THE EFFECT OF ALTERED METABOLISM ON UTERINE CONTRACTILITY

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Table of Contents

Acknowledgement **8**

List of Abbreviations **10**

List of Figures **15**

List of Tables **19**

List of Appendices **21**

THESIS ABSTRACT **22**

CHAPTER ONE **24**

1.1. The anatomy of the human uterus 25

1.1.1 Endometrium 26

1.1.2 Myometrium 27

1.1.3 The perimetrium 28

1.1.4 Vascularisation of the uterus 28

1.1.5 Innervation of the uterus 29

1.2 Overview of the physiology of human pregnancy 32

1.2.1 Progression towards labour 32

1.2.2 Parturition and labour process 33

1.2.2.1 First stage 33

1.2.2.2 Second stage 34

1.2.2.3 Third stage 35

1.2.3 Hormones involved in pregnancy. 35

1.2.3.1 Oxytocin 35

1.2.3.2 Oestrogen 36

1.2.3.3 Prostaglandins 36

1.2.3.4 Progesterone 37

1.2.3.5 Relaxin 38

1.2.4 The Scientific basis of labour 39

1.2.4.1 The regulation of the mechanisms of birth based on the endocrine/paracrine mechanisms and progesterone withdrawal. 39

1.2.4.2 The regulation of the mechanisms of birth based on the immune systems. 41

1.2.4.3 Target systems. 42

1.2.4.4 Human aspect of parturition. 44

1.2.4.5 The advantages and disadvantages of presently used animal models. 47

1.2.4.6 Theoretical contrast between human and animal models 49

1.2.4.7 Guinea Pig 50

1.2.4.8 Implication 55

1.2.4.9 The usefulness of the mouse uterus 57

1.3 Uterine myocytes 59

1.4 Mitochondria 60

1.5 Sarcoplasmic reticulum (SR) 60

1.6 Gap junctions 62
1.7 The physiologic role of uterine contractions 64
1.7.1 Background 64
1.7.2 Resting membrane potential (RMP) and action potentials 65
1.7.3 Excitation - contraction coupling (ECC)....68
1.7.4 Ion channels present in the myometrium and contributing to E-C couple 70
1.7.5 Voltage-dependent calcium channels 71
1.7.6 Calcium regulation 72
1.7.7 Calcium influx 72
1.7.8 Calcium exit 73
1.7.9 Potassium channels 73
1.7.9.1 Voltage-dependent potassium channels (Kv) 74
1.7.9.2 Large-conductance calcium dependent channels (BK) 75
1.7.9.3 Small-conductance calcium dependent channels (SK) 75
1.7.9.4 ATP-sensitive channels (K_{ATP}). 75
1.7.10 Sodium channels 77
1.7.11 Chloride channels 77
1.7.12 Ca^{2+}-Activated Cl^- Channels. 78
1.7.13 Plasma membrane Ca-ATPase (PMCA) 78
1.7.14 Na/Ca exchanger (NCX) 79
1.7.15 Sodium pump Na, K ATPase 79
1.8 METABOLISM IN THE SMOOTH MUSCLE OF THE UTERUS 81
1.8.1 Metabolites involved during uterine contraction. 81
1.8.2 Myometrial blood supply during labour 82
1.8.3 Defensive feedback machinery 83
1.9 Prolonged / dysfunctional labour 86
1.9.1 The reasons for delayed labour 87
1.10 Pathophysiology of uterine contractions 88
1.11 Role of glycogen 90
1.12 Glycogen structure 91
1.13 Glycolysis 92
1.14 Citric acid cycle 93
1.15 Oxidative phosphorylation 94
1.16 Glycolytic metabolism and lactate production 95
1.17. Glycogen and uterus 96
1.18 Glycogen and muscle contraction 97
1.18.1 Glycogen and muscle fatigue 97
1.18.2 Uterine metabolic reserves during pregnancy 98
1.18.3 Glycogen storage diseases 99
1.18.3.1 Introduction 99
1.18.3.2 Pathophysiology 100

1.18.3.3 Etiology. 100
1.18.3.4 Epidemiology 102
1.18.3.5 Management 102
1.18.4 Glycogen storage diseases and labour 103
1.18.5 Glucose infusion and labour 105
1.19 The rationale of this study 106
CHAPTER TWO 108
2.1 Preparation of animal tissues 109
2.1.1 Animal housing 109
2.1.2 Ethics and regulations..109
2.1.3 Availability of uterine tissues..110
2.1.4 Dissection..110
2.2 Preparation of human tissue 111
2.2.1 Ethics and regulations..111
2.2.2 Availability of uterine tissues..111
2.2.3 Dissection..112
2.2.4 Organ bath experiments 113
2.2.5 Solutions, drugs and gases used. 115
2.2.5.1 Physiological saline solution (PSS) 115
2.2.5.2 Glucose-free solution and experimental protocol 116
2.2.5.3 High K^+ solution 116
2.2.5.4 High K^+ zero glucose solution 117
2.2.6 Drugs used. 117
2.2.6.1 Oxytocin 117
2.2.6.2 Flavopiridol hydrochloride hydrate 118
2.2.7. Generation of hypoxic conditions..118
2.2.8 Analysis of contractility data 119
2.2.8.1 Sample size 119
2.2.8.2 Power analysis 119
2.2.8.3 Crude method..120
2.2.8.4 Analysis 122
2.2.8.4.1 Amplitude 122
2.2.8.4.2 Frequency 122
2.2.8.4.3 Area under the curve (AUC)..123
2.2.8.4.4 Statistical analysis 123
2.3 Structured literature review of research on glycogen storage 124
2.3.1 Literature search and strategy..125
2.3.2 Inclusion criteria 125
2.3.3 Exclusion criteria..126
2.3.4 Glycogen storage measurement..126
2.3.4.1 Tissue preparation and measurement 127

2.3.4.2 Glycogen standards for colorimetric detection 130
2.3.4.3 Result calculations 130
CHAPTER THREE 131
UTERINE GLYCOGEN STORAGE: A STRUCTURED LITERATURE REVIEW 132
3.1 OVERVIEW 132
3.2 INTRODUCTION..132
3.3 METHOD 134
3.4 LITERATURE SEARCH AND STRATEGY 134
3.4.1 Inclusion criteria 135
3.4.2 Exclusion criteria 135
3.5 RESULTS OF THE SEARCHES...136
3.5.1 Animal 138
3.5.1.1 Discussion 149
3.5.2 Human 151
3.5.2.1 Discussion 154
3.5.2.2 Critical analysis 157
3.5.3 Conclusion 159
3.5.4 The relevance of the review outcomes to my thesis 159
3.5.5 Limitations of these studies 159
CHAPTER FOUR 161
The effect of zero glucose on uterine contractility..162
4.1 The effect of zero glucose on uterine contractility in mice 162
4.1.1 INTRODUCTION..162
4.1.2 MATERIALS AND METHODS 164
4.1.3 EXPERIMENTAL PROTOCOLS..164
4.1.4. DATA ANALYSIS...165
4.1.5 RESULTS 165
4.1.5.1 THE EFFECT OF ZERO GLUCOSE SOLUTION ON UTERINE CONTRACTILITY 165
4.1.5.1.1 Spontaneous uterine response...165
4.1.5.1.2 Investigating the effects of zero glucose. 167
4.1.5.1.3 Investigating the effect of hypoxia. 170
4.1.5.1.4 Investigating the effect of zero glucose under hypoxic conditions. 173
4.1.5.1.5 Results summary...176
4.1.5.1.6 Investigating the effect of zero glucose on the OT-stimulated myometrium. 176
4.1.5.1.7 Results summary...182
4.1.5.1.8 Investigating the effect of zero glucose on the response to high K^+ 182
4.1.5.1.9 Results summary...186
4.1.5.1.10 Discussion 186
4.2 The effect of zero glucose on uterine contractility in humans 191
4.2.1 Introduction 191
4.2.2. Materials and method 192

4.2.2.1 Contractility measurement 192
4.2.2.1.1 Ethical consideration, patients, and tissue preparation 192
4.2.2.1.2. Preparation of solutions used. 193
4.2.2.1.3 Experimental protocols 193
4.2.2.1.4 Spontaneous uterine response 194
4.2.2.1.5. Comparison of spontaneous contractile activity between labouring and non-labouring tissues. .. 194
4.2.2.1.6 Analysis 196
4.2.3 Results 196
4.2.3.1 Investigating the effect of zero glucose solution. 196
4.2.3.2 Zero glucose results summary 201
4.2.3.3 Investigating the effect of hypoxia on uterine contractility. 202
4.2.3.4 Summary of hypoxia results 206
4.2.3.5 Investigating the effect of zero glucose under hypoxic conditions. 207
4.2.3.6 Results summary of the combined effect of zero glucose and hypoxia 211
4.2.3.7 Investigating the oxytocin response under zero glucose conditions. 212
4.2.3.8 Results summary of the effect of zero glucose on the response to oxytocin 216
4.2.3.9 Investigating the effect of zero glucose on t 217
he response to high concentration of potassium salt solution (high K^+) 217
4.2.3.10 Comparison of high K^+ and high K^+ zero glucose response between non- labouring and labouring myometrial tissues 220
4.2.3.11 Results summary of the effect of zero glucose on the response to high K^+ 221
4.2.4 Discussion 221
4.2.5 Limitations in this study 227
CHAPTER FIVE 228
5.1 Introduction 229
5.2 Objective 230
5.3 Methods 231
5.3.1 The effect of the DMSO solution on uterine contractility in pregnant and non-pregnant mice 231
5.3.2 Exploring the effect of flavopiridol hydrochloride hydrate on uterine contractility in non-pregnant and pregnant mice. 231
5.3.3 Exploring the effect of flavopiridol hydrochloride hydrate on uterine contractility in labouring and non-labouring myometria. 232
5.4 Results 233
5.4.1 Exploring the effect of flavopiridol hydrochloride hydrate on uterine contractility in non-pregnant and pregnant mice. 233
5.4.1.1 Flavopiridol influence on uterine contractility in non-pregnant mouse. 235
5.4.1.2 Flavopiridol influence on uterine contractility in pregnant mouse. 237
5.4.1.3 Flavopiridol response difference between non-pregnant and pregnant uteri 238
5.4.2. Exploring the effect of flavopiridol hydrochloride hydrate on uterine contractility in humans. 239
5.4.2.1 Flavopiridol influence on uterine contractility in non-labouring tissue. 241
5.4.2.2 Flavopiridol influence on uterine contractility in labouring tissue. 243
5.4.2.3 Comparison of the effects of flavopiridol on non-labouring and labouring myometria 244

5.4.2.4 Comparison of the effects between Zero glucose and Flavopiridol 245
5.4.3 Discussion 246
CHAPTER SIX 251
6.1 Introduction 252
6.2 Materials and methods 253
6.2.1 Patients and tissue preparation 253
6.2.2 Glycogen measurement 254
6.2.2.1 Glycogen assay 256
6.3 The results 257
6.3.1 Demographic information and obstetric history in non-labouring women 258
6.3.2 Glycogen time course (μg/mg) 258
6.3.3 Demographic information, obstetric history, and glycogen content in Labouring women 259
6.3.4 Demographic information, obstetric history, and glycogen content in Normal labour 260
6.3.5 Demographic information, obstetric history and glycogen content in Normal labour, excluding abnormal samples. 260
6.3.6 Demographic information, obstetric history, and glycogen content in Dysfunctional Labour 261
6.3.7 Demographic information, obstetric history, and glycogen content in Dysfunctional Labour excluding abnormal samples. 261
6.3.8 Discussion 262
CHAPTER SEVEN 264
7.1 The study design. 269
7.2 Limitations of the study 271
7.3 Future work 271
7.4 Conclusion 273
REFERENCES 275
Appendices 314
Appendix 1 315
Appendix 2 317
Appendix 3 318
Appendix 4 320
Appendix 5 323
Appendix 6 325
Appendix 7 328
Appendix 8 330

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List of Abbreviations

$[Ca^{2+}]$ I	Intracellular Ca2+
a.u.	Arbitrary units
ANOVA	Analysis of Variance
Apgar Score	Appearance, Pulse, Grimace, Activity, and Respiration score
ATP	Adenosine triphosphate
BKca	Large conductance calcium activated potassium channels
BMI	Body mass index
Ca^{2+}	Calcium ion
$CaCl_2$	Calcium chloride
CaM	Calmodulin
cAMP	Cyclic adenosine-3,5-monophosphate
CCE	Capacitative calcium entry
CICR	Ca^{2+} -induced Ca^{2+} release
Cl-	Chloride ion
ClCa	Calcium-activated chloride channel
ClVR	Volume-regulated chloride channel
CO2	Carbon dioxide
COX	Cyclo-oxygenase
C-section delivery	Caesarean section delivery
Cx26	Connexin 26
Cx43	Connexin 43
DMSO	Dimethyl sulfoxide
ECC	Excitation - contraction coupling
GDM	Gestational diabetes mellitus
GDPR	General Data Protection Regulation
GJα1	Gap Junction α1
GPCR	G-protein coupled receptor.
H2O2	Hydrogen peroxide

hCG	human chorionic gonadotropin
HEPES	4-(2-hydroxyethyl)-1- piperazine ethane sulfonic acid
IOL	Induction of Labour
IOM	American Institute of Medicine
IP3	Inositol triphosphate
IP3Rs	Inositol triphosphate receptors
IR	Insulin receptor
K^+	Potassium ion
K_{ATP}	ATP-sensitive potassium pump
KCl	Potassium chloride
KH2PO4	Monopotassium phosphate
Kirs	Potassium inwards rectifiers
Kv	Voltage-gated potassium channel
LGA	Large for gestational age
LMP	Last menstrual period
MgSO4	Magnesium sulfate
mV	Mini volt
MMP	Matrix metalloproteinase
MLC	Myosin light chain
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
PGE	Prostaglandin
MβCD	Methyl-ß-cyclodextrin
NA	Nicotinic acid
Na+	Sodium ion
$NaCl^-$	Sodium chloride
NCX	Na2+ /Ca2+ exchanger
NICE	The National Institute for Health and Care Excellence
N_2	Nitrogen
O_2	Oxygen

OD	Optical density
P value	Probability value
PGE2	prostaglandin E2
PIP2	phosphatidylinositol (4,5)-bisphosphate
PLC	Phospholipase C
PMCA	Plasma membrane Ca2+ -ATPase
PSS	Physiological saline solution real-time
ROCCs	Receptor-operated calcium channels
RyRs	Ryanodine receptors
SERCA	Sarco/endoplasmic reticulum Ca2+ ATPase
SFH	Symphysio-pubis fundal height
SOCCs	Store-operated calcium channels
SOCE	store-operated calcium entry
SK	Small-conductance calcium dependent channels
SR	Sarcoplasmic reticulum
VGSC	Voltage-gated Na+ channels
Vm	Resting membrane potential.
WHO	World Health Organization
High K^+	High concentration of potassium
pH	Potential hydrogen
N_2	Nitrogen
mM	Millimole
NPY	Neuropeptide Y
VIP	Vasoactive intestinal polypeptide
CGRP	Calcitonin gene-related peptide
SP	Substance P
PPH	Post-partum Hemorrhage
$[Ca^{2+}]$	Cytosolic Calcium
CICR	Calcium-Induced-Calcium-Release
BK	Calcium activated potassium channels.
STOCs	Spontaneous transient outward currents

SOCE	Store operated calcium entry.
cGMP	Cycling guanosine-3,5-monophosphate.
ADP	Adenosine diphosphate
VGSCs	Voltage-gated sodium channels
NALCN	Na^{+} leak channels
ClVr	Regulated chloride channels.
CaCC	Calcium activated chloride channels.
OTR	Oxytocin receptor
OT	Oxytocin
PLCß	Phospholipase-C beta
ml	Millimeter
Pcr	Phosphocreatine
G_1P	Glucose-1-phosphate
G_6P	Glucose-6-phosphate
GSDs	Glycogen storage diseases
IEM	Inherited inborn errors of metabolism.
GS	Glycogen synthase
GDE	Glycogen debranching enzyme
ERT	Enzyme Replacement Therapy
MPD	Myophosphorylase Deficiency
CTG	Cardiotocography
FFA	Free fatty acid
CRM	Calory restriction mimetics
$C0_2$	Carbon dioxide
mN	Millinewton
Gr	Gram
µM	Micromole
AUC	Area Under the Curve
H_2O_2	Hydrogen peroxide
HRP	Horseradish Peroxide
UDP	Uridine diphosphate
UTP	Uridine triphosphate

PPi	Pyrophosphate
ED	Oestradiol
mRNA	Messenger ribonucleic acid
μgr	Microgram
mg	Milligram
HIV	Human immune virus
AIDS	Acquired immune deficiency syndrome.
GP	Glycogen phosphorylase
CDK	Cycling dependent kinase.
AMP	Adenosine monophosphate
PROM	Pre-labour rupture of membranes
SROM	Spontaneous rupture of membranes

List of Figures

Chapter 1

Figure 1.1. Uterus anatomy: sagittal view of the female reproductive system………………….26

Figure 1.2. Uterus anatomy: the structure of the non-gravid human uterus……………………27

Figure1.3. Vascularisation of the uterus…………………………………………………………………… 29

Figure 1.4. The innervation of the uterus………………………………………………………………. ….31

Figure 1.5. Maternal serum progesterone levels through pregnancy ……………………………..50

Figure 1.6. Anatomical structure of mouse uterus (Basic)……………………………………………. 58

Figure 1.7. The structure of gap junctions…………………………………………………………….…63

Figure 1.8. Cardiotocography……………………………………………………………………………….…65

Figure 1.9. Schematic representation displaying membrane potential, calcium movement. and muscle contraction………………………………………………………………………………………..67

Figure 1.10. Myometrial ion movement…………………………………………………………………….68

Figure 1.11. A schematic representation explaining the excitation- contraction coupling…70

Figure 1.12. Uterine metabolism during labour……………………………………………………..….83

Figure 1.13. Blood flow and contractions in the uterus during labour…………………………..84

Figure.1.14. Glycogenolysis………………………………………………………………………………….…90

Figure 1.15. Glycogen molecule…………………………………………………………………………..…..91

Figure 1.16. Glycogen chemical structure………………………………………………………………. 91

Chapter 2

Figure 2.1. Tissue preparation in mice……………………………………………………………….111

Figure 2.2 Myometrial tissue’s dissection and organ bath mounting…………………………..113

Figure 2.3 A. Organ bath…………………………………………………………………………………… .113

Figure 2.3 B. Force transducer……………………………………………………………………………114

Figure 2.4 Uterine contractions…………………………………………………………………………..114

Figure 2.5 Hypoxic period generated by Nitrogen (N_2) on uterine contractility in mice (non-pregnant and pregnant) ……………………………………………………………………………119

Figure 2.6 The parameters measured during the myometrial contractile……………………..124

Figure 2.7 Glycogen measurement, tissue preparation procedure. Freeze-drying and homogenising processes, boiling and centrifugation………………………………………….128

Figure 2.8 Glycogen measurement procedure…………………………………………………………...129
Figure 2.9 Glycogen assay kit, colorimetric standard curve (Sigma Aldrich) ……………..…130

Chapter 3

Figure 3.1 The process of glycogenesis…………………………………………………………….……133
Figure 3.2 The selection procedure of articles included in the review…………………………..137

Chapter 4

Figure 4.1 Spontaneous contractions in pregnant (A) and non-pregnant mice (B)……………166
Figure 4.2 Effect of glucose depletion on spontaneous uterine contractions from pregnant and non-pregnant mice……………………………………………………………………………168
Figure 4.3 Effect of glucose depletion on spontaneous contractions of myometrial strips from pregnant and non-pregnant mice…………………………………………………………………169
Figure 4.5 Effect of hypoxia on spontaneous contractions of myometrial strips from pregnant and non-pregnant mice…………………………………………………………………………….171
Figure 4.6 The effect of hypoxia on pregnant and non-pregnant mice…………………………..172
Figure 4.7 The combined effect of zero glucose and hypoxia on pregnant and non-pregnant mice.……………………………………………………………………………………………174
Figure 4.8 The comparison of the combined effect of zero glucose and hypoxia on pregnant and non-pregnant mice…………………………………………………………………………..175
Figure 4.9 The effect of oxytocin on the myometrium in pregnant mice(A) and non-pregnant mice(B)……………………………………………………………………………………… .177
Figure 4.10 The effect of zero glucose on oxytocin-stimulated contractions in pregnant and non-pregnant mice…………………………………………………………………………………….179
Figure 4.11 The comparison of the effect of zero glucose in the presence of OT on pregnant and non-pregnant mice……………………………………………………………………180
Figure 4.12 Comparison of the effect of zero glucose and zero glucose in the presence of oxytocin in pregnant and non-pregnant myometrial tissue………………181
Figure 4.13 High concentration of potassium (K^+) in pregnant and non-pregnant mice (A) and high K^+ in zero glucose (B)……………………………………………………………184
Figure 4.14 The comparison of the effect of zero glucose on the response to high K^+…….185
Figure 4.15 Spontaneous contractions in non-labouring(A) and labouring(B) uterine tissues……………………………………………………………………………………………………194

Figure 4.16 Inhibitory effect of zero glucose on uterine contractility in
non-labouring and labouring myometria……………………………………………………………………198
Figure 4.17 Effect of zero glucose on uterine contractility in non-labouring myometria………199
Figure 4.18 Effect of zero glucose on uterine contractility in labouring myometrium………..200
Figure 4.19 Inhibitory effect of hypoxia on uterine contractility in non-labouring
and labouring myometrial strips (representative)…………………………………………………..203
Figure 4.20 Effect of hypoxia on uterine contractility in non-labouring myometria…………….204
Figure 4.21 Effect of hypoxia on uterine contractility in labouring myometria…………………..205
Figure 4.22 The effect of zero glucose on the response to hypoxia on uterine contractility
in non-labouring and labouring myometrial tissues…………………………………………………208
Figure 4.23 The combined effect of zero glucose and hypoxia on uterine contractility
in non- labouring myometria…………………………………………………………………………………209
Figure 4.24 Combined effect of zero glucose and Hypoxia on uterine contractility
in labouring myometrium………………………………………………………………………………………210
Figure 4.25 The increasing effect of oxytocin on uterine contractility
in non-labouring and labouring myometrial tissues………………………………………………….213
Figure 4.26 Effect of zero glucose on the response to oxytocin in non-labouring
myometria………214
Figure 4.27 The effect of zero glucose on the response to oxytocin in labouring
myometria………215
Figure 4.28 The effect of zero glucose on the response to high K^+ in non-labouring
myometria………218
Figure 4.29 Traces of high concentration of potassium (high K^+)
in non- labouring tissue from glucose and zero glucose (A&B)…………………………………218
Figure 4.30 The effect of zero glucose on the response to high K^+ in labouring
myometrium………………………………………………………………………………………………………..219
Figure 4.31 Traces of high concentration of potassium (high K^+) in labouring groups
from glucose and zero glucose (A&B) ……………………………………………………………………..219
Figure 4.32 Graph comparing the effect of zero glucose on the response
to high K^+ from non-labouring and labouring myometrial tissues……………………………………220

Chapter 5

Figure 5.1 The effect of DMSO and flavopiridol on uterine contractility in non-pregnant tissue……..234
Figure 5.2 The effects of flavopiridol on contractile parameters on uterine contractility in non-pregnant tissue…………………………………………………………………………….…235
Figure 5.3 The effect of DMSO on myometrial contractility in pregnant mice………………………236
Figure 5.4 The effects of flavopiridol on contractile parameters on uterine contractility in pregnant mice….……………………………………………………………………………………..237
Figure 5.5 Evaluation of flavopiridol impact between non-pregnant and pregnant uteri……..238
Figure 5.6 Flavopiridol effect on uterine contractility in a non-labouring uterus…………………. 240
Figure 5.7 The effect of flavopiridol on uterine contractility in non-labouring myometrium……………………………………………………………………………………………………….……241
Figure 5.8 The effect of flavopiridol on uterine contractility in labouring myometrium……….242
Figure 5.9 Effect of flavopiridol on uterine contractility in labouring myometrium……………..243
Figure 5.10 The comparison of the effect of flavopiridol between non-labouring and labouring myometria………………………………………………………………………………………………….…244

List of Tables

Chapter 1

Table 1.1 Reproductive features of animal models compared to human models…………..56

Table 1.2 Glycogen storage disease types and causal mechanism………………………………102

Chapter 2

Table 2.1 Physiological saline solution: components and composition……………………….115

Table 2.2 Zero glucose solution: components and composition…………………………………116

Table 2.3 High potassium solution: components and composition……………………………..117

Table 2.4. Zero glucose high potassium solution: components and composition…………..117

Chapter 3

Table 3.1 Key attributes of the animal studies selected, including the model, method, objectives, results, and conclusions……………………………………………………………………140

Table 3.2 Key attributes of the selected studies including the model, the method, the objectives, the results, and the conclusion in human……………………………………………151

Chapter 4

Table 4.1: The effect of zero glucose, hypoxia and zero glucose+ hypoxia in pregnant group………176

Table 4.2: The effect of zero glucose, hypoxia and zero glucose + hypoxia in the non-pregnant group…………………………………………………………………………………………176

Table 4.3 The effect of zero Glucose alone on uterine contractility and OT stimulated myometrium in pregnant mice………………………………………………………………………..182

Table 4.4 The effect of zero glucose alone on uterine contractility and OT stimulated myometrium in non-pregnant mice……………………………………………………………………182

Table 4.5 Effect of zero glucose on peak and plateau amplitude of the high K^+ response in pregnant myometrium………………………………………………………………………..186

Table 4.6 Effect of zero glucose on peak and plateau amplitude on the response to high K^+ in non- pregnant myometrium…………………………………………………..186

Table 4.7 Summary of the effect of zero glucose on the non-labouring group……………201

Table 4.8 The summary of the effect of zero glucose in the labouring group………………201

Table 4.9 Summary of the effect of hypoxia on the non-labouring group……………………206

Table 4.10 Summary of the effect of hypoxia on the labouring group…………………………206

Table 4.11. Summary of the combined effect of zero glucose and hypoxia in the non-labouring group……………………………………………………………………………………………………211

Table 4.12. Summary of the combined effect of zero glucose and hypoxia in the Labouring group……………………………………………………………………………………………………211

Table 4.13 The summary of the effect of zero glucose on the response to oxytocin in the non- labouring group……………………………………………………………………………………216

Table 4.14 The summary of the effect of zero glucose the response to oxytocin in the labouring group……………………………………………………………………………………………………216

Table 4.15 The effect of zero glucose on peak and plateau amplitude on the high K^+ response in non-labouring myometria…………………………………………………………….221

Table 4.16 The effect of zero glucose on the peak and plateau amplitude of the high K^+ response in labouring myometrial………………………………………………………………………221

Chapter 5

Table 5.1 Comparison of the effect of Zero glucose and Flavopiridol in non-pregnant mice……… 245

Table 5.2 Comparison of the effect of Zero glucose and Flavopiridol in pregnant Mice………..245

Table 5.3 Comparison of the effect of Zero glucose and Flavopiridol in Non-labouring……………………………………………………………………………………………….245

Table 5.4 Comparison of the effect of Zero glucose and Flavopiridol in labouring……….245

Chapter 6

Table 6.1 Glycogen standards preparation…………………………………………………………………256

Table 6.2 Sample preparation: dilution…………………………………………………………………………256

Table 6.3 Master reaction mix before plate reading………………………………………………… 257

Table 6.4. Demographic information of samples from elective C/S………………………………258

Table 6.5 Time course exhibiting the degradation of glycogen content in the myometrium in elective C/section samples over 24 hours……………………………………258

Table 6.6 Glycogen content in labouring myometrium…………………………………………………259

Table 6.7 Glycogen content in normal labouring myometrium……………………………………260
Table 6.8 Glycogen content in normal labouring myometrium excluding abnormal
samples……………………………………………………………………………………………………260
Table 6.9 Glycogen content in dysfunctional labouring myometrium…………………………261
Table 6.10 Glycogen content in dysfunctional labouring myometrium excluding
abnormal samples………………………………………………………………………………………261

List of Appendices

Appendix 1
Publications from this thesis……………………………………………………………………………316

Appendix 2
Animal handling (PPL) certificate……………………………………………………………………318

Appendix 3
Ethical approval for research at Liverpool Women Hospital Research
Ethics Committee………………………………………………………………………………………319

Appendix 4
Patient information sheet and consent form for the collection of human uterine
biopsy……………………………………………………………………………………………………321

Appendix 5
Patient information sheet and consent form for the collection of human uterine
Biopsy……………………………………………………………………………………………………324

Appendix 6
Patient information leaflet consent for storage and use of tissue for research
from women……………………………………………………………………………………………326

Appendix 7
Good clinical practice (GCP). A practical guide to ethical and scientific quality
standards in clinical research………………………………………………………………………329

Appendix 8
Glycogen assay curves…………………………………………………………………………………331

THESIS ABSTRACT

The effect of altered metabolism on uterine contractility

Tshikaya Kaleta

Among the challenges encountered in maternal and children health, uterine contractility issues remain one of the major obstacles. Uterine contractility problems may result in preterm birth, dysfunctional labour (one the reasons for cesarean sections) or post-partum hemorrhage, one of the leading causes of maternal death. The drugs that have been used to control these concerns have limited effect.

During labour, uterine contractions cause transitory ischemia, as a result, glucose provision to the myometrium is hampered. A direct causal link between glucose deficiency and impaired myometrial muscle function has not been established. How changes in glucose concentration affect uterine contraction is not known. To improve delivery outcomes and to decrease the frequency of emergency caesarean sections and post-partum haemorrhage, uterine contraction mechanisms and its impairments need to be made clear. I investigate this in vitro and compare the effect in mouse and human.

This study aims (1) Run a structured review to provide evidence from published works on myometrial glycogen storage during pregnancy and after delivery.(2) to clarify the effect of altered metabolism on uterine contractility by investigating the mechanism of the action of zero glucose (3) to determine the changes that it produces in myometrial glycogen storage using a mouse and human model in vitro (4) to investigate the effect of changes in glucose on labour progression.

A structured review on uterine glycogen storage was performed and found that under hormonal influence glycogen content increases during pregnancy for foetal survival and preparation of labour and decreases immediately after delivery. Therefore, it is suggested that glycogen storage is of major importance in the contractile activity of the uterus during labour.

Isometric contractility studies were performed exploring the effects of glucose depletion under hypoxic environment, oxytocin, depolarised conditions and Flavopiridol.

This thesis produced some evidence that zero glucose significantly inhibits spontaneous contractions, irrespective of how they are produced(t-test.) The decline in contractility was most pronounced with the combined action of zero glucose and hypoxia, with contractions frequently stopping altogether. In humans, there is evidence that the labouring myometrium is better able to withstand the effects of zero glucose than the non-labouring. This may be because of high reserves of glycogen in labouring myometrium.

The last step of this thesis explored the role of glycogen during labour and found that the content of glycogen in women labouring normally and women with dysfunctional labour was quite low, probably due to labour. The comparison of uterine glycogen content between women with dysfunctional labour and normal labour was not possible because of small sample size in these two groups, the duration of labour and the sample freezing time for glycogen measurement was not the same however, there were considerable disparities found in the level of glycogen in women at term, with some having high levels and others having very low. The findings indicate that women with low level of glycogen in the myometrium at term are likely to develop uterine dysfunction during labour. These results suggest that glucose deficiency may contribute to the pathway underlying contractility related disorders in vivo.

CHAPTER ONE

General introduction

CHAPTER 1.

General introduction

The uterus is an essential organ of the reproductive system that is responsible for successful gestation and childbirth. The uterus habitually receives the fertilised ovum and creates a safe territory for implantation in the endometrium. It then experiences phases of hyperplasia and hypertrophy to accommodate the growing foetus and its increasing metabolic needs. Throughout pregnancy, the uterus supplies nutritive elements, blood, and considerable mechanical support. As the pregnancy nears term, the uterus prepares for the onset of labour (March et al., 2012).

1.1. The anatomy of the human uterus

The uterus or womb is the main secondary sexual organ of both the human and animal reproductive system. The human reproductive organ is a hollow structure with thick walls in the shape of a pear located in the pelvic cavity, sandwiched between the rectum in the back and the bladder in front (Gartner and Hiatt, 2009). The uterus's normal position is anteverted (the uterine body leans forward) (Figure 1); however, in some women, it is retroverted and it may even change position after gestation (Ameer et al., 2021). The uterus in a non-pregnant adult woman is 7 to 9 cm long, 4.5 to 6 cm wide and around 1.5 cm thick. The non-gravid uterus weighs approximately 30 to 40 grams and gradually increases in weight to reach approximately 1 kg or more in a term pregnant woman (Umar et al., 2017).In the pelvic cavity, the womb is supported by numerous ligaments, including uterosacral, utero-ovarian, expansive, round and cardinal ligaments (Ameer et al., 2020). The human uterus consists of two main areas: the cervix and the corpus or body (Gartner & Hiatt, 2009). The corpus is the larger part of the superior part of the uterus. It is made up of three main parts: the corpus, the bottom, and the isthmus. The fundus of the uterus is the main part that connects to the fallopian tubes and the ovaries. The body, also known as the corpus, is the main part of the uterus that connects to the cervix via the uterine isthmus. The cervix is the lower, barrel-shaped portion that connects the uterus to the vaginal cavity. The cervix comprises connective tissues and glands that produce fluids (Sokol 2011). During ovulation, the cervix thins to allow sperm to easily enter the uterine cavity.

Throughout childbirth, the cervix gradually expands to let the foetus cross the birth canal. Furthermore, the uterus is composed of three major layers. This is the endometrium, which is the innermost layer; the thickest and firm muscle layer, called the myometrium; and the outermost layer, known as the serosa or uterine peritoneum/perimetrium (Aguilar and Mitchell, 2010).

1.1.1 Endometrium

The endometrium is the uterus's first layer. It is linked to numerous glands, blood vessels and lymphatic spaces. This is the uterus's deepest (innermost) layer, and it is made up of epithelial tissue. It also consists of a mucous membrane that lines the entire uterine cavity to the isthmus where it continues with the lining of the cervix. This mucus membrane is responsible for secreting mucus, which in turn supports the reproductive hormones in modifying the endometrial thickness with each uterine cycle.

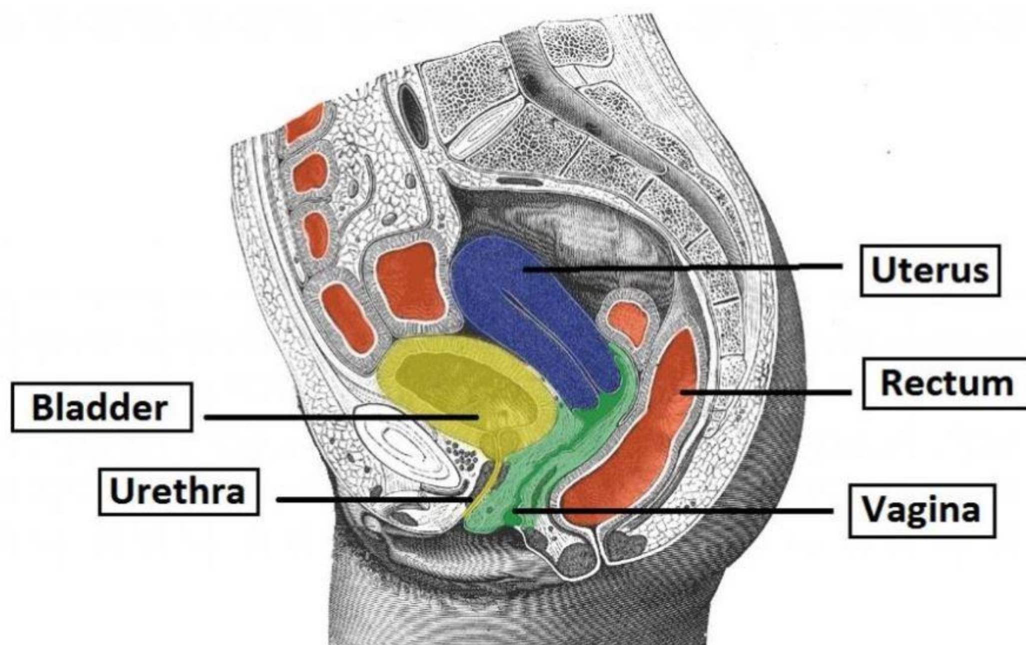


Figure 1.1 Uterus anatomy: sagittal view of the female reproductive system. Image depicting the position of the uterus compared to the bladder and the rectum and showing the anteverted position of the human uterus (Image from Teachme Anatomy Library).

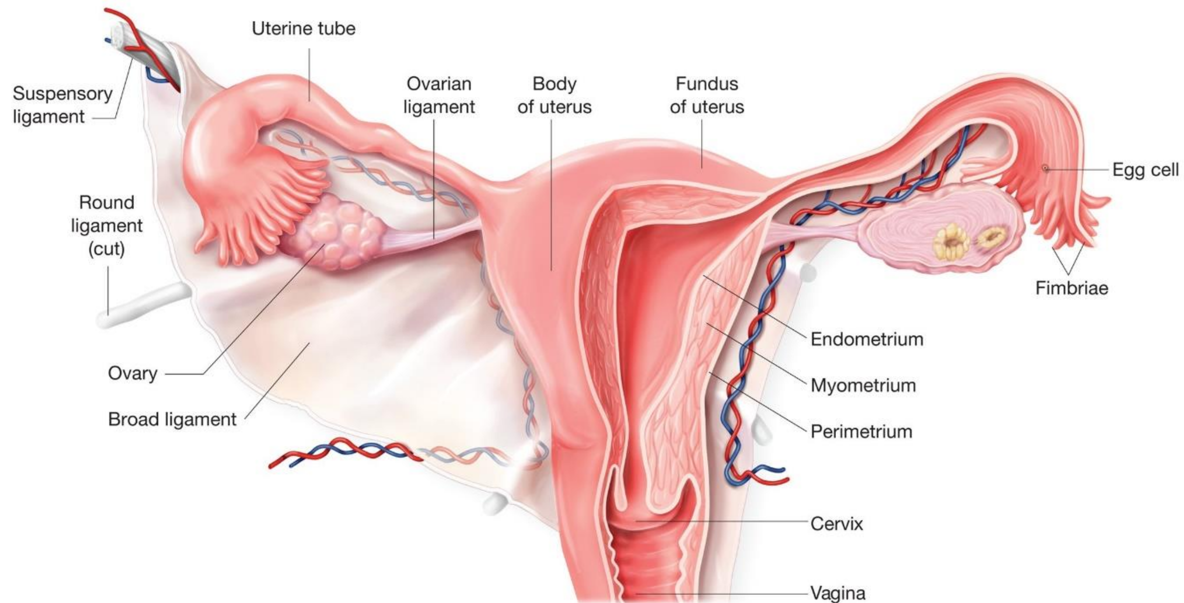


Figure 1.2. **Uterus anatomy**: **the structure of the non-gravid human uterus**. Image illustrating the 3 layers of the uterus: (1) endometrium (2) myometrium and (3) perimetrium, the cervix, the uterine tubes, ligaments, and annexes (Image from Biorender Library).

1.1.2 Myometrium

The myometrium is the thick central layer between the endometrium and the serosa or perimetrium. It consists of the uterine muscle cells that underlie the stroma and vascular tissues (Young, 2007). The myometrium is a key organ that causes uterine contractions during labour (Wray,1993).

There are three layers in the human myometrium that are poorly categorised. Each layer is ordered in an alternate fashion in the form of internal round fibres, central interlaced muscle filaments, and external longitudinal smooth muscle (Rudmann and Foley, 2013). The middle layer is made up of huge vessels referred to as the vascular layer. These huge vessels undergo marked thickening during pregnancy. Strong myometrial filaments are organized by forming a circle of spirals of different lengths with the aim of building their volume without exerting pressure on the developing embryo (Huszar and Naftolin, 1984; Henriquez Pino, 2017). In rodents and other non-primate species, the principal layers are organised into longitudinal and circular muscles (Huszar and Naftolin, 1984; Young, 2007).

During labour, the myometrium contracts, which causes the uterus to shorten, reduces the intra-uterine space, and effaces the cervix (Garfield and Maner, 2007).

1.1.3 The perimetrium

The perimetrium is an external serosa coat composed of connective tissues, which continue with the broad ligament (Figure 1.2). Its function is to provide a mechanical protective coat around the uterus.

1.1.4 Vascularisation of the uterus

The vasculature of the uterus is characterised by an organised anastomosing network and interconnection system around the surface of the uterus. This vasculature system is linked with organs to maintain a good supply of oxygenated blood (Osol, 2009). The uterus is mainly vascularised by an arcade linked to the internal hypogastric and iliac pathways on each flank of the uterus. It is also fed by the ovarian artery from the abdominal aorta. Besides, the myometrium is nourished by arching veins penetrating deeply from the uterine artery. These courses connect with other branches to the extended veins and sinuous arcade that end to feed the decidua endometrium and the placenta during gestation (Wray and Prendergast, 2019).
The uterine arteries have ramifications in the myometrium known as arcuate arteries situated in close proximity to the uterine surface. Radial arteries expand and cross the myometrium and reach the endometrium. At the boundary between the myometrium and the endometrium, the radial arteries divide to create spiral arteries and basal arteries. While gestation has no effect on basal arteries or the menstrual cycle, the spiral arteries are very sensitive to the action of oestrogen and progesterone. The radial and spiral arteries undergo a vasoconstriction in the menstrual cycle secretory phase, causing ischemia then necrosis, and resulting in a shedding of the functional endometrium during menstruation. Then the basal arteries generate new spiral arteries, and the new menstrual cycle begins (Wray and Prendergast, 2019).

During pregnancy, the uterine vasculature undergoes important adaptation. This includes the remodelling of the spiral arteries. They widen and become unable to respond to vasocontractile excitation. This is crucial to secure sufficient blood supply to the placenta as well as to the growing foetus for a successful pregnancy (Pijnenborg et al., 2006). The remodelling process involves the cells of the immune system. The uterine natural killer cells and macrophages activate the breakdown, disorganisation, and differentiation of the uterine vessel structures, giving rise to the invasion of the vessel walls by interstitial trophoblasts to the point that the whole endothelium is replaced by trophoblasts. Studies have revealed that the absence

of spiral artery remodelling is linked to pre-eclampsia and in utero foetal growth restriction (Chaiworapongsa et al., 2014).

Another major change is that the larger vessels such as radial, arcuate, and uterine arteries dilate in order to allow a very large amount of blood to flow to the uterus, with the flow rate increasing from 45 mL per min in non-gravid uterus to almost 750mL per min at term. An increasing fraction of the cardiac output goes to the pregnant uterus (Browne et al., 2015). This change in blood volume is accomplished by a growth in the of vessel and a diminished response to vasoconstrictive factors because of uterine denervation close to term in most species, including humans (Thorbet et al., 1979).

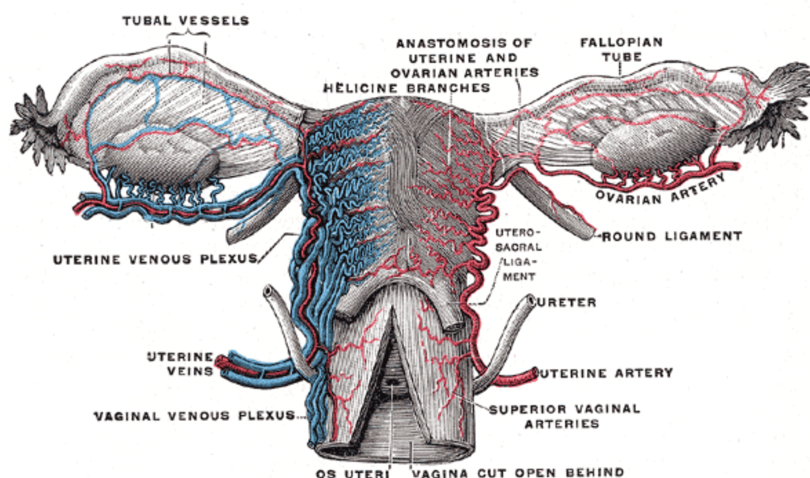


Figure1.3. Vascularisation of the uterus. The uterine arterioles form an anastomosing system with a very well-developed interconnection across and around the uterus for a good supply of oxygenated blood. The uterine arteries penetrate the myometrium with their branches; these are called arcuate arteries (Lumenlearning.com).

1.1.5 Innervation of the uterus

The uterus has a myogenic character. It contracts on its own without any nervous or hormonal stimulation (Wray 1993). The exact function of the nervous system on the uterine activity remains unclear (Tica et al., 2011). Although neurotransmitters are present in the myometrium, there is an absence of axons and contact between the endings of synapse and myocytes.

The pelvic organs are innervated by the autonomic nervous system, including the sympathetic and parasympathetic networks. The sympathetic supplies the uterus and the cervix and comes

from the inferior hypogastric plexus. The parasympathetic originates from the pudendal nerve and supplies the vagina and surroundings (Morizaki et al., 1989).

The uterus and the cervix insensitive to burns and cuts, therefore the cervix does not need anaesthesia during a cauterisation therapeutic procedure. On the other hand, the uterus and the cervix are respectively sensitive to expansion and dilation. These are the cause of pain associated with vaginal birth (Tong and Huo,1991). The uterine blood vessels are innervated by sympathetic and parasympathetic nerves as well as sensory neurons (Gnanamanickam et al., 2011). The sympathetic innervation generates a vasoconstriction effect by activating the α-adrenergic receptors, whereas the parasympathetic nerve produces a vasodilatation effect by activating muscarinic cholinergic receptors (Sato et al., 1996).

During pregnancy, the denervation of sympathetic nerves was noticed in the myometrium. In the non-pregnant or early pregnant rat, fluorescence histochemistry revealed an abundant presence of perivascular innervation but the nerve degeneration began around day 15 of gestation. This process carried on to the point that by term the perivascular nerves in the myometrium were almost absent (Klukovits et al., 2002; Monica and Smith, 2015).

Substance P (SP), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), and calcitonin gene-related peptide (CGRP) are all neuromodulators found in sensory nerves. The presence of nerves fibres for neuromodulators NPY, SP and CGRP has been identified in the myometrium of non-pregnant rat, but this innervation is affected by pregnancy (Gnanamanickam et al., 2011, Anouar et al., 1990). It has been discovered that the nerve fibres that contain the neuromodulators CGRP are more profuse in the myometrium of a non-pregnant rat than the pregnant at term (Anouar et al., 1990).

CGRP appears to relax non-pregnant myometrial tissues but has no impact on pregnant myometrial tissues. Although circulating CGRP levels in humans and animals rise during pregnancy, they plummet at term (Yallampalli et al., 2002). CGRP is a strong vasodilator. Besides, sensitivity to this neuromodulator augments during gestation (Gangula et al., 2003) and seems to be involved in the ripening of the cervix to facilitate the effacement and the dilatation of the cervix during labour. Its role is crucial in the vascular remodelling of the uterus during pregnancy (Kirby et al., 2005, Mowa and Pakpa 2004).

Substance P (SP) is also present in the nerve fibres of sensory nerves in non-pregnant rat myometrium. Its vasodilator effect on myometrial arteries seems not to change with gestation (Wimalasundera et al., 2005) but it has a positive effect on cervical ripening (Mowa and Pakpa 2004).

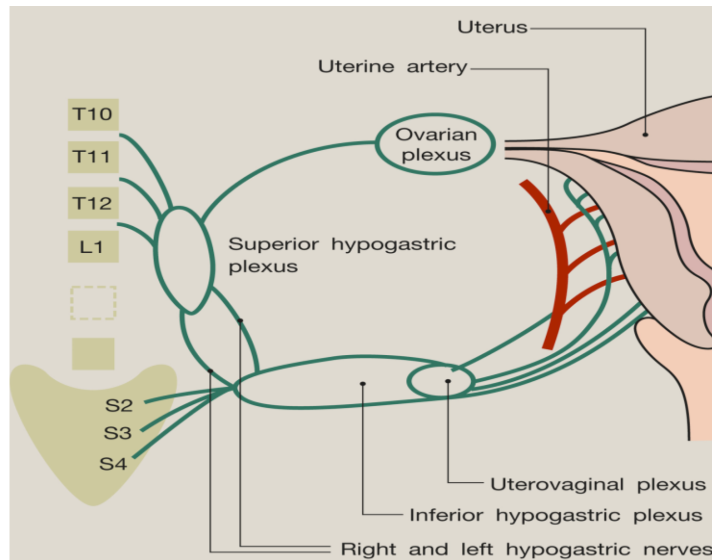


Figure 1.4 The innervation of the uterus. The uterus receives sympathetic and parasympathetic innervations. The autonomic nervous system is not needed for contractions since the uterus is myogenic. In the myometrium, there are no synapses nor direct contact between axons and myometrial cells. Nevertheless, neurotransmitters are released in the areas between muscles bundles (Aleksandrovych et al., 2019). Letters T, L and S refer to thoracic, lumbar, and sacral spinal vertebraes)

1.2 Overview of the physiology of human pregnancy

1.2.1 Progression towards labour

During pregnancy there are changes in ion channels. For instance, the gap junctions and their conductions increased and help make sure that changes in Ca^{2+} and their impulse are promptly communicated to the myometrium so that uterine contraction can be well coordinated. The ion channels that are present in the myometrium and contribute to the excitation and contraction coupling include calcium channels, potassium channels, chloride channels, store-operated channels, sodium pump (Na, K ATPase).

