	Millimetre
cm	centimetre
mm ²	Millimetre Square
ml	Millilitre
H & E	Haematoxylin and eosin
<i>Hfm</i>	Hemifacial microsomia strain mouse
HFM	Hemifacial microsomia
MHz	Mega Hz
pH	Negative logarithm of H ⁺ concentration expressed in molarity
RHBS	Residual bone host segments
PCNA	Proliferating cell nuclear antigen
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
OMENS	Orbital dystopia, Mandibular hypoplasia, Ear (external), Nerve (Cranial) and Soft tissue discrepancy
SAT	Skeletal-auricular and soft tissue

TMJ	Temporomandibular joint
kV	Kilovolt
mAS	milliampere per second
RC	Repeatability Coefficient
°A	Angström unit
3D	Three dimensions
%	Percentage
↑	Increase
↓	Decrease
↔	Remain the same