The process of contraction is dependent on the control of calcium entry, which is regulated by L-type Ca^{2+} channels. Cells are equipped with a variety of K^+ channels, which are triggered by voltage, Ca^{2+}, ATP and stretch. The opening of chloride channels results in the outflow of Cl^-. The plasmalemmal calcium flows are maintained by two pathways for efflux: plasma membrane Ca-ATPase (PMCA) and Na, Ca exchanger(NCX). The Na pump (Na, K ATPase) functions to eliminate $3Na^+$ in conjunction with the entry of $2K^+$.By using the sarco-endoplasmic reticulum Ca-ATPase (SERCA), the sarcoplasmic reticulum (SR) absorbs Ca^{2+}, and Ca^{2+} is only released via IP3-gated channels. STIM1 and Orai1 interact when SR Ca^{2+} is reduced, resulting in the creation of a Ca^{2+}-selective pore and the activation of non-specific cationic TRCP channels through this store-operated mechanism, which generates an inward current and enables the SR to regulate plasma membrane excitability. G-protein-coupled receptors (GPCR), such as oxytocin (OT), bind to agonists, which activate phospholipase C (PLC), generate IP3, contribute to depolarisation, promote Ca^{2+} entry, and boost contractility. The role of these ion channels is more described in section 1.8.4.

At the endocrine level, there is an increase in the production of prostaglandin, oxytocin, and their receptors (Brubaker et al., 2016). The growth in myocyte size and myofilament content, increased excitability of agonists in actin polymerisation. All these mechanisms contribute to the production of the vigorous contractions required for parturition, during which there will be an increase in intrauterine pressure which will gradually dilate the uterine cervix and finally expel the foetus (Arkinstall and Jones, 1990).

1.2.2 Parturition and labour process

Normal labour is defined as spontaneous activity and a full-term delivery. This means that it has commenced naturally, remained low risk from onset to delivery with a stable and constant presentation of the baby (vertex) in a natural birth position. Parturition according to the literature often implies the physiological procedure which results in delivering the baby from its mother's womb, including the placenta, the umbilical cord and the membranes which accompany it (all the products of conception).

Numerous quantifiable labour parameters are frequently used to measure both physiology and labour progress including the descent of the presenting part, cervical dilation, uterine contractions, and maternal and foetal observation records (partograms) (Gould, 2000). The process of labour is continuous but is marked by the first, the second and the third stage of parturition. These stages correspond mostly to the cervical and myometrial physiological modifications during childbirth resulting from biochemical, mechanical, and hormonal variations (Norwitz et al., 1999). Clinically there is a physiological difference in the duration and progression of labour from one woman to another, particularly in early parturition (Kominiarek et al.,2011).

1.2.2.1 First stage

The beginning of uterine contractions until complete cervical dilation marks the first stage of uterine labour. It usually lasts 8 in women giving birth for the first time and are unlikely to last over 18 hours. Second and subsequent parturitions last on average 5 hours and are unlikely to last over 12 hours (Nice 2022). The first stage of labour is typically split into two distinct phases: the inactive and the active. The inactive phase precedes the active. The cervix becomes soft, expands by 3 to 4cm, effaced and with irregular uterine contractile activity during the inactive phase (2-4 contractions per 10 minutes). Immediately after this phase is the active phase, during which uterine contractions become painful and regular (4-6 contractions per 10 minutes), lasting until the cervix is fully dilated. The inactive phase lasts longer and may take many hours; on the other hand, the active phase is shorter. It can last between 2 to 5 hours. During this phase, the usual cervical dilatation rate is 1 cm / hour (Friedman, 1995). However, this can vary considerably depending on the type of population, foetal weight, parity, the fetal presenting part, the type of labour (spontaneous or induced).

The assessment of all aspects of progress of labour needs to be performed if the delay in the first stage is suspected; the delay is diagnosed when the cervical dilatation is less than 2cm in 4 hours for first labours and less than 2cm in 4 hours or a slowing in the progress of parturition for second and subsequent labours. The descent and the rotation of the fetus head is slow and there are changes in the strength, frequency, and duration of uterine contractions (the contractile parameters become weak, irregular, and short in duration)

It is important to note that a prolonged inactive labour phase happens in 5 to 6.5% of parturient. The prolongation of this phase presages undesirable results for the mother and the foetus.

1.2.2.2 Second stage

Known as "pushing stage," this period characterises the second phase of labour. It usually begins with the complete dilation of the cervix and continues until the baby is delivered. The length of this stage varies depending on the case. It can last 1 to 2 hours for those who are giving birth for the first time and 0.5 to 1 hour for women who have already experienced childbirth. This stage can be divided into two stages: The prelude stage considered as early uninvolved period, which begins at the time of the complete dilation of the cervix until the descent of the presenting head of the foetus through the parturient pelvis. The second stage is the dynamic or the expulsion stage. During this stage, the contractions become expulsive, and the thrust becomes effective.

With epidural analgesia, a second prolonged phase of labour in women who have no experience of childbirth is characterised by a duration of approximately 2 to 3 hours (ACOG, 2003). For a nulliparous woman birth would be expected to take place within 3 hours of the start of the second stage in most women. The delay is suspected if progress (in terms of descent of the presenting part and/or rotation) is inadequate after 1 hour of second stage. Diagnose delay when it has lasted 2 hours. For a multiparous woman**,** birth would be expected to take place within 2 hours of the start of the second stage in most women. The delay is suspected if progress (in terms of descent of the presenting part and/or rotation) is inadequate after 30 minutes of active second stage. Diagnose delay in the second stage when it has lasted 1 hour. If full dilatation of the cervix has been confirmed in a woman without regional analgesia, but she does not get an urge to push, further assessment is required after 1 hour (Nice 2022)

1.2.2.3 Third stage

This is the last stage of labour. It begins from the completed or full neonatal delivery and continues until full expulsion of the placenta and membranes. Its duration is generally one hour if it is spontaneous and 5 to 15 minutes if it is manually facilitated. The evacuation of the placenta is in most cases combined with strong contractions and vaginal haemorrhage. The usual complication that occurs at this stage is postpartum haemorrhage (PPH). Intervention may involve appropriate clamping and section of the umbilical cord as well as the administration of uterotonic products such as oxytocin (Gungorduk et al., 2018).

1.2.3 Hormones involved in pregnancy.

Diverse modifications involving the uterus, the placenta and the foetus regulate pregnancy and labour. Major prerequisite conditions for a normal labour include changes from uterine quiescence to an active and regularly contracting state and changes involving the softening of uterine cervix from a rigid state (Weiss, 2000; Snegovskikh et al., 2006). An acceptable balance of these changes is essential for a better pregnancy outcome. A hormonal balance shift is required for the preparation of contractile activity in labour (Rivera et al., 1990; Holt et al., 2011).

1.2.3.1 Oxytocin

Oxytocin (OT) is a peptide hormone that is produced in the hypothalamus by the pituitary gland. This hormone attaches to the oxytocin receptor (OTR), a G-protein coupled receptor present in the uterine muscle (Kimura et al., 1992). When the OTR is activated, the Gαq family G-protein is stimulated, which activates phospholipase-C β (PLCβ). The catalysis of PLC will hydrolyse phosphatidylinositol biphosphate (PIP_2) resulting in the formation of inositol triphosphate (IP_3). In turn the IP_3 will bind to its receptor located on the SR to stimulate the release of Ca^{2+}, causing more Ca^{2+} influx in the cytosol. The action of oxytocin is facilitated by the presence of oxytocin receptors. The level of OTR is high in pregnant myometrium (Y Ku et al., 1995), and progressively increases toward the end of pregnancy in preparation for labour (Fuchs et al., 1984) (Arthur et al., 2007). Oxytocin triggers myometrial contractions directly through the PLC pathway described above, inducing the release of Ca^{2+} from SR (Rivera et al., 1990), and resulting in

uterine contractions (Strakova et al., 1998). The impact of OT during labour in the presence of glucose depletion was of interest to this thesis.

1.2.3.2 Oestrogen

Oestrogen plays a crucial part in uterine changes during the initial period of gestation. In mice, the secretion of oestrogen occurs during the first 2 days of pregnancy. During this phase, the glandular and luminal epithelial cells proliferate under the activation of pre-ovulatory oestrogen (Robertshaw et al., 2016). On the third day of gestation, the stromal cells proliferate under the action of progesterone after the formation of corpora lutea. The process is empowered by the secretion of oestrogen before implantation. Then, the implantation occurs on day 4 (Huet-Hudson et al., 1989). The secretion of oestrogen before implantation stops the proliferation of epithelial cells to allow the differentiation phase (Tan et al., 1999). Skarnes and Harper (1972) reported that ovariectomy in mice, leads to induction of preterm birth while progesterone intervention delays labour. In humans, oestrogen along with progesterone are all produced during the first ten weeks of pregnancy in the corpus luteum. After the implantation, the placenta takes over. Oestrogen is produced, gradually increases until it reaches its peak in the last three month of pregnancy, then drops after labour. Oestrogen exerts multiple effects on smooth muscle vessels by involving genomic mechanism and signalling pathways. These mechanisms contribute to the reduction of vascular resistance and increment of blood perfusion in the uterus during gestation. Oestrogen has been involved in the regulation of myometrial glycogen storage (Chapter 3)

1.2.3.3 Prostaglandins

Prostaglandins are bioactive lipid hormones produced by the decidua, the amnion, and chorionic membranes from arachidonic acid (AA), facilitated by prostaglandin synthase during pregnancy. Two forms of prostaglandins are expressed in the myometrium, PGE2 and PGF2α. The concentration of these hormones has been reported to rise progressively in the later stage of pregnancy. Vigorous and spontaneous contractions during labour are linked to an upsurge of PGE2 and PGF2α, leading to a biochemical process resembling an acute inflammatory reaction (Brown et al., 1998). The PGE2 receptor subtypes range from EP1 to EP4 and are all detected in the uterine muscle during gestation (Astle et al., 2005). The contractile activity is the result of PGE2 binding to either EP1 or EP3 receptors, promoting an inflammatory profile. When PGE2

binds to EP2 or EP4 receptors, relaxation and repression of inflammation is the result (Brown et al., 1998). This demonstrates the important role that PGE2 plays in keeping the uterus in a quiescent state during gestation, although the mechanism of action of prostaglandin behind the above uterine response is not yet well established (Hertelendy & Zakar 2004). The interaction between prostaglandins and oxytocin has been described by Vane, Williams and Chan. Prostaglandin inhibitors such as indomethacin reduced myometrial contractions induced by oxytocin in rats, and blocking prostaglandin biosynthesis reduced the oxytocin-induced stimulation in the uterine muscle (Vane and Williams 1973, Chan 1983). The ability of oxytocin to produce arachidonic acid from decidua cells is significantly higher during labour than in non-labouring or non-pregnant uteri. The oxytocin boosts prostaglandin biosynthesis by activating phospholipase enzymes, leading to a synergic effect of both oxytocin and prostaglandin (Wilson et al., 1988).

1.2.3.4 Progesterone

Progesterone plays a key role during gestation. It is a C-21 steroid hormone produced by the corpus luteum at the beginning of gestation (Garfield and Yallampalli, 1994). In mice, upon parturition, the level of circulating progesterone falls due to the death of the corpus luteum. This is not the case in humans during labour (Challis et al., 2000). The administration of progesterone reduced glycogen levels in all parts of the tract of the uterus (Gregoire et al., 1967). Additionally, Bowman and Rose results suggest that while oestrogen stimulates the accumulation of glycogen within the mink uterine tissue during oestrus and gestation, progesterone promotes the catabolism of glycogen in the uterus, providing the glucose that is required for early pregnancy's survival and implantation (Bowman and Rose 2017). In humans, a few weeks later, the placenta takes over and become the principal source of progesterone production during pregnancy and labour (Brown et al., 2004). The presence of progesterone in the system contributes to uterine quiescence during pregnancy (Mesiano, 2007). Progesterone has been involved in the down regulation of the oxytocin receptors. Progesterone also prevents the formation of calcium channels. These dispositions contribute to uterine quiescence during gestation (Garfield et al., 1998). While the catabolic action of progesterone on glycogen is needed for the survival of the foetus, the anabolic action of oestrogen on glycogen is needed for sufficient contractile uterine force during parturition. Progesterone has been involved in the myometrial glycogen storage metabolism (Chapter 3)

1.2.3.5 Relaxin

Relaxin has been reported to play a crucial function during pregnancy, for implantation, the development of the placenta, and the quiescence of pregnancy (Napso et al., 2018). It is considered to be a strong vasodilator and effective in controlling haemodynamics in non-pregnant and pregnant uteri (Conrad et al., 2004). O'Sullivan et al. suggested that relaxin is involved in renal function during gestation in mice, as its deficiency resulted in presence of proteins in the urine (O'Sullivan et al., 2017). Gooi et al. demonstrated that in mice, the uterine vessels become stiffer during gestation when relaxin is deficient (Gooi et al., 2013). Additionally, the level of circulating relaxin increased towards term in mice and other rodents (Marshall et al., 2018) and promoted blood flow. The deficiency in relaxin signalling in mice led to the increase of obstruction of delivery rate because of poor cervix ripping (Kaftanovskaya et al., 2015). In rats, injection of relaxin on the 15th day of pregnancy considerably increased uterine myometrial weight and glycogen levels in the non-pregnant horn. The rat had one pregnant horn and one non-pregnant horn. These data indicate that relaxin might be involved in the regulation and storage of glycogen in the myometrium in later gestation (Vasilenko et al., 1981).

1.2.4 The Scientific basis of labour

1.2.4.1 The mechanisms of parturition based on the endocrine mechanisms and progesterone withdrawal.

Animal models have been beneficial in investigating signaling pathways particularly endocrine or paracrine system determining the initiation of pre term or term labour. Based mostly on animal models that show a rapid progesterone withdrawal from the maternal circulatory system prior to labour, several researchers have preferred splitting uterine activity throughout gestation into three distinct stages: 1) uterine quiescence, which lasts almost the all duration of the pregnancy and is characterised by the uterus remaining moderately quiet and unresponsive to impulse; 2) uterine activation. During this phase, the uterus changes from a quiescent state to be able to respond to contractile stimuli and 3) The stimulation phase, which is characterised by vigorous contractions followed by delivery (Challis et al.,2000;Challis and Lye 1994). In summary, the findings show that oestrogen raises the expression of genes related with uterine activity whereas progesterone suppresses these genes while stimulating genes associated with uterine relaxation.

Animal models do not adequately portray the complexities of the physiological processes that govern uterine contractility in pregnant people. Early parturition research examined hormonal pathways by evaluating hormone concentrations in several fluid compartments, primarily the maternal serum. It has recently been shown that the uterine tissue has highly organised mechanisms for producing and metabolising a wide range of hormones. In most animal species progesterone has been recognised as the primary progestational hormone.

The use of animal models (rabbit and rat) gave birth to Csapo and others' (Capso and Wiest 1969) "progesterone block" idea. It was shown that progesterone withdrawal from the maternal serum was both essential to trigger the cervical and uterine alterations that resulted in labour (Fig.1.5).

Parturition was produced by surgical or pharmacological progesterone antagonism, which was stopped by progesterone supplementation, and this caused also the delay of natural birth. Progesterone, according to this idea, improves uterine quiescence through the stimulation of relaxant avenues and inhibiting stimulatory pathways. In animal models Parturition happens only after progesterone withdrawal.

However, arguably the most worrisome aspect of the progesterone blockade theory is that parturition occurs in women despite extraordinarily high and stable or growing progesterone

levels in the maternal serum (Boroditsky et al.,1978). Researchers have trying to prove the veracity of progesterone withdrawal but only one non verified study (Turnbull et al.,1974) was able to bring evidence of a fall in maternal progesterone before parturition. Despite the stable or increasing levels of progesterone in maternal serum, several research groups are still hunting the theory of "functional" progesterone withdrawal to explain the possibility that there might be a withdrawal of progesterone before labour.

To achieve functional progesterone withdrawal, four basic mechanisms have been suggested (Table 1).

1.The first thought, with strong evidence which exists, is the "paracrine hypothesis." According to this theory, the foetal membranes and the maternal decidua are responsible for the production and the regulation of progesterone levels in the myometrium. Other potentially essential hormones involved in parturition and produced by this intrauterine network include endothelin, oxytocin, prostaglandin, prostacyclin and nitric oxide (Olson et al.,1993; Camero et al.,1995).

That is the reason many researchers now consider that uterine contractility are regulated by this paracrine interactions and that these mechanisms could be more essential in humans than the hormone regulating pathways that dominate in the majority of animal.

2. The second mechanism of functional progesterone withdrawal is linked to the expression of progesterone receptor (PR) isoforms. In both animal and human uteri ,PR-A mRNA tends to rise dramatically at term before the onset of labour but PR-B remains steady or increases very modestly (Fanx et al.,200; Mesiano et al.,2002). Recent data reveals that this is also true at the protein level in humans (Merlino et al.,2007). The prospect of changing the PR-A:PR-B ratio to induce functional progesterone withdrawal has received a lot of interest.

3.A third proposed theory of progesterone withdrawal involves progesterone metabolites. The placenta, the membranes, and maternal decidua can metabolise progesterone into a wide range of metabolites. These metabolites have stronger relaxing action on the myometrium than progesterone (Perusquia 2001; Mitchel et al.,2005) and may thus play an essential role in “blocking progesterone" in pregnant women. These metabolites might also hinder the binding of progesterone to PR-B; however, this has never been shown experimentally.

4. A fourth possible site of regulation for progesterone activity can take place at the post receptor level, involving other transcription elements or transcriptional coregulators. A reduction in a key element in PR-B-mediated actions, or a rise in a factor that blocked PR-B's

efficacy, might result in progesterone withdrawal (Condon et al.,2003). Additionally, direct exchanges between PR and some other transcription factors like nuclear factor (NF)-B could alter myometrial contractility, however there is little agreement on the function these mentioned factors may play during labour (Mendelson 2009).

1.2.4.2 The regulation of labour based on the immune systems.

Labour, whether term or preterm, has long been recognised as an inflammatory process (Liggins 1981). Recent studies have focused on the link between labour and the immune system. Many recent reviews have addressed this (Keelan et al.,2003; Orsi and Tribe 2009). Despite this, this link is still poorly understood. When it comes to this connection, there are two critical factors to consider.

1.The first point to make is the difference between evidence of correlation and evidence of a cause-and-effect link.

2.The distinction between infection and an inflammatory response is the second, and possibly more essential, point. Significant intrauterine infection can result in premature birth, according to clear evidence (Goldenberg et al.,2000). Still, in this case, prompt delivery is advised, and parturition delay is not an option. This opposes "spontaneous" premature delivery when gestational prolongation may be favourable but prove for an immune system feedback is unclear.

The inflammatory response may play a significant role in parturition. Studies by Romero (Romero et al.,1992; Romero et al.,1993) and colleagues documented proinflammatory cytokine concentrations in women's amniotic fluid, in the presence or absence of positive amniotic fluid bacterial cultures in term or preterm pregnancy and discovered that the levels of Interleukin (IL)-1 were unusually high in women with preterm labour and negative cultures .Concentrations at term increased considerably (3- to 4-fold) with the commencement of labour (Opsjon et al.,1993)Additionally, in the presence of a positive culture, the concentrations increased fourfold more (Romero et al.,1992).

These findings significantly support the idea that parturition is accompanied by an inflammatory process. In the absence of infection, this process is of moderate proportion in usual term labour or spontaneous premature labour but in case there is intrauterine infection, the proinflammatory reaction is much heightened (Mitchell and Taggart 2009)

The study by Romero et al. highlights the syndrome's complex character and the probable participation of the immune system. However, one significant disadvantage of the cause-and-effect role of supernatural infection in this case is the fairly restricted ability of antimicrobial treatments to delay birth (Romero et al.,1992).

In the absence of premature ruptured membranes, a current metaanalysis of several randomised controlled studies assessing the efficacy of antibiotics used for spontaneous preterm labour, found no substantial maternal or fetal advantages (Hutzal et al.,2008). Conversely, antimicrobial treatment, could extend pregnancy and help newborns up to 30% of cases of premature labour that follow membrane rupture, where there is better prove for a role of bacterial engagement (Goldenberg et al.,2008; Sheenan and Chandiramani 2008).

The findings were mixed, with many favourable and some negative outcomes. Among the most rational results in recent investigations studying processes of labour, is an increased expression of immune-related genes (Breuiller-Fouche et al.,2007).

In conclusion, these findings confirm the link between immune system and parturition and give some ambiguous evidence for the cause-and-effect link. Numerous animal experiments were conducted to investigate a possible cause-effect link between the immune system (proinflammatory effect) and labour. The evaluation of these studies necessitates a particulier focus to the second point raised above: the contrast between an inflammatory process and infection. This difference is difficult to make. In normal pregnancies, many commensal microbes often occupy the lower genital tract. Inflammatory responses occur in almost every episode of labour. Aside from the existence of clinical signs manifestations of infection, it is sometimes difficult to distinguish between the physiological and pathophysiological environments (Mitchell and Taggart 2009)

1.2.4.3 Processes contributing to the last phase of labour.

The generation of uterine contractions and the cervical ripening constitute two crucial processes contributing to the last phase of labour and fetal expulsion. The first, relates to the modulation of uterine myocyte mechanisms, as well as pathways known to be vital in controlling uterine contractions. The second, involves the cellular machineries that transforms the rigid state of the cervix of early pregnancy into soft state that may be dilated to let the fetus to pass through the birth canal (Mitchell and Taggart 2009)

1. The generation of uterine contractions

In all species, myometrial contractile effort is intermittent and results from instinctive action potentials caused by instable membrane potential. This was measured in vitro in different species including humans, rats, rabbits, mice, and guinea pigs. The diverging ion channel expression or biophysical characteristics contribute to the configurational changes between species, It is widely assumed that their role is to increase intracellular Ca^{2+}, which is required for contractile stimulation (Well illustrated in section 1.7.3).

2. Cervical ripening

One of the most noteworthy occurrences in the parturition process is the morphological change of the cervix. This is a hard cylindrical structure that measures 4 -7 cm in length during most of pregnancy in human. The cervical canal is basically closed during the first pregnancy. It may be fairly shorter and around 1cm in diameter in pregnant women who had a vaginal birth before. The cervix's stiffness is partly attributable to its high collagen concentration. During late pregnancy, this stiff organ softens and becomes more distensible, a process known as cervical ripening. The cervix will totally undergo an effacement and dilatation during labour to allow the baby and the placenta to pass. The first cervical ripening research directed its attention to the alterations in conjunctive tissues (Danforth et al.,1960, Leppert 1995). The majority of researchers followed the same trend that have dominated contractility research: the involvement of progesterone and the immune system. Once again, rats and mice have been the most widely utilised animal models.During the ripening process, Matrix metalloproteinases (MMP) expression and activity increase around the time of parturition, resulting in gradual breakdown of the collagen pattern of the cervix and an increase in water content, hyaluronan and glycosaminoglycan concentrations (Uldbjerg et al.,1983; Granstrom et al.,1989). The local alterations in collagen, suggestive of cervical ripening after labour have been confirmed recently in the rats by the use of light-induced autofluorescence (LIF) measurements using a Collascope(Maul 2006). The cervical softening in mice occurs in mid-pregnancy, around gestational day 10-12 (Buhimshi et al.,2004). However, in the last two days of pregnancy, there is an accelerated phase known as cervical ripening.

The latter occurrence is followed by an invasion of cervical matrix by immune cells, mainly neutrophils (Xu et al.,2008). Intrauterine administration of LPS (Lipopolysaccharides)causes a comparable entry of neutrophils into the cervix, which may be avoided by pretreatment with a

synthetic progestagen (Yellon et al.,2008). Although it is unsurprising that a powerful inflammatory stimulation like LPS generates an entry of inflammatory cells into a neighbouring tissue, the findings revealed that there is a cause-and-effect relationship between inflammatory cells and cervical softening. In pregnant women, labour activity is escorted by an invasion of pro-inflammatory cells into the cervical matrix (Norman 2007).

1.2.4.4 Human aspect of parturition.

1. Progesterone withdrawal

The corpus luteum is the first location of progesterone synthesis in humans during pregnancy. Nevertheless, by five to six weeks following fertilisation, the placenta becomes the primary supplier of the exceptionally high production progesterone. Abortion can occur if the corpus luteum is removed before the "luteal-placental shift" stage (Csapo et al.,1973). The role of progesterone in the remaining stages of pregnancy is unknown.

Several studies conducted in large numbers of pregnant women indicate that in the maternal blood there is neither a decrease in progesterone nor a substantial increase in oestrogen at the time of parturition (Boroditsky et al.,1978). However, studies using rabbits, rats, and sheep established a vital function for progesterone signaling in the timing of labour.

This left a profound impact for researchers, leading to the already stated idea of functional progesterone withdrawal. In human, the role of progesterone during gestation is still a mystery that needs to be solved.

Several clinical trials have been conducted to investigate the possible effect of progesterone supplementation in the prevention of human preterm birth. Unfortunately, there is no statistically significant evidence of a better result with progesterone medication to prolong pregnancy. More research is being conducted, however progesterone therapy in women during pregnancy is unlikely to give a generally relevant tocolytic or preventive therapy (Mitchell and Taggart 2009)

2. Immune system

There is substantial prove of increased PGHS-2 expression and production of prostaglandin in the pregnant uterus when parturition is approaching mostly from foetal membranes and maternal decidua (Olson 1983). Their importance during labour, however, is unknown.

In animal, investigations have demonstrated that PGF2 produced by the endometrium plays a capital role in inducing luteolysis followed by progesterone withdrawal, resulting in labour.

However, in animals or humans the evidence of PGs contractile effect on myometrium is minimal (Rocca and Wehner 2009).
It also looks evident that the activation of PGHS is a significant component of the immunological reaction that takes place during labour, and it is likely that PG products are essential in regulating the immune system's effects on the parturition. One of the possible ways, which has some empirical support, is activation of myometrial gap junction production which allows for the rapid and effective spread of electrical currents through the myometrium to promote the vigorous and harmonious contractions typical of active labour (Garfield 1980).
In animal models where the corpus luteum is required for gestation, an immunological reaction, either it is triggered by infection or other impulses, might result in the production of PGF2, followed by luteolysis and parturition.
However, there is currently little evidence that this process is operational or physiologically significant in mammals where progesterone is primarily synthesised precisely in the placenta. This implies that the placenta should be the primary source of progesterone throughout gestation in an animal model of human labour. Unlike in progesterone-dependent mammal models, where antiprogestins terminate pregnancy at any time, there is comparatively little evidence on the impacts of antiprogestins during human gestation. Some evidence suggests that antiprogestins, while incapable of causing abortion on their own, stimulate cervix ripening and enhance sensitivity to uterine contractility by prostaglandins during induced abortions in the second trimester of human pregnancy (Thong and Baird 1992).

3. Cervical ripening

A review of clinical trials revealed that the antiprogesterone RU-486 doubled the rate of cervical softening and spontaneous delivery in term women within 48 hours (Nelson 2000).
Additionally, it has been shown that RU-486 needs substantially less OT to induce labour in term women when compared to placebo (Frydman 1992). Consequently, functional progesterone withdrawal whether pharmacologically induced or not may contribute to cervical ripening and boosting uterine sensitivity to contractile stimuli.

In view of this, it is worth noting that any benefits of progesterone-based treatment to delay preterm parturition may dwell not in tocolysis per se, but in stopping the shortening and ripening of the cervix, possibly through anti-inflammatory effects (Xu et al.,2008). Much

research has been conducted to examine the presence of contraction-associated proteins in the myometrium of pregnant women in late pregnancy, with varying and contradictory outcomes. They found peptide and estrogen-stimulated OT mRNA synthesis in decidua, with indications that production rate rise at the onset of labour (Chibbar et al.,1986; Chibbar et al.,1995).

Some researchers discovered that OTR concentrations in the decidua and myometrium rose before labour begins (Fuchs 1982), whilst others discovered that they did not change (Havelock et al.,2005) or were lowered (Phaneuf et al.,1998). Several studies have shown that proinflammatory cytokines enhance (Terzidou et al.,2006) or reduce (Schmid et al.,2001) the OTR gene expression in myocytes using in vitro methods. In some studies (Brodt and Myatt 1999), but not all (Grigsby et al.,2006), the FP receptor appears to drop throughout pregnancy and subsequently increase approaching labour.

Recent studies have used microarray methods to evaluate changes in the uterus after parturition. Data collected by this technology, as well as the enormous bio-informatical complexity connected with it, have offered only a restricted understanding of the physiology of labour.

Cervical ripening is an essential clinical phenomenon in human parturition. In preterm individuals with a history of premature labour, ultrasound examination of increasing cervical shortening is a good predictor of preterm labour onset (Crane and Hutchens 2008).

Any cervical failure to adequately ripen is a usual reason of unsuccessful vaginal birth, especially when induction of labour before term is recommended by the fetus's or mother's medical condition. Local administration of prostaglandin E or its analogue (Calder et al.,1977; Denison et al.,1999) is the most widely utilised drug to ripen the cervix. The mode of action is thought to be activation of MMP action or increased permeability of the cervical vasculature, allowing inflammatory cells to enter (Goshowaki et al.,1988). The human cervix, like the fundus, experiences an entry of neutrophils, during cervical ripening (Kelly 2002). These cells are most likely precursors of proinflammatory ILs and MMPs that aid in cervix redesigning (Leppert 1995)

1.2.4.5 The advantages and disadvantages of presently used animal models.

1. Sheep.

Liggins and others' pioneering investigations revealed the intriguing nuances of parturition control in this species. Progesterone is required for the maintenance of ovine pregnancy. The placenta is the primary fountain of progesterone, and the process governing progesterone withdrawal is quite complex yet intriguing. (Liggins 1981)

The fetus in sheep plays a vital function in the endocrine systems that govern the time of parturition. P450C17, a steroidogenic enzyme found in the placenta. The placenta uses this enzyme to convert progesterone to oestrogen. As a result, as progesterone is metabolised, the levels of maternal progesterone plummet and oestrogen concentrations rise.

One of the advantages of the sheep model for studies is the size of this animal. It allows instrumentation of the mother and its fetus during the late portion of pregnancy. This makes longitudinal sampling easier, which is required to quantify changes in physiology and investigate their control when parturition approaches. The ovine model, on the other hand, appears to have two main differences from the human model, limiting its use for parturition research. The first is its reliance on progesterone withdrawal. The second is the fetus' important function, which does not appear to be relevant in human gestation (Mitchell and Taggart 2009)

The ovine model will always be useful in understanding the control of fetal development during gestation but has some limitations in studying parturition in humans.

2. Mice, rats, and rabbits

These animal models differ from sheep in 2 major respects.1. Progesterone is generated by the corpora lutea during gestation rather than the placenta in all of these models. However, progesterone withdrawal is still required and adequate to induce labour.2. The fetus does not appear to be overriding.

The withdrawal of progesterone is caused by luteolysis, which is caused by the activities of PGF2 generated from the endometrium. Antiprogestins can cause preterm birth in all of these animals, and supplementary progesterone can prevent it (Chwalisz and Garfield 1994). The initial "progesterone block" notion was mostly backed by rabbit data (Mitchell and Taggart 2009). Rats and mice have recently become the most widely utilised.

Numerous categories of rats have been used in labour research. Some similarities have been noticed between the rat and humans with regard to particles that are upregulated (Eg.Cx43) or downregulated in uterine contractility (Arthur et al.,2008).
The main benefits of this approach are the comparatively short pregnancy time, affordable cost, and a proven way for inducing premature labour. Apart from progesterone, oestrogen appears to play an important role in parturition timing, since therapy with an oestrogen receptor antagonist delays labour by one day and could induce relative fetal development limitation (Fang at al.,1996).
Due to the absence of PGF2's luteolytic impact, FP receptor disruption resulted in extended gestation (Sugimoto et al.,1997). These animals delivered properly after ovariectomy, indicating that PGF2 was required for luteolysis and not for the ensuing procedure of labour.
These animal models have also been used to explore cervical ripening. In the rabbit, for example, vaginal administration of indomethacin extended RU-induced premature labour and decreased cervico-vaginal MMP action (Fortson et al.,2006). LPS-induced delivery and cervical expansion have been decreased in very limited trials by the possible antibacterial or anti-inflammatory activities of a recombinant human lactoferrin administered cervically (Hasegawa et al.,2005).
The rat has also been employed in a few studies to investigate the modulation of cervical alterations before to parturition (Chwalisz 1994; Wentz et al.,2007). Gene disruption experiments in mice revealed an intriguing aspect of cervical readiness for labour in mouse. Knocking down the gene encoding the steroidogenic enzyme 5-reductase isoform 1 resulted in a phenotype with prolonged and impaired labour due to failure of cervical dilatation (Mahendroo et al.,1999). This suggested the implication of this enzyme in the cervical ripening mechanisms, however it is unclear if this represents an vital metabolic way to lower the level of local progesterone or a direct involvement for 5-reduced steroids during cervical ripening. A natural occurrence of this condition in human pregnancy has not been seen.

In conclusion, genetic alteration of the mouse model has proven the relevance of certain putative pathways in parturition studies while casting uncertainty on others. Conversely, like with all of these animal models, labour procedure's reliance on progesterone withdrawal limits its applicability to human labour’s activity. The gene interruptions focusing on luteolysis control will probably have minimal significance to parturition in human. Likewise, genetic alterations

that change LPS or E. coli responses might or might not yield relevant data about the spontaneous premature labour in human.

Finally, given the significance of parturition, numerous modular pathways are expected to have evolved to govern the process, and single disruptions of gene could therefore offer facts without a system's framework. The sustained method of gene disruption will certainly provide significant information concerning potential roles of specific genes in certain situations, but it is probable to offer only partial information relevant to the general physiological pathways that control parturition in primate (Mitchel and Taggart 2009)

3. Higher order primates

Higher order primates continue to be the very close to humans in terms of pregnancy in a variety of aspects, including a shorter duration of pregnancy than rodents, a lack of progesterone withdrawal, the shape of the uterus(unicornuate) and electromyographic activity (Germain et al.,1982). As a result, they are still utilised in vivo research, notably to measure uterine electrical activity (Sadowsky et al.,2000), the parturient feedback to inflammatory cytokines or bacterial agents (Baggia et al.,1996; Sadowsky et al.,2000) and the tocolytic impact of novel medications (Reinheimer and Barusiban 2007),

The expense of such research is high and is regarded unaffordable by several grant-awarding authorities. Currently, such work is only available to a few academics at certain universities.

Research groups require access to more practical affordable alternative tissue sources for most of experimental studies, which has prompted the development of non-primate models of parturition and premature labour.

1.2.4.6 Theoretical contrast between human and animal models

Based on the preceding research, it was argued that there are two main theoretical flaws in commonly employed mammal models. The first is about critical progesterone withdrawal in these models but not in humans. The second is concerned with the chronological timing of the uterine activation procedure.

Progesterone withdrawal.

Parturition is the ultimate consequence of a seemingly linear sequence of physiological processes in all of the mammal models mentioned in the preceding sections (except for primates). Depending on the model, this may entail the onset of luteolysis or a shift in the aspect

of placental steroidogenesis, subsequently a considerable withdrawal of progesterone from the maternal blood (Fig. 1.5).

In contrast, the excellent data shows that progesterone from maternal serum has a little task in parturition process in human. Even though there could be some "functional progesterone withdrawal," as noted above, it looks like this is neither adequate nor, in fact, required for human parturition.

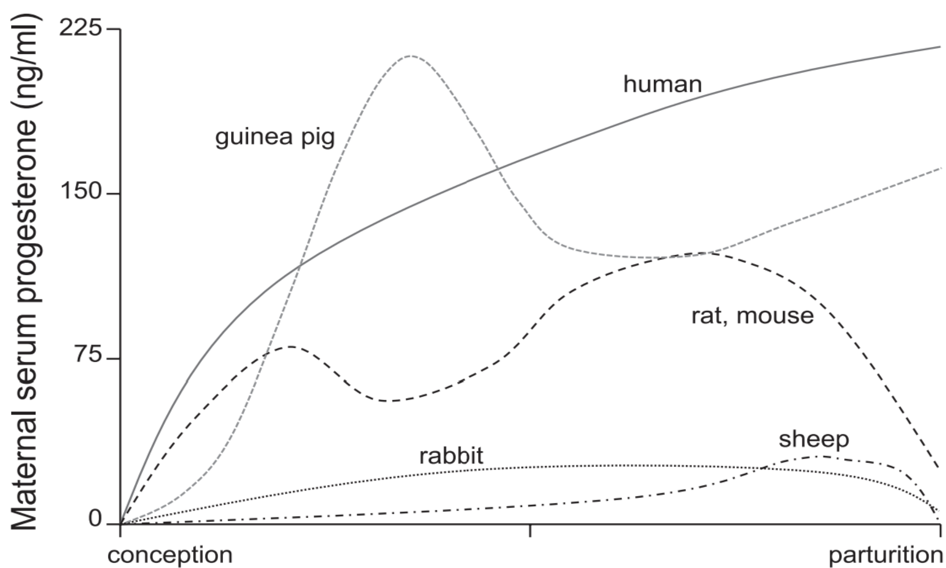


Figure.1.5 Maternal serum progesterone levels through pregnancy. Abrupt progesterone withdrawal is noticed from the maternal circulation before labour in most experimental animals. In contrast, only in guinea pig and human the levels of progesterone remained high and increasing throughout parturition (Mitchell and Taggart 2009)

1.2.4.7 Guinea Pig

1. Recommendation of the Guinea Pig as best animal model for human parturition studies

The following are the conditions for a better animal model. 1) Progesterone should be primarily synthesised in the placenta. Besides, withdrawal of progesterone from the maternal blood should not be the primary inducer of labour. 2) The change from uterine quiescence to activation should take place over a long enough period of time to allow for longitudinal evaluation of the characteristics and improvement of the physiological systems concerned. The normal incidence of spontaneous premature birth would corroborate the model's human

likeness.3) The animal's size must allow for longitudinal collection of hormonal measures in the same animals during pregnancy and labour. 4) The model's cost, which includes the buying price, maintenance costs, ease of time-mated breeding and testing costs, among other things, must allow for the collection of animal tissues at different time points during pregnancy in order to explore the physiological and biochemical phenomenon regulating normal labour. This issue is also vital for the creation of appropriate experimental models for preterm delivery. The guinea pig meets these characteristics better than current animal models, and this model ought to be investigated further. (Mitchel and Taggart 2009)

2. Progesterone levels in the guinea pig.

The guinea pig's resemblance to the human in terms of maternal blood progesterone concentrations in late gestation is perhaps its most significant benefit. In the guinea pig parturition happens when maternal progesterone levels are high and increasing (Fig. 1.5) (Challis 1971).The main supplier of progesterone during the first 4 weeks after conception is the ovary, then the placenta takes over for the rest of the gestation (Heap and Deanesley 1966). This luteoplacental change is strikingly analogous to human pregnancy (Csapo and Pulkkinen 1978). Luteal progesterone production seems to decrease after days 35-40 in the guinea pig, then accompanied by a considerable drop in serum progesterone level, with a nadir of 75-200 ng/ml taking place at days 50-55 (Rodriguez et al.,2003).

Following that, there is significant debate over the pattern of progesterone levels. According to one research, progesterone from placenta keeps on rising, resulting in higher maternal plasma levels over the following two weeks until parturition (Challis 1971); however, other studies show no substantial variation in maternal progesterone levels in the final two weeks of pregnancy (Alexandrova 1980). At parturition, maternal progesterone levels are 150-300 ng/ml, which is comparable to human pregnancy (Boroditsky et al.,1978). In the rat, maternal plasma progesterone concentrations peak at 100-150 ng/ml at the middle of pregnancy before dropping to 10 ng/ml during the next 2-3 days before parturition (Fuchs 1978). In mice, the trend is similar, but that peak levels are only 80 ng/ml (Virgo and Ballward 1974).

In rabbits and sheep, the levels peak at 10-20 ng/ml before quickly declining in the days before labour (Fuchs 1978). Large dosages of progesterone or progestins did not extend gestation in the guinea pig, as they did in the rabbit and rat (Schofield 1961).

Porter and colleagues conducted a large number of studies using different doses and routes of injection(intramuscular, intra-amniotic, intrauterine), and times of administration of progesterone or its analogues and found no important changes in uterine contractility or gestational length. The study concluded that in the guinea pig progesterone does not act as a myometrial blocker (Porter 1970)

Given the absence of prove for maternal blood progesterone withdrawal close to term or any influence of external progesterone on guinea pig pregnancy duration, the impacts of progesterone antagonist treatment are of great interest. These trials used mifepristone (RU-486). This product has antiprogestin and antiglucocorticoid action. In addition onapristone (ZK-98299) was used ,which is exclusively antiprogestin. Abortion occurs often when the corpora lutea is removed before day 28 of a guinea pig gestation, as it does in humans (Csapo et al.,1973).

These parallels between the two species support the idea that changes in progesterone- and OT-dependent signaling mechanisms have distinct but presumably consistent and modular effects during parturition. Although altered progesterone signaling may contribute to cervical ripening, OT as contractile agent is required to expel the fetus in a physiologically effective way. As previously stated, functional progesterone withdrawal could happen in primates and may potentially occur in the guinea pig (Welsh et al.,2009). It is also worth noting that if progesterone signaling is relevant in many species, probably, it will work in tandem with many other hormonal systems, such as oestrogen, CRH, and OT (Hang et al.,2008).

3. The length of pregnancy period in the guinea pig.

Goy et al. (89) meticulously chronicled the guinea pig's normal pregnancy development. The gestation period of this species is 67 days along with a standard variation of 1.5 days. The duration of pregnancy and pup birth weight change inversely with the quantity or number of fetuses, much as they do in humans. In guinea pigs, around 7-8% of pregnancies end in "premature" delivery of a live born pup which does not survive. Additionally, this mortality rate increases with litter size (Manjeli et al.,1998). These reproductive characteristics are quite similar to the human case, where the 5% rate of spontaneous (non-iatrogenic) premature delivery (37 weeks of gestation) increases with increasing multiple pregnancies (Goldenberg et al.,2008).

In contrast, as mentioned previously, spontaneous delivery before fetal viability (thus "preterm") is an extremely rare occurrence in animal that depend on progesterone withdrawal for labouring activity. The very widespread incidence of "preterm" birth in the guinea pig shows that, like in humans, the timing of labour is not nearly as finely regulated as in commonly regulated parturition models.

The guinea pig could give a good model of the MAPS notion of the progression of labour, which might be a significant step forward in comprehending the control of human labour and birth timing.

4. Other possible benefits of the use of guinea pig.

Despite the fact that other species have recently taken primacy as laboratory models of parturition, the guinea pig has a long history of making significant input to our understanding of uterine function (Karsdon et al.,2005). These include early microelectrode recordings of myometrial action potentials, myometrial gap junctional channel maturation, upregulation of L-type Ca^{2+} entry molecular pathways, G protein-coupled signaling mechanisms, and Ca^{2+} mobilisations from intracellular stores, as well as recent multiplex recordings of uterine electrical activity preceding labour onset and gene network analysis (Collins et al.,2000; Weiner et al.,2006).

Surprisingly few investigations have been conducted on the involvement of the immune system in guinea pigs labour process. Transcervical, extra-amniotic injection of LPS, TNF-, or IL-1 at mid-gestation or preterm induced labour within 24 - 48 h, which was considerably hampered by the indomethacin, a common prostaglandin synthesis inhibitor (Bokowsky et al.,1993). Activation of MAST cells in sensitised guinea pigs, either in vivo or in vitro, enhanced cervical and uterine contraction via a method facilitated by the histamine H1 receptor (Bytautiene et al.,2008).

Only a few research have looked at the functions of progesterone and the immune system in terms of cervical ripening in the guinea pig, as they have in humans. Chwalisz and colleagues discovered that an antiprogestin or the use of IL-1, IL-8, or TNF- may trigger cervical ripening. Other characteristics of the guinea pig make it a suitable laboratory species for parturition and gestational issues in global, not only premature labour. (Chwalisz etal.,1994) Dystocic labour, for example, is a typical characteristic of pregnant guinea pigs older than 7 months (North 1999), a condition that complicates up to 20% of labours in human and is a substantial contributor to emergency caesarean section incidence (Bugg et al.,2006).

Another resemblance between these two species is the considerable steroid synthesis and metabolism inside the fetal membranes.

These activators may have a major impact on uterine steroid levels and, as a result, uterine muscle contractile activity. The chorion and decidua in both species have sulfohydrolase action for estrone sulphate and synthesise increasing levels of estradiol after parturition (Mitchell and Wong 1993). Both species also have 17-hydroxysteroid dehydrogenase activity, which transforms estrone and estradiol and could participate in controlling oestrogen bioactivity in the uterus. Guinea pigs and human are also capable of reducing progesterone to reduced metabolites (Glasier et al.,1994).

Furthermore, there are similarities between humans and guinea pigs in terms of placentation and infant maturity. Both of these may be substantial benefits for using the guinea pig in perinatal investigations over other mammals.

This trait could be especially essential in preterm labour, implying that a premature birth model could be relevant for both processes of labour and experiments of neonatal treatment and results. The guinea pig, like humans, has a haemomonochorial form of placentation. In this paradigm, investigations of nutritional exchange between placenta and fetus may be more relevant (Sibley et al.,1982). These qualities imply that the model might be beneficial for research into the genesis of health and disease. Analysis in late pregnant guinea pigs show spontaneous development of preeclampsia, a disease characterised by malfunction at the placental-decidual interface (Manning et al.,1984).

The newborn guinea pig is also at a developed level of maturity, like the human neonate but different from other animal models (Carter 2007). Guinea pigs may be excellent models for developmental and reproductive toxicity investigations due to these qualities (Rocca and Weihner 2009). As a result, the model would be especially appealing for evaluating any potential medical drug created to prevent or stop premature labour.

Animal models used for labour.

Characteristics	Rabbit	Sheep	Rat	Mouse	Guinea pig	Human
Gestation days	32±3	147±4	22±1	20±1	67±3	266±14
Actual Inter size no.	8±4	1-2	10±6	10±5	3±2	1
Placental morphology	Hemodichorial Labyrinthine	Epithelial Chorial Cotyledonary	Hemotrichorial Labyrinthine	Hemotrichorial Labyrinthine	Hemomonochorial Labyrinthine, discoid	Hemomonochorial Villous, discoid
Source of progesterone	Corpus luteum	Corpus luteum Then placenta	Corpus luteum	Corpus luteum	Corpus luteum Then placenta	Corpus luteum, Then placenta
Progesterone Withdrawal?	**Yes**	**Yes**	**Yes**	**Yes**	**Partial at days 40-50 Not at parturition**	**No**
Induction of preterm birth	Antiprogestin Ovariectomy	Fetal ACTH glucocorticoid Antiprogestin	Antiprogestin Ovariectomy	Antiprogestin Ovariectomy LPS	Antiprogestin plus Oxytocin	Cervical ripening PGE_2 or antiprogestin) plus oxytocin

Table 1. Reproductive features of animal models compared to human model. There are similarities between Guinea pig and Human (Mitchell and Taggart 2009)

1.2.4.8 Implication

In view of the above, it transpires that over the previous decades, there has been regrettably little progress in understanding the processes that lead to spontaneous premature birth and term birth. It transpires that presently popular animal models will continue to be valuable in finding potential parturition regulating processes. Nevertheless, these models are restricted in terms of translation since they rely on parturition being initiated by hormonal changes that humans do not share. The literature suggests three important physiological aspects that should impact the choice of a more appropriate animal model for human labour (Mitchell and Taggart 2009).

1) It is improbable that the time of human parturition is influenced by a linear series of mechanisms resulting in maternal progesterone withdrawal. This is a significant shortcoming in current common animal models (mice, rats, rabbits, and sheep).

2) The notion generated from these models of a simple "trigger" mechanism that commences the cycle leading to progesterone withdrawal is probably incorrect for humans. Parturition in human takes place when many physiological elements work together to change the uterine state from quiescent to contractile. When a threshold number of components are triggered early, preterm labour ensues.

3) Parturition is accompanied with an immune system pro-inflammatory response. When there is no intrauterine infection, it is critical to clarify whether this is just an association link or a causative correlation. If the latter, it is necessary to identify which is the source and which is the result.

After thorough examination on the strengths and shortcomings of actual animal models, it was concluded that the guinea pig comes the closest to matching these criteria. Though there is scant information about its reproductive systems, it appears to be very similar to humans. The authors also believe that the guinea pig would be ideal for other studies on gestational implications on fetal growth and development. Additional definition of this model is necessary, and that the results will give a better comprehension of the systems that govern fetal development and birth timing.

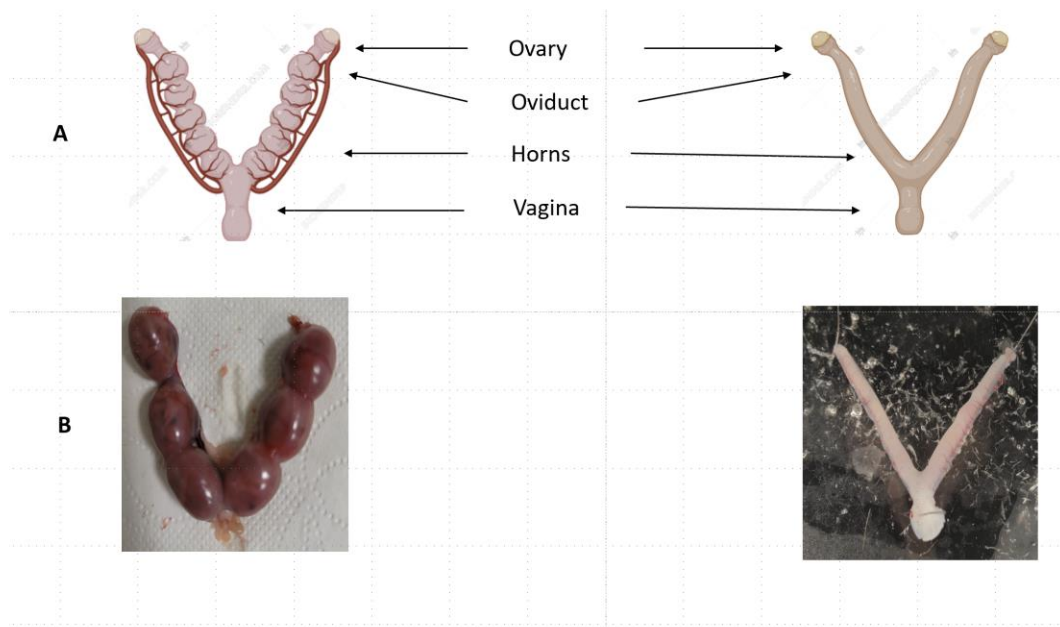
A basic component of this philosophy is the choice of the optimal animal species to solve the specific biological topic under investigation. Even though guinea pig physiology is not similar to human physiology, data suggests that it is the best model of labour for translational effect to the human environment. Reviving the research that use this model in tandem with human tissue investigations might improve knowledge of the processes driving parturition. This would represent a substantial advancement in physiological understanding as well as enormous advantages to general human health.

Due to unavailability of guinea pig and cost reason, the only alternative animal tissue source for my experimental work was mouse.

1.2.4.9 The usefulness of the mouse uterus.

The mouse is a much-used model for studying general physiology, including parturition. Much of the knowledge of the mechanism of uterine contractions comes from research conducted in animals such as mice (Carter, 2020). There are several advantages to studying animal uterine tissue during pregnancy over human uterine tissue, which can only be obtained during a caesarean section or uterine removal. Consequently, most of studies conducted on the human myometrial tissue come from elective (not-in-labour) caesarean sections, because it is extremely difficult to obtain laboratory samples from a woman in labour. Animal myometrial tissue can be obtained at different gestational ages and studies can also be conducted directly in vivo (Tribe, 2001). In this study, the experiments were first conducted on mouse, and then on human myometria, since the excitation-contraction (EC) coupling in the mouse myometrium is similar to that in humans (Matthew et al., 2004a). Mouse and human smooth muscles have similar ion channels, and all need the L-type calcium channel and proteins that control ionic balance e.g., exchangers and transporters. They both express proteins and hormones associated with uterine contractions, such as prostaglandin and oxytocin (Ratajkzak and Muglia 2008; Cook et al., 2000).

Mammalian species including rodents have different forms of uterus from the pear-shaped, single cavity structure of the human uterus, illustrated above (Figure 1.5). The mouse has a duplex uterus with 2 chambers which also allows more readily for several progeny (Croy et al., 2014). It is made up of 2 horns joined at the bottom by the cervix and running upwards into the ovaries. Despite this morphological difference, there are similarities between the structure of the uterine layers of the mouse and that of the human. They both have the inner endometrium, the central myometrium and the outer perimetrium or serosa. The mouse myometrium is made of circular and longitudinal layers separated by a vascular layer that contains blood vessels. The uterine artery provides blood to the uterus and gives ramifications through the myometrium and expends to the arcuate arteries following by radial and spiral arteries. The size of the uterine artery increases significantly during pregnancy in human and mice species (K. Griendling et al., 1985, Palmer et al., 1992).



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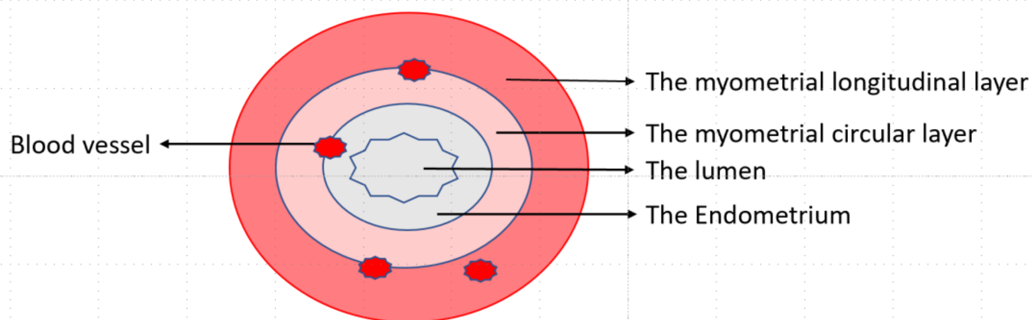


Figure 1.6. Anatomical structure of mouse uterus (Basic). A and **B:** Gross anatomical structure of pregnant and non-pregnant uteri (Biorender.com image library; images taken by Tshikaya Kaleta) **C:** Cross-sectional image of the non-gravid mouse uterus, illustrating different layers of the myometrium and blood vessels. (Drawn by Tshikaya Kaleta)

The average lifespan of a mouse is 24 months with a reproduction period of 7 to 8 months (Wilkinson et al., 2012). Numerous factors including strain, diet, age, breeding environment, pheromones, and general health can influence mouse reproduction (Baker et al., 1932; Keveme, 1983). Male and female mice reach sexual maturity between the ages of 4 and 7 weeks (Nelson et al., 1990). Mice have a short oestrous cycle (4-5 days), with ovulation occurring 8 to 12 hours after the cycle begins. In the event of fertilization, growing embryos can be felt by day 14 of gestation. The gestation period in mice ranges from 18 to 22 days, depending on the strains.

The gestation period of the C57BL/6J strain of mice used in this study is 18.5 days (Atchley, 1991). In human, the length of the cycle is variable, however an average duration is 28 days from the beginning of one menstrual period before the next. It starts at puberty and ranging from 10 to 16 years. Generally, the days of the cycle are determined by number commencing the first day of menstruation and the ovulation taking place between 13th and 16th days while the gestational length is 266±14 days (Coast et al,2019; Rostner et al,2022; Bryan et al,2009)

1.3 Uterine myocytes

In mice, as in humans, myocytes are the functional cells of the uterine smooth muscle. They are in charge of producing contraction forces, as well as propagating action potentials of uterine contraction control, and they are predominant in the uterus (Young, 2007). Structurally, they display specific features. As with other smooth muscle cells, they are elongated spindles with an oval core in the centre (Dawson and Wray, 1985). They vary in length that ranges from 30 to 50 µm in the non-gravid myometrium up to 500-600 µm in the gravid myometrium (Broderick, 1990; Blackburn, 2013). In the centre of the myocyte, there is a nucleus which takes a more or less oval shape during muscle constriction. When compared to their size at implantation, myocytes reach their maximum size at term (Blackburn, 2013). This makes them the body's largest smooth muscle cells. Gap junctions connect groups of myocytes to one another.

Myosin and actin are myofilaments found throughout the cell in the myocyte (Young, 2007, Wray, 1993). In smooth muscle cells, actin and myosin myofilaments are arranged obliquely across the cell, rather than in the rigid sarcomeric structure observed in striated muscle.

The myocyte plasma membrane is comparable to that in any cell of smooth muscle. It has ion channels, transporters, receptors, and proteins that provide ways for signal transmission.

The smooth muscle cell, in turn, contains three major proteins to regulate calcium: caldesmon, calponin and calmodulin. Calmodulin, a calcium binding protein that aids in contraction activation, is the most important protein for effective contractions (Aguilar and Mitchell, 2010). Calponin and caldesmon were linked to the regulation of contractile activity by influencing the binding and interaction between actin and myosin (Carmichael et al., 1994; Yilmaz et al., 2013). These cells contain several organelles which play a role in contraction, including the sarcoplasmic reticulum (SR) and mitochondria (Broderick and Broderick, 1990).

1.4 Mitochondria

Mitochondria are found in the cells of eukaryotic organisms. They are membrane bound organelles and provide the main place of aerobic respiration, hence their description as the cell's powerhouse (Broderick and Broderick, 1990). They have a diameter of approximately 0.5 to 1.0µm and make up 3 to 9% of the volume of smooth muscle cells. During normal contraction, in the smooth muscles, mitochondria play a key role in the control of intracellular concentrations of calcium $[Ca^{2+}]_i$ (Gravina et al., 2011; Gam et al., 2018). They can absorb, store and release Ca^{2+} (Gravina et al., 2010) and interact with the SR (Rizutto et al., 1998; Csordas et al., 2006).

Gravina et al. (2011) also demonstrated that oxytocin induces mitochondrial membrane potential (ψ_m) depolarisation in isolated myometrial cells. This effect was probably due to the fact that oxytocin is inciting a rise in cytosolic $[Ca^{2+}]$, increasing mitochondrial Ca^{2+} uptake and causing mitochondrial electrochemical gradient to disperse (Pandey et al., 2021). The amount of adenylate kinase and hexokinase were found to be higher in the pig uterine mitochondria than in the heart in a study performed by Clark et al. (1997). Patel et al. discovered that in older mice the copy number of myometrial mitochondria was reduced, but there was no age-related change in the enzymatic behaviour of the mitochondrial electron transport chain multiplexes. (Patel et al., 2017). Researchers have been interested in investigating to establish a link between changes in mitochondrial activity or phenotype and different pregnancy related disorders, but no mitochondrial changes have been noted in diabetes and obesity (Gam et al., 2017; McMurtrie et al., 1985) and minimal changes were recorded in pre-eclampsia (Shanklin and Sibai, 1990).

1.5 Sarcoplasmic reticulum (SR)

Muscle cells contain a sarcoplasmic reticulum (SR). The SR exhibits the properties of an endoplasmic reticulum. It is a network of interconnected tubules with running directions that are consistent with the direction of the major axis of the muscle fibres (Somlyo, 1985). The SR occupies about 5% of the total volume of smooth muscles (Wray and Burdyga, 2010). SR's functions include protein synthesis (coarse SR, whose volume increases during pregnancy) and calcium storage and release. (Ross and Klebanoff, 1971). The sarcoplasmic reticulum is an internal calcium store in all muscle types but with different size and role between smooth muscles. Its role may also vary with development, physiological status, or illness within the same smooth muscle (Wray and Burdyga, 2010). The myometrium has one of the largest sarcoplasmic

reticula. Different methodologies have been developed to show the distribution of the sarcoplasmic reticulum in the myometrium; to identify the Ca^{2+} release and Ca^{2+} uptake zones (confocal imaging); and to monitor changes in Ca^{2+} concentration ($[Ca^{2+}]$) in the cytoplasm (Ca^{2+}-sensitive fluorescent indicators). These methods demonstrate that the myometrium contains an SR that spreads throughout the myocyte near the myofilaments. Furthermore, the network is visible around the nucleus and near the plasma membrane (peripheral SR). (Ross and Klebanoff, 1967). Calcium ions are transported from the cytoplasm into the SR by the sarcoendoplasmic reticulum calcium transport ATPase (SERCA). SERCA has been found in both animal and plant cells.

At the protein level, more than ten different SERCA isoforms have been identified. This pump is encoded by genes from the SERCA family (SERCA1, SERCA 2, and SERCA3) which are well conserved but located on different chromosomes. The SERCA pump is essential for muscle contraction. Several studies on its role in cardiac smooth muscle and skeletal muscle diseases have been conducted. It was discovered that SERCA pump expression and activities decline with age and in some pathophysiological disorders such as heart deficiency. Recent studies have also shown that SERCA pump gene transfer is effective in restoring contractile activity in failing heart muscle, highlighting its importance in muscle physiology and capacity as a new therapeutic agent.(Periasamy et al., 2007).The SR is able to release Ca^{2+} through inositol triphosphate (IP_3)-mediated activation of IP_3 receptors or activation of Ca^{2+}-gated channels called Ryanodine receptors (RyR) situated on its membrane. RyR and IP_3 receptors are found in the myometrial SR. Ca^{2+}-induced Ca^{2+}release (CICR) was thought to be an important contributing factor to uterine physiology due to the presence of RyR.

However, researchers have not been able to demonstrate CICR in the myometrium (Matsuki et al., 2017). Moreover, it was found that an agonist at RyR, such as caffeine, was not able to stimulate the release of Ca^{2+}. On the contrary, it has a relaxatory effect on the myometrium through an increase in cAMP and inhibition of phosphodiesterase. In some smooth muscles, Ca^{2+} sparks, small spontaneous releases of Ca^{2+} from RyR, were able to activate Ca^{2+}-activated ion channels in the membrane, such as large conductance Ca^{2+}-activated K+ channels (BK), triggering spontaneous transient outward currents (STOCs) that reduce membrane excitability and muscle contractility (Burdyga and Wray 2002). However, Ca^{2+} sparks have not been observed in the myometrium (Burdyga et al., 2007). Different results from several researchers came to the conclusion that RyR in the myometrium were not functional (Matsuki et al., 2017).

Therefore, the absence of Ca^{2+} sparks likewise implies that Ca^{2+}-activated K^+ and Cl^- channels in the myometrial tissue are not stimulated in this way. The SR contributes to agonist activation of force in the uterine muscle by IP_3-gated Ca^{2+} release, decreasing SR luminal Ca^{2+} and activating Orai-Stim mediated store-operated Ca entry (SOCE). This entry of Ca^{2+}can generate a continuous depolarization and increase force. Therefore, in the uterus, the SR plays a pivotal role in contributing to membrane excitability, predominantly via excitation from SOCE (Noble et al., 2009).

1.6 Gap junctions

Connexins, or gap junctions, are well-established intercellular pores found between cells. They allow inorganic ions (such as Ca^{2+}) and small signaling molecules (less than 1000 kDa) (such as cGMP, cAMP, and IP_3) to pass directly through (Harris, 2007). They also allow electrical impulses to pass between adjacent cells' cytoplasm (Alberts, 2008). Connexins are integral membrane proteins that form hexagonal hemichannels with N- and C-terminal cytoplasmic zones that are linked by four transmembrane zones, an intracellular loop and two extracellular loops (Gerald and Elke, 2015). In other words, each gap junction is made up of two half-channels, each of which consists of six connexin proteins (Figure 1. 7).

The gap junctions are thus a type of intercellular canals between cells, but also known as gap junction proteins, which differ in their structures, tissue distribution, and role (Sakai et al., 1992). They are also unique in that they play a role in female reproduction physiology.

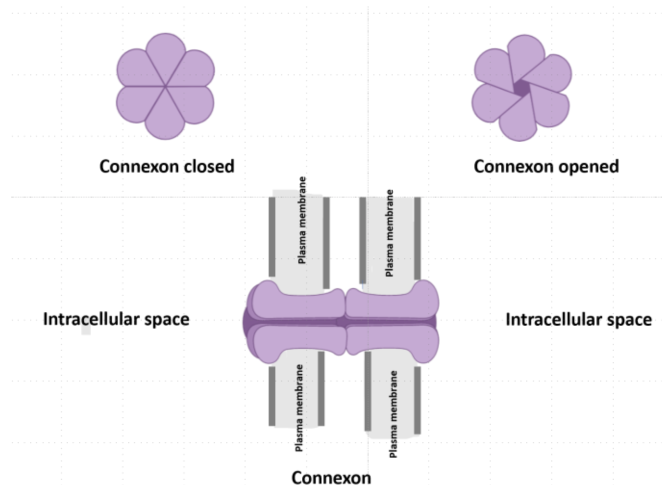


Figure 1.7. The structure of gap junctions. Every connexon contains six connexins and two semi channels unite to make a gap junction. Pores established by the connexin proteins allow communication and exchange of metabolites and ions between cells (adapted from Biorender library images).

The gap junction allows the uterus to function as a syncytium, allowing excitability propagation and the passage of ions and small molecules (Wray, 1993). The gap junction proteins' expression increases as the pregnancy progresses to term and peaks during childbirth, demonstrating their role in activating the myometrium during parturition (Sheldon et al., 2014; Xu et al., 2015). There is a link between increased uterine contractions frequency, increased cervical dilation and myometrial junction expression (Garfield et Hayashi, 1981).

Many studies have proved that as the number of gap junctions increases, so does metabolic and electrical coupling (Xu et al., 2015), and the myometrium's ability to induce action potentials improves during childbirth (Sheldon et al., 2014).

The majority of these gap junctions, however, are reduced to their initial number within 24 hours of giving birth. In human myometrium, gap junction connexins are a family of 21 genes and cognate proteins (Kidder and Winterhager, 2015). The gap junction permeability is controlled by GPCR agonists such as oxytocin, isoproterenol, and prostaglandin E2 (Laird, 2010). This permeability is similarly controlled by protons that could be linked with uncoordinated parturition (Swietach et al., 2007).

1.7 The physiologic role of uterine contractions

1.7.1 Background

The uterus is generally known for its function of guarding, protecting, and nourishing the foetus throughout pregnancy and ultimately contracting to expel the foetus during labour. There is low intensity contractile activity in the uterus throughout a woman's life. This contractile activity is present during menstruation, facilitating the evacuation of endometrial debris during fertilisation, and propelling the sperm from the vaginal cavity, then to the uterine cavity, and then to the fallopian tubes to reach the fertilised ovum (Prendergast and Wray 2019). The myometrium that is accountable for producing the electrical signals that cause uterine contractions. These signals can be recorded with electrodes placed directly on the pregnant woman's abdomen through a process called cardiotocography (CTG) (Garfield et al., 1998).

The CTG is performed during labour to monitor the fetal heartbeat and uterine contractions (Figure 1.8). It is a non-invasive procedure that helps assess the well-being of the baby and the progress of labour. This is crucial for ensuring the well-being of the baby and guiding proper management and care throughout the childbirth process. During a CTG, two sensors are placed on the mother's abdomen. One sensor records the baby's heart rate, while the other sensor measures the intensity and frequency of the mother's contractions. The readings from both sensors are displayed on a graph or monitor. CTG monitoring provides valuable information about the baby's oxygen supply and overall fetal health. It can detect any abnormal changes in the fetal's heart rate that may indicate distress or complications. It also helps assess the effectiveness of contractions in promoting labour progress and is especially important in high-risk pregnancies or when complications are suspected. It allows healthcare professionals to make informed decisions regarding interventions, such as administering medication, changing positions, or deciding on the need for further medical attention.

Cardiotocography monitoring

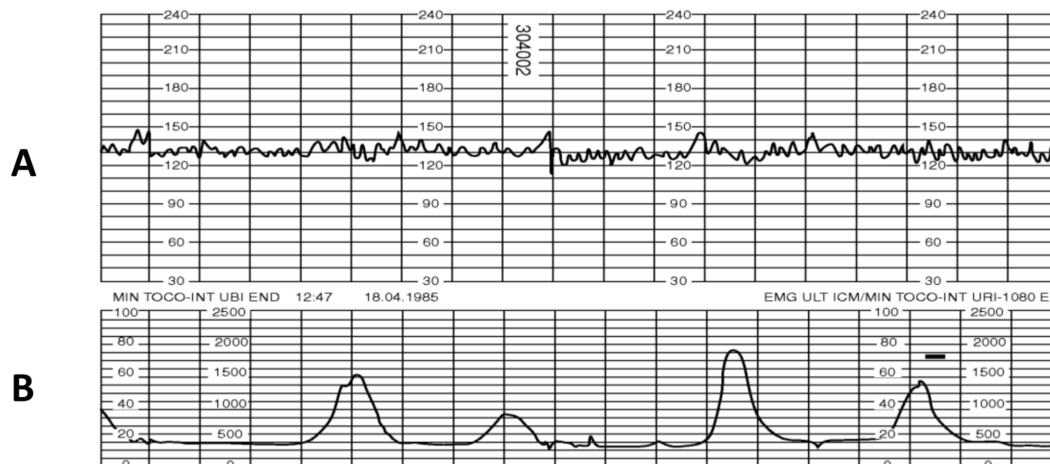


Figure 1.8. Cardiotocography. A. Fetal heart rate trace recordings. **B**. Uterine contractions trace recordings (Cambridge University Press)

In the context of *in vitro* measurement of myometrial contractions, these occur spontaneously and last for many hours in the absence of hormonal or neuronal stimulus (Wray ,1993; Gullam et al., 2009). In my thesis, the contractile activities were recorded in an *in vitro* organ bath system using myometrial biopsies from women undergoing caesarean section and from mice. Myometrial strips were connected to a force transducer and appropriate software were used for the recording of data (Well illustrated in chapter 2).

Understanding the physiology of uterine excitability and contraction during pregnancy and birth is paramount for tackling myometrium related complications, such as preterm birth and dysfunctional labour. I find it useful to next describe the nature of excitability in the myometrium, and then the mechanism of contraction.

1.7.2 Resting membrane potential (RMP) and action potentials

The voltage or electric potential difference (discrepancy) between the outside and inside of a cell is called the resting membrane potential and is determined by the balance of activities of the ion channels. Its usual values vary between -40 and -80 mV. The myometrium is a smooth muscle and is able to contract spontaneously. These contractions are produced as a direct effect of action potentials generated within the myometrial cells. Great variations have been observed in the measurement of action potential time course. The measurement outcome depends on

the zone of the uterus measured, the gestational condition and the species. Complicated action potentials which are characterised by continuous or prolonged plateau of depolarisation were recorded in the myometrial tissues of pregnant guinea pigs, mice, and rats where initial spikes result in a constant depolarisation estimated at -30 mV in rat; the length of these myometrial contractions is determined by the duration of this plateau. (Parkington and Coleman 1988)

When the membrane potential of myometrial cells depolarises and reaches the threshold, the L-type Ca^{2+}channels open, followed by the entry of Ca^{2+} into the cell. The Ca^{2+} channel threshold potential has been estimated to be -40 mV. The inward Ca^{2+} current reach their high point at around 10ms before gradually decreasing (Perez-Reyes,2003). The Ca^{2+} dependent deactivation of voltage-dependent L-type Ca^{2+} channels and the opening of voltage dependent K^+ channels initiating hyperpolarisation of outward K^+ current, both contribute to repolarisation of the cell membrane. It is believed that the RMP of the uterine myocytes become more depolarised while the pregnancy is progressing towards term (Sanborn,2000). A study conducted on the last three months of pregnancy in women revealed that the membrane potential progressively rises from -70 mV at 29 weeks of pregnancy to -55 mV at labour. This progressive depolarisation process was associated with a progressive increase of uterine contraction frequency. A reduction in the RMP's negativity would ease the Ca^{2+} entry through L-type Ca^{2+}channels. In late pregnancy, the decrease of potassium channels in the myocyte membranes contribute to the prolongation of myocytes action potentials and boost uterine contractility (Parkington et al., 1999).

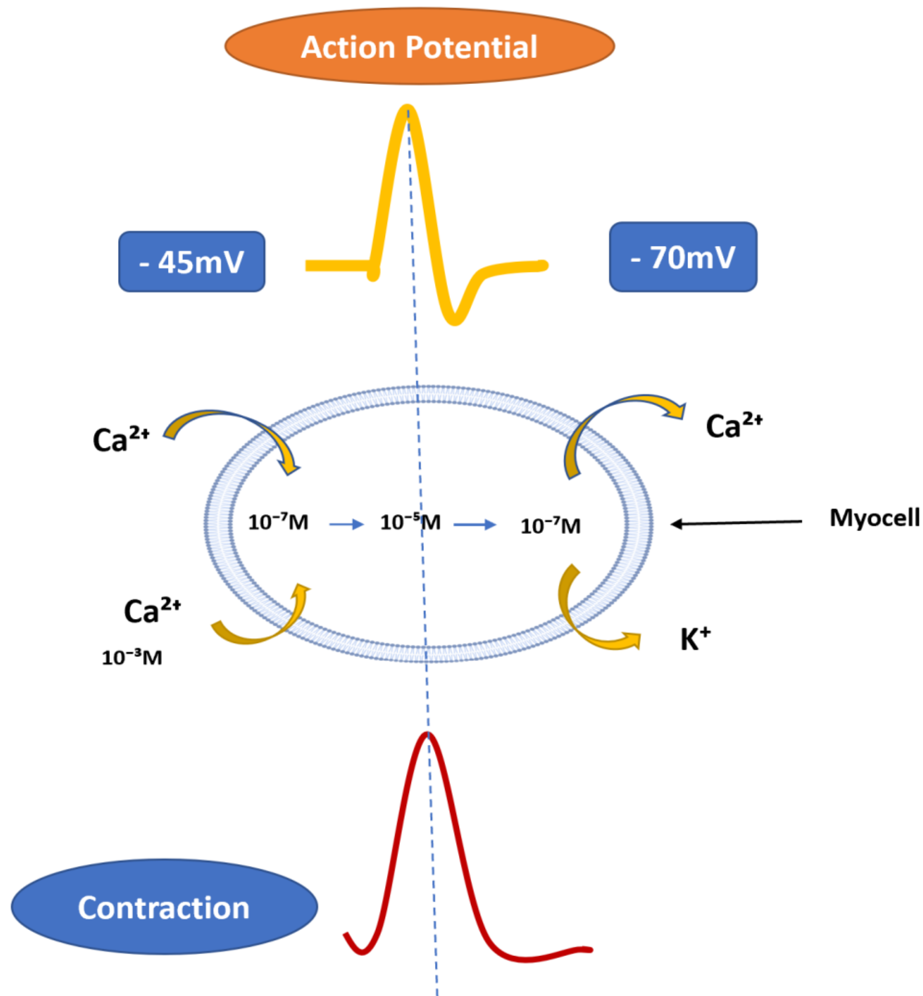


Figure 1.9. Illustration displaying membrane potential, calcium movement and muscle contraction. The movement of ions through the membrane causes the membrane potential to gradually rise and fall without crossing the threshold point. Depolarisation of the membrane happens when the calcium channel opens followed by the influx of calcium into the cell, causing the production of action potential. Repolarisation happens when the potassium channels open followed by the exit of potassium from the cell and the closing of calcium channels.

1.7.3 Excitation - contraction coupling (ECC)

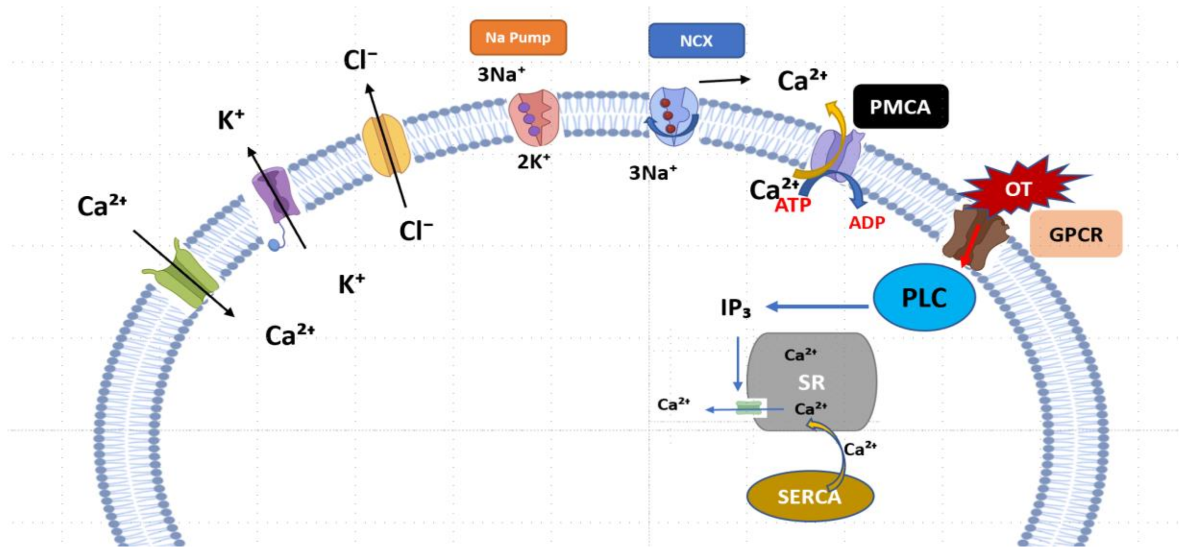


Figure 1.10. Myometrial ion movement: channels, exchangers, and pumps. Calcium influx is regulated by voltage gated Ca^{2+} channels. This is indispensable for uterine contractions. Calcium exit is controlled by two pathways: sodium-calcium exchange (NCX) and plasma membrane Ca-ATPase (PMCA). The sodium pump eliminates $3Na^{+}$ associated with $2K^{+}$ the entry. The SR stores Ca^{2+} through SERCA and liberates Ca^{2+} through the IP_3 gated channel. Oxytocin, as an agonist, binds to G-protein-coupled-receptors and through phospholipase-C (PLC) activates IP_3, followed by the release of Ca^{2+} into the cytoplasm and improved uterine contractility.

The excitation-contraction coupling (ECC) is caused by the sequence of activities that occur after an action potential and the onset of muscle contraction, which is controlled by $[Ca2+]_i$ (Pehlivanoglu et al., 2013). The depolarization of the plasma membrane is the most important cause of the increase in $[Ca2+]_i$. The surge in $[Ca2+]_i$ is required for myometrial contractions to occur. As soon as Ca^{2+} enters the cell, it binds calmodulin in such a way that four Ca^{2+} bind each calmodulin molecule then activate MLCK. In turn, on the regulatory light chains of myosin, MLCK phosphorylates serine 19. This process activates the interconnection of phosphorylated myosin with actin microfilaments. Contraction occurs as a result of the subsequent cross-bridge cycling. This is an active mechanism that necessitates ATP hydrolysis. Once MLCK is inhibited, contractions will be abolished. Relaxation follows when Ca^{2+}channels are deactivated along causing the membrane potential to come back to the resting state so that as Ca^{2+}levels decline, MLCP dephosphorylates the myosin light chain (Stephens et al.,1992).

Depolarisation is frequently linked to pacemaker cell activity but the existence of pacemaker cells in the uterine muscle is still under discussion (Shmygol et al., 2007). Interstitial cells of Cajal (ICC) were implicated in the pacemaker activity in some smooth muscles where permanent peristaltic waves exist. These include, for example, the gastro-intestinal tract and the urinary tract. These Interstitial cells of Caja-like cells, usually called telocytes, have been discovered in the myometrium (Duquette et al., 2005) and were believed to play a pacemaker function (Othman et al., 2016); however, this theory is still under discussion. Besides, according to the results available from the literature, Roatesi et al. (2015) indicate that the ICC pacemaking function is not even needed in the myometrium (Campeanu et al., 2014); however, they can play a role in the smooth muscles as they are having a constant peristaltic wave to evacuate their content. The absence of permanent slow waves of depolarisation in the telocytes located in the myometrium has been proven (Cretoiu et al.,2013) and corroborate this point of view. In humans, the location and the role of pacemaker cells in the uterus during labour are still being hunted by researchers (Lammers 2012). The traditional theory stipulates that the uterine contractile waves originate from the fundus and progress down to the lower segment of the uterus (Buhimshi, 2009). However, based on recent discoveries, it has been suggested that in rat and guinea pig uteri the pacemaker function takes place more regularly near the ovarian end of the horns (Lammers et al., 2015).

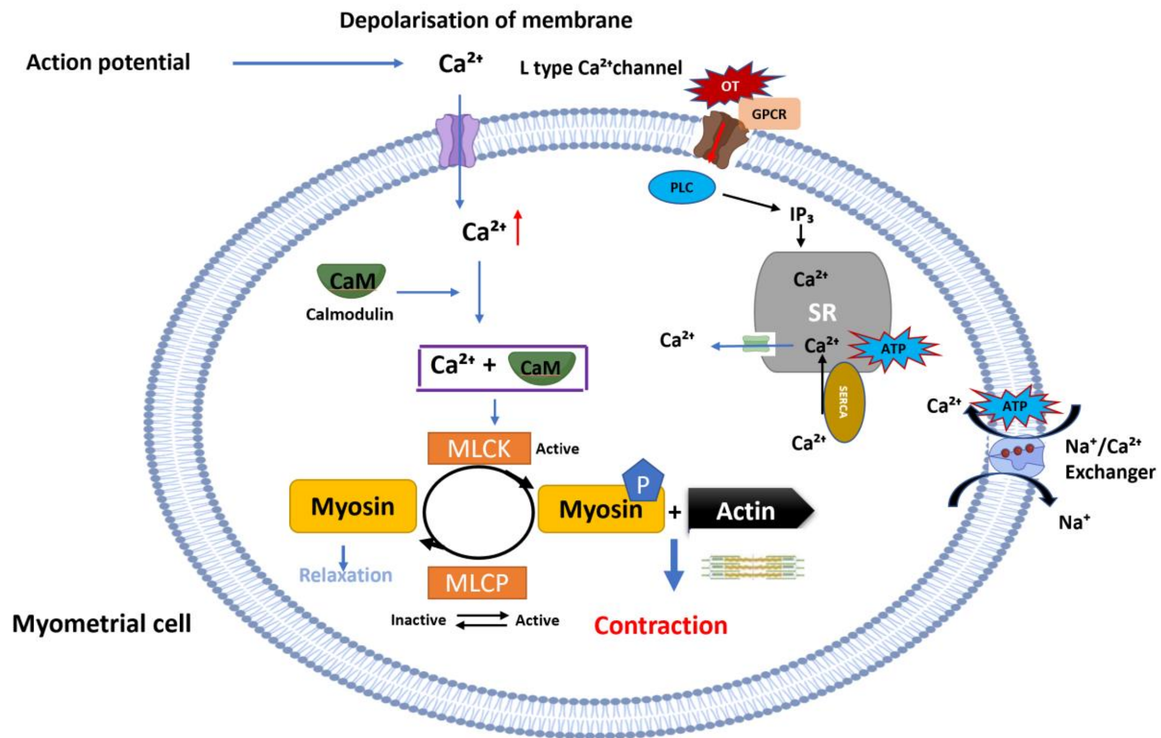


Figure 1.11. A schematic representation explaining the excitation- contraction coupling. This procedure starts with an action potential and depolarisation of the membrane followed by some changes in the ionic permeability of the membrane. The opening of the voltage gated Ca^{2+}channels is followed by the influx of Ca^{2+} across the plasma cell. Ca^{2+} binds to calmodulin (CaM) to creäte a complex Calcium-CaM. This complex in turn, set in motion myosin light chain kinase (MLCK) so that it phosphorylates myosin light chains (P). The phosphorylated myosin connects to actin then, triggers the cross-bridge cycling, resulting in uterine contractions. The dephosphorylation of the myosin light chain results in relaxation.

1.7.4 Ion channels present in the myometrium and contributing to E-C couple.

Ion channels are pore-forming proteins allowing movement of ions through either plasma membranes or intracellular organelle membranes (Hille,2001). Cells contain a wide range of ion channels. Ion channels are classified based on their structure; some channels, such as L-type Ca^{2+} channels, are voltage gated, while others are voltage-insensitive, such as IP_3 receptors, and are gated by intra or extracellular mediators or messengers. (Yu et al., 2005). Nowadays, ion channels represent the second greatest target for current drugs, following G protein coupled receptors (Overington et al., 2006).

1.7.5 Voltage-dependent calcium channels

The L-type and T-type calcium channels are two types of voltage-dependent calcium channels found in the myometrium (Young et al., 1993; Wray and al., 2003). The third type, N, is mostly found in neurons. Each channel consists of four distinct subunits: 1) Alpha (α); 2) Beta (β_{1-4}) ;3) Gamma (γ) and 4) Delta(δ). The first subunit is an ion conducting pore subunit, while the other three are regulatory subunits. (Dolphin, 2006). The subunit α contains especially useful pharmacological characteristics of the Ca^{2+} channels, relating to ion permeability, drug binding and voltage detection, while the other subunits are considered to be auxiliary. The most predominant and important subtype of voltage-dependent calcium channels in the myometrium is type L, which is required for the coupling E-C (Lipscombe et al., 2004). They provide stable and durable high voltage activated flux. In this way, they constitute a major part of the current of calcium which is contained in the myometrium. Thus, fluctuations in the expression and regulation of the L-type calcium channels may be expected during pregnancy and labour (Tezuka et al., 1995). The sex hormones have been shown to direct their capacity (Batra, 1987). The inhibition of L-type Ca^{2+} channels (e.g., by drugs such as nifedipine) suppresses the myometrial action potentials, calcium transients and myometrial contractility (Coleman et al., 2000).

While the $\alpha_2\delta$ sub-unit supports the channel's surface expression, the sub-unit β inhibits the ubiquitination, as well as proteasomal breakdown of the channel protein and facilitates the channel movement to the membrane (Altier et al., 2011). The voltage threshold required to activate Ca^{2+}current is around minus 50 to minus 40 mV. When depolarisation conditions were induced by a holding potential of minus 80 mV, the average maximum current density was almost equal to 6.3 pA/pF (Inoue et al., 1991, Miyoshi et al., 1991). Hyperpolarisation conditions cause these channels to close. A fast Ca^{2+}-sensitive inactivation is also present, which is a negative feedback process that prevents the cell from becoming Ca^{2+} overloaded. When voltage gated L-type Ca^{2+}channels are deactivated, uterine contractions cease. During pregnancy, the expression of voltage gated Ca^{2+}channels vary. It was found in the rat that the number of α_1-subunit increased four times by day 14 of pregnancy and this expression was maintained throughout until labour (Mershon et al., 1994). In the same way, the α_1-subunit's expression

increased gradually throughout gestation and then decreased during parturition (Tezuka et al., 1995), while β-subunit expression increased rapidly on day 22 then decreased during labour. In human myometrium a similar increase of expression was observed with α_1-subunit and L-type Ca^{2+}channel function (Longo et al., 2003). The T-type form of Ca^{2+}channels have also been identified and their expression (mRNA) has been established in the myometrial tissue (Ohkubo et al., 2005; Blancks et al., 2007). Interestingly, electrophysiology studies have detected a nickel-sensitive T-type Ca^{2+} current (Longo et al., 2003), unfortunately the physiological relevance and the T-type Ca^{2+} channels role has not yet been proven.

1.7.6 Calcium regulation

Intracellular calcium regulation can be split into three different stages: i) the maintenance of basal concentration in order to sustain the resting tone of uterine muscle; ii) a spike in intracellular calcium following the spontaneous generation of an action potential or stimulation by an agonist, resulting in the generation of contractions; and iii) the restoration of intracellular calcium ions to the resting state after activation. These stages are mainly managed by changes in the permeability of the membrane to calcium via the opening or closing of calcium channels, the release of calcium by SR, and mechanisms of exchangers and pumps to maintain and restore intracellular [Ca^{2+}] (Aguilar and Mitchell, 2010)

When the L-type calcium channels open, after depolarization, calcium enters down both its electrical and concentration gradients. This is the main source of calcium provision. As free intracellular Ca^{2+} changes from a resting state of from, for example 100 nM to 1000nM, it triggers contraction. To produce spontaneous contractions, extracellular calcium mainly enters the myocyte via the voltage gated calcium channels in the myometrium.

1.7.7 Calcium influx

The main route of calcium penetration into the cell remains the L-type calcium channels. Calcium is also able to enter the cell via receptor operated calcium channel or store operated calcium channel (Putney & Ribeiro, 2000). Thromboxane, oxytocin, and prostaglandin FP are all agonists of these channels.

1.7.8 Calcium exit

Two mechanisms are responsible for Ca^{2+} elimination in the myometrium: the plasma membrane Ca^{2+}-ATPase pump (PMCA) and the Na^{2+}/ Ca^{2+}exchanger (NCX). (Shmigol et al., 1998).

While the entry of Ca^{2+} takes place down the electrochemical gradient, the extrusion of Ca^{2+} follows at the same time in opposition or against the electrochemical gradients, requiring ATP as energy. This type of energy can be generated directly (in the case of PMCA) or indirectly (in the case of NCX). However, if these mechanisms are abolished, calcium recovery at rest can be eliminated. (Shmigol et al., 1999). PMCA accounts for 65% to 70% of calcium efflux, with NCX accounting for the remaining 30% to 35%. Calcium is forced out of the cell by PMCA and NCX (Taggart et al., 1997).

1.7.9 Potassium channels

Potassium ions are the major intracellular ions and will efflux from the myocyte when the channels open. When this happens, the cell will hyperpolarise. Thus, activation of K^+ currents occurs when the membrane repolarises following an action potential. The main function of potassium channels is to keep the membrane potential at rest near the K^+ ion reversal potentials (which is approximately – 84 mV). Thus, K channels represent a major mechanism for suppressing the generation of the action potential as well as the cellular excitability and contractions (Khan et al., 2001).

The RMP, which is the voltage difference between the inside and outside of a cell, is significantly influenced by potassium channels. Contractions are caused by action potentials generated within myometrial cells.The activation or inactivation of K^+ channels affects the repolarisation or the time course of the action potentials; that explains why K^+ channel action is frequently linked to the quiescence of the uterus and why variations in potassium channels are associated with the beginning of labour (Smith et al., 2007).The uterine muscle cells contain a large number of potassium channels. These include 1) voltage- dependent channels (K_V);2) large-conductance calcium-dependent channels (BK);3) small-conductance calcium dependent channels (SK);4) ATP-sensitive channels (K_{ATP}). It is important to mention that the key factor of the resting membrane potential under resting conditions is the K^+ conductance (Wood, 2005).

The large-conductance channels (BK) are the most abundant in both non-pregnant and pregnant in human myometrium (Khan et al.,1997) and have minor functional role (Noble et al.,2010). However, the K_{ATP} and the K_V channels have been suggested to take part in maintaining uterine quiescence and the induction of uterine contractions (Brainard et al.,2007)

1.7.9.1 Voltage-dependent potassium channels (Kv)

These channels are largely represented in the myometrium. These channels are activated by the depolarisation of the plasma membrane. The efflux of K^+ from cells results in the repolarisation of the RMP. When these channels were inhibited by a channel blocker (for instance, tetraethylammonium), uterine contractility increased (Brainard et al., 2007).A variety of subtypes of voltage-dependent K^+ channels have a vital part in sustaining the membrane potential of uterine myocytes and contribute to uterine quiescence during gestation or regulating the transition from quiescence to parturition (Knock et al., 1999).K_V7 and K_V11 channels have been involved in the regulation of uterine contractility (Greenwood and Tribe, 2014). Parkington et al. (2014) revealed that ERG activities (K_V11) result in the inhibition of uterine contractions. The ERG's inhibitory β -subunit's expression increases during parturition, resulting in a fall in ERG activity and initiation of uterine contractions.

(Greenwood et al., 2009). Parkinton et al. (2014) went on to demonstrate that in obese women, the inhibitory β-subunit level is low; as a result, this will increase ERG action and K^+ conductance, then, apparently leads to weaker uterine contractions. Studies have already shown that the elevated level of cholesterol in women with obesity increases the outward K^+ current. Obese women's increased outward K^+ conductance may be a key factor in understanding why they are more likely to require caesarean section delivery (Smith et al., 2005).

The $K_V4.3$ channels is another part of the Kv family that has been identified in the myometrium and seems to play a role in the quiescence of the uterus and transition to labour. In mouse, the expression of these channels is considerably decreased in the myometrium at term (Smith et al., 2007).

1.7.9.2 Large-conductance calcium dependent channels (BK)

In all smooth muscles, BK channels are present. In the myometrium they are expressed with quite high density. Because of their large conductance of almost 200 pS, they were expected to play a significant role but blocking these channels had a minimal impact on either calcium signalling or uterine contractions (Aaronson et al., 2006). This lack of effect could be attributed to the fact that the myometrial cells do not generate Ca^{2+} sparks (Burdyga et al., 2007). This means the stimulation of BK channels could only be minimal.

1.7.9.3 Small-conductance calcium dependent channels (SK)

The stimulation of SK channels controls membrane excitability by triggering the hyperpolarisation of the membrane (Church et al., 2015). In contrast, the small conductance channel (SK) blockers reduce the outward current, resulting in the depolarisation of membrane in uterine myocytes and increased myometrial contractility throughout pregnancy (Noble et al., 2010). Studies on transgenic mice revealed that the myometrial overexpression of the SK3 channel was able to delay parturition (Pierce et al., 2008).

1.7.9.4 ATP-sensitive channels (K_{ATP}).

The K_{ATP}, or ATP-sensitive, potassium channels also have a vital role in the regulation of uterine quiescence during gestation; they are downregulated before parturition (Brainard et al., 2007). K_{ATP} channels are stimulated by MgADP but inhibited by intracellular ATP. As a result, they participate in coupling the metabolic condition to the excitability of myocytes. In their experiment, Robinson, and Wray (2012) used cyanide to explore the role of potassium efflux on myometrial contraction in non-pregnant and pregnant rats and found that cyanide considerably decreased spontaneous uterine contractions. Myometrial contractions reappeared when depolarisation of membranes was induced by raising external K^+. They suggested that the inhibition of uterine contractile force previously achieved was attributed to the lack of membrane excitation. Uterine contractions were reduced by increasing the K^+ efflux from the myometrium (Robinson and Wray 2012).

As an antagonist of the K^+ATP channel, glibenclamide (20 µM) was able to reduce the cyanide-induced elevation of 86Rb efflux by approximately 50%.Therefore, Heaton and colleagues assumed that metabolic depression generated by cyanide, increases the K^+ efflux from the

myometrial tissue and results in hyperpolarisation of the membrane, and therefore decreases myometrial membrane excitation and causes the decline of force in hypoxia, particularly the preliminary loss of spontaneous uterine contractions(Heaton et al., 1993).Stretch-activated K^+ Channels are also found in myometrial cells (Bai et al., 2005; Buxton et al., 2010). Researchers discovered TREK-1 channels that are activated during gestation and parturition. (Buxton et al., 2010). In response to stretch, TREK-1 was found to be stimulated and to enhance outward current in both animal (mouse) and human myometrial cells (Heyman et al., 2013). The gene expression of TREK-1 increases gradually during pregnancy and falls at labour. The channels played a vital role in maintaining probably the uterine quiescence as the foetus grows and the uterus expands (Heyman et al., 2013). The ATP-sensitive potassium channels are constituted of sulfonylurea receptors (SUR) which give selectivity for drugs, ATP, and potassium inward rectifiers (Kirs). These channels (KATP) open when [ATP] declines and are regulated by [ADP] as well as pH (Teramoto, 2006). It has been suggested by Xu and colleagues that in animal and human myometria, functional K_{ATP} channels expression declines at term (Sawada et al. 2005, Xu et al.2011). Besides, it has been reported that as women age, there is a modulation of these channels. Changes in Kir and SURs decreasing levels of expression may be a contributing factor to more difficult parturition in older nulliparous women (Du et al.2013). The inhibition of these channels results in the improvement of force and duration of uterine contractions (McCloskey et al., 2014). As these channels are ATP-dependent, they are not likely to be effective during resting conditions or contribute to RMP. The antagonistic effect of glibenclamide was found to be minimal on either spontaneous or oxytocin-induced contractions even in in vivo conditions (Pipa et al.1990). In pregnant uterine muscle, KATP channel openers decrease either spontaneous or oxytocin-induced contractility; however, this inhibition effect is less in labouring women compared to non-labouring (Longo et al.2003, Xu et al.2011). Within channel groups, subunit expression differences have been detected. Kir6 tends to be less abundant in parturition; SUR1 increased, while SUR2B did not change (Xu et al.2011). It could be useful to explore the role of these channels in uterine arteries during labour as there is vascular compression due to transient ischemia at each contraction. In these conditions, Adenosine diphosphate [ADP] and [H^+] increase and [ATP] declines leading to the opening of channels (Lacombe et al.1999). It was reported that the decline in contractile force was explained by hyperpolarisation caused by the increase of K^+ efflux via KATP channels (Heaton et al.1993).

Experiments in human tissue confirmed that nitric oxide decreases myometrial contractility by direct activation of KATP channels (Modzelewska et al.1998).

1.7.10 Sodium channels

Voltage-gated Na^+ channels (VGSCs) have been shown to be capable of mediating phasic contractility maintained over prolonged periods in the myometrium of non-gravid rats (Seda et al. 2007). The fact remains however that, unlike voltage-gated calcium channels, there is little compelling evidence for a predominant role of Na^+ in myometrium. The Na^+ leak channel (NALCN) produced the Na^+ dependent leak currents inside the uterine muscle cells (Reinl et al., 2015). They revealed that NALCN contributes ∼50% of the Na+ leak in non-labouring women's myometrial cells and that mice lacking uterine NALCN had action potentials with shorter burst periods and fewer spikes than wild-type controls and suffered dysfunctional labours.
Yoshino et al. reported the presence of fast sodium channels in rat uterine muscle (Yoshino et al., 1997) and Na^+ current expression was found to be increased in mid and late pregnancy in human uterine muscle (Boyle & Heslip 1994).
In the rat, studies conducted on variation in Na^+ and Ca^{2+} channel current densities in rat during pregnancy, suggested that the role of fast Na^+ channels was crucial in spreading excitation in uterine smooth muscle and this seems to be more enhanced as the pregnancy approaches term (Inoue and Sperelakis,1991).

1.7.11 Chloride channels

Chloride (Cl^-) channel expression was confirmed in a different smooth muscle including vascular (e.g., mesenteric artery, portal vein (Klockner, 1993, Hogg et al., 1994) and myometrium (Adaikan,2005). Two forms of Cl^- channels were detected in smooth muscles. These are volume regulated chloride channels (CIVr) and calcium activated chloride channels (ClCa) and can be stimulated by a combination of pH changes, ligands, or intracellular calcium (Shi et al., 2007). Smooth muscle cells have a higher intracellular Cl^- concentration than most other cells, according to research (Aickin and Vermue ,1983). The value found (of around 50mM) suggests that there should be an active system keeping intracellular [Cl^-] higher and the balance potential of Cl^- at around -20 mV (Aickin and Vermue ,1983). This means that the exit of chloride ions causes depolarization, which causes the L-type channels to open and the entry of calcium. Negative ions leaving the cell are electrically equal to positive ions that enter the cell, so

depolarisation occurs. During gestation myometrial cells increase in volume and it is believed that chloride volume regulated channels may contribute to this growth. The calcium activated chloride channels regulate excitability in rat myometrium and trigger membrane depolarisation and subsequently the opening of voltage gated calcium; the calcium influx that follows produce uterine contractions. Once these channels are inactivated, inhibition of contractions will ensue by repolarisation of the cell membrane (Jones et al., 2004). This has been confirmed by Adaikan and Adebiyi, by blocking ClCa channels with niflumic acid in a pregnant rat myometrium (Adaikan and Adebiyi, 2005). The chloride channels help to produce contractile force and spontaneous contractions in smooth muscles, the uterus included (Yarar et al., 2001). It is important to be empowered with information concerning chloride channels to better understand their excitability in the myometrium.

1.7.12 Ca^{2+}-Activated Cl^- Channels.

In addition to volume regulated Cl^- channels, there is another Cl^- channel expressed in the myometrium, the Ca^{2+}-activated Cl^- channel (CaCC), that is sensitive to voltage as well as Ca^{2+}. CaCC-mediated responses are activated by release of Ca from intracellular stores. For example, agonists such as acetylcholine, angiotensin II, ATP, or noradrenaline, acting via their G protein-coupled receptors (GPCR), stimulate the leaving of Ca^{2+} from the SR through IP_3 receptors. It is this internally released Ca^{2+} which is accountable for the activation of CaCCs (Large and Wang 1996). The expression of CaCCs has been detected in both rodent and human myometrium (Elble et al., 2002, Daniel et a.,2018). In the myometrium, the role of these channels was explored by using the myometrial strips and isolated cells; it was found that a CaCC current was present in 30% of newly isolated muscle cells from rats (Jones et al., 2004). When these channels were blocked with niflumic acid, the uterine contraction's frequency decreased considerably in both spontaneously contracting or oxytocin-stimulated myometrial strips.Further studies have demonstrated that Ca^{2+}-activated Cl^- channels are upregulated at term and suggest that these channels may play role in boosting myometrial contractility during labour (Song et al., 2009).

1.7.13 Plasma membrane Ca-ATPase (PMCA)

PMCA is a P-type Ca-ATPase and plays a key function in preserving Ca^{2+} signalling and homeostasis by expelling Ca^{2+} that enters the cytosol during excitation. PMCA has 10 transmembrane spanning zones and is regulated by calmodulin. It has 4 types of isoforms (PMCA

1, 2, 3, and 4) with 80 to 90% amino acid sequence similarity. PMCA 1, 2b and 4 were identified in the myometrium (Penniston and Enyedi,1998). It is worth noting that the PMCA and the Na/Ca exchanger (NCX) are responsible for Ca2+ expulsion from isolated myometrial cells during the decline of a calcium transient. (Shmigol et al., 1999). All calcium efflux is prevented by the inhibition of these mechanisms. Studies from the rat uterus revealed that 65% of calcium released by SR was expelled by the PMCA and 35% by the NCX (Taggart and Wray 1997). This confirms the crucial role that the PMCA plays during calcium efflux procedure in the myometrium. Additionally, some studies revealed that the contribution of NCX to calcium efflux is altered during gestation (Clodagh and Wray 2019).

1.7.14 Na/Ca exchanger (NCX)

NCX is an antiporter membrane protein with 9 transmembrane zones and 938 amino acids that uses the Na^+ gradient supplied by the sodium pump to expel Ca^{2+} from uterine smooth muscles. Typically, one Ca^{2+} ion is exchanged for three Na^+ ions during this electrogenic process. This exchanger mechanism may happen in any direction depending on the $[Na^+]$ inside and outside of the myometrial cell. Nevertheless, it is possible that NCX brings Ca^{2+} into the myometrial cell due to the existence of plasma membrane micro-domains where NCX reposes next to SR and links with isoforms of Na, K ATPases (Juhaszova and Blaustein1997). It is suggested that both the PCMA and the NCX may work in a compartmentalised way, with the low resting $[Ca^{2+}]$ inside the uterine myocyte being maintained by the PMCA, while the micro-domains between the plasma membrane and the SR are regulated by the NCX (Juhaszova and Blaustein 1997)

1.7.15 Sodium pump Na, K ATPase

The sodium pump is made of 1) two α-subunits; 2) two β-subunits and 3) an FXYD subunit. There are numerous isoforms for each subunit. The expression of these isoforms within different tissues allows the Na, K ATPase role to be strictly controlled (Shull and Lingrel 1987, Martin et al., 1989).

The Na, K ATPase channel is known for maintaining a low intracellular level of Na^+ ($[Na^+]$). The channel moves 3 Na^+ out of cells, in exchange for the entry of 2 K^+ (Blaustein et al., 2016). In the uterine myocytes, the contribution of Na^+ pump current to the negative resting membrane potential is minimal (Nakamura et al., 1999). The transport of ions (Cl^-, Ca^{2+} and H^+) which are

vital to the excitability and function of the uterine muscle are linked to the produced sodium gradient (Wray 2015).It has been found in smooth muscles that NCX and Na, K ATPase channels reside in the same location (Moore et al., 1993). Furthermore, in the intracellular space, [Na^+] impacts on the activities of the NCX and the SR Ca^{2+} content (Golovina et al., 2003). In the myometrium, the SR, the caveolae and the plasma membranes are side by side. Therefore, the existence of Na^+, K ATPase in these micro-domains might affect uterine force (Wray and Shmygol 2007). In addition, the Na^+, K ATPase could contribute to myometrial excitability and gestational changes (Floyd et al., 2003). The inhibitory role of prostaglandins was observed through stimulation of Na^+, K ATPase generating hyperpolarisation of cell membrane (Parkington et al., 1999).

During gestation, the expression of isoforms changes in either animals or humans (Maxuell et al., 1998; Esplin et al., 2003; Floyds et al., 2017). Changes in isoform expression can have a functional effect in myometrium (Tsai et al., 2003). Floyd et al revealed that α2, α3 and β2 expression increased during gestation and as pregnancy progressed, sensitivity to pump inhibitors changed and their action of improving contractile frequency became larger, along with the accompanying Ca^{2+} transients. Furthermore, differences in the expression of α-isoform were found between pregnant and non-pregnant myometria in human (Maxwell et al., 1998, Esplin et al., 2003). Moreover, the declined expression of α3 isoform corresponded to a reduced uterine contractility in a rat treated with oestradiol (Tsai et al., 2003). The expression of isoforms, as well as the distribution of Na^+ pumps, are able to promote uterine activity. They have also been linked to pregnancy problems such as pre-eclampsia. Any deficiency or disorders in their activities mediated by inhibitors may result in the rise of [Na^+], thus contributing to the development of pre-eclampsia (Graves 2007)
In conclusion, ATP is the molecule responsible for storing and supplying energy in our cell. Glucose is broken down through a process call glycolysis to generate ATP. When glucose levels are low, it means there is limited fuel available for glycolysis. In the absence of glucose, muscles may undergo anaerobic metabolism, leading to the accumulation of lactic acid. Increased lactic acid can alter the pH balance within the cell and reduce calcium uptake into the cell and affecting uterine contraction.

1.8 METABOLISM IN THE SMOOTH MUSCLE OF THE UTERUS

During pregnancy, the uterus undergoes enormous changes. The growth of the uterus is extraordinary, and this is due to an enlarged myometrium. From the size of a pear, the uterus extends up to the epigastric region; its size increases from about 7 to 20 cm; its usual capacity of about 10ml increases in volume to accommodate even up to about 5000 mls; and its weight ranges from about 70 g to reach about 1000g. The uterine wall that was thin at the start of pregnancy gradually thickens as the foetus grows, then becomes thin again towards the end of pregnancy. These changes occur from the beginning of the pregnancy to accommodate the growth of the foetus (Prendergast and Wray, 2019). These changes always start with the hyperplasia of the myocytes stimulated by hormonal changes and the implication of growth factors. To this the stretching of the uterine wall is added later. In addition, uterine myocytes can synthesise certain basic elements like collagens, and they appear well filled with contractile proteins around the third trimester of pregnancy. It is worth mentioning the increase in the number of gap junctions, changes in ion channel expression and increased level of both hormones and receptors (Prendergast and Wray, 2019). The physical, biochemical, and metabolic changes are essential for the generation of the powerful, regular, and intermittent contractions necessary for the expulsion of the foetus during parturition. Among these changes there are modifications of the glycolytic and oxidative enzymes in the myometrial (Wynn 1967). Throughout parturition, the uterus needs energy to overcome the hypoxic challenges it will encounter. This is the reason the uterus establishes large reserves of free glycogen and free fatty acids during the gestational period. Immediately after childbirth, the uterus gradually returns to its initial state of before pregnancy (involution) due to hormonal changes and progressive suppression of mechanical stretching (Wray 1980).

1.8.1 Metabolites involved during uterine contraction.

For uterine contraction to occur, the myometrium needs ATP. ATP is essential in the cross-bridge cycle and for the phosphorylation of the myosin light chain which is one of the proteins necessary for uterine contraction. Uterine myocytes also contain a reserve of phosphocreatine as a buffer to resupply ATP, but it is present at much lower concentrations (~5 mM) in myometrium, compared to striated muscles (~40 mM) (Dawson and Wray 1985). The glycogen reserve in the myometrium increases gradually during pregnancy, presumably for an effective

parturition. It is worthy to mention that contractions are slower in smooth muscles (including the uterus) compared to skeletal muscles; this is partly attributable to the liberation of inorganic phosphate, and partly to the fact that the phosphorylation of myosin is regulated by Ca^{2+} rather than the troponin Ca^{2+} system. This is what causes the bridge cycle and its length of fixation time to be slower but more durable. In addition, it should be mentioned that oxygen uptake is low in smooth muscles compared to skeletal muscles, so metabolism can continue to occur with contractions during usual activity without an increase in oxygen uptake. Like any type of muscle, effective control of Ca^{2+} and pH is essential to accomplish effective contractions. Both regulatory systems have energy requirements that always involve ATP (Almohana and Wray,2018). One of the major differences between smooth muscles and other types of muscle is metabolic compartmentation. Contractile activities are strongly supported by oxidative phosphorylation while ionic control, more particularly Na, K ATPase, is backed by ATP produced from anaerobic metabolism (Lynch and Paul,1987) (Paul et al., 1984). This theory of compartmentation has been empirically established and confirms that glycolytic enzymes appear to be membrane-bound and secured by F-Actin (Dhar-Chowdhury et al., 2007)

Even in anaerobic conditions the myometrium is able to use anaerobic pathways to produce ATP along with lactate (Wray 1990).Under anaerobic conditions, glycolysis couples to fermentation reactions to generate lactate.

In the myometrium, under hypoxic conditions, lactate exit increases drastically (Wray 1990). A recent contractility study conducted on the rat uterus to explore the effects of lactate found that lactate has an inhibitory effect on myometrial contraction due to the transient inhibition of the entry of Ca^{2+} into the myocyte. This inhibition is the result of lactate acidifying myometrial cytoplasm (Hanley et al., 2015). It has therefore been suggested that the accumulation of lactate in the extracellular environment, by inhibiting myometrial contractility, could be a crucial cause of dysfunctional parturition (Wray et al., 2021).

1.8.2 Myometrial blood supply during labour

During labour, the strong, intermittent, and rhythmic contractions generated by the myometrium are sufficient to dilate the cervix so that the foetus can completely pass through the birth canal. At each contraction, uterine blood vessels are temporarily compressed (~45 -60 seconds) and the blood supply to the myometrium is reduced (Ischemia). As a result, glucose and oxygen levels are reduced accordingly (Larcombe et al., 1999). This is a normal parturition

phenomenon. However, if these contractions become more tonic (hypertonic)than phasic then the uterine vessel compression may lead to foetal asphyxia (foetal distress).

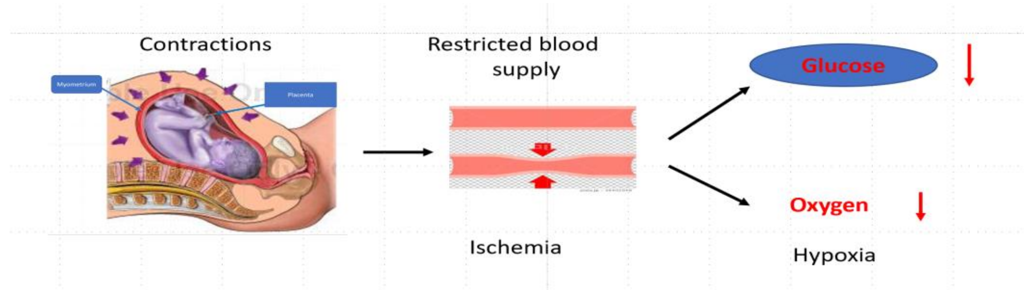


Figure 1.12 Uterine metabolism during labour. The restriction of glucose supply to the uterus is due to restricted blood supply to the uterus following the compression of uterine blood vessels at each contraction.

1.8.3 Defensive feedback machinery

During labour, at each uterine contraction, as glucose supply to the uterus is reduced, the concentration of ATP ([ATP]) should normally drop. The pH drops due to the intracellular acidification caused by hypoxia. This acidification of the cytoplasm of uterine myocytes results in a remarkable decrease in the entry of calcium current ([ca^{2+}]) into the cytoplasm, due to membrane hyperpolarization resulting in the inhibition of the activation of action potentials. When the uterine contractions reach their peak, the myocytes gradually become much more acidic, then uterine contractions gradually begin to wane and return to their initial state.

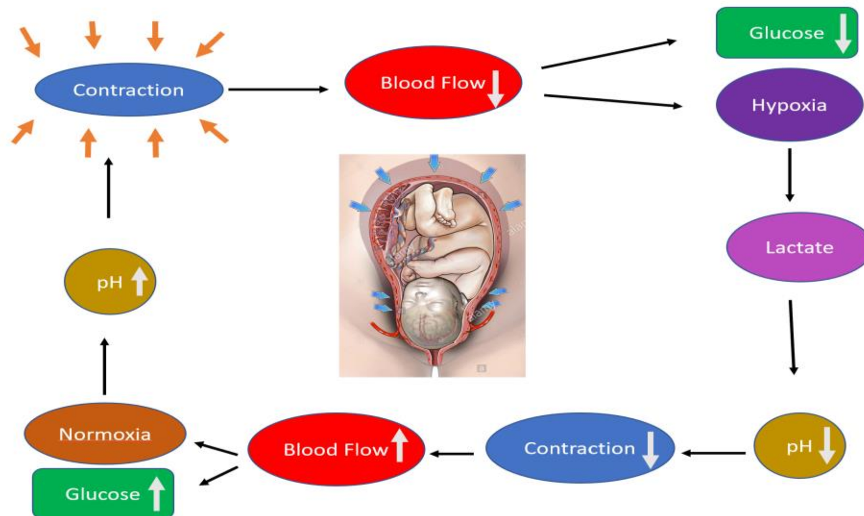


Figure 1.13 Blood flow and contractions in the uterus during labour. Myometrial contractions during labour lead to compression of the myometrial blood vessels. This compression results in hypoxia which increases lactate, leading to the acidification of the myocyte cytoplasm. The decline in pH reduces calcium entry; consequently, the myometrial force decreases. This alleviates the compression on the uterine blood vessels, followed by the restoration of the myometrial environment, and another powerful contraction arises. This cycle helps protect and prevent hypoxic harm to the myometrium and the baby.

This is the resting period. During this resting period, metabolic shifts produced by ischemia and hypoxia are restored along with [PCr], [ATP] and pH. The restoration of oxygen supply and the improvement of the environmental conditions activate the firing of action potentials again and cause $Ca^{2+}$to enter myocytes, triggering strong contractions again (Taggart et al., 1996, Wray and Prendergast, 2019).

There is an intrinsic mechanism of negative metabolic feedback in the myometrium thus limiting the duration and the amplitude of the contraction. This feedback helps prevent hypoxic and ischemic damage in the myometrium; that is the reason this critical period is shortened.

This feedback mechanism is essential for the foetus as well because it helps avoid transmitting ischemia and hypoxia from the myometrium to the foetus through the placenta.

The sequence of brief hypoxia followed by foetal heart rate stimulation is an integral and normal part of parturition. Clinically, the peak of contraction may be identified by changes in the heart rate of the foetus. The hypoxia is identified by the increase in foetal heart rate. To cope with hypoxia, the foetus defends itself by increasing its heart rate, but if the ischemia and hypoxia continue, this will lead to a decrease in the foetal heart rate, which is a warning sign of foetal distress (Wray and Prendergast,2019). During labour, the foetal heart rate is a particularly

crucial element in monitoring the status of the baby during labour, as it helps detect a foetus in distress.

The phenomenon that limits the contractile activity of the uterus contributes to the protection of the foetus

1.9 Prolonged / dysfunctional labour

Although prolonged or dysfunctional labour generally refers to failure of the dynamic labour progression, the exact definition has been controversial. For instance, according to the National Institute for Excellence in Health and Care (NICE), delayed labour is defined as cervical dilation of less than 2 cm within 4 hours of a first-time labour. In addition, The American Pregnancy Association opines that the duration of delayed labour is at least 20 hours or more in women who have never given birth to a living child (nulliparous), and more than 14 hours in women who have already had several deliveries of viable children (Albers, 1999).

In nulliparous women, it is defined as a dilation rate of less than 1 cm per hour (Neal et al., 2010). However, according to WHO, it is defined as a failure of the baby's presenting part to advance into the birth canal despite effective uterine contractions during labour (Hofmeyr, 2004). It is estimated that nearly 8% of all women who give birth will have a prolonged labour, with the majority of them being first-time mothers. However, this estimate varies depending on the population type (Wray, 2007), and depends on information such as timing and uterine contractions reported by parturient themselves. Dysfunctional labour is parturition characterised by weak and uncoordinated contractions. Initially uterine contractions may start normally and then gradually become weak and uncoordinated and eventually disappear. This slows down and then stops uterine dilation (Wray 2015). The delayed progression of labour can be diagnosed using a partograph which is a graphic recording of major changes in mother and foetus during labour. It is usually plotted against time (Lavender et al., 2013).

Prolonged labour increases the risk of foetal dystocia, maternal fever, and other negative neonatal outcomes (Harper et al., 2014).

Other known undesirable outcomes include maternal dehydration, sepsis, ketoacidosis and a greater risk of post-partum haemorrhage, obstetric fistulae, uterine rupture, stillbirth, and maternal death. These are prominent in low resource settings.

Furthermore, Sandstrom et al. discovered that prolonged labour and maternal pushing are linked to a considerable risk of neonatal adversity (Sandstrom et al.,2017). However, work by Cheng et al reported no adverse neonatal outcomes amongst women who have never carried a child or given birth but experienced prolonged active labour. Nevertheless, there was a

higher rate of caesarean deliveries amongst these women (Cheng et al.,2010). A minimal rise in maternal and neonatal morbidity was observed as well as low perinatal death (Laughon et al., 2014). These have been associated to several factors influencing the length of labour, including the age of mother, parity, weight, pelvic shape and size, foetal position, the length of pregnancy and increased gestational weight (Nesheim, 1988; Allen et al., 2009)

1.9.1 The reasons for delayed labour

The causes of delay in labour can be related to one or more of the following factors, also known as the four Ps: power, passenger, passage, and psyche.

Power refers to the strength and frequency of the uterine contractions, as well as the maternal expulsive efforts in the second stage of labour. Inadequate power can lead to prolonged labour, especially in the first stage. Insufficient power may occur when the myometrium is not well loaded with glycogen prior to parturition otherwise the uterus will run out energy and leads to myometrial dysfunction. (Swer 2021). Passenger refers to the size, presentation, and position of the baby. A large baby, an abnormal presentation (such as breech or transverse lie) or a malposition (such as occipito-posterior) can cause difficulty in passing through the birth canal and result in obstructed labour (Swer,2021). Obstructions constitute obstacles and obstacles to the descent of the foetus will necessarily need more power or energy. This may be the cause of myometrial glycogen depletion resulting in delayed labour (Failure to progress, 2020). Passage refers to the shape and size of the maternal pelvis and soft tissues. A narrow pelvis, a pelvic tumour, a fibroid or excessive swelling of the cervix or vagina can obstruct the descent of the baby and cause prolonged or obstructed labour. (Swer,2021). The obstruction caused by an abnormal passage may need more energy as well for a smooth achievement of the outcome, the delivery. The resistance opposed by an abnormal passage may definitely deplete the myometrium of its glycogen and this may result in uterine dysfunction during parturition. Psyche refers to the psychological state and emotional well-being of the woman. Fear, anxiety, stress, or lack of support can inhibit the release of oxytocin, which is the hormone that stimulates uterine contractions. This can also lead to prolonged labour, especially in the first stage. This can be assessed by talking to the woman and providing reassurance, encouragement, and comfort measures. (Nice,2022)

The management of delay in labour depends on the cause, the stage and phase of labour, and the condition of the mother and the foetus. Some possible interventions (Nice,2022) include artificial rupture of membranes to stimulate stronger contractions, giving oxytocin infusion to augment labour, changing position, walking or using a birthing ball to facilitate descent of the baby, providing adequate hydration, nutrition and pain relief to the woman, monitoring the fetal heart rate and liquor colour to detect fetal distress, performing an operative delivery (forceps, ventouse or caesarean section) if there is evidence of obstruction or fetal compromise.

Another issue associated with poor contractility in labour occurs in the third stage. Uterine atony is one cause of post-partum haemorrhage, when the uterus fails to contract following delivery of the placenta. 10.5% of labouring mothers that have a live birth will suffer post-partum haemorrhage as a consequence of poor contractility (Healthengine, 2005). Issues with contractility account for more than 50% of pregnancy and childbirth complications (Healthengine 2005). In clinical practice, oxytocin is prescribed in the treatment of poor contractions and poor progress in labour, but there are still discussions on the success rate of oxytocin (Arrowsmith et al.,2010; Bugg et al.,2013; Lukasse et al.,2020). Therefore, prolonged labour is the most widely recognised cause for emergency caesarean section deliveries (Nystedt and Hildingsson, 2014; Wray, 2007). Considering the issues highlighted above, it is vital to undertake further investigation into uterine contractility, with a specific interest on regulating uterine contractility for a good management of cases clinically.

1.10 Pathophysiology of uterine contractions

Myometrial contractions are the basis of dysfunctional labour if the labour is not hindered by foetal or maternal anatomical disturbances. Metabolic needs can be easily balanced in a mother in good health. In the presence of prolonged labour, the myometrium will depend on its stores of glycogen and ATP. However, because of ATP deficiency, anaerobic metabolism, and general ketoacidosis, this can result in an acidic environment (Neilson et al., 2003). Research has shown that myometrial lactic acidification reduces and/or abolishes uterine contractility in rats and humans (Taggart and Wray, 1993; Taggart and Wray, 1995, Hanley et al., 2015). In addition, reduction of Ca^{2+} in the myocyte have been linked to reduction in muscle contractility (Pierce et al., 2003). It is still difficult to understand the pathophysiological mechanism underlying dysfunctional labour. Lactic acidosis in the

myometrial capillary blood is associated with dysfunctional labours, along with a significantly reduced myometrial capillary blood pH, see below (Quenby et al., 2004). Furthermore, the local increases in myometrial lactate spill into the amniotic fluid. Thus, lactate in the amniotic fluid is actually used as a marker and predictor of uterine dysfunction during labour (Wiberg-Itzel et al., 2014; Sterpu et al., 2018), but it does not reveal what the underlying cause of the dysfunctional labour is.

After analysing a capillary blood sample from the myometrium during the first incision at a caesarean delivery, Wray's group proved for the first time a link between pH, lactate, and dysfunctional labour. Women who experienced caesarean section for a reason other than dysfunctional labour had a pH of around 7.3 in their myometrial capillary blood. However, women who underwent a caesarean section for labour dysfunction had a statistically lower pH level of approximately 7.1. In addition, the lactate level was two times higher in their myometrial capillary blood (Quenby et al., 2004). It should be noted that there was no systemic hypoxia and other indicators of maternal health were normal. Thus, this group had obtained evidence to link poor contractility in vivo with acidity and elevated lactate. The Wray group went on to hypothesize that these finding could be explained by a disruption of the feedback mechanism. In other words, if the myometrial blood flow is not restored in an adequate way during the resting period between contractions and the pH and lactate do not return to their normal values, then uterine contractions will gradually become weak, and labour will be dysfunctional. This anomaly was corrected by oral ingestion of bicarbonate in a small randomised clinical trial, which led to a marked reduction in the number of caesarean sections in women diagnosed with dysfunctional labour (Wiberg-Itzel et al., 2018). The group considered that the oral bicarbonate had helped to reduce the level of acidity around the uterine myofibrils and in this way improved contractile strength and allowed many of these women to proceed with a normal labour and delivery. Interesting as these data are, they do not shed much light on the origins of the poor contractions and are unlikely to explain all causes of dysfunctional labour. I want therefore to introduce another important factor in myometrial biochemistry – glycogen.

1.11 Role of glycogen

Glycogen is a complex glucose polymer observed in all animals and humans. It is a type of glucose storage form found primarily in the liver and skeletal muscles. Glycogen is broken into glucose via glycogenolysis pathway (Figure 1.12). It is essential in providing a mechanism for rapid ATP production in muscles, which can have a high and rapidly fluctuating energy turnover. (Jensen et al., 2012).

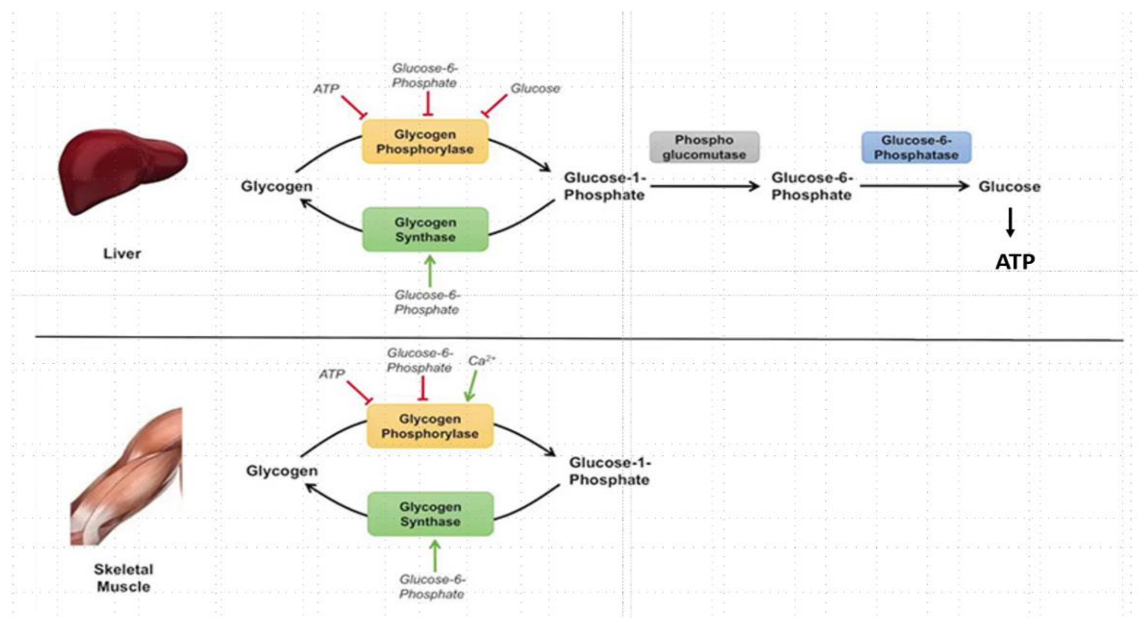


Figure.1.13 Glycogenolysis. This is a process where glycogen(storage) is broken down into glucose. This is facilitated by three principal enzymes: glycogen phosphorylase transforming glycogen to glucose-1- phosphate(G_1P), then phosphoglucomutase converting G_1P to glucose -6-phosphate(G_6P), then, again, glucose-6-phosphatase converting G_6P into glucose. The ATP is produced through glycolysis (Healthjade images).

The rate of ATP regeneration decreases when glycogen is depleted. As a result, the human skeletal muscle is unable to maintain an adequate global energy supply to the excitation and contraction coupling process (Sahlin et al.1998). However, this energy deficit theory has been challenged by studies in skeletal muscles revealing a reduced muscle activity even after recovery periods where ATP content would be normal (Chin et al., 1998). The experiment stipulated that the cause-and-effect link between low glycogen and impaired muscle function still needs to be confirmed (Chin and Allen, 1997).

1.12 Glycogen structure

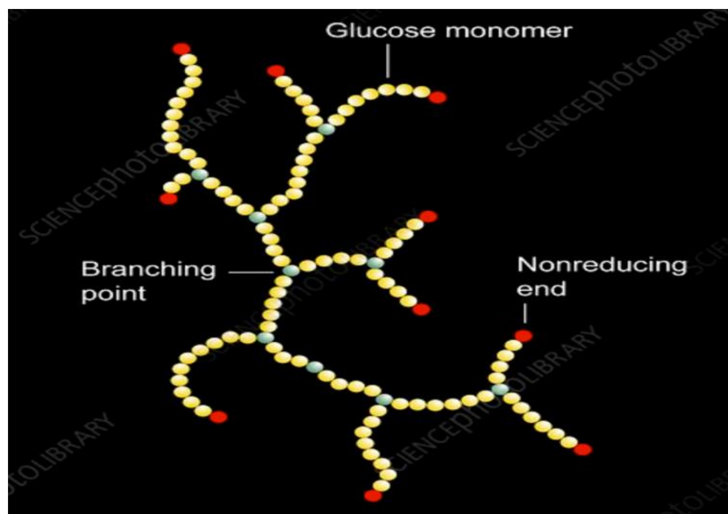


Figure 1.14 Glycogen molecule. Glycogen is a polymer composed of numerous glucose molecules (in yellow) connected to one of two forms of glycosidic bonds. Branching in glycogen structure is caused by the least common of the two bonds, shown in grey (Image from Science Photo Library).

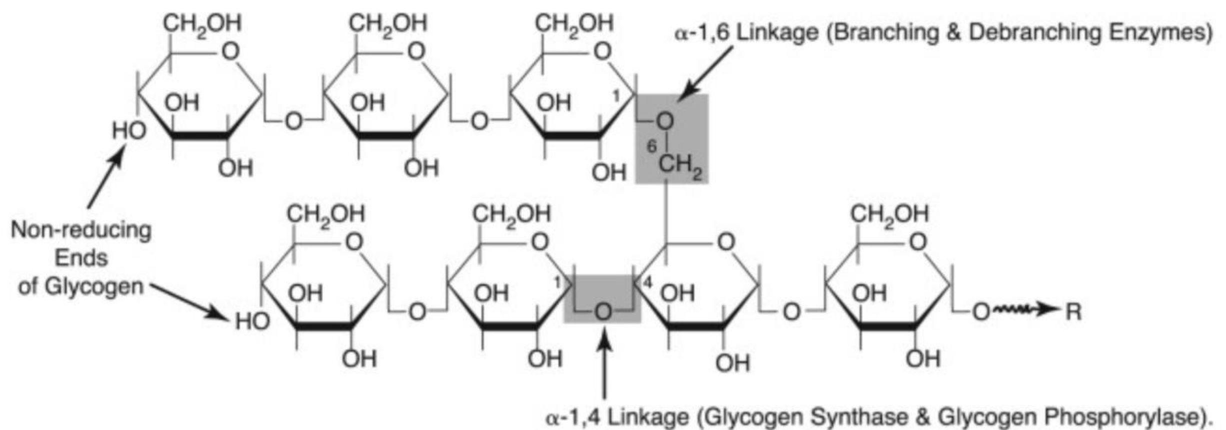


Figure 1.15 Glycogen chemical structure. The glycogen α-1,6 glycosidic bonds are generated by a non-regulatory branching enzyme (then broken by the debranching enzyme), whereas the glycogen α-1,4 glycosidic bonds are produced by the regulatory glycogen synthase (and broken by glycogen phosphorylase). (Image from Science Direct Library).

1.13 Glycolysis

Glycolysis is a metabolic pathway occurring in the cytoplasm of cells and is the first step in the breakdown of glucose to produce energy. It is a central pathway in both aerobic and anaerobic organisms, and it plays a crucial role in providing energy for various cellular activities.

During glycolysis, one molecule of glucose (a six-carbon sugar) is transformed into two three-carbon molecules called pyruvate. This process involves a series of ten chemical reactions, each facilitated by specific enzymes. Glycolysis can be split into two phases: 1) The energy investment phase and 2) The energy payoff phase (Akram 2013)

In the energy the first phase of glycolysis, two molecules of ATP (adenosine triphosphate) are consumed to phosphorylate glucose, making it more reactive. The glucose molecule is then divided into two glyceraldehyde-3-phosphate (three-carbon molecules). This phase requires energy input but is necessary for the further breakdown of glucose. In the second phase (The energy payoff), the two molecules of glyceraldehyde-3-phosphate are oxidized, releasing energy. This energy is used to produce NADH (nicotinamide adenine dinucleotide), an energy-rich molecule, and ATP through a series of reactions. Ultimately, each glyceraldehyde-3-phosphate molecule is converted into pyruvate, yielding a net gain of two ATP molecules. (Chaudhry & Varacallo 2023)

While glycolysis produces a small amount of ATP, its main purpose is to generate pyruvate, which can go on to produce more ATP in the presence of oxygen through oxidative phosphorylation. In anaerobic conditions (the absence of oxygen), glycolysis is followed by fermentation, which involves the conversion of pyruvate to lactate or ethanol, depending on the organism (Granchi 2010)

Glycolysis is a fundamental process and is considered one of the most ancient metabolic pathways, believed to have evolved early in the history of life on Earth. It is essential for both simple and complex organisms as it serves as a main source of energy in the form of ATP (Akram 2013)

Understanding glycolysis has important implications in various fields, including medicine and biotechnology, as alterations in this pathway can lead to metabolic disorders and diseases like diabetes and labour dysfunction as the myometrium needs energy during parturition.

1.14 Citric acid cycle

The citric acid cycle is also known as the Krebs cycle or tricarboxylic acid (TCA) cycle. It is a key metabolic pathway taking place in the mitochondria of eukaryotic cells. It plays a pivotal role in cellular respiration and energy production by facilitating the oxidation of acetyl-CoA, a two-carbon molecule obtained from the breakdown of carbohydrates, proteins, and fats (Haddad & Mohiuddin 2023)

This cycle starts with the condensation of acetyl-CoA and oxaloacetate (a four-carbon molecule), forming a six-carbon molecule known as citrate. This reaction is activated by the enzyme citrate synthase. Through a series of enzymatic reactions, citrate is continuously converted into isocitrate then α-ketoglutarate, succinyl-CoA and succinate, fumarate, and malate. These reactions involve the transfer of electrons and hydrogen ions to electron carriers, such as NAD^+ and FAD, thereby generating reducing equivalents in the form of NADH and $FADH_2$.The Krebs cycle serves multiple functions in cellular metabolism. Firstly, it serves as a major source of electrons for ETC (the electron transport chain) which ultimately leads to the generation of ATP through oxidative phosphorylation. The NADH and $FADH_2$ generated during the cycle donate their electrons to the ETC, leading to the production of an electrochemical gradient across the inner membrane of the mitochondria (Sheeran et al.,2019)

Secondly, the citric acid cycle is involved in the generation of precursor molecules for other metabolic pathways. Several intermediates of the cycle, such as oxaloacetate and α-ketoglutarate, can enter various anabolic pathways to synthesize amino acids, nucleotides, and other biomolecules needed for cell growth and repair. Furthermore, the citric acid cycle helps maintain cellular homeostasis by regulating the production of energy-rich molecules. It is highly regulated by allosteric control mechanisms and hormonal signals to ensure that the cycle functions optimally based on the cell's energy needs (Alabduladhem & Bordoni 2022)

In summary, the citric acid cycle is a central metabolic pathway that plays a vital role in energy production and the synthesis of important biomolecules. It is a highly regulated and interconnected part of cellular metabolism, ensuring the efficient utilisation of energy and the maintenance of cellular homeostasis.

1.15 Oxidative phosphorylation

Oxidative phosphorylation is a metabolic process that occurs in the mitochondria of eukaryotic cells, responsible for generating the majority of ATP (adenosine triphosphate) needed for cellular energy. It is a vital process for sustaining life as it fuels energy-demanding processes like muscle contractions, brain function, and active transport of molecules across cell membranes. The process comprises the transfer of electrons from electron transporters, NADH and FADH2 to a series of protein complexes embedded in the inner mitochondrial membrane called the ETC (Boyman et al.,2019)

As the electrons travel through the ETC, they produce a proton gradient across the mitochondrial membrane. This proton gradient acts as an electrical current that powers ATP synthesis. As protons move back across the membrane through ATP synthase, a complex enzyme, ATP is generated from ADP (adenosine diphosphate) and inorganic phosphate. This process is known as chemiosmosis and is the final step in oxidative phosphorylation (Matlin 2016)

There are five protein complexes involved in the ETC: complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome bc1 complex), complex IV (cytochrome c oxidase), and complex V (ATP synthase). Each complex plays a critical role in accepting and donating electrons, and complex IV also utilizes oxygen as the final electron acceptor, producing water as a byproduct. Additionally, oxidative phosphorylation requires several coenzymes and cofactors such as coenzyme Q, cytochrome c, and iron-sulfur clusters, which help facilitate electron transfer between the complexes (Letts & Sazanov 2017)

Notably, oxidative phosphorylation is a highly efficient process, with each NADH molecule resulting in the synthesis of approximately three ATP molecules, and each FADH2 molecule generating about two ATP molecules (Nath 2019)

Overall, oxidative phosphorylation is crucial for energy production in eukaryotic cells, allowing them to carry out their fundamental functions. By harnessing the energy stored in electron carriers and utilizing oxygen as the final electron acceptor, this process efficiently generates ATP, the currency of cellular energy, to meet the cell's energy demands.

1.16 Glycolytic metabolism and lactate production

Glycolysis is the metabolic pathway in cells that breaks down glucose to generate energy. Usually, glycolysis proceeds in the presence of oxygen, leading to the complete oxidation of glucose and production of carbon dioxide and water. However, in the absence of oxygen, glycolysis switches to a process called anaerobic glycolysis, which results in the production of lactate (Granchi et al.,2010)

During anaerobic glycolysis, glucose is still broken down into two molecules of pyruvate through a series of enzymatic reactions. However, since there is no oxygen available for the pyruvate to enter the oxidative phosphorylation pathway (part of cellular respiration), it is converted into lactate instead. This conversion is carried out by the enzyme lactate dehydrogenase. (Peek et al., 2017)

Lactate production serves an essential purpose in the absence of oxygen. By regenerating the molecules of NAD+ (nicotinamide adenine dinucleotide) needed for glycolysis to continue, it allows cells to produce a small amount of ATP, which is the energy currency of cells. Although anaerobic glycolysis yields significantly less ATP compared to aerobic respiration, it provides a quick energy source when oxygen availability is limited, such as during intense exercise or in certain tissues like red blood cells. (Rabinowitz & Enerbäck 2020)

Once oxygen becomes available again, the accumulated lactate can be transported to the liver, heart, or other tissues where it can undergo a process called the Cori cycle. In the Cori cycle, lactate is converted back into pyruvate in the presence of oxygen, and subsequently undergoes gluconeogenesis to reform glucose. The glucose can then be released into circulation to provide energy to other tissues, completing the cycle (Katz & Tayek 1998; Soeters et al.,2021)

In summary, glycolysis in the absence of oxygen leads to the production of lactate, which helps maintain a minimum energy supply through anaerobic glycolysis. This process serves as a vital adaptation for cells and tissues that experience oxygen deprivation, ensuring their survival and function.

1.17. Glycogen and uterus

Glycogen is mainly stored in the liver and skeletal muscles, but it is also found in other tissues, such as the brain, heart, kidney, and uterus. Glycogen in the uterus and fallopian tubes is an important source of glucose during early pregnancy, as it supports embryonic development and decidualization of the endometrium(Dean,2019)

The endometrium is the inner lining of the uterus that undergoes cyclic changes during the menstrual cycle. The endometrium consists of two layers: the stratum basalis and the stratum functionalis. The stratum basalis is the permanent layer that attaches to the myometrium, the muscular layer of the uterus. The stratum functionalis is the layer that is shed during menstruation and regenerated during each cycle.(Cornforth 2023)

The myometrium is the thick layer of smooth muscle that surrounds the endometrium and contracts during labour and delivery. The myometrium also contains blood vessels, nerves, and connective tissue (Cornforth 2023)

Glycogen synthesis and storage in the endometrium and myometrium are regulated by ovarian hormones, such as oestrogen and progesterone, as well as insulin. Estrogen stimulates glycogen synthesis in both endometrial and myometrial cells, while progesterone enhances glycogen accumulation in the endometrial glands. Insulin regulates glycogen synthesis in the endometrial glands by activating glycogen synthase, the enzyme that catalyzes the formation of glycogen from glucose(Flannery et al.,2018)

Glycogen storage in the endometrium varies throughout the menstrual cycle. During the proliferative phase, estrogen causes the thickening and vascularization of the stratum functionalis and increases glycogen synthesis in both epithelial and stromal cells. During the secretory phase, progesterone causes further enlargement of the endometrial glands and increases glycogen accumulation in the glandular cells. The glycogen content of the endometrium reaches its peak at the time of implantation[4].

Glycogen storage in the myometrium also changes during the menstrual cycle. Oestrogen increases glycogen synthesis in both longitudinal and circular muscle layers of the myometrium during the proliferative phase. Progesterone decreases glycogen synthesis in the longitudinal muscle layer but maintains it in the circular muscle layer during the secretory phase. The glycogen content of the myometrium is higher in the circular than in the longitudinal layer throughout the cycle(Lakna,2018). Glycogen stored in the endometrium

and myometrium can be mobilised to provide glucose for various metabolic processes. Glycogen breakdown is catalysed by glycogen phosphorylase, which cleaves glucose units from glycogen molecules. Glycogen phosphorylase is activated by various stimuli, such as glucagon, epinephrine, and calcium(Flannery et al.,2018)

Glycogen degradation in the endometrium occurs mainly during menstruation, when glucose is needed for tissue repair and regeneration. Glycogen degradation in the myometrium occurs mainly during labour when glucose is needed for muscle contraction(Lakna,2018)

In summary, glycogen storage in human myometrium and endometrium is a dynamic process that depends on hormonal regulation and physiological conditions. Glycogen serves as a glucose reservoir that supports uterine function and pregnancy outcome.

1.18 Glycogen and muscle contraction

1.18.1 Glycogen and muscle fatigue

Traditional understanding associating low levels of glycogen in the skeletal muscle and defective contractile muscle function is that the depletion of glycogen will directly result in diminution in the level of ATP regeneration. Therefore, the muscle is not able to sustain a sufficient global supply of energy to the procedures implicated in excitations and contractions, resulting in a failure to convert the motor drive into an acceptable force, and ensuing tiredness (Ørtenblad et al.2013). This was endorsed by the findings of Sahlin et al. (1997) when observing the decrease of phosphocreatine (PCr) in conjunction with an elevation in free adenosine diphosphate (ADP) and inositol monophosphate (IMP) after long glycogen-exhausting physical activities; but the concept of energy shortage in striated muscle and fatigue has also been questioned (Chin and Allen 1997).Therefore, the findings from the experiments on the whole body or on particular muscles have not been able to produce evidence for the precise role of glycogen during muscle function. This has been explained by the fact that variations in total level of glycogen in the muscle cause other metabolic changes that can impact the excitation-contraction coupling mechanism (Allen et al.2008). Nevertheless, there is no well-known correlation between glycogen exhaustion during fatigue and striated muscle dysfunction. The direct cause and effect remain to be established.

In this thesis, I am therefore aiming to establish the direct cause and effect between glucose deficiency and myometrial dysfunction both in animals (mice) and humans.

1.18.2 Uterine metabolic reserves during pregnancy

The metabolic reserves that drive contractions are also established during pregnancy. These reserves enhance phosphocreatine (PCr) concentration, the direct backup for ATP, while glycogen and fatty acid deposits likewise increase (Gruber et al., 2011, Dawson et al., 1985). Glycogenolysis (the breaking down of glycogen) is a process whereby glycogen stored in the liver or skeletal muscle is transformed first into glucose-1-phosphate, then into glucose-6-phosphate. This process is regulated by two hormones: epinephrine from the adrenal gland and glucagon from the pancreas. Both hormones are responsible for the stimulation of glycogen phosphorylase to begin glycogenolysis (epinephrine) or to stop glycogenesis by inhibiting glycogen synthetase (glucagon); glycogenesis is the formation of glycogen from glucose. The conversion of glucose to glycogen is achieved through the glycogenesis pathway, whereby glycogen is synthesised depending on the demand for glucose and ATP. In cases where both are present in reasonably high amounts, the surplus of insulin stimulates the conversion of glucose into glycogen for storage in liver and muscle cells(Ophardt, 2003).The increase in blood flow to the uterus is equivalent to the growing of the product of conception for most of the pregnancy but reduces towards the end of the term. Compression of the vessels within the myometrium decreases blood flow to the uterus. Subsequently, cyclic variations occur in [PCr] [ATP], and pH with every uterine contraction (Larcombe et al., 1999). The extent of these changes has been found to be proportional to the level of vascular occlusion; additionally, uterine force decreases concurrently with decreased blood supply (Larcombe et al., 1998).

Contraction of uterine muscles requires energy produced by ATP; the energy demand for smooth muscles is considerably lower compared to striated muscle, but it is required for prolonged periods (e.g., 24 hours for labour). However, as mentioned, in smooth muscles the stores of high energy phosphates are small. Therefore, for smooth muscles to function efficiently they have a higher demand than striated muscles for a constant supply of metabolic energy.

1.18.3 Glycogen storage diseases

1.18.3.1 Introduction

Glycogen storage diseases (GSDs) are genetic disorders involving the metabolism of carbohydrate. These are part of inherited inborn errors of metabolism (IEM). These errors are frequently due to single and specific gene mutations that encrypt specific proteins. These disorders may first reveal themselves in early childhood. Overall, IEMs are a result of an absence or deficient number of particular enzymes required to transform carbohydrates or fat into energy or breakdown other metabolites or amino acids, leading them to build up and then become harmful. Depending on the particular type, glycogen storage diseases result from a failure to transform glycogen into energy or a toxic build-up of glycogen. Therefore, all glycogen storage diseases are caused by a deficiency to store or use glycogen (Hicks et al.2011).

Glycogen is a polymer composed of monomeric glucose units. After meals, the glucose levels in the plasma rises and triggers the storage of surplus of glucose as glycogen in the cytoplasm. The storage process is facilitated by glycogen synthase. By weight, the liver has the highest amount of glycogen (around 10%), while the muscles can store only around 2%. (Jensen et al.,2011) The total amount of glycogen in the muscles is greater than the total amount stored in the liver since the entire bundle of muscle is greater than the total mass of the liver. When required, glycogen can disintegrate into glucose and used to generate energy. Enzymes and transporters for this pathway are the main key to the origin of glycogen storage diseases.

An important number of GSDs have been detected and named after their discovering physician, but some are extremely scarce. They range from type 0 to 6 (Stone et al., 2021), as detailed below.

1.18.3.2 Pathophysiology

The inability to synthesise glycogen or the incapacity to appropriately break down glycogen into glucose results in hypoglycaemia, along with exercise intolerance. This failure to degrade glycogen results in an unusual accumulation of glycogen in the liver, leading to hepatomegaly. In striated muscles, GSD can prevent muscles functioning efficiently and this may lead to exercise intolerance and the breakdown of muscle tissue (rhabdomyolysis). Signs/symptoms would include hypoglycaemia, hepatomegaly, failure to grow, low muscle tone, exercise intolerance, heat intolerance, acidosis, and hyperlipidaemia. In glycogen storage disease type 1, glycogenolysis (the breaking down of glycogen) in the liver leads to an increased production of lactic acid due to an intracellular build-up of glucose-6-phosphate (G-6-P), which induces the glycolytic path (Stone et al.2021).

1.18.3.3 Etiology.

To understand the cause of glycogen storage diseases, we must follow Kannourakis' metabolic steps leading to glycogen synthesis and degradation (Kannourakis,2002). All glycogen storage diseases may be due to a genetic deficiency in enzymes involved in glycogen synthesis (glycogenesis) or breakdown (glycogenolysis). Glycogen synthesis is achieved by glycogen synthase (GS). There are two different types of glycogen synthase. The first one is encoded by the GYS1 in the striated muscles and the second one is encoded by the GYS2 in the liver.

Any failure or absence of glycogen synthase in the liver due to mutations in the GYS2 gene or in the muscle due to mutations of GYS1 gene will stop the synthesis of glycogen. This explains the origin of GSD type 0a in the liver and GSD type 0b in the muscle.

In the same way, mutations in the branching enzymes (GBE1 gene) may lead to the generation of glycogen with an aberrant structure, called polyglucosan bodies: this is the origin of GSD type 4. The polyglucosan bodies do not efficiently undergo glycogenolysis and can significantly build up in the muscles (causing myopathy and muscle weakness) or build up in the liver (causing hepatomegaly).

Whereas GSD 0a and GSD 0b are caused by inadequate glycogen storage, the majority of GSDs are due to inabilities to break down glycogen into glucose, resulting in excess in the storage of glycogen in the tissue. The first step in glycogenolysis is glycogen phosphorylase breaking down glycogen into glucose-1-phosphate (G-1-P). Mutation or defect in the glycogen

phosphorylase gene in the muscles (PYGM) will cause GSD type 5 and in the liver (PYGL) will cause GSD type 6. The glucose-1-phosphate (G-1-P) is converted into glucose-6-phosphate (G-6-P) by the effect of phosphoglucomutase. In the liver, the G-6-P is in turn degraded into glucose via glucose-6-phosphatase. As a result, glucose is discharged into the bloodstream as energy for various organs (Kanduras ,2002). Muscles do not release glucose into the blood stream because they lack glucose-6-phosphase. Genetic abnormalities or gene mutation in the metabolism of G-6-P are causes of GSDs type 1 (von Gierke disease) (Parikh et al.2021).

In the hepatocyte, the synthesis of G-6-P is performed in the cytoplasm, then transported to the endoplasmic reticulum (ER), and broken down into glucose by glucose-6-phosphatase. The GLUT2 transporter is responsible for transporting glucose from the cytoplasm to the bloodstream. Fanconi-Bickel disease is an extremely rare glycogen storage disease caused by the deficiency of GLUT2. This deficiency is a result of a failure to export glucose outside the hepatocyte and this leads to an increase of the intracellular level of glucose and a reduced glycogen breakdown. Consequently, glycogen will accumulate and cause hepatomegaly.

During glycogenolysis, a glycogen debranching enzyme (GDE) is needed to act at glycogen polymeric branch points. This enzyme is called “ammylo-alpha-1,6-glucosidase,4-alpha-glucanotransferase”. AGL is the gene encoding this enzyme; mutations of the AGL gene cause GSD type 3, leading to a reduced or absent function of GDE (Dagli et al.2010).

Another type of GSD, categorised as lysosomal storage disease (LSD), is GSD type 2. All lysosomal storage diseases are caused by a non-functional or absence of a lysosomal enzyme. In GSD type 2, the lysosomal enzyme responsible for the degradation of glycogen into glucose is called “lysosomal acid alpha-glucosidase, encoded by the GAA gene. Mutations in this gene may lead to a toxic build-up of glycogen in the lysosome (Chan et al.2017).

Glycogen storage disease (GSD) types	Absence, mutation, or defect	Gene encoding
GSD type 0 (A)	Glycogen synthase (liver)	GYS2
GSD type 0 (B)	Glycogen synthase (muscle)	GYS1
GSD type 1	Glucose-6-phosphatase	G6PC
GSD type 2	Lysosomal acid alpha- glucosidase	GAA
GSD type 3	Amylo-alpha-1,6 glucosidase 4-alpha - glucanotransferase	AGL
GSD type 4	Branching enzymes	GBE1
GSD type 5	Glycogen phosphorylase (muscle)	PYGM
GSD type 6	Glycogen phosphorylase (liver)	PYGL

Table 1.2 Glycogen storage disease types and causal mechanism. Any absence or failure of enzymes involved in glycogenesis or glycogenolysis (due to mutation or defect of the encoding gene) will lead to an inability to synthesise glycogen or an inability to break down glycogen into glucose.

1.18.3.4 Epidemiology

Glycogen storage diseases (GSD) are extremely rare conditions, with an incidence estimated between 1 in 20,000 and 1 in 40,000. Very mild forms of GSD can go undiagnosed and foetal forms can result in neonatal death. Some differences have been observed in terms of ethnicity. The GSD type 6 is frequently found in people of Jewish descent of North Africa, while GSD type 3 is found in the Mennonite population (Stone et al.2021).

1.18.3.5 Management

At present, no cure has been found for any type of glycogen storage diseases. The principles of management are to alleviate signs or symptoms, by treating or avoiding hypoglycaemia, hyperuricemia, hyperlactatemia, or hyperlipidaemia. Consuming starch helps avoid hypoglycaemia; allopurinol helps reduce hyperuricemia; and statins helps combat hyperlipidaemia. Recently enzyme replacement therapy (ERT) has been used to cure GSD type 2 (Ferreira and Gahl 2017), and researchers are exploring avenues in order to use ERT for other types of GSD.In case of hepatic failure or malignancy, liver transplantation is preconised.

1.18.4 Glycogen storage diseases and labour

Mc Ardle's disease

Dr. Brian McArdle, who practised in London, England, first described McArdle's disease, also known as myophosphorylase deficiency (MPD) or Type V GSD in 1951.

The disorder is a recessive inherited condition marked by an inability to metabolise glycogen. This results in a diverse range of musculoskeletal and urinary tract symptoms. Muscle fatigue is common in McArdle's disease patients (McArdle,1951). Intolerance of physical exercise and tiredness are most typical signs and symptoms of McArdle's disease.

Muscle pain, muscle contractures, rigidity, spasm, rhabdomyolysis, and myoglobinuria.

Cochrane and Alderman reported on a case of a 31-year-old woman previously investigated and diagnosed with myophosphorylase deficiency (Cochrane and Alderman 1973). At 36 weeks of pregnancy, she was referred to Liverpool Maternity Hospital

The pregnant woman and her doctor were concerned that muscle spasms would make labour difficult and force a Caesarean section. On the advice of Dr. Mc Ardle, a consultant chemical pathologist it was decided to wait for the onset of parturition in the hope of a normal delivery. The contractions became regular after 10 hours of labour, and the cardiotocography (CTG) appeared to be completely normal.

18 hours later, the uterine contractile activities were still short and inefficient, so an infusion of 5% dextrose (500 ml) with 0.5 units of oxytocin was started. The 1st phase of labour lasted 21 hours. A slow progression to the second stage was most likely due to the epidural's lack of a pushing reflex. After a 45-minute second stage, the delivery was completed with Keilland forceps.

The post-partum period was normal for the mother and the baby. Pregnancies complicated with Mc Ardle's disease are not common. Several studies identified women with confirmed or suspected MPD; however, no details of any pregnancies were given (Schmid and Hammaker (1961); Tobin and Coleman (1965); Dawson, Spong and Harrington (1968).

The Cochrane and Alderman case study (1973) described above was the first to describe MPD in pregnancy. Cochrane and Alderman attributed the inefficiency of uterine activity to the presenting part of the foetus in the pelvic cavity (occipito-posterior position); myophosphorylase deficiency appeared to have no effect on uterine activity, and pregnancy and labour were both normal. (Muscular symptoms did not change). This indicated the

presence of compensatory mechanisms. In the case of myopathy, glucose and fructose administration has been linked to a reduction in muscular symptoms and increases in muscle blood flow and arterial levels of free fatty acids (FFA) have the same favourable results (Pernow et al.1967).

Sinclair (1963) states that muscle blood flow increases during pregnancy, which may help alleviate symptoms. Furthermore, there is a rise in plasma FFA levels due to increased mobilisation from adipose tissue (likely influenced by placental lactogen) (Myant, 1970).

During pregnancy, the maternal metabolism of carbohydrates and lipids takes on diabetic characteristics, with fat providing a large amount of energy than carbohydrates. Hormones from the placenta can sometimes interfere with how insulin works in the body. This condition is known as insulin resistance. Consequently, the pancreas may not be able to produce enough insulin to overcome the resistance, leading to high blood sugar levels (Sonagra et al.,2014; Kampmann et al.,2019). As a result, the demands on the glycolytic system would be lowered.

In the 1973 case study described above, Cochrane and Alderman suggested that normal labour in myophosphorylase deficiency could be explained by a compensatory effects of increased blood flow and the diabetic character of maternal metabolism of carbohydrates and lipids occurring during pregnancy. The fact that the uterine activity was continuous, it was suggested that the uterine glycogen phosphorylase was normal or probably the intervention of a different glycolytic enzyme was possible. This supports the findings of Eyerman, Engel, and Williams (1962), who discovered a normal glycogen phosphorylase activity in blood vessel muscle in a Mc Ardle's disease patient.

Recently, similar cases have been reported. Giles and Maher (2011) described myophosphorylase deficiency in a patient with normal pregnancy followed by a normal pregnancy outcome. Another case report came from Stopp et al. (2018) on pre- and peripartum management of a woman with McArdle’s disease, resulting in a C/section delivery on parturient request after labour had started. Furthermore, McArdle disease causing rhabdomyolysis following vaginal delivery was described by McMilan et al. (2019).

In conclusion, the authors have established that women with myophosphorylase deficiency should not expect their condition to worsen or their symptoms to worsen during pregnancy. The precise mechanism linking glycogen to muscle function is unknown.

1.18.5 Glucose infusion and labour

There is some evidence to suggest that glucose infusion may improve uterine contractions during labour, but the results are mixed, and more studies are still needed to fully comprehend its effects. The use of glucose infusion as a therapeutic intervention for improving and sustaining uterine contractions is still being studied and not widely practiced.

Glucose infusion, also known as dextrose infusion, involves the administration of glucose intravenously to a labouring woman. It is believed that glucose can provide an additional energy source for the uterine muscles, which may help to enhance the strength and frequency of contractions.

Several research have explored the effects of glucose infusion on uterine contractions during labour, but the results have been conflicting. Two studies published in 2017 and 2018, found that glucose infusion was associated with increased uterine contractility and improved labour outcomes in women experiencing suboptimal contractions. The researchers observed some increase in the strength and frequency of contractions during labour following glucose administration (Pare et al.,2017; Riegel et al,.2018)

Dawood and colleagues review did not produce strong evidence to suggest usual administration of intravenous dextrose (Dawood et al.,2013). These results were upheld by Dapuzzo et al. who found no significant difference in labour outcomes between women who received glucose infusion and women who did not (Dapuzzo et al.,2016)

On the other hand, another study conducted by the Cochrane Collaboration in 2018 concluded that there is insufficient proof to support the routine use of glucose infusion during labour. The review analysed the results of five randomised controlled trials involving 283 women (Cochrane 2018).

One of the concerns regarding glucose infusion is the potential for hyperglycemia, or high blood sugar levels, in both the mother and baby. Hyperglycemia can lead to complications such as gestational diabetes, fetal macrosomia (abnormally large baby), and neonatal hypoglycemia (low blood sugar levels in the newborn). Therefore, careful monitoring of blood glucose levels is essential when administering glucose infusion during labour (Dekker 2022)

In conclusion, the effectiveness of glucose infusion in improving uterine contractions during labour remains uncertain. While some studies have suggested potential benefits, larger and more robust trials are needed to establish its efficacy and safety. Considering the potential

risks of hyperglycemia, careful monitoring of blood glucose levels is crucial if glucose infusion is administered. It is recommended that healthcare providers individualize the decision to use glucose infusion based on the specific needs and circumstances of each labouring woman.

1.19 The rationale of this study

Issues with contractility account for more than 50% of pregnancy and childbirth complications (Health Engine 2005). Due to the lack of treatment for poor labour progress and its poor outcomes, there has been an increasing trend in caesarean section rates worldwide and such treatments pose a risk to both mother and baby.

Uterine contractions cause transient ischemia during labour, limiting glucose delivery to the myometrium. (Lacombe et al., 1998). Glucose deprivation and muscle dysfunction relationship still needs to be proved in the myometrium (Chin et al., 1998, Ørtenblad et al.2013).

The uterus is not able to synthesise glucose. Therefore, all glucose comes straight away from maternal circulation, as required, or is temporarily stored as glycogen which acts as a glucose reservoir. (Dean, 2019).

During pregnancy, it can be expected that glycogen content stored in the myometrium will progressively increase to comparatively higher degrees close to term. As a significant energy component, glycogen (reserve) needs to build up during pregnancy to withstand metabolic challenges during labour (Wray 1990; Dawson and Wray 1985). The level of glycogen in the myometrium was found to be low just after labour compared to the glycogen level before parturition (at term). This means that glycogen was used as source of energy during labour. Glycogen deposits in the myometrium was supposed to be reduced through this procedure (Karri et al., 2011). Previous studies have directly implicated altered uterine metabolism due to ischemia as a cause of uterine dysfunction by stimulating anaerobic metabolism, increasing lactate and decreasing pH. Ischemia also reduces delivery of metabolites to the myometrium, especially glucose. Glycogen is a storage entity in both skeletal and smooth muscles and can be broken down to release glucose moieties to the muscles in times of need. Despite many studies in striated muscle investigating the effects and mechanisms of glucose deprivation on muscle contractility there is a lack of similar information for the myometrium. This is despite a clear clinical need to better predict, prevent and treat conditions such as dysfunctional labour.

The main question that remains is about how changes in glucose concentration affect uterine contraction. Myometrial contraction mechanisms and their impairment must be better understood in order to improve delivery outcomes and reduce the need for emergency caesarean sections and related maternal and neonatal complications. My thesis was therefore developed to obtain fundamental and novel data concerning altered metabolism on uterine function with a focus on the effect of the depletion of glucose on uterine contractility.

Research questions:

1. Does glucose depletion in the myometrium contribute to labour dysfunction?
2. Do women with dysfunctional labour have lower levels of glycogen in the myometrium compared to women labouring normally?

Hypothesis: Glucose depletion occurring in the myometrium during labour may contribute to labour dysfunction; women with labour dysfunction have lower glycogen levels in the myometrium than those labouring normally.

This study has never been conducted before. The partial results of this study were submitted to the University of Liverpool for my master's thesis (Kaleta and Wray 2018) then expanded on for this thesis. Other partial findings of this thesis were published by Acta physiologica. (Kaleta and Wray 2019)

This study aims to:

- Run a structured literature review on myometrial glycogen storage and seek evidence for glycogen content before parturition and immediately after labour.
- Clarify the effect of altered metabolism on uterine contractility by exploring the effects and mechanism of the action of zero glucose.
- Determine the changes that zero glucose produces in myometrial glycogen storage, using a mouse and human model in vitro.
- To Investigate the effect of changes in glucose on labour progression.

CHAPTER TWO

Methodology

CHAPTER 2
Methodology

In this chapter, I will describe in a general way the methodology, equipment, chemicals, and drugs used to support my thesis work.

2.1 Preparation of animal tissues

2.1.1 Animal housing

In the work conducted in this thesis, I have exclusively chosen female CBL57 / 6J mice (Charles River, UK). These animals were subjected to light and dark cycles of 12 hours under a standard laboratory diet; calorie restriction mimetics (CRM) was used in the form of pelleted seeds according to nutrient requirements for mice (49% fat, 20% proteins and 15% of carbohydrate, SDS Ltd, UK) and the animals had voluntary access to as much food and water as they wanted. The pregnant mice were housed separately in a normal cage. Non-pregnant mice were kept in groups of up to 3. As stipulated in the UK Home Office regulations, the temperature in the room was maintained at 21±2°C, and the humidity was kept between 40 and 55%.

2.1.2 Ethics and regulations

Animals were handled in accordance with the Code of Practice for the Slaughtering of Animals under Section 1 of the Scientific Procedure Act 1986. The mice were anaesthetized with an increasing concentration of CO_2. The death was confirmed by cardio-respiratory arrest by placing two fingers on the mouse thoracic cage to assess the breathing and heartbeat. Then the abdominal cavity was opened, puppies were removed from the uterus and beheaded according to home Office legislation. As soon as the mice were killed, the uterine tissues were dissected.

2.1.3 Availability of uterine tissues

Uterine tissue was obtained from virgin non-pregnant females weighing 20-25g, 8-10 weeks old and from time-mated 18 days pregnant (near-term) mice. Male and female mice were time-mated with males placed in the cage on Day 0 and taken out of the chamber on Day 1. Parturition in mice takes place between 19th and 20th days of gestation.

2.1.4 Dissection

The process started by the preparation of the dissection area and the instruments needed included small and large dissection scissors, dissecting forceps, a dissecting microscope, dissection dish, pins, and 5-0 silk sutures. The preparation of tissues was performed as described by Arrowsmith and Wray (2014). Myometrial tissue strips were collected from 18-day pregnant and non-pregnant C57BL6 mice. The mice were euthanised by administering a rising concentration of CO_2 and cervical dislocation according to Schedule 1 of the Animals Act 1986(scientific procedure). Immediately after a mouse was killed, its uterus was removed and then immersed in physiological saline solution (PSS) at a pH of 7.4. (154 mM NaCl; 5.6 mM KCl; 1.2 mM $MgSO_4$; 10.9mM Hepes; 8mM glucose; and 2mM $CaCl_2$ prepared in one litre of distilled water). The placenta, fat, foetal membranes, and excess blood were all removed, and the uterine tissue was flushed and meticulously cleaned for myometrial dissection under a dissecting microscope. The uterine horns were cut open and pinned in placed with the endometrial aspect facing downwards. This allowed the direction of the myometrial muscle fibres on the posterior aspect of the tissue to be observed. Full thickness myometrial strips were dissected, following the direction of the muscle fibres, and measuring roughly 1-2mm x 5-6 mm. The strips were tied at each extremity with surgical silk sutures. A long piece of suture (roughly 30 cm) was tied at one end of a uterine strip with a double knot. A small piece of suture was tied to the other end with a double knot. The tissues were then transferred in a 5ml organ bath (Linton, UK) between a fixed hook at the bottom and a transducer at the top (World Precision Instruments, UK). Approximately 45 minutes elapsed between the excision of the sample and the mounting in an organ bath. 5 mN tension was placed on the tissue and sufficient time allowed for spontaneous contractions to develop and become stable (40-60min). Tissues were superfused with physiological saline solution (PSS) at a rate of 5ml per

minute, kept at 37°C, and bubbled with pure oxygen simulating the physiologic environment (Babiychuck et al., 2004).

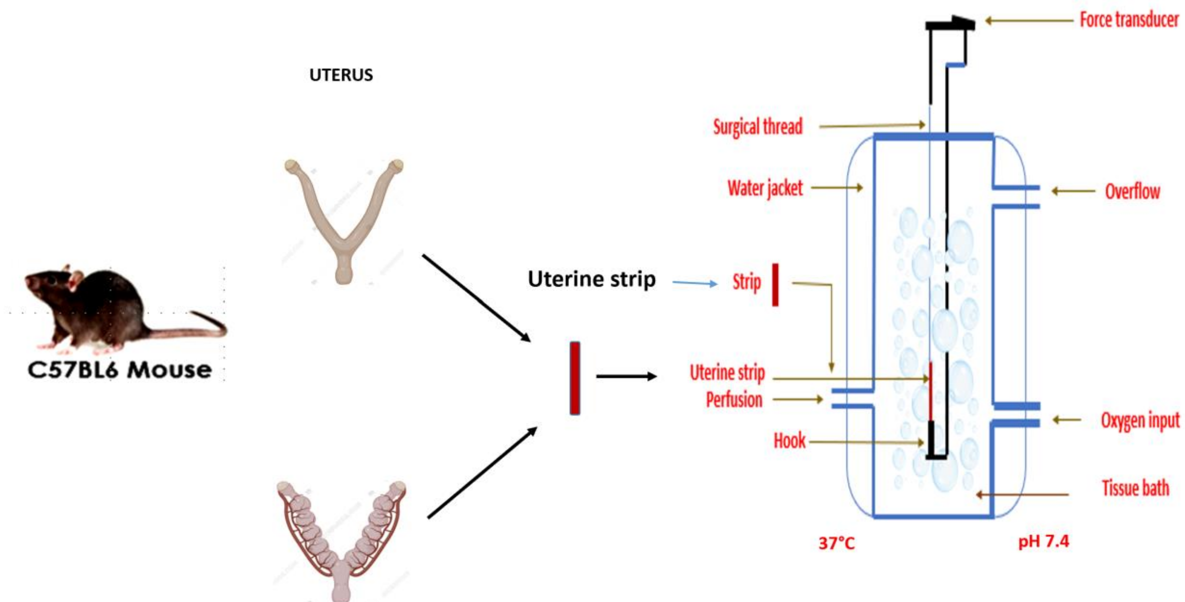


Figure 2.1 Tissue preparation in mice. Non-pregnant and pregnant mice were used to obtain myometrial strips. then mounted vertically in the organ bath, super fused with PSS solution (pH 7.4) at 37°C and bubbled with O_2. (Images from Biorender library)

2.2 Preparation of human tissue

2.2.1 Ethics and regulations

All work on human tissue was performed in accordance with UK law. The ethics approval for studies exploring the pathophysiological effects "of substances or any other agents on human uterine tissue (reference 10/H1002/49 of 26 August 2010), along with written informed consent, were obtained and are provided in appendices 4 and 5.

2.2.2 Availability of uterine tissues

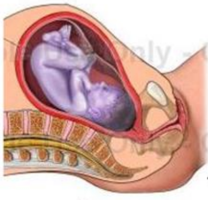
Human samples were obtained from women undergoing caesarean section at Liverpool Women 's Hospital. Human tissue samples with a thickness of 1 cm x 1 cm were acquired from the middle of the superior border following a uterine incision of the lower segment is made

at the time of delivery by Caesarean section. According to Lucas and Wray, in this zone, there is no difference in terms of contractility between lower and upper segments (Lukas and Wray, 2000). Immediately after the C-section, biopsies were placed in Hanks balanced salt solution (HBSS), (137 mM NaCl; 0.44 mM KH_2PO_4; 5.1 mM KCl; 0.26mM $Na2HPO_4$; 10mM HEPES; 5mM glucose; pH 7.2) to maintain tissue viability, then sent to our department. This solution has been used to clean or wash cells and tissue and maintain them in a viable state. It is buffered with phosphate and prepared to maintain a physiological osmotic pressure. Care was taken to ensure that the samples were kept in the fridge until used.

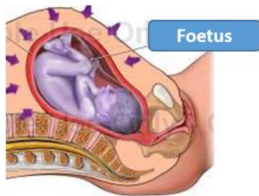
2.2.3 Dissection

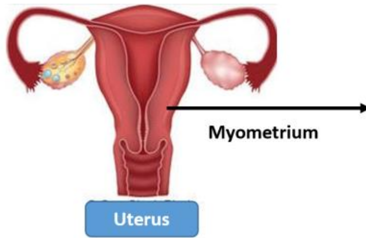
The myometrial biopsy was placed on a dissection dish filled with PSS under the dissection microscope and rinsed. Using forceps, the biopsy was cleaned of blood, endometrium, perimetrium and any obvious blood vessels. This helped identify the myometrial fibres within the biopsy. The biopsy was fixed on the base of the dissection dish with pins, under a gentle stretch. Blunt dissection (using blunt round-ended forceps) was used to gently open up gaps between the muscle bundles. Under the dissection microscope, the direction of muscle fibres could be identified. Tissue strips of 2mm wide x 8mm long x 1mm thick were cut longitudinally in the direction of the myometrial fibres using small scissors. Several strips were dissected from each biopsy. Following dissection, strips were attached to a 30 cm long suture at one end of the strip and another 5cm suture at the other end of the strip, which was then transferred to a dish filled with physiologic saline solution for mounting in the organ bath (Figure 2).

Non-labouring



Labouring





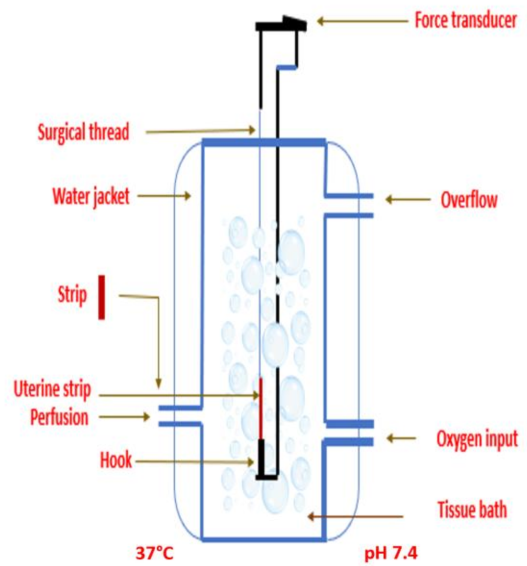


Figure 2.2 Myometrial tissue's dissection and organ bath mounting. Myometrial strips, obtained from non-labouring and labouring women, then mounted vertically in the organ bath, super fused with PSS at 37°C, pH 7.4 and fizzed with O_2. Spontaneous contractions and parameters were measured. (Nucleus Medical Media Inc/Alamy Stock Photo)

2.2.4 Organ bath experiments

A

Figure 2.3 A. Organ bath. The myometrial strips were super fused in the organ bath with PSS by the way of a peristaltic pump **(1)**. Strips were mounted vertically in an organ bath **(2)**. The circulating water bath maintained the reservoir at a set temperature **(3).**

B

Figure 2.3. B. Force transducer. The contraction measurement was obtained by the mean of a force transducer (B).

C **D**

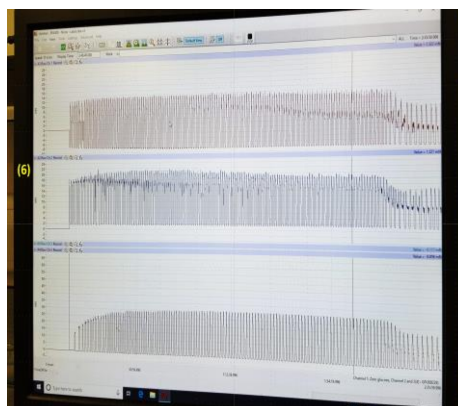


Figure 2.4. Uterine contractions. During contraction, the transducer recorded the variations in tension which was amplified through an amplifier **(4)** then converted into a digital signal by a converter device **(5)**. **C**. Through an acquisition software, the computer recorded signals as expressed on the screen **D (6)**

Contractions were isometrically recorded under 2mN tension in a 5ml organ bath super fused with PSS at 37°C, under 100% oxygen, at a pH of 7.4. Strips were mounted in the bath by attaching one end of the strip to the lower hook and the other end to the transducer's upper hook which measured the myometrial contractions. It was important to make sure the entire tissue strip was submerged in the PSS contained in the bath for the experiment. All the contractility data were recorded through a world precision device, a voltage transducer (Aston UK World Precision Instruments). The contractions were amplified by a trans-bridge amplifier connected to the computer using appropriate software (Labscribe2). The recorded contractions were stored in a data acquisition software program. As mentioned in chapter 1,

the myometrial tissue has a myogenic character, and contracts without stimulation. On the screen the uterine contractions appeared spontaneously. With the mouse tissue, uterine contractions appeared immediately after mounting myometrial strips in the organ bath and applying tension and were allowed to stabilise for a period of 30 minutes(minimum) to achieve constant baseline activities. With the human tissue, it took 1 to 2 hours before uterine contractions were observed. The values of these spontaneous contractions, once they were stable, were taken as 100% control for the parameters measured when the tissue was exposed to any condition of treatment. 100% oxygen (O_2) was always applied during experiments, but nitrogen(N_2) was applied to induce hypoxia. For all the solutions prepared and applied, HEPES was utilised as a buffer rather than bicarbonate. All the control experiments were completed concurrently. The effect of any agent, drug or condition applied to the myometrium and their respective control were analysed by calculating the amplitude, the frequency, and the area under the curve before and after treatment by means of OriginPro 9.0 software. When using multiple myometrial strips for the same treatment, the mean value was calculated and used as a single value.

2.2.5 Solutions, drugs and gases used.

Unless otherwise specified, drugs, solutions and all other chemicals used were purchased at Sigma Aldrich (Poole Dorset, UK).

2.2.5.1 Physiological saline solution (PSS)

On the day of an experiment, a physiological salt solution was freshly prepared and equilibrated to pH 7.4 using NaOH. For the composition of PSS, see table 2.1.

Salts	Molarity
Sodium chloride (NaCl)	154 mM
Potassium chloride (KCl)	5.6 mM
Magnesium sulphate ($MgSO_4$)	1.2 mM
HEPES buffer (HEPES)	10.9 mM
Glucose	8mM
Calcium chloride ($CaCl_2$)	2mM

Table 2.1 Physiological saline solution: components and composition

2.2.5.2 Glucose-free solution and experimental protocol

Testing the effect of zero glucose solution on uterine contractility

In this experiment, uterine strips were dissected as described above, mounted in the tissue bath, tensioned, and allowed to stabilise and produce spontaneous and stable contractions with steady amplitude, regular frequency, and duration. These contractions served as the control (100%) baseline activity. Once stable spontaneous contractions were established, strips were exposed to zero-glucose PSS solution (PSS without glucose) until a maximal effect was achieved (20-40 minutes).

The glucose-free solution was prepared using the subsequent composition.

Salts	Molarity
Sodium Chloride (NaCl)	154mM
Potassium Chloride (KCl)	5.6 mM
Magnesium Sulfate ($MgSO_4$)	1.2 mM
HEPES Buffer (HEPES)	10.9mM
Calcium Chloride ($CaCl_2$)	2mM

Table 2.2 Zero glucose solution: components and composition

2.2.5.3 High K^+ solution

Testing the effect of zero glucose under high K^+:

I used high K^+ solution as an experimental means of causing depolarisation of the myometrial membrane and thereby producing a larger force response as calcium flooded into the cell (Arrowsmith 2018). The general index of tissue integrity and the measure of tissue response can be achieved using high K^+ solution. With a high K^+(40mM) contraction, the contraction amplitude quickly reaches a peak before declining and more or less stabilising into a plateau phase, and then returning to baseline when the high K^+ solution is replaced with PSS. The aim of this test was to obtain the tissue's response while being treated with either high K^+ solution or with high K^+ zero glucose solution and to compare the results. The effects were tested for 20 to 40 minutes.

High potassium solution was prepared according to the following formula:

High K^+	Molarity (mM)
NaCl	120
KCl	40
MgSO4	1.2
HEPES	10.9
Glucose	8
$CaCl_2$	2

Table 2.3 High potassium solution: components and composition

2.2.5.4 High K^+ zero glucose solution

High potassium solution without glucose was prepared according to the following formula:

High K^+ zero glucose	Molarity(mM)
NaCl	120
KCl	40
$MgSO_4$	1.2
HEPES	10.9
$CaCl_2$	2

Table 2.4. Zero glucose high potassium solution: components and composition

2.2.6 Drugs used.

2.2.6.1 Oxytocin

Oxytocin was used as a stimulatory agonist; it is well known to increase the force and frequency of uterine contractions. The effect was sought and compared in glucose and zero glucose conditions, to determine if agonists protected the tissue from the effects of zero glucose. A 1mM oxytocin solution was made by dissolving the lyophilised powder of lyophilized oxytocin acetate in distilled water. Subsequently, as indicated in the manufacturer's prospectus, a 10µM preparation was made in physiological salt solution from

the 1mM stock solution kept at -20 °C. In order to increase the frequency and strength of contraction without inducing tonic contraction in the myometrial strips of pregnant and non-pregnant mice and humans, a concentration was chosen of 0.5 µM for mouse tissue and 1 µM for human tissue.

2.2.6.2 Flavopiridol hydrochloride hydrate

In my experiment, flavopiridol hydrochloride hydrate was used to stop the action of glycogen phosphorylase, an enzyme involved in the breakdown of glycogen into glucose in order to explore the effect on myometrial contractility. This product was purchased from Sigma Aldrich.11.41 mM of flavopiridol hydrochloride hydrate solution was made by dissolving 25mg powder of flavopiridol hydrochloride hydrate in 5ml of dimethyl sulfoxide (DMSO) according to the manufacturer's instructions. A 3 µM solution was made from the 11.41 mM stock solution and stored at 4°C. For the myometrial strips of pregnant and non-pregnant mice, and labouring and non- labouring human tissues, a concentration of 12 µM was used. Less than 1% of DMSO was in the bath when flavopiridol was used. A control of DMSO was performed and proved that it had no effect on uterine contraction.

2.2.7. Generation of hypoxic conditions

During organ bath experiments, the myometrial tissues were supplied with 100% oxygen. Bubbling the tissues with nitrogen, instead of oxygen, produced hypoxic conditions in the bath. Hypoxic conditions were induced while exposing tissue to zero glucose solution to compare the response to zero glucose under normoxic conditions (Alotaibi, Arrowsmith and Wray 2015)

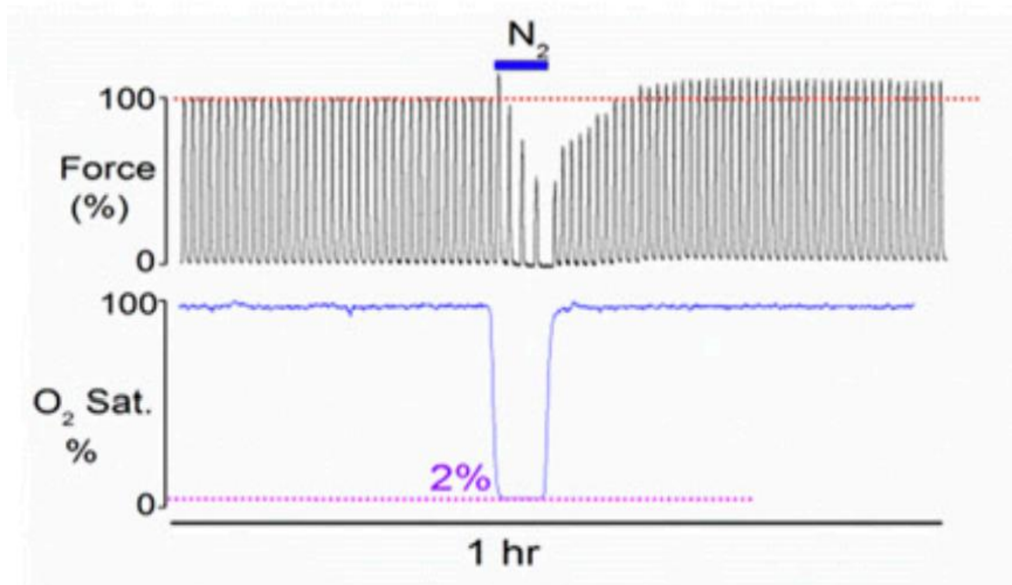


Figure 2.5 Hypoxic period generated by Nitrogen (N_2) on uterine contractility in mice (non-pregnant and pregnant). The saturation in oxygen decreased down to 2% and resulted in inhibition in uterine amplitude contractility (Alotaibi, Arrowsmith and Wray, 2015).

2.2.8 Analysis of contractility data

2.2.8.1 Sample size

According to Charan and Kantharia (2013), there exists two scientific methods for calculating sample size in animal experiments. (Crude method and power analysis). Power analysis is more reliable and is similar to the method used in clinical studies. Calculations can be performed manually using formulae, but more complex calculations require statistical software or the assistance of a statistician.

2.2.8.2 Power analysis

Researchers must first understand the effect size and decide how much minimum variation between the means of two distinct groups can be considered clinically significant before calculating sample size using power analysis. In the case of quantitative studies, information about variation or difference should be taken (if possible) from previously conducted research, along with standard deviation. The type 1 error is normally fixed at 5% (*P = 0.05)* calculated by significance level. This is a subjective value that can be increased or decreased depending on the research question. This concept can be applied to the power of the study which is the possibility of finding the expected result and may be kept between 80 and 99% but generally kept at 80%. The direction of the effect determines the type of test researchers

are required to use while exploring the effect. They may use a one-tailed test if the effect is observed all in the same direction or a two-tailed test if they feel that the effect is in both directions**.** In most animal studies, two tailed tests are frequently used. The calculation of sample size requires researchers to have insights about the statistical test they will apply to the data. For a simple test, such t-test, calculation may be done manually using a formula; but for a more complicated test like analysis of variance (ANOVA), appropriate software is needed, or the help of a statistician may be required. To obtain the actual sample size, the calculated final sample size should be adjusted based on the expected attrition (corrected sample size = sample size/ (1 [% attrition/100])

2.2.8.3 Crude method

Also called the "resource equation" method, this method is used when previous information or findings about standard deviation or effect size are not available. This method can also be used when hypothesis testing is not the principal purpose of the study but when the researcher is only interested in the results. The method is based on the analysis of variance, where "E" is measured as a degree of freedom.

E should be in the range of 10 to 20. If E is less than 10, more animals must be added to increase the likelihood of a significant outcome; if E is greater than 20, more animals are not required for a significant outcome. This method is appropriate to all animal research and all sample sizes. Keeping the E-value between 10 and 20 should be considered as acceptable and is calculated according to the following procedure:

E = Total number of animals − Total number of groups

This formula is a simple method but cannot be counted as strong as the power analysis process. Based on the crude method, numerous researchers consider 6 animals per group as a satisfactory sample size. Power calculation is often used in clinical studies in order to have a sufficient number of samples to have the desired effects. The principle is generally to have a large number of samples for reliable results, taking into account UK Home Office legislation regarding the use of animals and humans. In this thesis the sample size refers to the number (N) of mice or humans. For this study, the sample size varied between different experiment protocols depending on the availability of the tissues. Although the power calculation was used, previous animal and human studies conducted in our department and elsewhere guided the determination of the number "N" of samples needed for the study. According to UK

legislation, it was ideal to slaughter the smallest number of animals capable of producing reliable results. Based on the power analysis the following formula was used to calculate the sample size (N)

$$\text{Sample size} = \frac{(\text{Level of Confidence})^2 * (\text{Standard Deviation})^2}{(\text{Error rate})^2} \qquad N = \frac{Z^2 * \sigma^2}{E^2}$$

$$\text{Where} \quad E = \frac{Z\,\sigma}{\sqrt{n}}$$

Z = Confidence level

E = Margin of error

σ = Standard deviation

The above formula produced the minimum number of myometrial samples needed to ensure that the margin of error did not surpass "E" in confidence of interval.

With a margin error of 5% (0.05), a confidence level of 95%, and a significant level of 0.05, the previous sample calculation based on the power analysis gave at least a number (n) of at least 6 animals with an attrition rate of 5%. The same formula was applicable to the human tissue used.

2.2.8.4 Analysis

Myometrial tissues contracted spontaneously and were allowed to equilibrate until contractions were stable and regular. For mouse tissue, the contractions appeared immediately within 5 minutes. The 5 minutes immediately prior to starting an experimental protocol was designated as the control period and assigned the value of 100%. Subsequent responses (e.g., to hypoxic conditions or zero glucose conditions) were expressed relative to this control period. For human tissues, a 20-minute control period was chosen. If the same treatment was applied to more than one tissue strip from a particular animal/woman, then the results were meaned so as to generate a single value. Data capture was performed by LabScribe/Datatrax software.

The parameters of contractions measured while investigating contractile activity included the amplitude of contraction, frequency of contraction, and AUC (Integral force). The AUC is a measure of how much work the tissue strip does in a given amount of time. The three main contractility parameters were examined using the Origin Pro 2018 software: amplitude, frequency, and area under the curve.

2.2.8.4.1 Amplitude

The amplitude (mN) represents the height of the concentration obtained by subtracting the baseline value from the peak of the uterine contraction waves. The uterine contractions were measured for a 5-minute control period for mouse tissue and for a 20-minute control period for human tissue. The average control amplitude value was obtained, then compared to the mean amplitude value from the subsequent myometrial tissue treatment and expressed as a percentage in relation to the control value.

2.2.8.4.2 Frequency

Frequency is the interval between the start of one contraction and the start of the next contraction. The number of uterine contractions during a specific period was measured. The frequency of uterine contractions was measured; their mean value was calculated; this was then compared to the average frequency value obtained from the subsequent treatment used to the myometrial strips and expressed as a percentage in relation to the control value.

2.2.8.4.3 Area under the curve (AUC)

The area under the curve is a mean integral of force or the combined effect of frequency, duration, and amplitude, providing a measure of overall myometrial contractility. AUC was measured over the 5-minute control period and in a 5-minute period after the treatment had reached its maximum response in mouse tissue. In human tissue the AUC was measured over a 20-minute control period and for 20 minutes after the treatment had reached its maximum response.

2.2.8.4.4 Statistical analysis

Uterine contractions were captured by a force transducer (World Precision Instruments, UK) connected to a computer system, recording data via Labtrax software (WPI). During the exploration of contractile activity, the following parameters were measured: amplitude, frequency and area under the curve (AUC). A pre-treatment control period of 5 minutes was selected, and parameters measured. The parameters were measured again at the end of the treatment period (5 minutes) and compared to the initial control period. The treatment effect was expressed as a percentage of the internal control parameters. Origin Pro 2018 software (Origin lab corporation, USA) and Microsoft Excel 365 were used to analyse data. The data were presented as Mean Standard Error Mean (SEM), with N representing the number of mice in each group. One sample t-tests were performed to determine significance between the internal control period and the treatment. The unpaired tests were used to compare one treatment response between different groups by using Graph Pad Prism 8 software).

For high K^+ data, a 5-minute control period was chosen before treatment. The means of the peak amplitude and plateau amplitude were measured compared to the control; this was determined in high K^+ solution and in high K^+ zero glucose. The effects of zero glucose on the response to high K+ were then compared using an unpaired t-test. Peak amplitude with high K^+ solution was compared to peak amplitude with high K^+ zero glucose solution, and plateau amplitude with high K^+ solution was compared to plateau amplitude with high K^+ zero glucose solution, using Graph Pad Prism 8 software. A p-value of <0.05 was considered significant ($p< 0.05$, $p< 0.01$ and $p< 0.001$ were denoted by *, **, and ***, respectively).

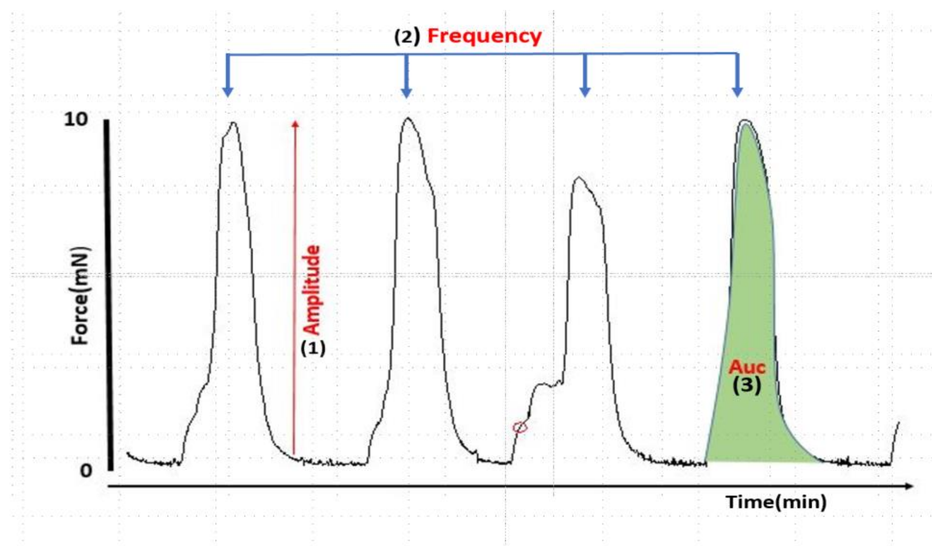


Figure 2.6 The parameters measured during the myometrial contractile activity :(1) The contraction amplitude (mN) is the magnitude of the contractions from baseline to peak. (2) The number of myometrial contractions in a given time period is referred to as the frequency. (3) AUC denotes the total activity over a given time period

2.3 Structured literature review of research on glycogen storage

This structured literature review was conducted for providing proof of the research that has already been conducted on the topic of myometrial glycogen concentration before parturition and immediately after parturition. The results from the present review gave me insights before measuring myometrial glycogen content in women with dysfunctional labour. Studies on these topics were identified. The collected material was methodologically investigated, summarised, analysed, interpreted, and their results and findings were discussed. Moreover, the limitations of the research were also identified, and suggestions for upcoming studies were also made. This review was conducted using the systematic framework suggested by Khan et al. (2003) for the conduction of a structured literature review, comprising the following 5 steps: definition of the research question, identification of relevant research, assessment of the document quality, summary of the outcomes of the research and interpretation of the results. The primary question of this structure review is:

What evidence exists to demonstrate the amount of glycogen storage in the myometrium immediately before parturition and immediately postpartum?

2.3.1 Literature search and strategy

For the conduction of this research, the research papers that provided insights regarding the concentration of myometrial glycogen immediately post-partum and before parturition were investigated. Pieces of research that were published were investigated during the period of July to September in the year 2022. The search engines used for searching the articles were PubMed, Google Scholar on the 25th of July. Moreover, a thorough review of the reference list of extracted papers was performed as well. While the papers were researched, no limitations were applied in the terms of the publication date, language, and location. The key terminologies that were used for the search included:

glycogen [Title/Abstract]) OR glycogen storage [Title/Abstract]) OR glycogen content [Title/Abstract]) OR glycogen level [Title/Abstract])

AND myometrium [Title/Abstract] AND pregnancy [Title/Abstract]. Experts from the Liverpool Cochrane database for pregnancy were consulted throughout this time.

2.3.2 Inclusion criteria

The pieces of research included in the analysis had to fulfil certain eligibility criteria. The criteria of research included the journals and articles that evaluated the concentration of glycogen present in the myometrium in the pregnancy course, before and after delivery. Moreover, the criteria of research also included animal or human models, in vivo and ex vivo, all languages, all kinds of research and clinical trials, abstracts and complete documents, systematic reviews and case studies, and unpublished and published literature. The criteria of eligibility for the research papers included research conducted following an experimental design which compared the concentration of glycogen post and pre-delivery.

2.3.3 Exclusion criteria

The research studies that were not covered by the inclusion criteria included research describing experiments which investigated the storage of glycogen in myometrium. Moreover, commentaries and editorials were also not included in the research.

2.3.4 Glycogen storage measurement

Glycogen is a branched polymer of glucose, serving as the main short-term energy reserve molecule in animals. It is predominantly synthesised in liver, where it represents 10% of the weight of the liver, and in muscles, where glycogen constitutes 1-2% of the muscle weight and is used locally. In the liver, glycogen acts as a buffer by storing excess glucose as glycogen(glycogenesis) when blood glucose levels are high and releasing glucose from glycogen stores(glycogenolysis) when blood glucose levels are low, hence, to keep blood glucose levels within a normal range. Its metabolism is dysregulated by glycogen storage diseases and diabetes. Diabetes can dysregulate glycogen metabolism in multiple ways. In type 1 diabetes, the pancreas fails to produce insulin, resulting in high blood sugar levels. This leads to increased breakdown of glycogen into glucose, as insulin normally inhibits glycogen breakdown. In type 2 diabetes, the body becomes resistant to the effects of insulin, leading to elevated blood sugar levels. This can also disrupt glycogen metabolism, as impaired insulin signaling can lead to increased glycogen breakdown and reduced glycogen synthesis. Furthermore, high levels of blood glucose can stimulate glucagon release, promoting glycogen breakdown. Overall, diabetes can disrupt the delicate balance of glycogen metabolism (Ashcroft 2017).

a. Glycogen Assay Kit

A glycogen assay kit was bought from Sigma Aldrich. It is a regular colorimetric assay measuring the content of total glycogen contained in a biological tissue sample using a 96-well microtiter plate format. Technically the kit uses a coupled enzyme reaction. That reaction hydrolyses the glycogen contained in the uterine samples into glucose. Then, the glucose is oxidised to produce a colorimetric substance proportional to the concentration of glycogen contained in the sample (observed at 570nm), after removal of background signals.

The lysates were analysed using a glycogen assay kit (Merck, Catalog.MAK016, Sigma-Aldrich, St. Louis, MO). They were diluted ten and twenty-fold before hydrolysis to line up with the optical density (OD) range of the assay standard curve. The regular concentration of glycogen ranged between 0.4 and 2 mg/well. The colorimetric product was measured by means of a plate reader. The glycogen level in a sample was determined by comparison with a glycogen standard of known concentration. The detection sensitivity limit of the glycogen assay kit was 3.8 µM glycogen. Every tissue sample replicate needed two assays; one assay was treated with amyloglucosidase and the other without amyloglucosidase. From the two wells of the reading plate, glycogen content was measured based on the difference in OD readings.

b. Glycogen determination

In this thesis, the myometrial samples were assayed based on the manufacturer's instructions and the colorimetric product was measured using a micro plate reader (Thermo Multiskan Ascent 354).

2.3.4.1 Tissue preparation and measurement

This procedure was subsequent to methods already established by Steingrímsdóttir et al. (1999) and Shabihkhani et al. (2014)

From emergency C/section cases, uterine biopsies were instantly snap frozen in liquid nitrogen then stored at -80°C. The samples were freeze dried prior to analysis in a Modulyo 4K freeze dryer for 24 hours (Edwards, Crawley, UK). The aim of this process was to remove water from the tissues and enable a dissection of roughly 10mg aliquots of the myometrial sample.

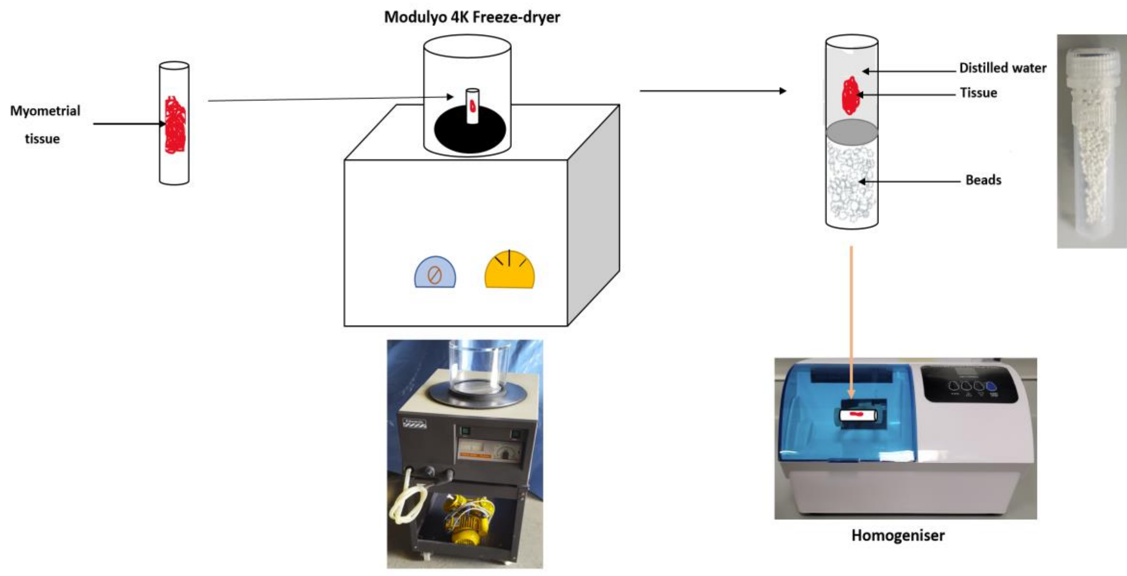


Figure 2.7 Glycogen measurement, tissue preparation procedure. Freeze-drying and homogenising processes, boiling and centrifugation. (Figure drawn by Tshikaya Kaleta)

The aliquot was homogenised in 1 ml of distilled water mixed with 1 ml of beads for 3 minutes using a mini-bead beater machine from Biospec (Bartelsville-Oklahoma, US) at a single speed brushless motor operating at 4500 revolutions per minute (rpm) according to the manufacturer instructions. Each homogenate was then boiled for 5 minutes in a QBD_2 dry

block heater, in order to get rid of enzyme activity, and then centrifuged for 5 more minutes at 4°C at a speed of 13000 rpm in an Eppendorf Centrifuge 5424R. In each well, 10µL of sample (lysate) was placed, then 40µL of hydrolysis buffer and 2µL of enzymes were added before taking the well plate to the well plate reader for the glycogen reading.

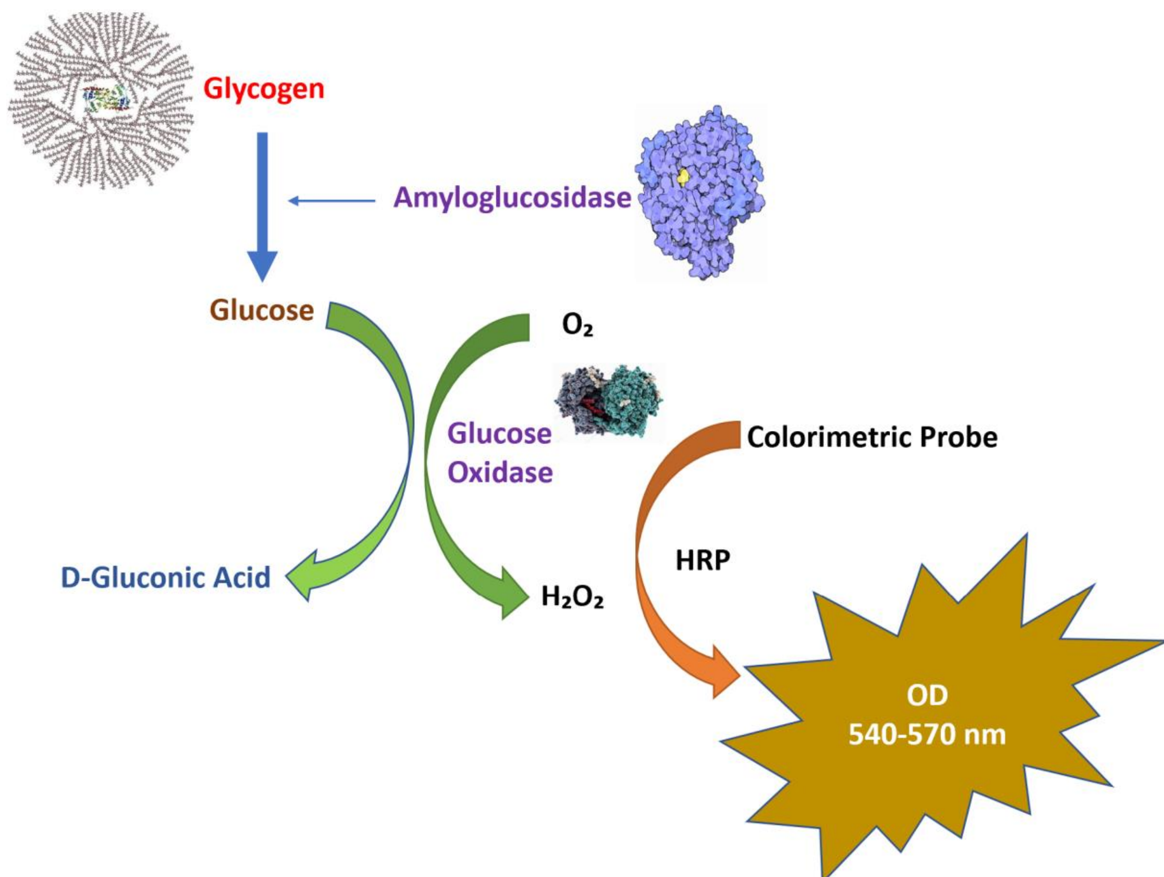


Figure 2.8 Glycogen measurement procedure. First, glycogen was broken down into glucose by amyloglucosidase. Then, glucose was oxidised by glucose oxidase into hydrogen peroxide (H_2O_2) and D-gluconic acid. The H_2O_2 was detected by a precise colorimetric probe. The reaction between the probe and H_2O_2 was catalysed by horseradish peroxidase at a ratio of 1:1. For glycogen determination, the tissue sample was compared to a well-known concentration of glycogen standard. Tissue samples and glycogen standards were finally incubated for 45 minutes, then a reading was made with a 96-well colorimetric plate reader. Glycogen (Wikimedia image) Amyloglucosidase (Protein data bank image), Glucose oxidase (stock image, science photo library), Figure drawn by Tshikaya Kaleta

2.3.4.2 Glycogen standards for colorimetric detection

For colorimetric detection, the dilution of 10mL of 2mg/mL glycogen standard was done with 90mL of ultrapure water to make a 0.2mg/mL solution. Then 0, 2, 4, 6, 8, or 10mL of the 0.2mg/mL standard solution was added into a 96 well plate to produce 0 (assay blank), 0.4, 0.8, 1.2, 1.6, and 2.0 mg/well standards. Then, to bring the total volume to 50 µL, hydrolysis buffer was added to each well.

The free glucose level in each sample was measured using the glucose standard curve. For glycogen determination, the absorbance calculated for free glucose in non-treated samples was subtracted from the absorbance calculated for amyloglucosidase-treated samples.

The glycogen concentration in each sample was determined based on the glycogen standard curve. The glycogen level was measured by a coupled enzyme assay which generated a colorimetric (570nm) product equal to the glycogen contained in a tissue (Catalog MAK016, Sigma-Aldrich, St. Louis, MO). This procedure was linear.

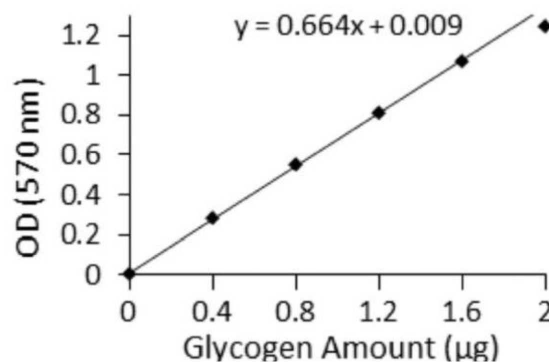


Figure.2.9 Glycogen assay kit, colorimetric standard curve (Sigma Aldrich)

2.3.4.3 Result calculations

The obtained value for the 0-glycogen standard was the background for the assays. The correction for the background was done by deducting the assay blank value from all readings. The glycogen standard dilution values were used to create a standard curve. Every time the assay was run, a new standard curve had to be created, and the assay blank background reading had to be deducted from the sample readings each time to find the appropriate measurement. Finally, by using the corrected measurement, the level of glycogen in the tissue sample was determined from the standard curve.

CHAPTER THREE

CHAPTER 3

UTERINE GLYCOGEN STORAGE: A STRUCTURED LITERATURE REVIEW

3.1 OVERVIEW

This is a structured literature review of the storage of uterine glycogen. Evidence was sought on uterine glycogen content both prior to parturition and immediately after labour. An extensive search was performed for the existing published literature and research papers from the search engines PubMed and Google Scholar, followed by a hand search. After the search, 17 studies passed the eligibility criteria and were included in this review. The result of this structured literature review demonstrates that under hormonal influence, glycogen content is present in the myometrium and increases during pregnancy for fetal survival and preparing for labour. Glycogen level decreases immediately after delivery. Therefore, it is suggested that glycogen storage is of major importance as a main provenance of energy for the uterine contractile activity during labour.

Keywords: Glycogen, myometrium, pregnancy.

3.2 INTRODUCTION

Glycogen is an homopolymer polysaccharide deposited in the tissues and stored in the tissues as a carbohydrate. Glycogen is formed from glucose through a process called glycogenesis. Glycogen is synthesised according to the demand for energy. When energy demands are high or when the body is not receiving enough glucose from the diet, glycogen is rapidly broken down into molecules of glucose into the blood stream and used as fuel for cells**.** Insulin is a polypeptide hormone produced primarily by beta cells in the pancreatic islets of Langerhans. This hormone may coordinate with glucagon to regulate blood sugar levels; insulin acts through an anabolic pathway, while glucagon plays a catabolic role. In the blood stream, glucose level is regulated by insulin and stimulates glucose storage in the liver, muscles, and fat tissue, leading to overall weight gain (Rahman et al., 2021). Normally, when the amount

of insulin is sufficient in the organism, excess glucose is not used and will be stored or saved in the form of glycogen. The purpose of glycogenesis is to ensure that the body does not run out of glucose which is the primary source of energy in the body (Patino and Orrick, 2022). The phosphorylation of glucose into glucose-6-phosphate is the first step of glycogenesis. This process is catalysed in the liver by glucose kinase and in the muscle by hexokinase. In the second step, glucose-6-phosphate is transformed into glucose-1-phosphate (G-1-P). This process is catalysed by an enzyme called phosphoglucomutase. In the third step, glucose-1-P attaches to uridine triphosphate (UTP) to create uridine diphosphate glucose (UDP-Glc). This mechanism is catalysed by an enzyme called UDPGlc pyrophosphorylase (Galvão et al., 2010). The following step involves a small existing fragment of glycogen serving as a primer to activate the synthesis of glycogen. UDP-Glc is then attached to a glycogen primer called glycogenin (Dean et al., 2014). See Figure 3.1. In the final step the enzyme glycogen synthase synthesises unbranched glycogen by transferring the molecule of glucose from UDP-Glc to glycogen, creating alpha 1,4-linkages (Bueno et al., 2010).

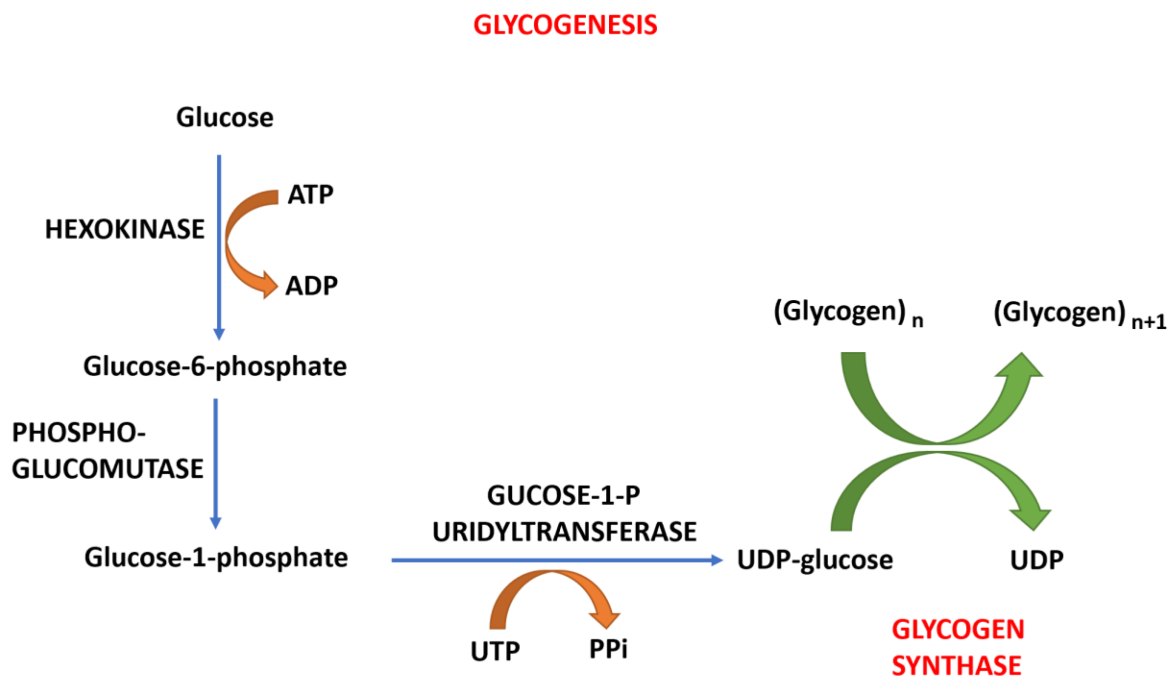


Figure 3.1 The process of glycogenesis

This structured literature review was conducted to provide evidence of the research that has already been conducted on the topic of myometrial glycogen concentration immediately post-partum and before parturition, to inform the research in this thesis on measuring myometrial glycogen content in women with dysfunctional labour.

3.3 METHOD

The collected material was methodically investigated, summarised, analysed, and interpreted, and the results and findings were discussed. This research was conducted on a systematic framework that was based on five strategical implementations for the conduction of the review, as suggested by Khan (2003). These strategies consist of 1) Framing questions concerning the review.2) Identifying relevant publications. 3) Evaluating the quality of selected publications. 4) Summarising all the evidence found.5) Interpreting the results. The first strategy of this structured literature review was to find out if there are pieces of evidence of research responding to the following question:

Do glycogen levels differ at certain time points of pregnancy and immediately post-partum?

The next two strategies of Khan et al. (2003) are discussed in the methodology section, while the fourth strategy is discussed in the result section on findings and results. The fifth and final strategy is stated in the section 3.5.1.1 of this review.

3.4 LITERATURE SEARCH AND STRATEGY

Studies of Sarah Neidler on medical search engines (2022) reveals that PubMed is considered to be the best search engine for medical research due to several reasons. Firstly, it is a trusted and reliable database that is managed by the National Library of Medicine in the United States. It specialises in providing comprehensive coverage of biomedical literature, including research articles from various medical disciplines. PubMed also offers advanced search options, allowing users to filter and refine their searches based on specific criteria such as article type, publication date, and study methodology. Additionally, PubMed's indexing system is highly efficient, ensuring that relevant articles are easily discoverable.

While PubMed is widely recognised as the go-to search engine for medical research, there are other notable options available. The Cochrane Library is another prominent database that focuses on evidence-based medicine and systematic reviews. Embase is a comprehensive

biomedical literature database that offers extensive coverage of drug-related research and pharmaceutical science. Google Scholar is also frequently used by researchers, as it provides access to a wide range of scholarly articles across various fields, including medicine. These search engines, along with PubMed, provide researchers with multiple options to access, retrieve, and study relevant medical literature. In my thesis, the searching was performed with PubMed, Google scholar, manual or hand search. Additionally, Cochrane library was consulted for this structured literature review.

Research papers that provided evidence on the concentration of myometrial glycogen immediately post-partum and before parturition were investigated between August and October 2022. A manual examination of the retrieved research pieces was performed, and a thorough review of the reference list of extracted papers was carried out. No limitations were applied on the terms of the publication date, language, or location. The key search terms that were used for the search were glycogen [Title/Abstract]) OR glycogen storage [Title/Abstract]) OR glycogen content [Title/Abstract]) OR glycogen level [Title/Abstract]) AND myometrium [Title/Abstract] AND pregnancy [Title/Abstract]. As this study was researching glycogen content before labour and after labour, human non-pregnant myometrium was considered outside the scope of this study.

Experts from the University of Liverpool library and Cochrane library were consulted in choosing the search terms.

3.4.1 Inclusion criteria

To be included in the review, the research had to fulfil the eligibility criteria. The inclusion criteria were journals and articles that evaluated the concentration of glycogen present in the myometrium during pregnancy and after delivery. The research criteria included animal or human models, in vivo and ex vivo, all languages, all kinds of research and clinical trials, abstracts, complete documents, systematic reviews, case studies, and published literature.

3.4.2 Exclusion criteria

Studies that did not meet the inclusion criteria.

3.5 RESULTS OF THE SEARCHES

Extensive research was conducted using PubMed, Google Scholar, and hand search. In total, 43 research articles were found and 31 pieces of research were extracted from a reference check. In total, 7 pieces of research were excluded because they were duplicates and 23 papers were excluded due to irrelevance to the topic. After the evaluation, from the remaining 44 articles, 28 were excluded as their contents did not meet the inclusion criteria. 17 pieces of research met the criteria for this structured review. The flowchart below illustrates the literature search and the outcomes gained from the search.

Prisma flow chart of selected articles

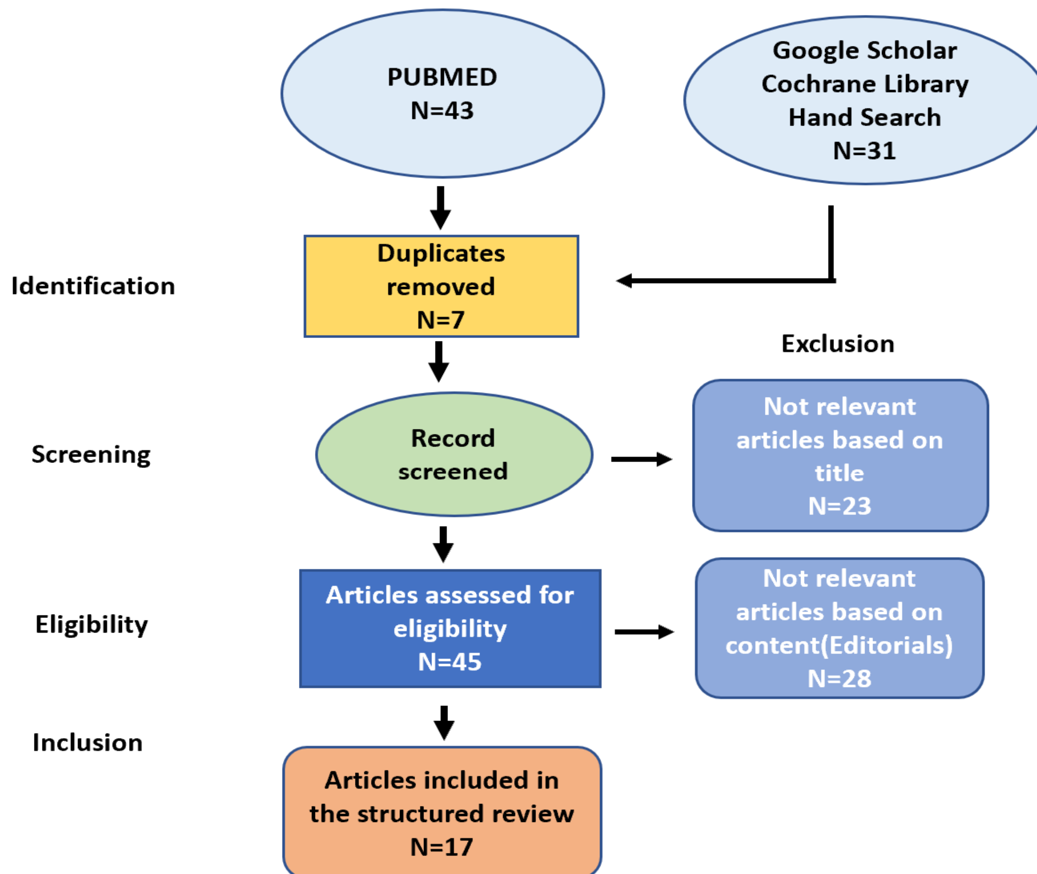


Figure 3.2 The selection procedure of articles included in the review: The chart explains how the articles were selected for the review.

3.5.1 Animal

Articles	Model, glycogen determination methods, number in study	Objectives	Results	Conclusion
1.West & Cervoni 1955	Rat Anthrone method (Seifer *et al.,* 1950) n= 10 (treated) n= 11(control)	Evaluating citric acid, lactic acid, and glycogen content through hormonal influence (oestrogen and progesterone) in the uterus of ovariectomised rats.	Hormonal pre-treatment led to an increase in glycogen content in the uterus by 4-5 times. **Control: 2.4 µmol/g** **Treated: 11.5 µmol/g**	Glycogen storage within the myometrium is regulated by the hormones.
2. Kostyo 1957	Rat Glycogen level not reported	Investigating hormonal control of glycogen levels in the rat uterus during pregnancy	**Only oestrogen was effective for stimulating glycogen deposition**.	The data indicate the vital role played by oestrogen in glycogen deposition.
3. Vasilevskaia 1960	Rat Glycogen amount not reported	Exploring the glycogen activity of myometrium in pregnant rat with one pregnant uterine horn and one non-pregnant uterine horn	The level of glycogen was significantly higher in the myometrium containing fetuses than in the myometrium with no fetuses. The quantity of glycogen increased along with pregnancy growth. The glycogen level was maximal near term.	The data indicates that the increasing level of glycogen relates to the development of the fetus during pregnancy. The physiological importance of this uterine function is in turn connected to the requirements of the developing fetus.
4. Bo & Smith 1966	Rat McManus method (1948) for glycogen determination: Takeushi & Kuriaki method (1955) for synthetase and phosphorylase activity. n= 13 (term pregnant) n= 6 (post-partum)	The histochemical determination of glycogen synthetase, phosphorylase activity, and the glycogen content in pregnant rats and pseudo-pregnant myometrium.	It was observed that the content of glycogen was higher in the external muscular layer than in the inner layer, with increasing activity in synthesising enzymes. A decline was observed after delivery. **Term pregnant: ++++(glycogen)** **Post-partum: ± (glycogen)**	It was suggested that there is more correlation between myometrial glycogen content and glycogen synthetase activity compared to phosphorylase activity. The authors found that relaxin has a possible role in increasing the level of myometrial glycogen.
5. Grégoire et al., 1967	Rat Anthrone method (Seifer *et al.,* 1950) n= 20 (oestrogen-treated) n= 10 (progesterone-treated)	To study the effect of different oestradiol 17-beta dosages on glycogen accumulation in the uterus, cervix, and vagina of rats.	Oestradiol benzoate administered into muscles for 3 consecutive days results in the build-up of glycogen within the uterine tissue and cervices of castrated Wistar rats **(129 ± 10 µg/100 mg of tissue).** **Progesterone reduced the level of glycogen (35.8 ± 5 µg/100 mg of tissue).**	Oestrogen promotes glycogen deposition; progesterone decreases glycogen levels in all uterine parts.

6. Murakami 1969	Rat Anthrone method (Seifer *et al.,* 1950) n= 18	Studying the effect of oestrogen on glycogen synthesis in the rat uterus.	When estradiol-17 was injected subcutaneously, uterine glycogen levels increased linearly 4–12 hours after administration, from **58 ± 10 µg/100mg**, reaching a maximum level after about 18 hours **(273 ± 34 µg/100 mg)**, and then gradually decreased after 24 hours.	These results indicate that the transport of glucose in the myometrial cells is stimulated and hence the glycogen synthesis promotion is caused by substrate glucose, without increasing the activity of glycogen synthetic enzymes. Oestrogen stimulates glycogen deposition.
7. Demers et al.,1972	Rat Montgomery colorimetric method (1957) n= 6	The goal of this study was to look at the levels of glycogen in the uterus, the activities of glycogen phosphorylase and glycogen synthetase, and the metabolism of the glycogen in the uterus in early pregnant, ovariectomized, and intact cycling rats.	An augmentation in turnover of myometrial glycogen, synthetase and phosphorylase activity during early pregnancy: **Day 5: 150 mg/100 g wet weight tissue** **Day 8: 240 mg/100 g wet weight tissue.**	The results suggest a crucial role played by the progressive increase of glycogen during pregnancy in preparing for labour.
8. Chew et al., 1979	Rat Anthrone method (Seifer *et al.*,1950) n= 10	Exploring glycogen content before and after labour.	The glycogen content in the myometrium of pregnant rats significantly increased immediately before birth. The level of glycogen in the myometrium reduced after delivery. **Before labour: 665 ± 70 mg/100 g tissue.** **After labour: 423 ± 40 mg/100 g tissue.**	For the purpose of sustaining uterine contractions during labor, glycogen may be a vital source of energy. When blood flow to the uterus is restricted due to intense myometrial contractions, a significant supply of glycogen can be very important.
9. Vasilenko et al.,1981	Rat Walaas method (1952) n= 6	Comparing the uterine size and glycogen content of a cycling rat and a pregnant rat in response to relaxin, the pregnant uterine horn and the non-pregnant uterine horn in the same rat.	Uterine glycogen content **increased (Day 5: 100 mg/100 g)** and reached the **peak at day 23(1000 mg/100 g)** then **decreased rapidly after delivery (Day 1: 200 mg/100 g).** The increase was considerably greater in the pregnant horn than in the non-pregnant horn. The injection of relaxin on the 15th day of pregnancy substantially increased uterine weight and myometrial glycogen content in the non-pregnant horn.	These data indicate that relaxin's role might be vital in the regulation and the storage of glycogen in the uterus in later pregnancy.

10. Beatty et al., 1969	Monkey Method previously described by Beatty, Peterson, Basinger & Bocel (1966). Data is reported in terms of non-collagenous protein nitrogen (NCN) rather than numbers.	The carbohydrate metabolism of myometrium from pregnant rhesus monkeys is being studied.	The amount of glycogen in the myometrium nearly doubled during the first half of pregnancy, rising from **120± 8 mg/g NCN (n= 5) at 54 days to 209± 27 mg/g NCN (n= 6) at 92 days**. **The glycogen level was 204 ± 24 mg/g (n= 9) at 155 days.** No further change was noticed during the latter half of pregnancy.	The storage of glycogen is vital as energy reserve for the growth of the myometrium. Lipids are the primary source for resting myometrium instead of carbohydrate.
11. Wicklund & Hillemann 1970	Hamster McManus & Mowry method (histochemistry) n= 5	Assessing the patterns of glycogen distribution in the uterus of early pregnancy and lactating golden hamsters.	The outer longitudinal myometrium of the pregnant uteri largely contains more glycogen content (**very dense glycogen granules**) than the inner circular myometrium (glycogen **granules: diffuse**). However, they usually contain equal amounts in the uteri of lactating hamsters.	The results indicate that in pregnant myometrium the glycogen content tends to be higher than in lactation uteri, clarifying the role played by glycogen content during gestation and labour.
12.Sype & Hillemann 1970	Hamster McManus & Mowry method (histochemistry) n= 17 (cycling) n= 12 (post-partum)	Investigating the changing patterns of glycogen distribution in the uterine tissue of the immature virgin hamster, along with the cycling virgin and the post-partum hamster.	The level of glycogen in all stages of the cycle was higher **(dense)** than that which was present in the immature hamster uterus. In the post-partum period, the glycogen deposits in the myometrium decreased **(diffuse)**.	These findings suggest that the distribution of glycogen content in uterine tissue depends on hormonal influence. The decreased level of glycogen in post-partum justifies glycogen consumption during labour.
13. Bowman & Rose 2017	Mink Modified method of Good et al. (1933) (Histochemistry, spectrophotometry) n= 5	Exploring glycogen synthesis in response to oestrogen and progesterone in the uterus of the American mink.	Oestradiol stimulated the synthesis of glycogen, but progesterone decreases glycogen storage in the uterus: 3 days treatment with: **Oestrogen: 21.91 ± 3.09 mg/g** of tissue (dry weight) **Progesterone**: **3.79 ± 0.54 mg/g** of tissue (dry weight).	The results suggest that while oestrogen promotes the accumulation of glycogen by the mink in uterine tissue during oestrus and gestation, progesterone induces glycogen catabolism in the uterus, releasing the glucose that is required to sustain gestation.

N.B. Text in bold denotes the change in glycogen amount found in animal myometrial tissues.

Table 3.1 Key attributes of the animal studies selected, including the model, method, objectives, results, and conclusions.

1. **WEST TC, CERVONI P. Influence of ovarian hormones on uterine glycogen in the rat; glycogen requirements for contractility under varying environmental conditions in vitro. Am J Physiol. 1955 Aug;182(2):287-91. doi: 10.1152/ajplegacy.1955.182.2.287. PMID: 13258804.**

West and Cervoni intended to expand contractility work to studies of metabolic intermediates. They sought to determine the levels of citric acid, lactic acid, and glycogen in the uterus of ovariectomised rats. Animals were treated with a combination of progesterone and oestradiol or did not receive hormonal treatment. Hormonal pre-treatment led to a 4-to-5-fold increase in glycogen content in the uterus. After contracting for 2 hours in an O_2 bubbled glucose-free Tyrode medium, the uterine tissues had lost around 60% of their glycogen and a significant amount of lactic acid was detected in the bath solution. Given these results, the authors suggested that ovarian hormones have an impact on the synthesis of glycogen and glycogen is an important energy storage for adequate contractility during labour.

2. **KOSTYO JL. A study of the glycogen levels of the rat uterus and certain skeletal muscles during pregnancy. Endocrinology. 1957 Jan;60(1):33-7. doi: 10.1210/endo-60-1-33. PMID: 13384381.**

The main goal was to study the changes in glycogen in the myometrium; The report discusses uterine glycogen changes during pregnancy as well as changes caused by epinephrine at the end of pregnancy. The experiment was carried out on the non-pregnant horns of unilaterally gravid rats. These experiments also allowed for the investigation of glycogen changes in the "stretched" muscles of the abdomen and the rectus femoris of the leg. Studies on the hormonal control of glycogen levels in the rat uterus revealed that only oestrogen is effective at stimulating glycogen deposition. Moreover, epinephrine treatment and subsequent extraction of the pituitary gland rapidly decreased glycogen levels in the uterus. In addition to this, it also demonstrated that almost all glycogen is concentrated in the myometrium compared to the endometrium.

3.VASILEVSKAIA NL. On the "glycogenic function" of the myometrium during pregnancy. Biull Eksp Biol Med. 1960 Apr; 49:101-4. Russian. PMID: 13841441.

This review investigated the myometrial glycogen activity in pregnant rats. It was estimated from this research that non-gravid animals have a similar glycogen content as individual fetuses. The myometrial level of glycogen is multiple times higher in the presence of fetuses. The removal of fetuses three, five and fifteen days before examination reduced glycogen levels. Growing level of glycogen in the myometrium was observed along with pregnancy growth. The glycogen level seemed to be maximal near the end of pregnancy. The data indicated that the increasing level of glycogen relates to the development of the fetus during pregnancy, and that the physiological importance of this uterine function is connected to the requirements of the developing fetus and the importance of glycogen as energy storage during labour.

4. BO WJ, SMITH MS. A histochemical study on the glycogen synthesizing enzyme in the myometrium of the pregnant and pseudo-pregnant rat. J Reprod Fertil. 1966 Oct;12(2):237-42. doi: 10.1530/jrf.0.0120237. PMID: 5951115.

The purpose of this study was to better understand the relationship between the amount of glycogen in the myometrial tissue during gestation and the activity of the enzyme's phosphorylase and glycogen synthase. Therefore, a histochemical determination was performed in pregnant and pseudo-pregnant rat uterine smooth muscle, in the absence and presence of deciduomata. The highest levels of enzymatic activity and glycogen content was determined in the absence of deciduomata in the pseudo-pregnant rat, with peaks on the eleventh and fourteenth day. Phosphorylase activity was high in both muscle layers. However, glycogen synthetase activity was only found in the external longitudinal layer of myometrial muscles. Glycogen content was higher in the external muscular layer in comparison to the inner layer. In the myometrium with deciduomata, glycogen and the activity of the enzymes mentioned above, were highest on the 12th and 13th day of pseudo-pregnancy. In the pregnant rat, an increase of glycogen content and the two enzymes was observed in the

myometrium from day 18 to day 21. Glycogen levels and glycogen synthase activity were significantly reduced in postpartum from day 1 to day 5, although phosphorylase levels in the circular muscle layer barely changed. The uterus of both pregnant and pseudo-pregnant rats could be found to have glycogen derived from G-1-phosphate and uridine diphosphate glucose, according to in-vitro experiments. Additionally, a stronger link between glycogen concentration and glycogen synthase activity than phosphorylase activity was found; but the question was to find out whether the effect of oestrogen and progesterone was responsible for the surge in glycogen content and the movement of synthase and phosphorylase. The possible intervention of relaxin in the increase of glycogen concentration and the activities of the two enzymes in the myometrial tissue was discussed.

5. **GREGOIRE AT, RAMSEY H, ADAMS A. The effect of various doses of oestradiol 17-beta on glycogen deposition in the rat uterus, cervix, and vagina. J Reprod Fertil. 1967 Oct;14(2):231-4. doi: 10.1530/jrf.0.0140231. PMID: 6053771.**

This study's objective was to investigate any possible connections between the formation of glycogen in the genital system and increasing oestradiol doses in all genital tract segments. The daily intramuscular administration of oestradiol benzoate for three days in a row led to the uterine accumulation of glycogen and the cervix of castrated Wistar rats. However, not all areas of the reproductive tract responded in the same way, and there was no linear link between the amount of oestrogen administered and the amount of glycogen deposited. Although oestradiol supplementation did not affect vaginal glycogen levels, progesterone reduced glycogen levels in all parts of the tract. The results suggest that glycogen deposition increases with oestrogen and that glycogen can be hydrolysed to a straightforward product and used as source of energy for the activity of the reproductive system either during gestation or labour.

6. **MURAKAMI S. [Effect of oestrogen on glycogen synthesis in the rat uterus]. Nihon Naibunpi Gakkai Zasshi. 1969 Feb 20;44(11):1249-59. Japanese. doi: 10.1507/endocrine1927.44.11_1249. PMID: 5815060.**

This article describes the mechanism of glycogen synthesis in the rat uterus in the early period following oestrogen administration. The following results were obtained. When estradiol-17 (ED, 50 pg/rat) was injected subcutaneously, uterine glycogen levels increased linearly 4–12 hours after administration, reaching a maximum level at about 18 hours and then gradually decreasing after 24 hours. When the rats fasted overnight for 16 hours, the uterine glucose level decreased, and the glycogen content decreased simultaneously to 2/3 of the control.
At 4 and 8 hours after the administration of ED, the glucose content in the uterus increased by about 30% without a significant change in serum glucose. With an increase in glucose concentration, glycogen synthesis accelerated in proportion to the glucose concentration even with the castrated uterus. Cycloheximide, a protein synthesis inhibitor, was administered, and this caused the liver to prompt glycogenolysis.
After hyperglycaemia, which caused glycogenolysis and an increase in the level of glucose and glycogen in the uterus, the above results convincingly indicated that the key factor regulating uterine glycogen content is the amount of glucose in uterine cells. The probable mechanism of increased synthesis of uterine glycogen in the early stage (4-8 hours) following the administration of ED is that the transport of glucose in the myometrial cells is stimulated and hence glycogen synthesis promotion is caused by substrate glucose without increasing glycogen synthetic enzyme activity.

7. DEMERS LM, YOSHINAGA K, GREEP RO. Uterine glycogen metabolism of the rat in early pregnancy. Biol Reprod. 1972 Oct;7(2):297-304. doi: 10.1093/biolreprod/7.2.297. PMID: 4631227.

The objective of this research work was to explore the metabolism of the glycogen present in the uterus in early pregnant, ovariectomised and intact cycling rats. In various endocrine environments, the amount of glycogen in the uterus as well as the activities of glycogen phosphorylase and synthetase were assessed.
An increase in turnover of glycogen was observed, as evidenced by increased synthetase and phosphorylase activity during early pregnancy, which coincided with the process of egg implantation. Such changes in the metabolism of the glycogen present in the uterus suggest a crucial role played by a progressive increase of glycogen in preparation of labour.

8. CHEW CS, RINARD GA. Glycogen levels in the rat myometrium at the end of pregnancy and immediately postpartum. Biol Reprod. 1979 Jun;20(5):1111-4. doi: 10.1095/biolreprod20.5.1111. PMID: 476244.

The aim of this study was to examine the glycogen levels in the rat myometrium before and after labour. A pregnant rat's myometrium was shown to contain considerably more glycogen right before giving birth. At that time, glycogen concentrations were like skeletal muscle concentrations and were much higher than concentrations in the uterus of rats at 18 and 20 days of pregnancy and in the uterus of non-pregnant rats and ovariectomised rats or those treated with oestrogen. Delivery, when allowed to occur in a natural manner or when caused by an infusion of oxytocin, reduced glycogen in the myometrium by half (from 665 ± 35mg /100mg of tissue at day 22 to ~ 450mg/100mg of tissue immediately after normal delivery). These consequences proposed that glycogen may be a crucial energy source for maintaining contractions during labour. A significant supply of glycogen can be vital when blood flow to the uterus decreases due to strong contractions of the myometrium.

9. VASILENKO P 3RD, ADAMS WC, FRIEDEN EH. Uterine size and glycogen content in cycling and pregnant rats: influence of relaxin. Biol Reprod. 1981 Aug;25(1):162-9. doi: 10.1095/biolreprod25.1.162. PMID: 7197174.

The purpose of this study was to look into the effect of relaxin on uterine size and glycogen levels in pregnant and cycling rats. The weight of the uterus and glycogen concentration were determined at various times during the oestrus cycle and in the pregnant and non-pregnant horns of unilaterally pregnant rats. Between the oestrus-diestrus stage and the fifteenth day of pregnancy, the response to a purified formulation of swine relaxin was evaluated. Before and during oestrus, uterine weight and glycogen content were highest, and at diestrus, they were lowest. The loss of uterine weight and glycogen mass was postponed when 0.1 mg of relaxin was given at the start of oestrus and over a 24-hour period. During pregnancy, the weight of the uterus decreased before implantation and increased rapidly until day 22, after which the size of the uterus decreased due to childbirth. The uterine glycogen content changed in a similar manner, but the peak occurred on the 23rd day, then decreased rapidly. The pregnant horn's increase in glycogen content and uterine weight was noticeably greater than that of the non-pregnant horn's, with the myometrium on delivery day exhibiting the highest glycogen concentration in either horn. The 15th day of pregnancy saw the injection of relaxin, which had no impact on the pregnant horn's variable parameters, considerably increase uterine weight and myometrial glycogen content. These findings suggested that relaxin had an influence in the regulation and storage of glycogen in the uterus in later pregnancy because uterine tissue and glycogen growth profiles are similar to those recently reported for relaxin concentrations in rats rather than changes in steroid hormone levels.

10. BEATTY CH, BASINGER GM, BOCEK RM. Carbohydrate metabolism of myometrium from the pregnant rhesus monkey. J Reprod Fertil. 1969 Aug;19(3):443-54. doi: 10.1530/jrf.0.0190443. PMID: 4980217.

To study the carbohydrate metabolism of the myometrium, the authors collected uterine muscle samples from pregnant rhesus monkeys and incubated them in a solution containing [1-^{14}C] glucose and [6-^{14}C] glucose. The production of CO_2, lactate, glucose uptake and the Qo_2

were the same at ninety-two days (55% of pregnancy) and at 155 days (93% of pregnancy). The level of glycogen increased between the 54th and the 92nd day of pregnancy and there was no evidence that the increase continued until near term. According to the findings, the glycolysis pathway is more important in pregnant rhesus uterine muscle than it is in striated muscle. Nonetheless, only about 0.2% of glucose uptake may be attributed to oxidation via pentose phosphate pathway. The glycogen storage appears to be fundamental as an energy reserve for the development of the myometrium.

11. **WICKLUND TG, HILLEMANN HH. Glycogen: in uterus of early pregnant and lactating hamster. Trans Am Microsc Soc. 1970 Jan;89(1):1-6. PMID: 5420437.**

This research elaborates the histochemical identification of the varying glycogen patterns within the uteri of golden hamsters (Mesocricetus auratus Waterhouse) that were pregnant or lactating. Uteri were examined on days one through five of gestation, and days one through ten, twelve, and fourteen of lactation. The sections were compared with adjacent marked sections to assess the glycogen pattern. The presence of glycogen and its amount within uterine tissues of animals that were either lactating or pregnant were inconsistent. The outer longitudinal myometrium of the pregnant uteri largely contained more glycogen content than the inner circular myometrium, however, they usually contain equal amounts in the uteri of lactating animals. In the endometrium, granular glycogen was seen solely during pregnancy and especially on the fifth day, on which it was constrained to the locular level of the uterine tissue and in very compact deposits. In the uterus, the epithelium held granular glycogen in tiny amounts on some days of pregnancy, but not during lactation. Even though the connected basement membrane never had granular glycogen, its diffuse form was present, but somewhat inconsistent. The uterine epithelial border in gestation contained glycogen granule patches with more frequency compared to during lactation. In the uterine lumen, it was rare or in patches. The uterine gland lumen and the epithelium had tiny amounts of granular glycogen on some days of gestation but none of these areas had granules during lactation.

Diffuse glycogen in changing amounts was detected in the basement membrane connected with the glands of the uterus whereas during gestation, the tunica of the veins had more granular glycogen compared to the tunica media of the arteries. Only diffuse glycogen was

observed in these tunics and in equal amounts in the lactation uteri. An evaluation is made between present opinions on the hamster and observations made by others in other species.

12. SYPE WE, HILLEMANN HH. Glycogen: distribution in the estrus and postpartum hamster uterus. Trans Am Microsc Soc. 1970 Jan;89(1):6-11. PMID: 5463288.

In this study the changing patterns of glycogen distribution were explored in the uterine tissue of the immature virgin hamster, along with the cycling virgin and the post-partum hamster. A small amount of glycogen was accumulated in the uterine epithelium and inner myometrium during oestrus and remained moderate in outer myometrium. In all regions of the uterus, there was a decrease in glycogen during metestrus. However, during diestrus, glycogen levels increased slightly in inner myometrium but remained unchanged in outer myometrium. During the first 6 days post-partum, the amount of myometrial glycogen increased above the level found in both immature and cycling hamsters. The content of glycogen in all stages of the cycle surpassed that was present in the immature hamster uterus. In the post-partum period, the glycogen deposits in the myometrium decreased.

13. BOWMAN K, ROSE J. Oestradiol stimulates glycogen synthesis whereas progesterone promotes glycogen catabolism in the uterus of the American mink (*Neovison vison*). Anim Sci J. 2017 Jan;88(1):45-54. doi: 10.1111/asj.12564. Epub 2016 May 12. PMID: 27170562; PMCID: PMC5107167.

In this report, it was hypothesised that progesterone (P_4) was accountable for the catabolism of glycogen stored in the uterus to secure reproductive success, meeting the energy needs for pre-embryonic development as well as implantation. Minks were treated with P_4, oestradiol (E_2) or vehicle control for three days and uterine tissues collected 24 h (oestradiol, progesterone, and the vehicle) and then 96 h (oestradiol) later. In a separate experiment to assess E_2 priming, mink was treated with E_2 for three days and then P_4 for another three days ($E_2 \rightarrow P_4$); collection of uteri occurred 24h later. At E_2 + 96 h, the concentration of glycogen in glandular and luminal epithelium was superior to E_2 +24 h and each of them were superior to controls. $E_2 \rightarrow P_4$ treatment reduced glycogen content in the myometrium to levels similar to controls, while simultaneously increasing catabolic enzyme gene expression and the volume

of phosphor-glycogen synthase protein in myometrial homogenates. Curiously, $E_2 \rightarrow P_4$ treatment amplified glycogen synthase 1 mRNA, hexokinase 1 mRNA and proteins. The results suggested that while oestrogen promotes the accumulation of glycogen by the mink uterine tissue during oestrus and gestation, progesterone induces glycogen catabolism in the uterus, providing the glucose needed to sustain pre-embryonic existence and implantation.

3.5.1.1 Discussion

This study is an expanded structured literature review on the storage of uterine glycogen in animal and human myometrium. Articles on the content of glycogen were explored for the existing evidence prior to parturition, during parturition and immediately after labour. A large and deep search was performed for the current published literature and research papers from PubMed, Google Scholar, and Cochrane library as search engines along with a hand search. 17 articles passed the eligibility criteria and were included in this review. There were 13 articles on animals (nine on rats, two on hamsters, one on mink, and one on rhesus monkeys) and four articles on humans.

This structured literature review on glycogen storage stresses that extensive work has been conducted on rats (9 out of 13). The aims included the exploration of glycogen content, glycogen enzyme activity, the hormonal influence on glycogen metabolism in non-pregnant and pregnant rats, and glycogen in post-partum. Glycogen determination methods were similar, although the sampling and glycogen values were not mentioned in some of the articles. These methods include anthrone reaction, phosphorolysis, glucosidase, and hydrolysis. Glycogen values measured and obtained from all these methods were comparable and reliable (Passonneau and Lauderdale, 1973).

The results from this review confirm that myometrial glycogen content increased progressively along with the age of gestation in the rat (Chew et al., 1979; Vasilenko et al., 1981; Bo & Smith, 1966; Beatty et al., 1969; Vasilevskaia 1960). The findings were corroborated by the research outcome from hamster, mink, and monkey (Wicklund & Hillemann, 1970; Bowman & Rose, 2017; Beatty et al., 1969).

The decline in the content of myometrial glycogen immediately after labour was described in (Chew et al., 1979; Vasilenko et al., 1981; Bo & Smith, 1966).

The authors indicated that 1. Glycogen might be a vital energy source or reserve for the sustainability of contractions during parturition and that the decline of the myometrial glycogen level relates to the use of glycogen as energy during parturition.2. Glucose is more utilised as principal metabolite by the uterine smooth muscle of pregnant women, compared to lipids. 3.Glucose plays a major role as the principal fuel to produce ATP during labour. A huge amount of glycogen could be specifically vital when blood supply to the uterus decreases due to strong contractions of the myometrium during labour.

The impacts of oestrogen, progesterone and relaxin on glycogen storage were reported as follows. Oestrogen had an anabolising effect on myometrial glycogen storage (West & Cervoni 1955; Gregoire et al.1967; Murakami 1969; Bowman & Rose 2017). Relaxin had a similar effect (Vasilenko et al.1981; Bo & Smith 1966). In contrast, the catabolising effect of progesterone was also described (West & Cervoni 1955; Gregoire et al.1967; Bowman & Rose 2017). Thus, the findings from the literature review suggest that hormonal involvement in the building up or degradation of myometrial glycogen content is not negligible.

3.5.2 Human

Articles	Glycogen Quantification Method	Objectives	Results	Conclusion
1.Brody 1958	Method of Carrol, Longley, and Roc (1956): Colorimetric Method (Spectrophotometry) Non-Pregnant: n=8 Pregnant: n=7 Post-Partum: n=2	Exploring the myometrial concentration of glycogen in human at different stages of ovarian hormone activities	The highest amounts of glycogen were found during gestation and these values were increasing towards term. **From 19.7 µg/mg to 23.3µg/mg** There was a tremendous decline of glycogen content immediately in post-partum**(10.3µg/mg)**	The results indicate that the myometrial glycogen content was increasing along with the growth of the fetus. The decline of glycogen content relates to the use of glycogen as energy during labour.
2. Midwisky & Gutman 1983	Method of Carrol, Longley, and Roc (1956): Colorimetric method (spectrophotometry) Non-pregnant: n = 32 Pregnant: n = 4	Attempting to measure glycogen concentrations and glycogen metabolism enzyme activity within the myometrium of postmenopausal and premenopausal women, pregnant women, and myomas.	The glycogen concentration in the muscles of non-pregnant females was around **3 mg /g** and raised **to 13 mg/g** inside tissue obtained through caesarean section	It is suggested by these evaluations that metabolism of glycogen within myometrium is controlled by the hormones and enzyme activity
3. Steingrimsdottir et al., 1995	Glucose Oxidase Method and Enzymatic Method n = 10	Examining the energy metabolite pattern in term pregnant women's uterine muscle and striated rectus muscle	It was revealed that high content of glucose respectively **12.9 µmol/g and 5.9 µmol/g** dry weight and an increased ratio of lactate/pyruvate exists in the myometrium compared to the rectus muscle. Adenosine was found in 36-fold higher concentration in the uterine tissue than in the rectus muscle. Free uridine **Myometrium** :**0.61 µmol/g** **Rectus muscle: 0.05µmol/g**	These outcomes demonstrate that glycogen is more utilised as principal metabolite by the uterine pregnant smooth muscle, compared to lipids. Furthermore, in comparison to the myometrium, the striated rectus muscle is less active in the anaerobic pathway of glucose metabolism. Furthermore, glucose serves as the primary fuel for the production of ATP.
4. Steingrimsdottir et al., 1999	Glucose Oxidase Method n = 10	Before labour, the glycogen content of the myometrium was compared to that of the rectus muscle in term pregnant women.	The content of glycogen in the myometrium was double the content present in striated muscle, respectively **13.6 and 5.81 µmol/gr dry weight)**	The results indicate the vital role played by the high level of glycogen as probable source of energy prepared for labour.

N.B. Text in bold denotes the change in glycogen amount found in animal myometrial tissues.

Table 3.2 Key attributes of the selected studies including the model, the method, the objectives, the results, and the conclusion in human.

1. BRODY S. Hormonal influence on the glycogen content of the human myometrium. Acta Endocrinol (Copenh). 1958 Mar;27(3):377-84. doi: 10.1530/acta.0.0270377. PMID: 13507994

The goal of this study was to explore the myometrial concentration of glycogen in human at different stages of ovarian hormone activities. Uterine samples were from non-pregnant, pregnant, parturient, and post-menopausal women. Menopausal women had the least glycogen in their uteri. The glycogen level was slightly higher in the non-pregnant group. (4-fold increase). The highest amounts of glycogen were found during gestation and these values were increasing towards term (from 19.7 µg/mg to 23.3µg/mg). At term, myometrial glycogen levels were roughly 50 times higher than in the post-menopausal group. There was a tremendous decline of glycogen content immediately in post-partum (10 µg/mg). These results indicated that the increasing glycogen content of myometrium pertains to the growth of the foetus and readiness for labour. The decline of glycogen content relates to the use of glycogen as energy during labour.

2. MILWIDSKY A, GUTMAN A. Glycogen metabolism of normal human myometrium and leiomyoma--possible hormonal control. Gynecol Obstet Invest. 1983;15(3):147-52. doi: 10.1159/000299405. PMID: 6403415.

This study sought to determine the glycogen concentrations and enzyme activities involved in glycogen metabolism within the uterine muscle of postmenopausal and premenopausal women, pregnant women, and myomas. The authors found that the glycogen concentration in the muscles of non-pregnant females was around 3 mg/g and was raised to 13 mg/g in term pregnant tissue collected through caesarean section. The activities of glycogen synthase and glycogen phosphorylase were also found to be significantly higher in the pregnant myometrium. Among 8 myomas, the content of glycogen and enzymatic activity of 5 were elevated to a similar extent as that found at the end of pregnancy. There was no significant increase in the other 3 myomas. It was suggested by these evaluations that metabolism of glycogen within myometrium is controlled by the hormones. Furthermore, the hormonal environment of fibromyomas may differ from that of surrounding myometrium tissues.

3. STEINGRÍMSDÓTTIR T, RONQUIST G, ULMSTEN U, WALDENSTRÖM A. Different energy metabolite pattern between uterine smooth muscle and striated rectus muscle in term pregnant women. Eur J Obstet Gynecol Reprod Biol. 1995 Oct;62(2):241-5. doi: 10.1016/0301-2115(95)02195-d. PMID: 8582504.

The goal of this study was to compare the energy metabolite pattern in the human pregnant myometrium at term to that of skeletal muscle. By comparing striated (rectus abdominis) and myometrial muscles at term, it was revealed that a higher concentration of glucose respectively 12.9 µmol/g and 5.9 µmol/g dry weight and larger ratio of lactate/pyruvate exists in the uterus smooth muscle, but a lower triglyceride metabolite level is present compared with rectus abdominus. Adenosine was found in 36-fold higher concentration in the uterine tissue than in the rectus muscle. Free uridine in the myometrium was exceedingly high (0.61 µmol/g) at term compared to with the rectus abdominus (0.05µmol/g). These outcomes indicate that glucose is more utilised as principal metabolite by the myometrial tissue of pregnant women as compared to lipid. Moreover, in comparison to the myometrium, the striated rectus muscle is less active in the anaerobic pathway of glucose metabolism.

4. STEINGRÍMSDÓTTIR T, RONQUIST G, ULMSTEN U, WALDENSTRÖM A. Low myometrial glycogen content compared with rectus muscle in term pregnant women before labour. Gynecol Obstet Invest. 1999;47(3):166-71. doi: 10.1159/000010086. PMID: 10087410.

The purpose of this study was to compare the glycogen content of skeletal muscle to that of uterine smooth muscle in pregnant women prior to parturition. Purines, nucleosides, glucose, and glycogen derivatives were tested in biopsies from ten pregnant women at term who had elective caesarean sections before labour. A comparison of concentrations in biopsies that were extracted from the uterus and rectus abdominis was made. The concentration of glucose in the myometrium was double the content present in striated muscle (respectively 13.6 and 5.81 µmol/gr dry weight). This high glucose content in term pregnant uterine smooth muscle versus striated muscle suggests that the pregnant myometrium has high energy demands in preparation for parturition.

3.5.2.1 Discussion

Sam Brody stated in his article that no quantitative studies on the level of glycogen in the human myometrium had been attempted (1958). Six decades have passed; published papers on glycogen content in human uterine smooth muscles remain rare. For this thesis, only four suitable papers on humans could be found. Biopsies were obtained either from the fundus or the lower segment of the uterus from C-sections and immediately frozen in dry ice or liquid nitrogen.

Brody focused his study on the content of glycogen at different moments of ovarian hormonal function, non-pregnant women, pregnant, post-partum and post-menopausal. although the sampling was not specified. Brody was the first to demonstrate the evolution of the levels of glycogen during gestation and post-postpartum in humans. In pregnant women, a progressive increase of glycogen toward term was noticed, and a tremendous fall was observed in post-partum within the first four days (10 µg/mg). But in his conclusion, Brody stated that “the increasing glycogen content of the myometrium pertains to the growth of the fetus and readiness for labour, the decline of glycogen content relates to the use of glycogen as energy during labour”. The statement is in contraction with what is known today, about the role of the endometrial glycogen content during pregnancy:

The role of myometrial glycogen

The myometrial glycogen storage refers to the accumulation of glycogen in the myometrium, the muscular layer of the uterus. While glycogen is primarily known for its role in providing energy, its impact on the growth of the fetus is not well-established (McEvoy & Sabir 2022)

During pregnancy, the myometrium undergoes significant changes to accommodate the growing fetus. It experiences hypertrophy, or an increase in cell size, and hyperplasia, or an increase in the number of cells. These changes are influenced by various factors including hormones, growth factors, and maternal nutrition, but the specific role of myometrial glycogen has not been widely studied (Ciarmela et al.,2011)

Glycogen is stored in tissues as a readily accessible source of energy. However, it is primarily utilized within the tissue that stores it. The placenta and maternal blood provide the main

sources of nutrients for fetal growth, rather than relying on glycogen stored in the myometrium.

Therefore, it is unlikely that myometrial glycogen storage directly impacts the growth of the fetus. The growth of the fetus is primarily influenced by factors such as the availability of nutrients and oxygen, placental function, hormonal balance, and genetic factors. (Herrick & Bordoni 2023)

In conclusion, while the myometrial glycogen storage is an interesting aspect of pregnancy physiology, its direct impact on fetal growth is unclear. Further research is required to better understand the role of myometrial glycogen and its potential influence on various aspects of pregnancy.

The role of endometrial glycogen

Endometrial glycogen storage does have an impact on the growth of the fetus during pregnancy. The endometrial lining of the uterus undergoes changes every month to prepare for the potential implantation of a fertilized egg. One crucial aspect of these changes is glycogen storage. Glycogen is a complex carbohydrate stored in organs like the uterus, liver, and muscles (Burton & Fowden 2015)

During pregnancy, the endometrial glycogen stores increase significantly. This increase in glycogen is important for providing nourishment and energy to the developing fetus. Glycogen serves as a readily available source of glucose, which is crucial for the fetus's growth and development (Burton & Jauniaux 2023)

The glycogen stores in the endometrium are converted into glucose and transported to the placenta. The placenta acts as a vital link between the mother and the fetus, allowing the exchange of nutrients and oxygen. The glucose derived from glycogen is transferred to the fetus through the placenta, supporting its energy needs and facilitating proper growth (O’Brien & Wang 2023)

Therefore, adequate endometrial glycogen storage is essential for the optimal growth of the fetus. Insufficient glycogen storage in the endometrium could result in compromised nutrient delivery to the fetus, potentially leading to developmental issues or growth restrictions. (Madunić et al.,2021)

Milwidsky et al. (1983) determined by similar methods that the level of glycogen found in non-pregnant women's myometria was considerably lower but rose at term from tissues obtained by C-section. Although the value of glycogen at the beginning of gestation was not mentioned, however I believe that logically this value is close to that of non-pregnant. Besides, Milwidsky and colleagues confirmed the hormonal implications of glycogen metabolism in human myometrium. It was demonstrated that while oestrogen and relaxin are promoting glycogen deposition in the myometrium, progesterone decreases glycogen by releasing the glucose that is required to sustain gestation. It could be understood that high energy demands by the product or products of conception during pregnancy might interfere with the building up of the amount of glycogen necessary for sustaining the metabolic challenges of labour. Through this evaluation it was suggested that the metabolism of glycogen within myometrium is controlled by hormones.

Steingrímsdóttir et al. (1995) compared the pattern of several energy metabolites between the myometrium and the rectus abdominus muscle in 10 pregnant women just before parturition. The biopsies were collected from elective C-section and immediately plunged in liquid isopentane at -170°C and surrounded with liquid nitrogen. Glucose level was determined by glucose oxidase procedure or method. Glycerol, pyruvate, and lactate were determined by enzymatic method while free fatty acids content was measured by both enzymatic and colorimetric method. The difference between metabolites were statistically analysed. Two tailed paired student's *t*-tests as well as Pearson's correlation test were used to measure correlations metabolites. The level of glucose in the myometrium was found to be twice the level found in the rectus abdominus muscle. Although glycogen content was not directly measured in this study, the level of free uridine that was found in the myometrium was extremely high at term compared to the one found in the found in the rectus muscle. This high level of free uridine suggested that the level of glycogen was consequently high since uridine is an intermediate metabolite in the glycogenesis pathway. A higher ratio of lactate/pyruvate and a reduced triglyceride metabolite content found in the myometrium indicates that glucose was used in a pregnant uterus as the main fuel.

3.5.2.2 Critical analysis

1.Animal studies

1.Research Topic: Out of the 13 animal studies selected, nine were conducted on the rat, one on the mink, two on the hamsters and one on the monkey. All the research subjects were presented in a clear manner.**2. Introduction:** The research topics were well explained in the introduction and provided general information of the subjects. In addition, objectives were well defined.**3. Literature Review:** The literature review was well-organised but not extensive. It covered a large range of different studies on glycogen storage in the myometrium during pregnancy and post-partum. Although the literature used appeared to be old, it provided information that was needed for this review. This is understandable because of the scarcity of studies around this specific topic However, it could benefit from including more recent studies and providing a more critical analysis of the existing literature.**4. Research Methods:** The research methods were clearly described and appropriate for the research questions. 8 out of 9 studies conducted on the rat, used anthrone method to measure the levels of glycogen in the myometrium with large ample size. These strengths enhanced the robustness, the validity and reliability of the study's findings. These studies were therefore comparable compared to the mink (1 study), Monkey (1 study) and hamster (2 studies). Researchers used anthrone reaction for the mink and the monkey. In these two species, insufficient publications made them not be comparable although their sample sizes were good. Despite the fact that studies with hamsters were comparable and the same technic used for the determination of myometrial glycogen storage (Staining method) and large sample size, the lack of enough publications in the hamsters' group was a limitation although the results were similar. When analysing all the animal studies selected, it become evident that the rat group holds stronger evidence due to its rigorous methodology, same species, and large sample size, therefore comparable. It is essential to note that in a structured literature review, multitude of studies are needed to draw a comprehensive conclusion.**5. Results:** The results were clearly presented and easy to understand. The study found that glycogen level in the myometrium increased progressively along with the age of gestation and declined immediately after parturition. The metabolism of glycogen in the myometrium is regulated by hormones. **6.Discussion**: The discussion section provided a good analysis of the study's results. The authors did not acknowledge the limitations of the studies. Only one (Mink) provided recommendations for future research. However, they could benefit from discussing some

future work.**7. Conclusion**: The conclusions of the selected studies summarised the main results of the studies and provided some implications for future research and practice. However, it could be beneficial from providing more specific recommendations for future.

2.Human studies

1. The topic was clear.**2. The introduction** of their research topics was clear and provided an overview of the issue**.3. The literature review** is comprehensive and well-structured. It covers a broad range of studies that have examined the myometrial glycogen storage during pregnancy and in postpartum. However, these studies look old. More recent studies could be beneficial and provide a more critical analysis of the existing literature.**4. The research methods** are clearly described and appropriate for the research. In the first 2 studies glycogen content was determined using anthrone method. Although the method is old, but it was suitable and applicable in those days and could provide comparable results between studies. For many years, methods used for glycogen analysis were based on glycogen isolation from interfering substances in the samples, after which the glycogen was determined by anthrone reaction. Since seventies, enzymic methods have been developed. In the other 2 studies, glycogen content was measured by enzymic reaction. These procedures involve the degradation of glycogen using diazyme followed by glucose measurement with glucose oxidase. Enzymic procedures are conducted with greater ease and in less time compared to anthrone although the results are similar. However, it could be useful to include studies that have used the same glycogen measurement methods to minimise bias.**5. The results** are presented in a clear and concise manner. In general, all the articles confirmed the increased level of glycogen in the myometrium throughout the pregnancy and a drastic drop of myometrial glycogen immediately after parturition. This decline of glycogen was perceived to be the use of glycogen as source of energy during labour. The studies confirmed the role of enzyme activities and hormones in the regulation of glycogen metabolism. The sample size in each study was acceptable, the first 2 studies (Brody 1958 and Midwisky & Gutman 1983) were comparable between them considering their sample size and methodology. The third and the fourth studies were comparable as well. However, the size of the studies was small. It is essential to note that the systematic review should consider a multitude of studies to draw comprehensive conclusions.

6.The discussion section provides a thorough analysis of the study's findings, but the authors did not either acknowledge the limitations of the study nor provide suggestions for future research.**7. The conclusions** summarised properly the main findings of the studies.

3.5.3 Conclusion

This is the first structural review exploring the metabolism of glycogen in the myometrium during pregnancy and immediate post-partum. Considering study designs, sample sizes, methodologies, and the quality of evidence presented, it transpires that only publications from rat experiments could help draw a valuable conclusion about glycogen metabolism in the myometrium during pregnancy and post-partum. Despite insufficient publications from other species, it emerges that in either animal or human studies, the selected articles reported a gradual increase of myometrial level of glycogen during gestation and a huge glycogen decline just after labour; the hormonal regulation in the metabolism of glycogen; and the enzymes involved in this process. The authors mentioned the crucial role played by glycogen as the main source of energy and its major importance in the contractile activity of the uterus during labour. In addition to other metabolic changes occurring in the myometrium during pregnancy, it transpires that the increase in the amount of glycogen during gestation prepares the uterus for an effective labour by storing energy.

3.5.4 The relevance of the review outcomes to my thesis

This review supports the hypothesis of my thesis, which suggests that since glycogen is the principal energy for power production, glycogen is indispensable in the contractile uterine activity, and needs to be used during labour, glucose depletion in vivo during parturition may contribute to labour dysfunction, and that enough glycogen storage is needed to overcome the metabolic challenges associated with labour.

3.5.5 Limitations of these studies

In this structural review, most of the studies selected are becoming old. There can be various reasons for studies becoming old, not necessarily indicating a halt in research. Firstly, it takes time for research to be conducted, published, and disseminated. Secondly, certain fields may have reached a point of saturation, with foundational studies already established, leading to a reduction in further investigations. Additionally, limited funding, lack of interest, or shifting

research priorities may divert attention away from certain areas. Furthermore, researchers may be focusing on other aspects of a field or novel emerging topics. Lastly, legal, ethical, or practical constraints can restrict further studies in certain areas. These factors collectively contribute to the aging of studies; however, it does not imply a discontinuation of research, as science continuously evolves and addresses new challenges. Uterine related studies are still a vast field to explore. Researchers are still trying to comprehend the reasons behind dysfunctional labour, the results of my thesis will be a contribution to uterine studies.

Except for studies with rats, the small sample size of some different animal species (Mink, Hamster, Monkey) and human tissues made some studies not comparable nor reliable.

CHAPTER FOUR

The effect of zero glucose on uterine contractility

CHAPTER 4

The effect of zero glucose on uterine contractility

4.1 The effect of zero glucose on uterine contractility in mice

4.1.1 INTRODUCTION

Hypoxia can be defined as a diminution of uterine blood flow by occlusion of blood vessels during contractions. This is a normal process happening to the myometrium during parturition. However, if hypoxia is prolonged, this will result in dysfunctional labour or poor contractions leading to slow progress of labour (Wray et al., 2021). Hypoxia leads to increased lactate production and a fall in pH. These metabolic changes contribute to a decrease in strength and duration of uterine contractions. During relaxation, the myometrial blood supply is restored along with its metabolites and pH. If blood supply does not fully recover and myometrial metabolites and pH are not restored, uterine contraction will fail, and labour dysfunction may occur. As the decrease of blood flow during each contraction at labour is accompanied with a reduction of glucose supply to the myometrium, this thesis examines the combined effect of glucose depletion and hypoxia on uterine contractility.

Glucose is the principal source of energy for cellular metabolism. Glucose is successively catabolised in three processes: glycolysis, then the Krebs cycle, and finally oxidative phosphorylation to generate ATP. ATP is commonly known as the energy exchange component that links anabolism and catabolism and fuels procedures such as phosphorylation, active transport, and motile contraction. Additionally, ATP plays an important role as a signaling molecule at purinergic receptors (Pollard-Knight D et al., 1982; Bonora, Massimo et al., 2012).

Creatine is a nitrogenous organic acid produced naturally. Its main metabolic role is to combine with free inorganic phosphoryl groups to produce phosphocreatine, which is utilised to stimulate the production of ATP. Creatine phosphate operates as an energy buffer. When a muscle (striated or smooth) begins to contract and requires energy, creatine phosphate passes on its phosphate back to ADP to generate ATP and creatine through creatine kinase (Sahlin & Harris 2011).

As a smooth muscle, the myometrium needs the production of ATP for contractions, even though the energy requirement for smooth muscles is significantly lower than for skeletal

muscles (Ishida, Y. et al., 1994). At each contraction during labour, blood supply to the myometrium is reduced; consequently, glucose delivery to the myometrium is also reduced. In this chapter the effects of this glucose fluctuation on uterine contractility will be explored. The outcome from this exploration should provide more insights on establishing the direct cause and effect correlation between glucose deficiency and smooth muscle (myometrial) dysfunction. This relationship has not been clarified thus far in either smooth or striated muscle.

The effect of agonists on uterine contractility should not be neglected. Oxytocin (the word originates from Greek and means swift birth) is a hormone naturally synthetised in the hypothalamus and released principally from the neurohypophysis; it has a range of different functions in the body, including augmenting myometrial contractile amplitude and frequency in human tissue and breast milk production (Akerlund et al., 1983; López-Ramírez et al., 2014).

In clinical settings, oxytocin has been prescribed by obstetricians to improve contractility during labour when uterine contractions are poor, or when labour fails to progress. Therapeutic management with this drug relies on its activation of IP_3, which sequentially causes the delivery of Ca^{2+} into the cytosolic space from the sarcoplasmic reticulum (Arrowsmith and Wray, 2014; Matthew et al., 2004b). It is not known if glucose depletion during labour could contribute to OT limitations.

In this thesis, the effect of zero glucose solution in the presence of oxytocin was sought to give some understanding of the degree to which oxytocin-stimulated myometrial smooth muscle can resist the effects of glucose depletion, as might occur during labour in vivo. This approach was used to provide more insight on the metabolic factors which could affect normal labour, since the effect of glucose depletion on uterine contractility has not received much attention yet, nor has the efficacy of oxytocin in a glucose depleted environment been studied.

In this chapter, a mouse model was used *in vitro*, to determine the effect of zero glucose on spontaneous uterine contraction, and on the response to hypoxia, oxytocin, and high K^+ depolarisation.

4.1.2 MATERIALS AND METHODS

4.1.2.1 Contractility measurement

4.1.2.1.1 Tissue preparation

For details on tissue preparation, please see chapter 2, section 2.1.

4.1.2.1.2 Preparation of solutions used.

All the chemicals used for solutions preparations were obtained from SIGMA UK.

For details on solution preparation, please see chapter 2, section 2.2.5

4.1.2.1.3 Physiological saline solution (PSS)

Physiological saline solution was freshly prepared before each experiment.

4.1.2.1.4 Zero glucose solution

This was a physiological saline solution made without glucose.

4.1.2.1.5 high K^+ solution

This was PSS in which KCl was elevated to 40 mM and NaCl reduced to 120 mM.

4.1.2.1.6 high K zero glucose

This was high K^+ solution prepared without glucose.

4.1.2.1.7 Oxytocin zero glucose solution

For this, oxytocin (0.5 µM) was added to physiological saline solution made without glucose.

4.1.3 EXPERIMENTAL PROTOCOLS

4.1.3.1 The effect of zero glucose solution on uterine contractility

Following the establishment of spontaneous, stable contractions, achieved after 30-40 minutes equilibration, myometrial strips were exposed to zero glucose solution (PSS without glucose) until the achievement of a maximal effect (20 minutes). Pregnant and non-pregnant tissues were returned to PSS to allow recovery of contractions.

4.1.3.2 Effect of zero glucose on uterine contractility under hypoxic conditions

In subsequent experiments, the combined action of zero glucose and hypoxia was sought. Myometrial tissues were exposed simultaneously to zero glucose solution and hypoxic conditions for 20 minutes. Once a maximal effect was achieved, tissues were returned to PSS and to normoxia to allow recovery of contractions.

The organ baths were bubbled with 100% N_2, instead of O_2, to induce hypoxia. Initial experiments were conducted to determine the effects of hypoxia on uterine contractility in pregnant and non-pregnant mouse myometria.

4.1.3.3 The effect of zero glucose in OT stimulated contraction.

The effect of 20- minute zero glucose was assessed in 0.5µM oxytocin stimulated contraction as a control (one sample *t*-test).

4.1.3.4 The effect of zero glucose on response to high K^+

Once stable contractions were achieved, high K^+ solution was applied to the tissues for a period of 40 minutes. This was compared to the application of a zero-glucose high K^+ solution for the same amount of time.

4.1.4. DATA ANALYSIS

For the data analysis, please refer to chapter 2, section 2.2.8.

4.1.5 RESULTS

4.1.5.1 THE EFFECT OF ZERO GLUCOSE SOLUTION ON UTERINE CONTRACTILITY

4.1.5.1.1 Spontaneous uterine response

Spontaneous contractions were produced and remained stable for 3 to 4 hours' duration when myometrial strips (pregnant and non-pregnant) were mounted in an organ bath containing a PSS solution at 37ºC and a pH of 7.4, bubbled with O_2. This was sufficient time for all protocols to be completed and enabled the clear identification of changes to contractions induced by the experimental protocols applied. The control data was set as 100% (spontaneous contractions) and the values obtained were expressed as a percentage of this control. The spontaneous contractile activity of pregnant and non-pregnant mouse myometrium was compared in vitro. Traces representing spontaneous contractions in pregnant and non-pregnant mice are displayed in the figure below (Figure 4.1).

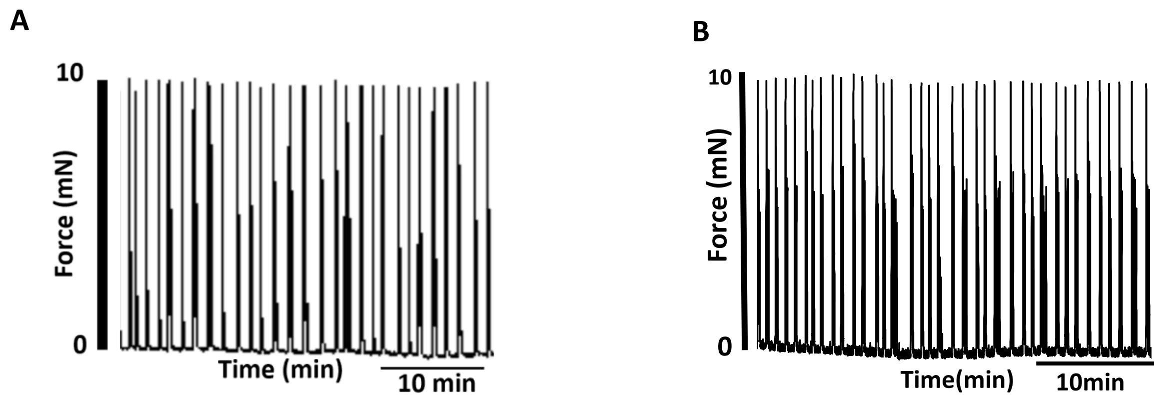


Figure 4.1. Spontaneous contractions in pregnant (A) and non-pregnant mice (B). No significant difference was found in the parameters measured between A & B

The mean amplitude, the mean frequency, and the mean area under the curve (AUC) were calculated and compared for 6 pregnant and 6 non-pregnant mice for a period of 5 minutes.

a. Force amplitude

The mean amplitude for spontaneous contractions in pregnant mice was 10.5 ± 1mN while in non-pregnant mice the mean force was 10.4 ± 1mN. There was no significant difference between myometrial spontaneous contraction force amplitudes in pregnant and non-pregnant mice ($p > 0.05$).

b. Frequency

The number of myometrial contractions in a selected period of 5 minutes was calculated. In pregnant mice, the mean frequency was 7.5 ± 0.5, while in non-pregnant mice the mean frequency was 7.2 ± 0.8. There was no significant difference between myometrial frequency of contraction in pregnant and non-pregnant mice ($p > 0.05$)

c. The area under the curve (AUC)

The mean integral force of contraction was analysed over 5 minutes. AUC was 8.2 ± 2 in pregnant mice and 8.4 ± 2 in non-pregnant mice, demonstrating no significant difference ($p > 0.05$).

Overall, therefore, no significant difference was found between spontaneous myometrial contractions in pregnant and in non-pregnant mice.

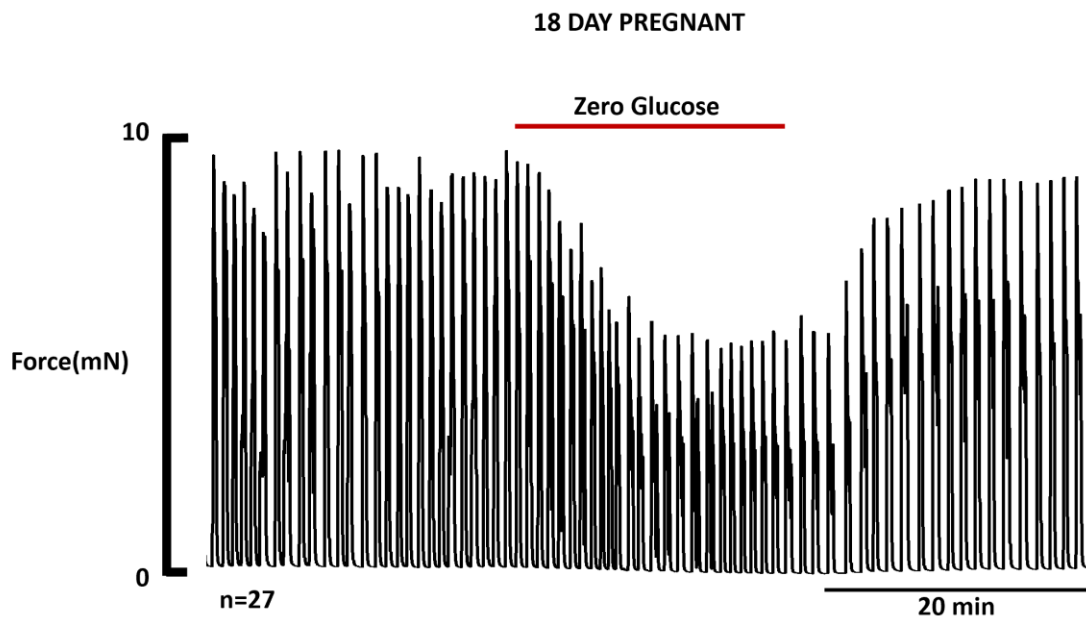
4.1.5.1.2 Investigating the effects of zero glucose.

The objective of this set of experiments was to examine the effect of glucose depletion on spontaneous uterine contractions and compare the results between pregnant and non-pregnant mice in vitro. Myometrial tissues were obtained from 8 non-pregnant and 27 pregnant mice. When zero glucose solution was applied to myometrial strips, a significant decrease in contractility was observed (Figure 4.2). In pregnant mouse myometrium, contractile amplitude significantly decreased to 60 ± 4% of the control level (one-sample *t*-test, $p < 0.0001$, n= 27); and the AUC significantly decreased to 54 ± 7% (p < 0.0001) (Figure 4.3 A & B). Contractile frequency decreased to 91 ± 7%, although this did not achieve significance ($p = 0.2790$).

In non-pregnant mouse myometria, contractile amplitude significantly decreased to 82 ± 3% of the control values (paired *t*-test, $p = 0.0022$, n= 8, Figure 4.3); AUC significantly decreased to 59 ± 3% ($p < 0.0001$) (Figure 4B); and contractile frequency decreased to 82 ± 7% ($p = 0.0496$) of control values.

While application of zero glucose solution inhibited contraction in both pregnant and non-pregnant myometria, the decline in amplitude was significantly greater in pregnant than in non-pregnant myometrial strips (unpaired *t*-test, $p = 0.0278$, Figure 4.3). There was no significant difference in the effect of zero glucose solution on AUC and frequency in pregnant and non-pregnant myometrium (AUC, $p = 0.8356$; frequency, $p = 0.5397$, Figure 4B). In both tissues, contractions recovered fully once conditions returned to normal (PSS containing glucose, Figures 2 & 3)

A



B

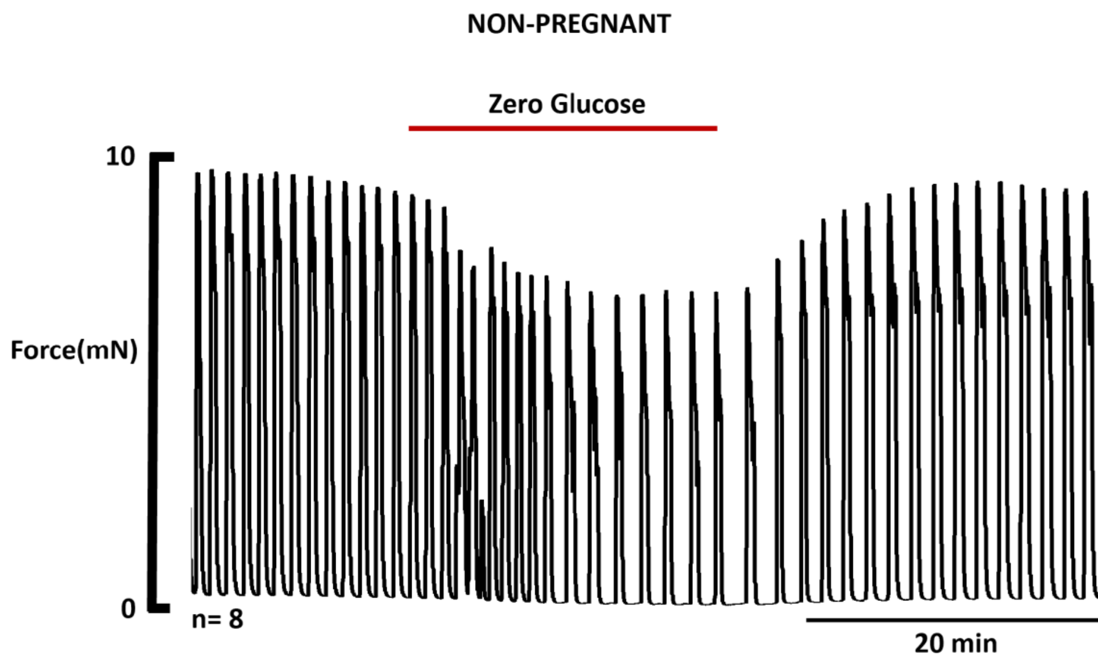


Figure 4.2 The effect of glucose depletion on spontaneous uterine contractions from pregnant and non-pregnant mice. Representatives displaying myometrial contractile inhibition. Amplitudes are significantly decreased (A & B).

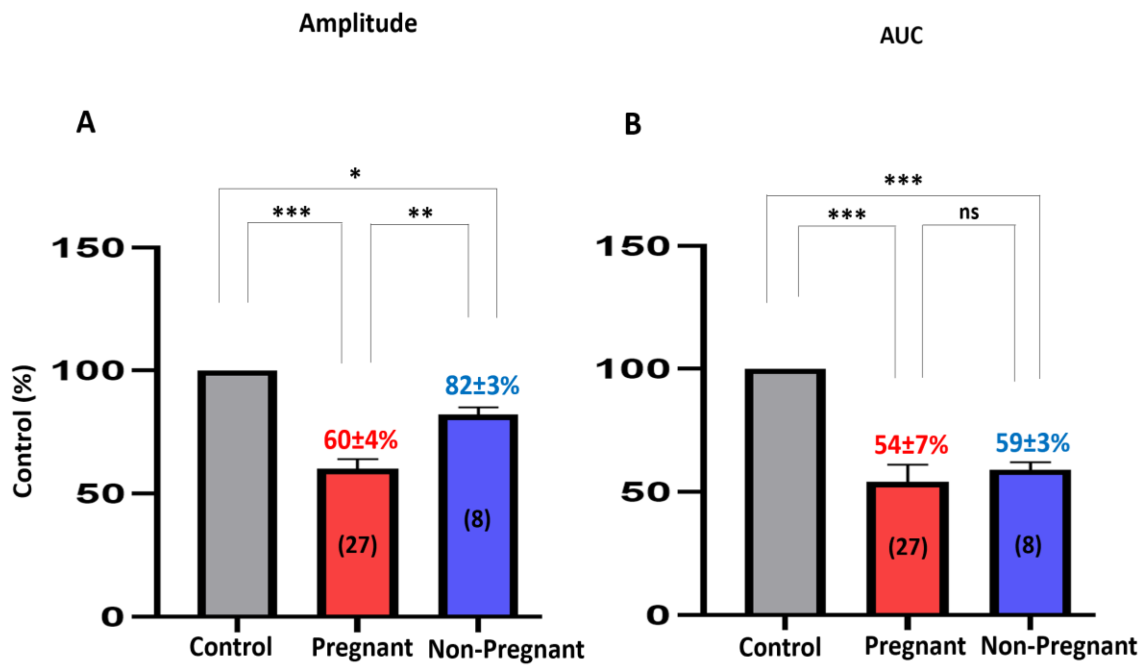


Figure 4.3. The effect of glucose depletion on spontaneous contractions of myometrial strips from pregnant(n=27) and non-pregnant(n=8) mice. The pregnant myometrium contraction amplitudes are more significantly affected than non-pregnant (A & B).

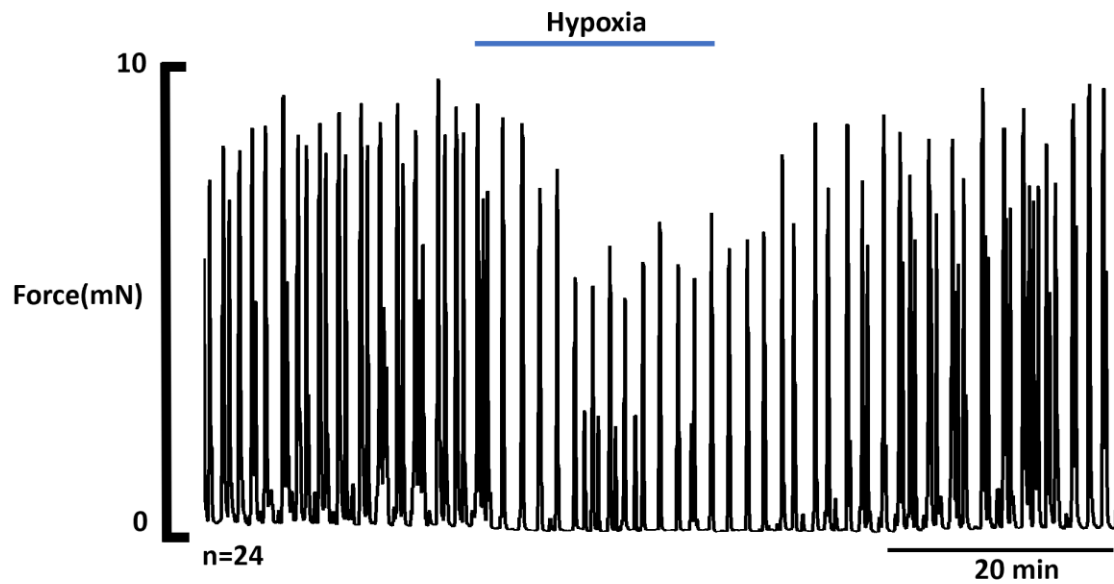
4.1.5.1.3 Investigating the effect of hypoxia.

The objective of this set of experiments was to explore the effect of hypoxia on uterine contractility and compare the effects between pregnant and non-pregnant mice.

When hypoxia was applied to myometrial strips, a significant decrease in contractility was observed (Alotaibi et al., 2015) (Figure 4.5). Pregnant myometrial tissues that were exposed solely to hypoxia demonstrated a significant decline in contractile amplitude to 52 ± 4% of the control level (n= 24, one sample *t*-test, $p < 0.0001$), while AUC and frequency fell to 48 ± 4% ($p < 0.0001$) and 81 ± 5% ($p = 0.0042$) of control values, respectively.

The overall decline in contractility was more pronounced in non-pregnant tissues with amplitude decreasing to 57 ± 9% of the control level, AUC to 31 ± 7% (n= 8, $p < 0.0001$) and frequency to 50 ± 12%. The decrease in AUC and frequency in non-pregnant tissue was significantly greater than in pregnant tissue (AUC, $p = 0.1560$; frequency, $p = 0.0564$), although the change in contractile amplitude was similar in both tissues ($p = 0.8419$). Contractility recovered once normoxia was restored at the end of the protocol (Figure 4.6).

A



B

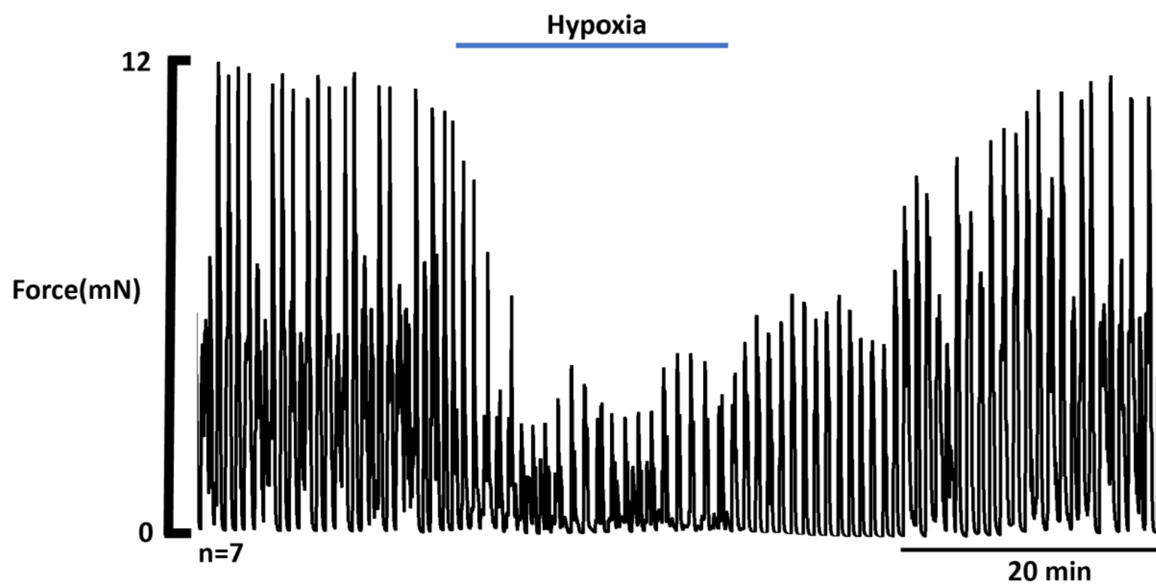


Figure 4.5 The Effect of hypoxia on spontaneous contractions of myometrial strips from pregnant and non-pregnant mice. The non-pregnant myometrium contractile amplitudes are significantly affected (Representative isometric recording).

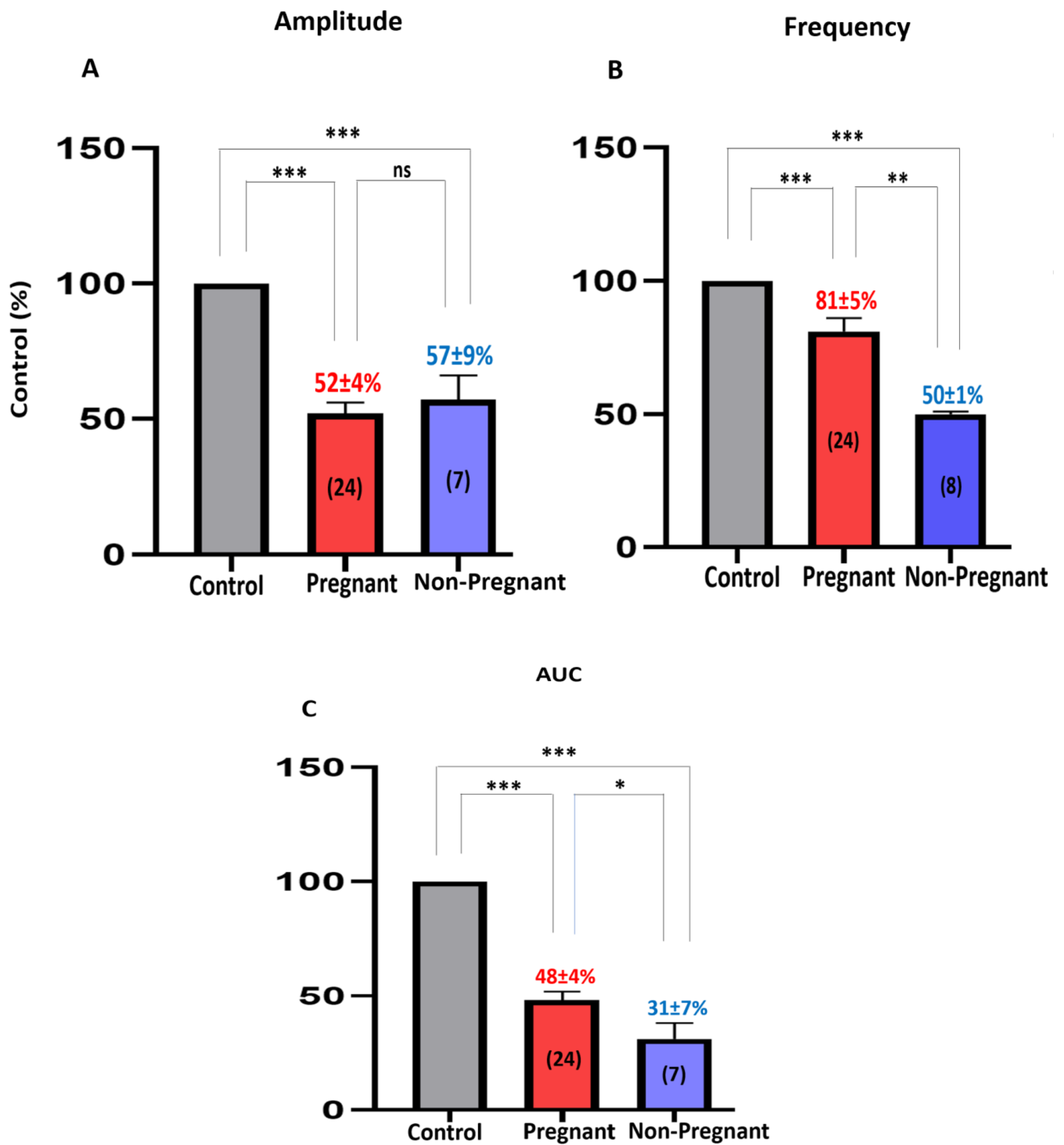


Figure 4.6 The effect of hypoxia on pregnant and non-pregnant mice. The effect of hypoxia appears to be greater on the non- pregnant myometrium(n=7) than on the pregnant myometrium(n=24)

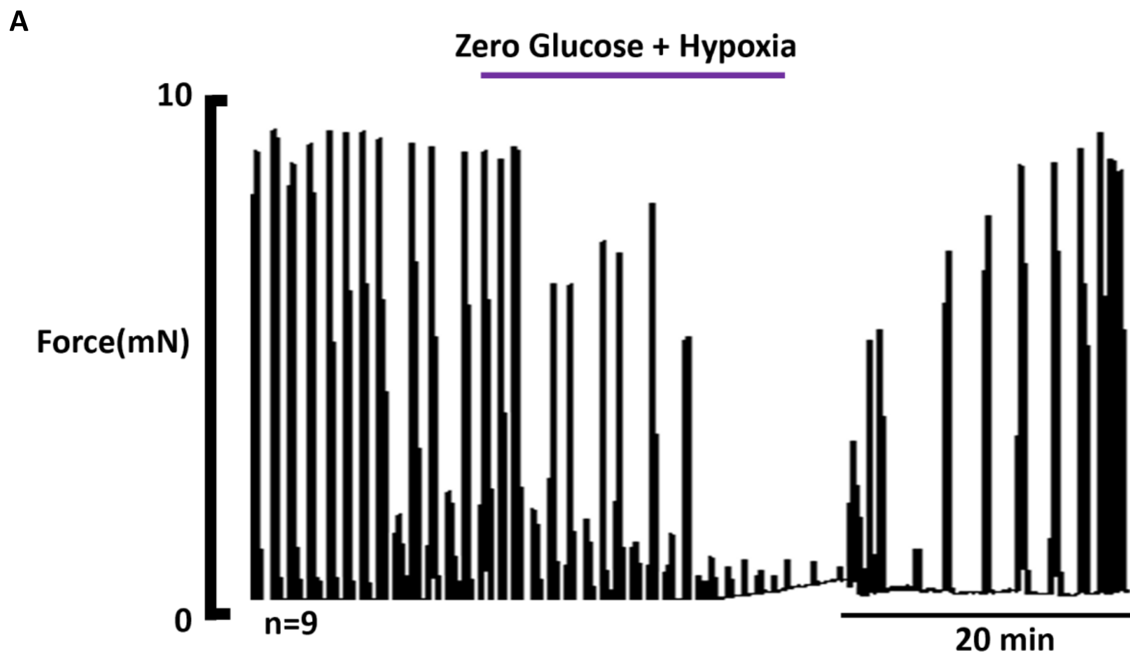
4.1.5.1.4 Investigating the effect of zero glucose under hypoxic conditions.

The objective of this test was to explore the effect of zero glucose under hypoxic conditions on uterine contractility and compare the effects between pregnant and non-pregnant mice. Contractile decline was pronounced with the combined action of zero glucose solution and hypoxia, with contractions frequently halting completely, although they did recover once normoxia and glucose levels were restored (Figure 4.7).

In pregnant myometrium, the mean amplitude dropped to 16 ± 10% of the control level ($p < 0.0001$, n= 9) and frequency down to 20 ± 14% ($p = 0.0005$, n= 9) in the pregnant group, while AUC fell to 19 ± 15 ($p < 0.0001$, n= 9).

In non-pregnant mice, the mean amplitude fell to 17 ± 6%, frequency to 25 ± 11% and AUC down to 10 ± 4% of control values (n= 6, $p < 0.0001$). These values are not significantly different to the effect in pregnant tissue (Figure 4.8A & B).

18 DAY PREGNANT



NON-PREGNANT

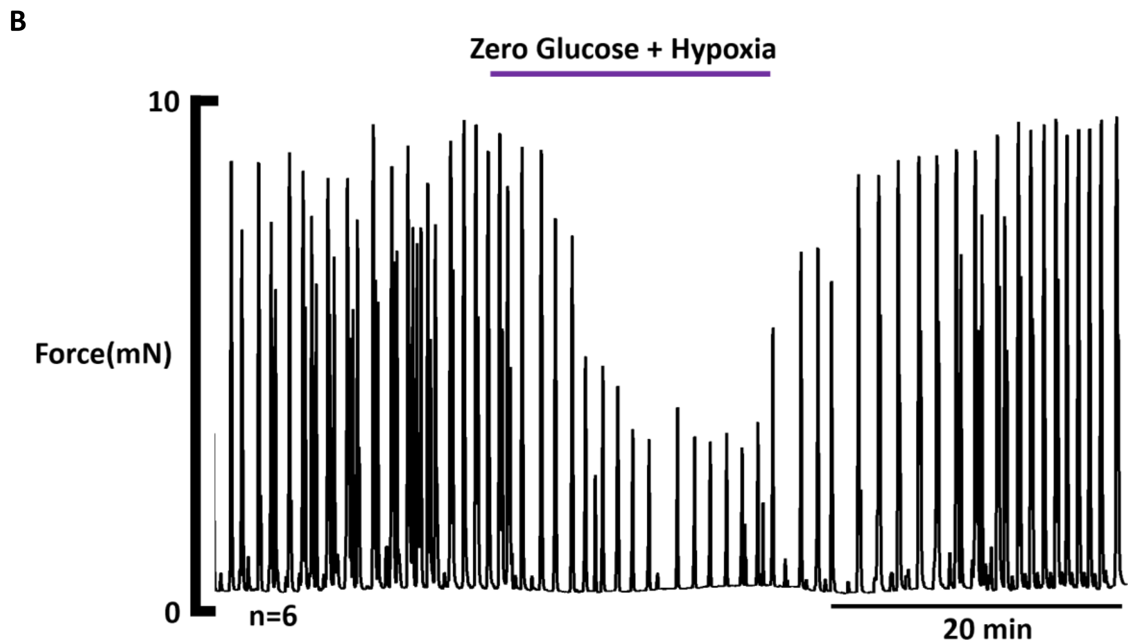


Figure 4.7 The combined effect of zero glucose and hypoxia on pregnant and non-pregnant mice. Representative isometric traces showing contractile decline. The decline in contractility was greater than in zero glucose or hypoxia alone (A & B).

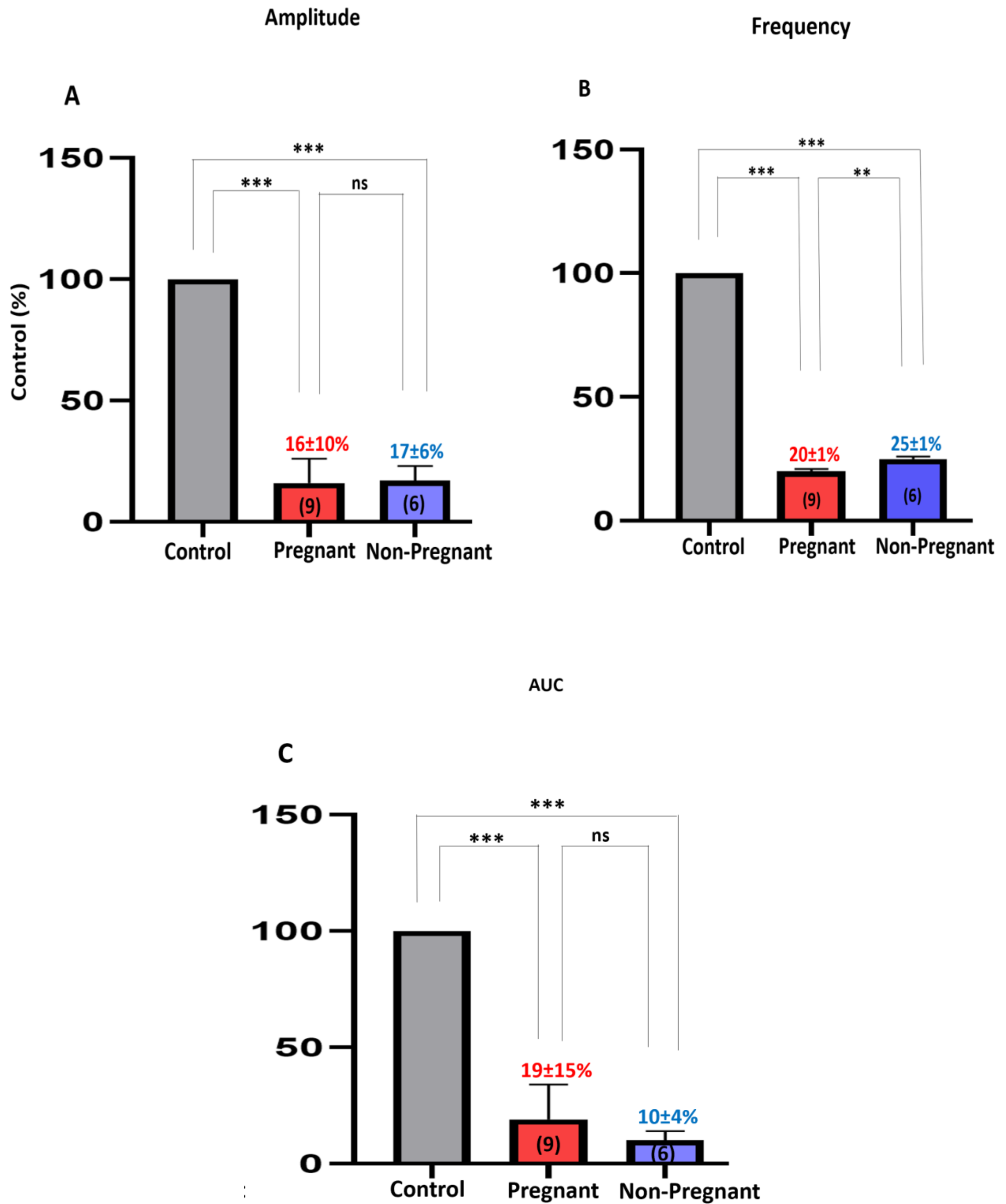


Figure 4.8 The comparison of the combined effect of zero glucose and hypoxia on pregnant and non-pregnant mice. No difference was noticed between pregnant(n=9) and non-pregnant(n=6) myometria.

4.1.5.1.5 Results summary

Pregnant group	**Zero glucose**	**Hypoxia**	**Zero glucose + hypoxia**
Amplitude	60 ± 4% ($p < 0.0001$, n = 27)	52 ± 4% ($p < 0.0001$, n = 24)	16 ± 10% ($p < 0.0001$, n = 9)
Frequency	91 ± 7% ($p = 0.2790$, n = 27)	81 ± 5% ($p = 0.0042$, n = 24)	20 ± 14% ($p = 0.0005$, n = 9)
AUC	54 ± 7% ($p < 0.0001$, n = 27)	48 ± 4% ($p < 0.0001$, n = 24)	19 ± 15 ($p < 0.0001$, n = 9)

Table 4.1: The effect of zero glucose, hypoxia and zero glucose+ hypoxia in pregnant group. P values result from one sample *t*- test comparing each treatment with its own internal control period.

Non-pregnant group	Zero glucose	Hypoxia	Zero glucose+ hypoxia
Amplitude	82 ± 3% ($p = 0.0022$, n = 8)	57 ± 9% ($p < 0.0001$, n = 7)	17 ± 6% ($p < 0.0001$, n = 6)
Frequency	82 ± 7% ($p = 0.0496$, n = 8)	50 ± 12% ($p < 0.0001$, n = 7)	25 ± 11% ($p < 0.0001$, = 6)
AUC	59 ± 3% ($p < 0.0001$, n = 8)	31 ± 7% ($p < 0.0001$, n = 7)	10 ± 4% ($p < 0.0001$, n = 6)

Table 4.2: The effect of zero glucose, hypoxia and zero glucose + hypoxia in the non-pregnant group. The *p*-values result from one sample *t*-test comparing each treatment with its own internal control period.

4.1.5.1.6 Investigating the effect of zero glucose on the OT-stimulated myometrium.

Oxytocin has been known to increase uterine contractility. (Arrowsmith and Wray,2014, Matthew et al., 2004b). When the myometrium is stimulated by oxytocin, contractility increases in both non-pregnant and pregnant mice as shown in Figure 4.9 A & B.

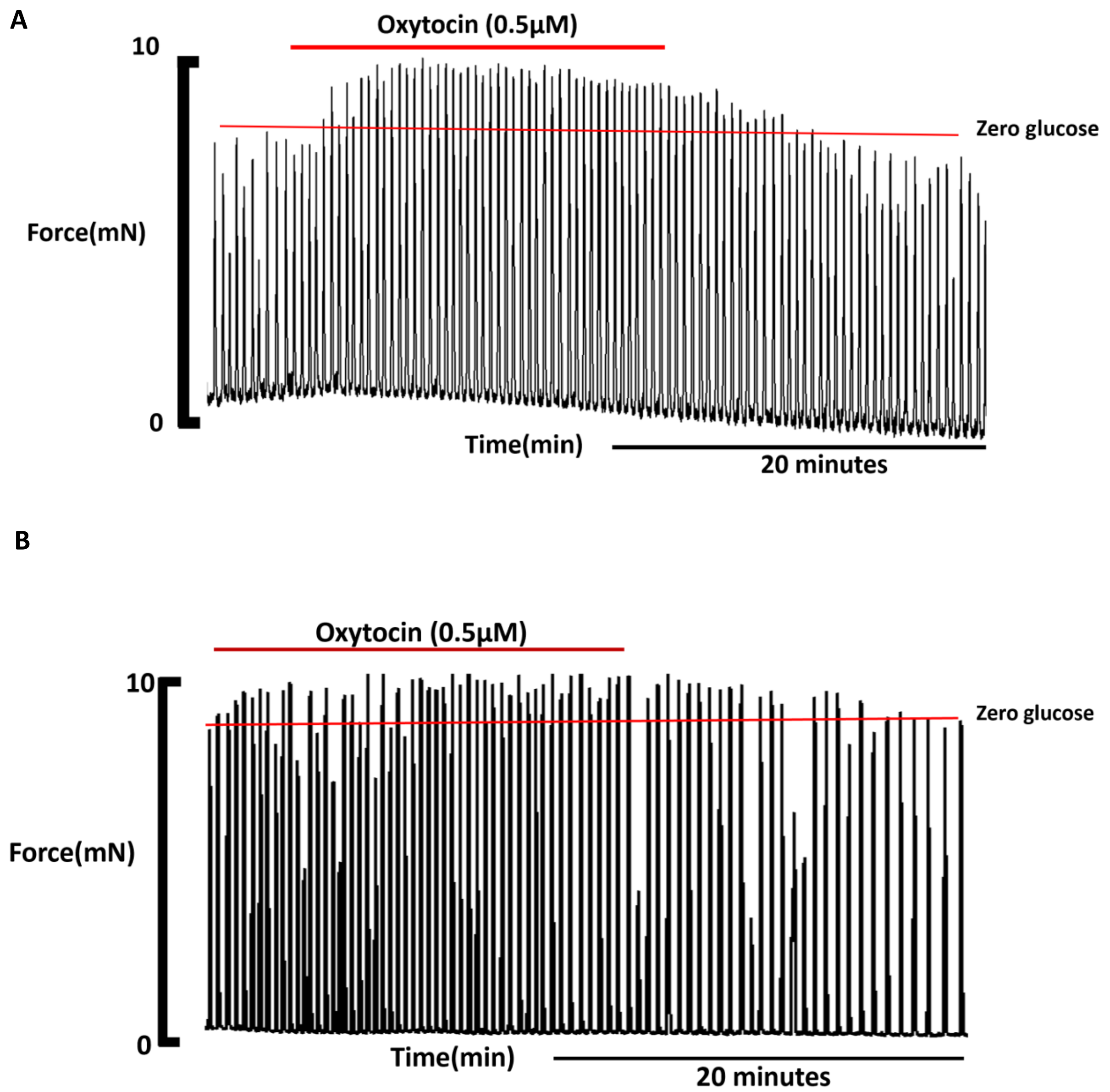


Figure 4.9 The effect of oxytocin on the myometrium in pregnant mice(A) and non-pregnant mice(B). Representative recordings displaying an increase in contractility noticed in the myometrium (A&B)

The aim of this set of experiments was to explore the effect of zero glucose in OT-stimulated myometrium and compare the results between pregnant and non-pregnant mice.

When zero glucose solution was applied to oxytocin-stimulated myometrium, contractility was significantly reduced in both pregnant and non-pregnant mice (Figure 4.10 A & B) In both group, contractility recovered to its previous OT-stimulated level once normal glucose levels were restored.

In pregnant myometrium, amplitude fell to 61 ± 5% ($p < 0.0001$, n= 13) and AUC to 61 ± 6% ($p < 0.0001$, n= 13) compared to initial OT-stimulated values. Frequency of contraction did not change 105 ± 6%, (n= 13, $p = 0.4256$). In the non-pregnant group, the amplitude, AUC, and frequency declined significantly respectively down to 67 ± 7% (n= 6, $p = 0.0077$), and 56 ± 6% (n= 6, $p < 0.0001$) and 86 ± 9% (n= 6, $p = 0.2042$) of initial OT-stimulated values. (Figure 4.11 A & B). But the decline in frequency was not significant. The size of the response to zero glucose is similar whether oxytocin is present or not.

The unpaired *t*-test revealed that no difference between zero glucose and oxytocin in zero glucose in non-pregnant and pregnant mice except for myometrial frequency in pregnant ($p = 0.0142$)

In pregnant mice, the *p*-values were respectively: Amplitude ($p = 0.8477$), Frequency ($p = 0.0142$), AUC ($p = 0.4415$).

In non-pregnant mice the *p* -values found were Amplitude ($p = 0.0749$), Frequency ($p = 0.7452$), AUC (0.6939)(Figure 4.12A & B).

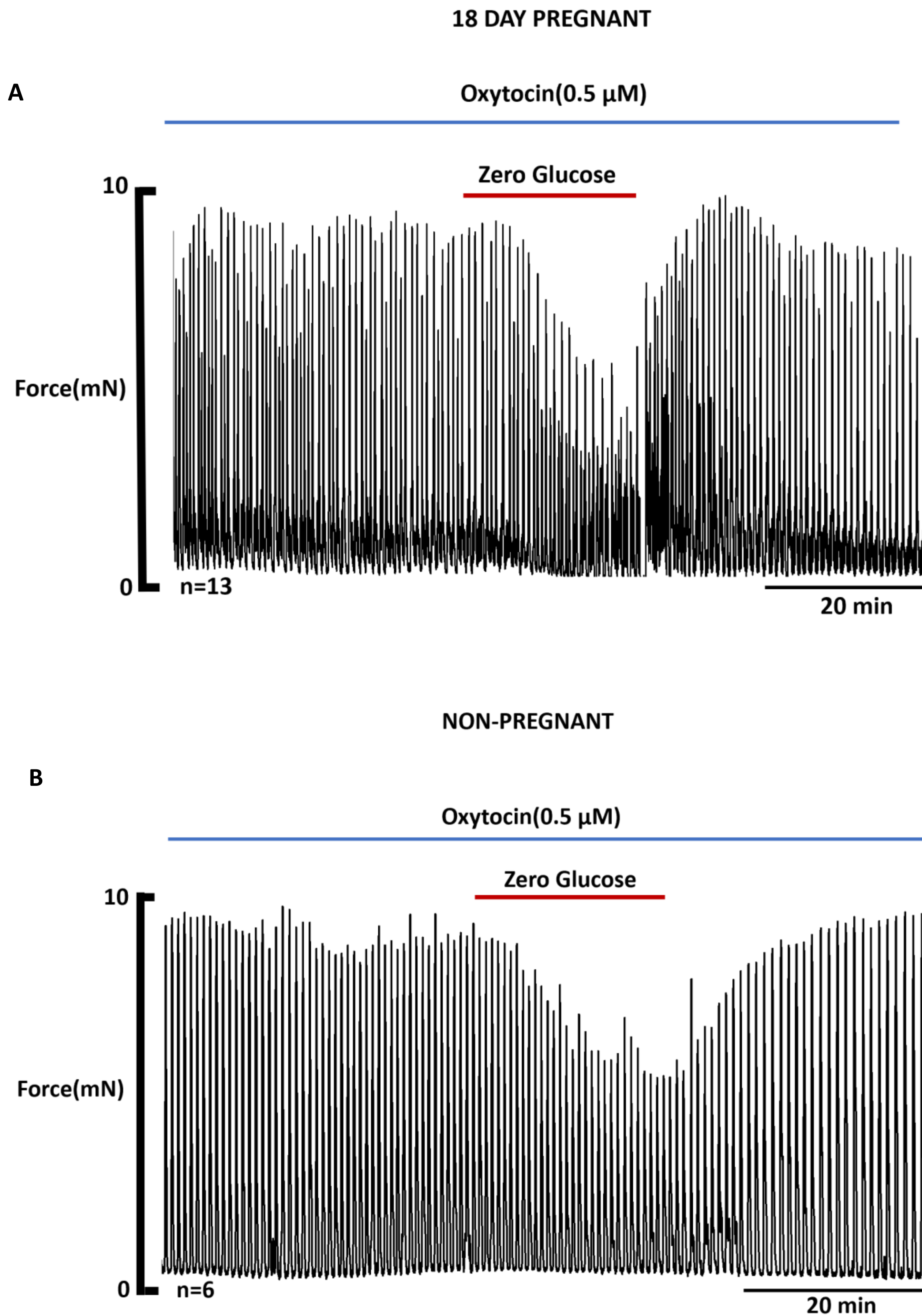


Figure 4.10 The effect of zero glucose on oxytocin-stimulated contractions in pregnant and non-pregnant mice. The decline of uterine contractility was observed (A&B) (Representative of traces recorded).

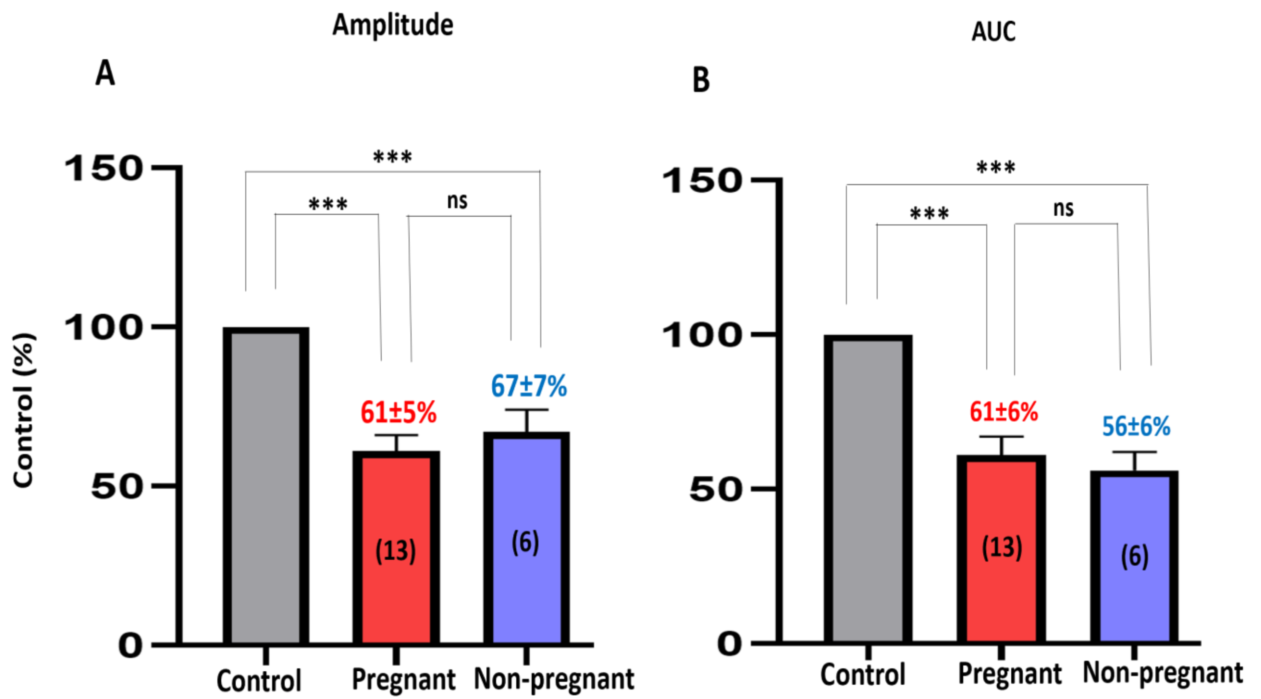
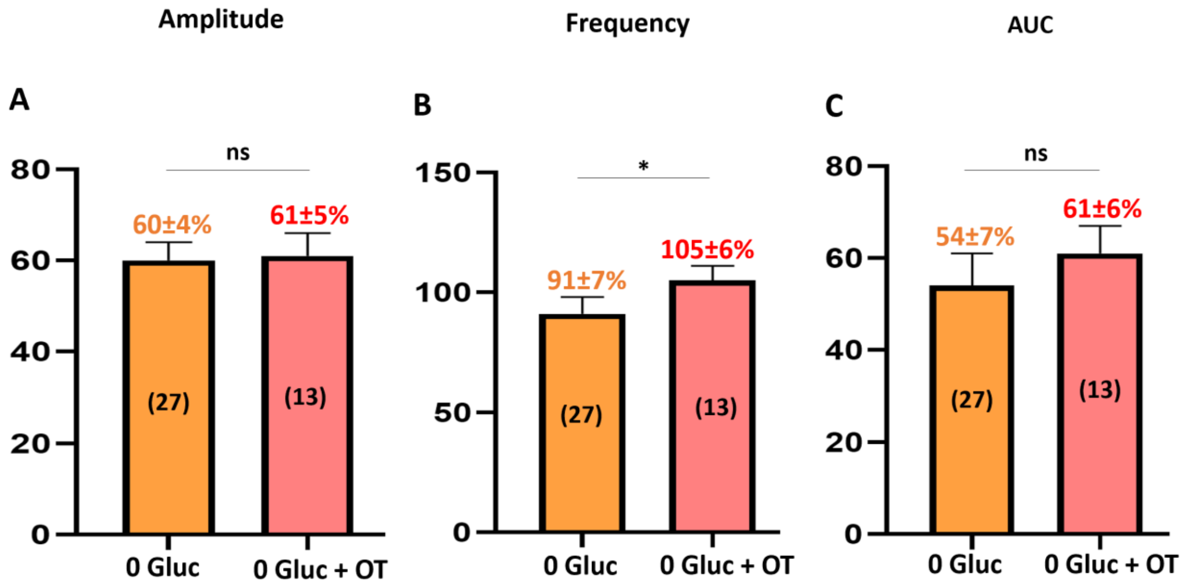


Figure 4.11 The comparison of the effect of zero glucose in the presence of OT on pregnant and non-pregnant mice. Under zero glucose and oxytocin, there are no differences between pregnant(n=13) and non-pregnant (6) uterine mice strips (A&B)

PREGNANT MOUSE

I.



II.

NON-PREGNANT MOUSE

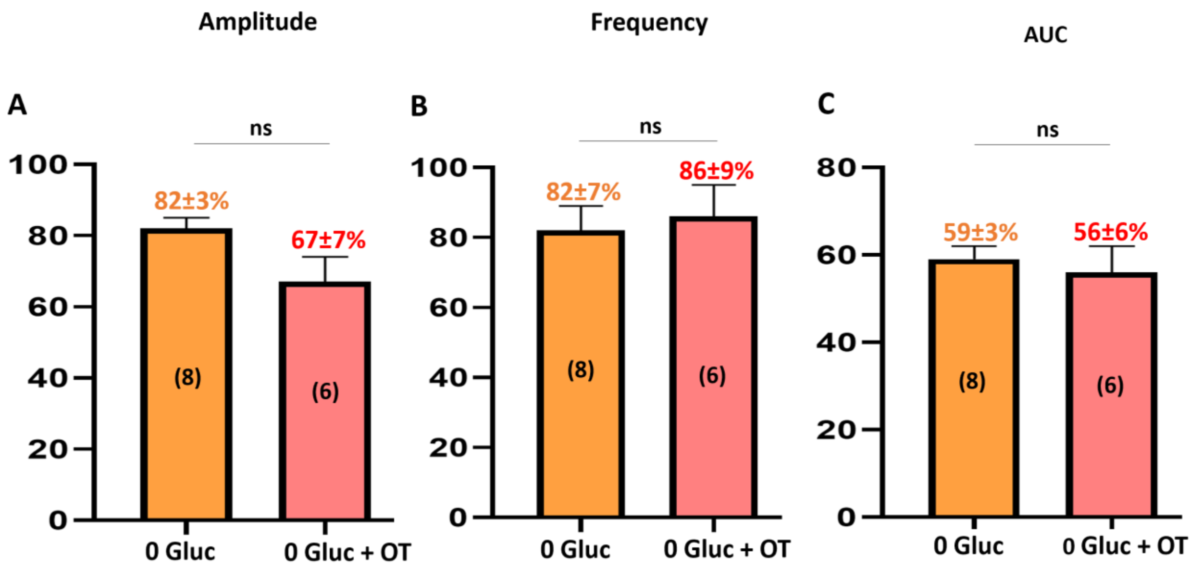


Figure 4.12 Comparison of the effect of zero glucose and zero glucose in the presence of oxytocin in pregnant and non-pregnant myometrial tissues. There are no differences between zero glucose and zero glucose + oxytocin except for frequency in pregnant group (IB). Pregnant: 0 Gluc(n=27),) Gluc+ OT(n=13). Non-pregnant: 0 Gluc(n=8),) Gluc+ OT (n=6)

4.1.5.1.7 Results summary

Pregnant group	Zero Glucose	Oxytocin+ Zero glucose
Amplitude	60 ± 4% ($p < 0.0001$, n = 27)	61 ± 5% $p < 0.0001$ n = 13
Frequency	91 ± 7% ($p = 0.2790$, n = 27)	105 ± 6% $p = 0.4256$ n = 13
AUC	54 ± 7% ($p < 0.0001$, n = 27)	61 ± 6% $p < 0.0001$ n = 13

Table 4.3 The effect of zero Glucose alone on uterine contractility and OT stimulated myometrium in pregnant mice. *P* - values result from one sample *t*- tests comparing each treatment with its own internal control period.

Non-Pregnant group	Zero Glucose	Oxytocin+ Zero glucose
Amplitude	82 ± 3% ($p = 0.0022$, n = 8)	67 ± 7% $p = 0.0077$ n = 6
Frequency	82 ± 7% ($p = 0.0496$, n = 8)	86 ± 9% $p = 0.2042$ n = 6
AUC	59 ± 3% ($p < 0.0001$, n = 8)	56 ± 6% p < 0.0001 n = 6

Table 4.4 The effect of zero glucose alone on uterine contractility and OT stimulated myometrium in non-pregnant mice. *P*-values result from one sample *t*- tests comparing each treatment with its own internal control period.

4.1.5.1.8 Investigating the effect of zero glucose on the response to high K^+

Myometrial strips were exposed to a high K^+ solution containing raised levels of potassium salt (40mM) for 40 minutes, before they were returned to normal PSS solution. In both pregnant and non-pregnant tissues, the response to high K^+ solution comprised of an initial increase in amplitude (peak), before falling and reaching a stabilised plateau (Figure 4.13)

The purpose of this experiment was to the explore the action of glucose depletion on the response to high K^+ from pregnant and non-pregnant mice. The contractile peak and plateau amplitudes were compared between high K^+ and high K^+ zero glucose expecting a contractile inhibition from high K^+ zero glucose.

In pregnant myometrium, the peak and plateau amplitudes of the high K^+ zero glucose response are 118 ± 7% (n = 10) and 14 ± 5% (n = 10), respectively. The peak response was not significantly different to high K+ alone(126 ± 6%,n = 12) the unpaired *t*-test revealed a *p* value of 0.4134) (Figure 4.14, A & B), but **the plateau amplitude was significantly lower with**

high K^+ zero glucose 14 ± 5%, than high K+ alone (33 ± 3%) (unpaired *t*-test, *p* = 0.0046, **) (Figure 4.14, C & D).

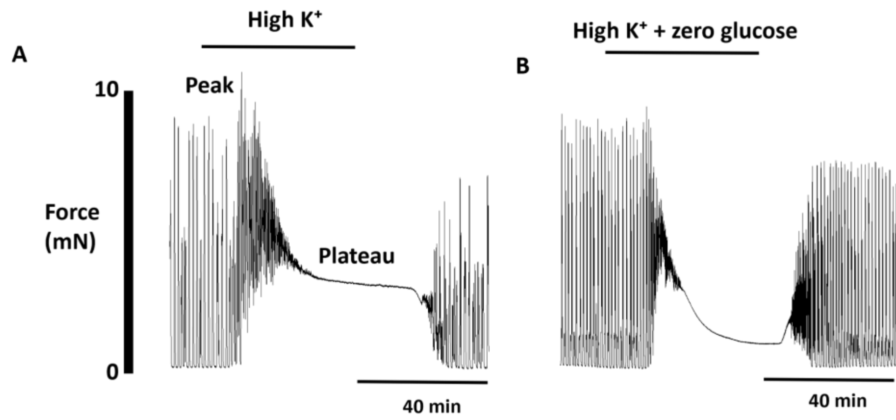
In non-pregnant myometrium, the peak and plateau of the high K^+ zero glucose response are 110 ± 2% (n = 8) and 31 ± 8% (n = 8), respectively. Like in pregnant tissue, the peak amplitude of the high K+ response (110 ± 8%) was not significantly different in the absence (110 ± 2%) or presence of glucose in the solution (unpaired *t*-test, *p* = 0.9860) (Figure 4.14, A & B) and the plateau amplitude was lower in the high K^+ zero glucose group (31 ± 8%) than high K^+ (48 ± 7%) but with no significant difference (unpaired *t*-test, *p* = 0.1321). (Figure 4.14, B & D)

In comparing the pregnant and the non-pregnant, it was found that the peak amplitude was higher in pregnant than in non-pregnant while the plateau amplitude was lower in pregnant than in non-pregnant (Figure 4.13, A & C).There was no significant difference between peak amplitudes from pregnant (126 ± 6%, n= 12) and non-pregnant (110 ± 8%, n= 8) tissues with high K^+ alone (unpaired *t*-test, *p* = 01631), but a significant difference was noticed with plateau amplitudes (pregnant 33 ± 3% and non-pregnant 48 ± 7% (unpaired *t*-test, *p* = 0.0488).

The peak amplitude was greater in pregnant tissue than in non-pregnant , while the plateau was lower in pregnant than in non-pregnant tissue(Figure 4.13, B & D).When tissues were stimulated with high K^+ under zero glucose conditions, no significant difference in peak amplitudes was found between pregnant (118 ± 7%, n= 10) and non-pregnant (110 ± 2%, n= 8) (unpaired *t*-test, *p* = 0.4282). No significant difference was found with the plateau amplitudes (pregnant 14 ± 5% and non-pregnant 31 ± 8%) (unpaired *t*-test, *p* = 0.0831).

18 DAY PREGNANT

I



II

NON-PREGNANT

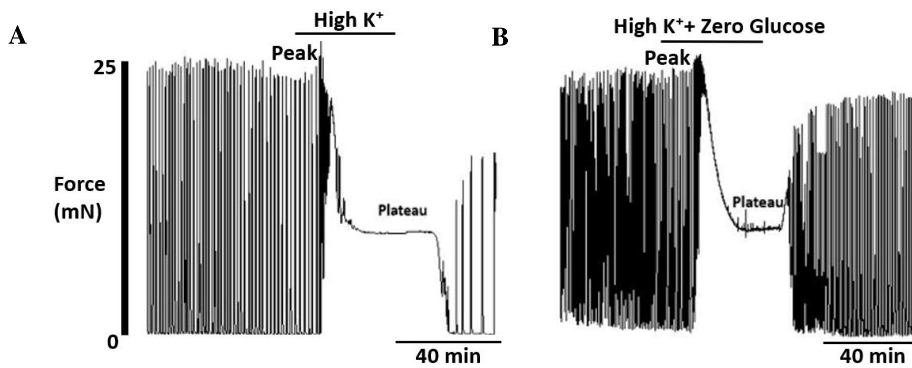


Figure 4.13 High concentration of potassium (K^+) in pregnant and non-pregnant mice (A) and high K^+ in zero glucose (B). Representative recordings of High K^+ response. The point the amplitude reaches is the peak before decreasing and stabilising in a plateau. Both, peak and plateau amplitudes were measured from the baseline of contractions and compared to control spontaneous contractions (I&II).

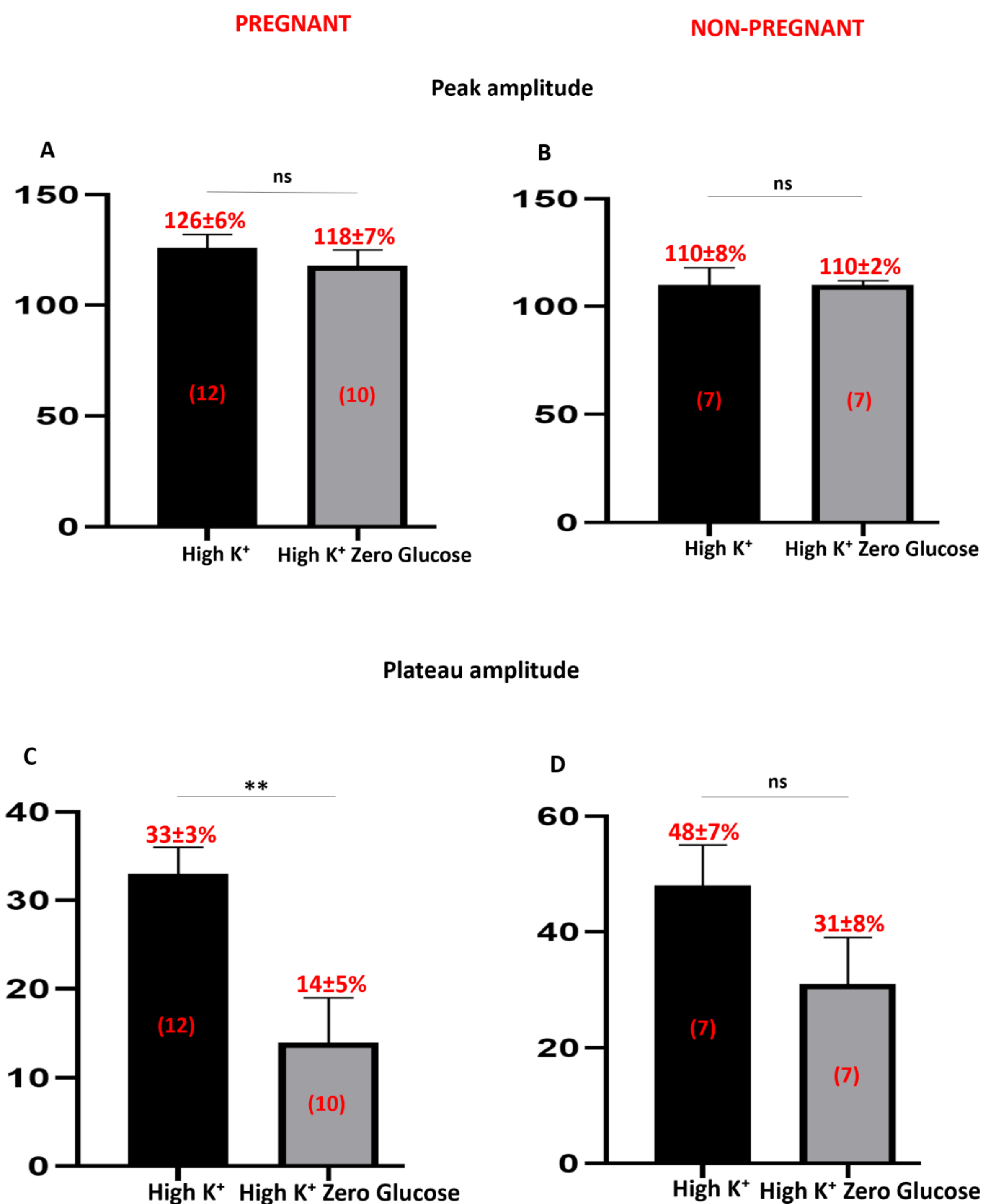


Figure 4.14 The comparison of the effect of zero glucose on the response to high K^+
There was no significant difference found in peak amplitude under zero glucose (A, B, D); however, zero glucose significantly decreased the plateau amplitude under high K^+ in pregnant tissue (C), probably due to a deceased calcium sensitivity of contractile proteins. Pregnant: Peak amp (High K^+(n=12), High K^+0 Gluc(n=10). Non-pregnant: Peak amp (High K^+(n=7), High K^+0 Gluc(n=7).). Pregnant: Plateau amp (High K^+(n=12), High K^+0 Gluc(n=10). Non-pregnant: Peak amp (High K^+(n=7), High K^+0 Gluc(n=7)

4.1.5.1.9 Results summary

Pregnant group	high K^+	high K^+ zero glucose
Peak amplitude	126 ± 6% ($p = 0.0018$, n = 12)	118 ± 7% ($p = 0.0424$, n = 10)
Plateau amplitude	33 ± 3% ($p < 0.0001$, n = 12)	14 ± 5% ($p < 0.0001$, n = 10)

Table 4.5 Effect of zero glucose on peak and plateau amplitude of the high K^+ response in pregnant myometrium

Non-pregnant group	high K^+	high K^+ zero glucose
Peak amplitude	110 ± 8% (n = 8)	110 ± 2% (n = 8)
Plateau amplitude	48 ± 7% ($p < 0.0001$, n = 8)	31 ± 8% ($p < 0.\ 0001$, n = 8)

Table 4.6 Effect of zero glucose on peak and plateau amplitude on the response to high K^+ in non- pregnant myometrium

4.1.5.1.10 Discussion

The effect of glucose depletion was examined on uterine contractility in non-pregnant and pregnant mice under different experimental conditions: hypoxia, oxytocin, and depolarising conditions.

Results revealed that the depletion of glucose has an impact on uterine contractility in the mice, exhibiting a significant contractile inhibition in amplitude and area under the curve, but not frequency, compared to control values. This was true for both non pregnant and pregnant mice. The unpaired *t*-test between the two groups found that there was a significantly greater decrease in amplitude in the pregnant group than in the non-pregnant, but no difference was found in the area under the curve.

Studies conducted by Souhrada and Loader, while examining the role played by glucose in contractility in the smooth muscle(trachea) of guinea pigs, revealed that the contractile response to bronco-constrictive agents was significantly reduced under zero glucose conditions (Souhrada and Loader 1979). These findings are consistent with a similar study in human and animal (rat) smooth muscles, showing that the depletion of glycogen resulted in a decrease in ATP and suppression of smooth muscle contractility (Shi et al., 2020). My data and the previous literature in other smooth muscles demonstrate the importance of glucose as the major energy source powering smooth muscle contraction. As previously mentioned,

myometrial glycogen levels increase during the course of gestation, and this increase is influenced by hormonal metabolism (Demers et al., 1972; Gregoire et al., 1967).

During normal gestation, mothers undergo exceptional functional challenges to adapt to glucose metabolism in preparation for metabolic stress due to the evolution of the fetus. This metabolism is characterised by an increase of insulin resistance, glycolysis as well as a decrease of glucose aerobic oxidation. (Di Cianni et al., 2003; Drynda et al., 2015). These changes during pregnancy seem to be vital and a beneficial metabolic reaction to meet the energy requirement for the development of the fetus and to supply supplementary energy storage for labour (Di Cianni et al., 2003). It is understandable that pregnant tissue is more susceptible to loss of glycogen than unpregnant tissue, as shown in the data presented in this chapter.

As previously mentioned, during labour, at each contraction, the blood flow is reduced due to compression of vessels within the myometrial tissues, thus resulting in a transient hypoxia followed by the fluctuation of pH, phosphocreatine, and ATP. To induce hypoxia experimentally in this study, the organ bath was bubbled with nitrogen rather than oxygen. Previous work from our research group demonstrated that this produces below 95% decrease in O_2 levels in the organ bath (Alotaibi et al., 2015). This study demonstrated that when the myometrial tissues were exposed to hypoxia alone, this had a significant inhibitory effect on uterine contractility; and the amplitude and the area under the curve (AUC) decreased considerably in both non-pregnant and pregnant tissues. (Alotaibi et al., 2015). This is in agreement with Wray et al. who found that uterine contractility was drastically suppressed in non-pregnant and pregnant rats when myometrial strips were exposed to hypoxia induced by cyanide, and that recovery occurred after reoxygenation (Wray et al.1992). Similarly, a study on the guinea pig ureter demonstrated an inhibition of contractility when hypoxia was induced with cyanide, resulting in a rapid and considerable alteration of contractile force (Taggart and Wray, 1998).

With regard to hypoxia, an interesting experiment was performed in vivo by Harisson et al (1994) on uterine smooth muscle. The intra-uterine pressure measurement and 31P-NMR and spectroscopy were used to explore concurrently the impact of ischemia, the pH_i and the contractile force. A portion of uterus (1-2cm) was exteriorised from anesthetised rats from day 1 postpartum and the NMR surface coil was placed on the tissue. A balloon catheter was introduced in the lumen of the uterus to record the intra-uterine pressure changes. Occluders

were placed around uterine arteries. The NMR results revealed a significant decline in pHi, ATP, and phosphocreatine when arteries were occluded. Concerning contractile force, it was noted that artery occlusion provoked ischemia and produced a decline and then abolition of uterine contractions. The force was restored along with metabolites upon reperfusion. The authors suggested that the fall of pHi during ischemia was responsible for the decline of contraction (Harisson et al.1994). Relakov and colleagues have demonstrated that hypoxia induced the inhibition of calcium entry into the cell in guinea pig taenia caeci, producing smooth muscle relaxation.

Under hypoxic conditions, Hanley et al. reviewed the effect of lactate on the myometrium of a term-pregnant rat and demonstrated that lactate in a normal range effectively decreased pH_i and reduced uterine contractility due to its inhibition of Ca^{2+} entry into the cell. Additionally, the authors indicated that the build-up of extracellular lactate will decrease uterine force and could consequently contribute to dysfunctional labour. (Hanley et al. 2015). These data show that hypoxia inhibits non-pregnant contractions more than pregnant. This could be explained by the fact that, at term, the uterine smooth muscle is well prepared for hypoxia by laying down glycogen as metabolic reserve as well as fatty acid (Milwidsky & Gutman 1983). During hypoxia, glucose is generated from glycogen via glycogenolysis, and lactate is produced from glucose via pyruvate production. A shift happens with the expression of lactate dehydrogenase isoforms which at term are slightly inhibited (Makkonen et al.,1982). In addition, Dawson and Wray demonstrated that at term there is a moderate rise in ATP and PCr in the rat myometrial tissue (Dawson & Wray ,1985). As a result, term-pregnant myometrial tissue is better prepared to resist the hypoxic challenges than non-pregnant.

Reduction of blood supply caused by vessel compression at each contraction during labour, inducing transient hypoxia, means that glucose supply to the myometrium is reduced concomitantly. My findings reveal that the combined inhibitory effect of hypoxia and zero glucose was extremely pronounced, causing the uterine contractions to be abolished. The pronounced inhibitory response was expected, since the effect of hypoxia, combined with the situation where phosphocreatine and ATP are depleted (zero glucose), causes the uterine contractions to stop.

The impact of glucose depletion on the response to oxygen depletion on smooth muscles contractility have been previously reported (Vanhoutte 1976 & Altura 1970, Pessina et al 1997), and are similar to my results on the myometrium. Among them, Pessina and partners

examined the impact of anoxia and deprivation of glucose on contractility of strips from guinea-pig detrusor; it was described that the size of contractile response declined rapidly upon induction of anoxia, while glucose depletion led to a slow and progressive decreased contractile response. The combined conditions (oxygen and glucose depletion) resulted in a rapid decline and abolition of contractile response within sixty minutes (Pessina, F. et al., 1997). The decrease of ATP production caused by glucose depletion associated with lactate produced during hypoxia interfere with Ca^{2+}entry into myometrial cell may explain the suppression of the uterine contractions.

The exploration of the effect of the depletion of glucose in the presence of OT is of importance since OT is a natural hormone in activity during labour and is a product that clinicians prescribe to induce or improve uterine contraction during labour. I examined the effect of zero glucose on uterine contractility, in the absence and presence of OT in both non-pregnant and term pregnant mice. As previously described, my results confirmed that OT alone improved spontaneous uterine contractile force in both myometrial tissues, but OT stimulation could not overcome the inhibitory effect of zero glucose conditions. These findings indicate that the process through which OT increases myometrial contractions was unable to compensate for the loss of the tissue's main energy source, glucose. There is no difference between zero glucose and oxytocin in zero glucose in non-pregnant and pregnant mice. This was not surprising since more studies are still needed on the role of oxytocin on murine myometrium during labour as oxytocin receptor knockout mice deliver normally (Lee et al.,2008)

It is possible that a proportion of women in labour who do not respond to OT treatment are suffering from glucose depletion in the myometrium, as well as hypoxia-induced acidosis, so contractions can't be rescued by OT (Arrowsmith et al.,2010; Bugg et al.,2013; Lukasse et al.,2020)

Considering the high K^+ experiment, the maximal influx of Ca^{2+} into the cell through the opening of the voltage dependent L-type Ca^{2+}channel can be reached by depolarising the cell membrane using high concentrations of potassium (high K^+); this activates muscle contraction (Karaki et al 1984). This process is regularly used experimentally to test the integrity of muscle tissues. The trace resulting from exposure of the tissue to high K^+ (high concentration of potassium (40mM) displays a progressive increase of contractile amplitude until it reaches a peak (beyond 100% of control) followed by a decline to stabilise in a steady

state plateau (Arrowsmith 2018). In this thesis, the objective was to measure the uterine tissue's response while treated with high K^+ solution and high K^+ zero glucose solution and compare the results in the attempt to find a significant decline in peak and plateau amplitudes. This study demonstrates that the amplitude of the steady state plateau was significantly reduced under zero glucose conditions. The contractile peak response did not yield a significant difference between high K^+ alone and high K^+ zero glucose in the pregnant myometrial tissue, however the plateau amplitude was significantly lower with high K^+ zero glucose than high K^+ alone. The peak amplitude is attributed to the massive entry of Ca^{2+}into the myometrial cell in the presence or absence of glucose. This explains may explain the non-significant difference between the two states. Besides, the contractile amplitude decreases and stabilize in plateau. The fact that the plateau amplitude was significantly lower with zero glucose still remains unclear.

The high K^+ peak amplitude response was not significantly different in the absence of glucose in non-pregnant myometrial tissue. The plateau response was lower with high K^+ zero glucose than high K^+, but with no significant difference, while in pregnant myometrial tissue the difference was considerable.

In conclusion, glucose depletion significantly inhibits uterine contractions, irrespective of how they are produced. The decline in contractility was most pronounced with the combined action of zero glucose and hypoxia, with contractions frequently stopping**.** The stimulatory effect of OT was unable to overcome the glucose depletion-induced inhibition of contractions and If glucose depletion occurs in the myometrium during labour, it will contribute to the development of contractile dysfunction and potentially lead to poor labour outcomes.

4.2 The effect of zero glucose on uterine contractility in humans

4.2.1 Introduction

Animal studies have always played a crucial role in medical progress. Whenever possible, however, the use of human specimens is vital to advance the knowledge and understanding of human physiology, pathophysiology, and treatment of disease. Researchers can use human tissue to create *in vitro* models and explore illnesses in the tissue that is really affected, instead of simulating the process in animals (Frame 2019). The use of tissues that are biologically and physiologically appropriate may help speed up the development of therapy, by bypassing pre-clinical animal studies which frequently generates results that are irrelevant in humans; increasing the likelihood of success in clinical settings; and decreasing the number of animal use in studies (Roberts et al., 2002). The first uterine biopsy in research was performed in the early 1900s by John Sampson. He used it to study uterine cancer and endometriosis. The procedure has since become a common diagnostic tool for evaluating the health of the uterus and identifying any issues or abnormalities (Gastur & Tank, 2011).

Uterine smooth muscle biopsies can be taken from women undergoing caesarean sections (C/S) with informed consent. During C/S, a piece of myometrial tissue (~1cm x 1cm) is resected from the upper edge of the incision made in the lower segment of the uterus. To obtain contractility in vitro in the lab, dissections of tiny myometrial strips are performed; the strips are then clipped or tied with sutures and attached to a transducer within a bubbled organ bath and continually superfused with PSS at 37C and pH of 7.4. In these conditions, strips produce spontaneous uterine contractions within 2 to 3 hours under set tension and remain constant for several hours. Myometrial strips can also be stimulated to obtain contraction in the lab by using endogenous hormones, vasopressin, and oxytocin, which cause the development of contractile parameters closely resembling to contractions obtained during labour (Arrowsmith et al., 2018). Non-labouring biopsies are obtained from consenting women planned for C/S (elective) while labouring are taken from consenting women during labour.

As described in chapter 4, it was important to evaluate the effect of glucose depletion on uterine contractility in humans and compare the results between non-labouring and labouring tissues.

4.2.2. Materials and method

4.2.2.1 Contractility measurement

4.2.2.1.1 Ethical consideration, patients, and tissue preparation

49 non-labouring and 19 labouring women undergoing Caesarean section deliveries were recruited from Liverpool Women Hospital from women undergoing C/S. From all the recruited women, 3 non-labouring and 5 labouring myometrial tissues failed to contract.

The ethical approval for this study was obtained from the local research committee of the University of Liverpool. 'Non-labouring' refers to women who delivered at term (39 - 41 weeks pregnant) through planned CS before labour (elective). 'Labouring' refers to women at term who delivered through CS during labour (emergency). The mean maternal age at the time of delivery was 32 years (± 4.3 years) in the non-labouring group. The indications for C/S included previous C/S, previous difficult vaginal delivery, foetal reasons, breech, and request from mother. The mean maternal age at the time of delivery in the labouring group was 29 years (± 5.5 years). The indications for C/S included foetal distress, failure to progress, undiagnosed breech, cephalopelvic disproportion, and planned C/S but starting labour before the C/S booked date. Women with medical conditions such as high blood pressure, diabetes, maternal infection, HIV/AIDS, hepatitis, or multiple pregnancy, were excluded. This exclusion was important in this study for standardisation of samples and some factors that could impair the contractile activities in a myometrial tissue (e.g., Diabetes). Standardising samples was an essential step in my study to ensure consistency, comparability, and accuracy in data analysis and interpretation.

It was worth mentioning that it was difficult to determine with exactitude the time between the beginning of labour and the time the C/S was performed (Duration) and the dilatation parturient reached the time C/S was decided (information not recorded). During labour, the level of glycogen in the myometrium is decreasing as glycogen is being used as source of energy. The duration of labour between the starting time and the C/S time may be different for each sample. Consequently, the myometrial glycogen storage may not be the same for each sample the time C/S was performed.

Women who were able to give written and informed consent for the collection of samples regarding the study before their C/S were included. The ethical consideration and preparation of tissues was performed as explained in chapter 2, section 2.2 of this thesis.

4.2.2.1.2. Preparation of solutions used.

All the chemicals used for the solution preparations were purchased from SIGMA UK; the preparation process is clarified in chapter 2, section 2.2.5.

4.2.2.1.3 Experimental protocols

a. Effect of zero glucose solution on uterine contractility

After the establishment of spontaneous, stable contractions were achieved after 2 hours equilibration, myometrial strips were exposed to zero glucose physiologic solution (physiologic solution without glucose) until the achievement of a maximal effect (40 minutes). They were then returned to physiologic salt solution to allow the recovery of contractions.

b. Effect of zero glucose on uterine contractility under hypoxic conditions

100% nitrogen(N_2) was used in the organ bath instead of oxygen to induce hypoxia. Initial experiments were conducted to determine the effects of hypoxia in non-labouring and labouring myometrial tissues**.** These tissues were exposed to hypoxic conditions for 40 minutes and then returned to normoxia (switched back to oxygen to allow recovery).

c. Effect of zero glucose on response to oxytocin

Treatment with oxytocin induces the production of inositol triphosphate (IP_3) which causes Ca^{2+} release from the sarcoplasmic reticulum; this in turn raises cytosolic calcium (Ca^{2+}). This process increases uterine contractile force and frequency.

The effects of 1µM of oxytocin in PSS and oxytocin in zero glucose PSS were tested once contractions developed and stabilised. These treatments were applied for 40 minutes.

d. Effect of zero glucose on response to high K^+

Once stable contractions were achieved, high K^+ solution was applied to both non-labouring and labouring myometrial tissues for 40 minutes. This was compared to the application of high K^+ zero glucose solution for the same amount of time. The peak and plateau amplitudes were measured from the contraction baseline.

4.2.2.1.4 Spontaneous uterine response

Spontaneous contractions were generated and remained stable for 3 to 4 hours' duration when I mounted labouring and non-labouring myometrial strips in an organ bath which contained a bubbled physiologic solution at 37ºC and a pH of 7.4. This time was enough for all protocols to be completed and allowed me to identify changes that occurring during the experiments after applying agents to myometrial strips. The control data was set as 100% and the values obtained were expressed beneath each solution or treatment as a percentage of this control.

4.2.2.1.5. Comparison of spontaneous contractile activity between labouring and non-labouring tissues.

Myometrial samples were obtained from 6 non-labouring and 6 labouring women. Their spontaneous contractile activities were compared in vitro to their control contractile activities. Myometrial strips were mounted in the organ bath and left to contract for 40 minutes. Traces representing spontaneous contractions in non-labouring and labouring tissues are displayed in the figure below. (Figure 4.15)

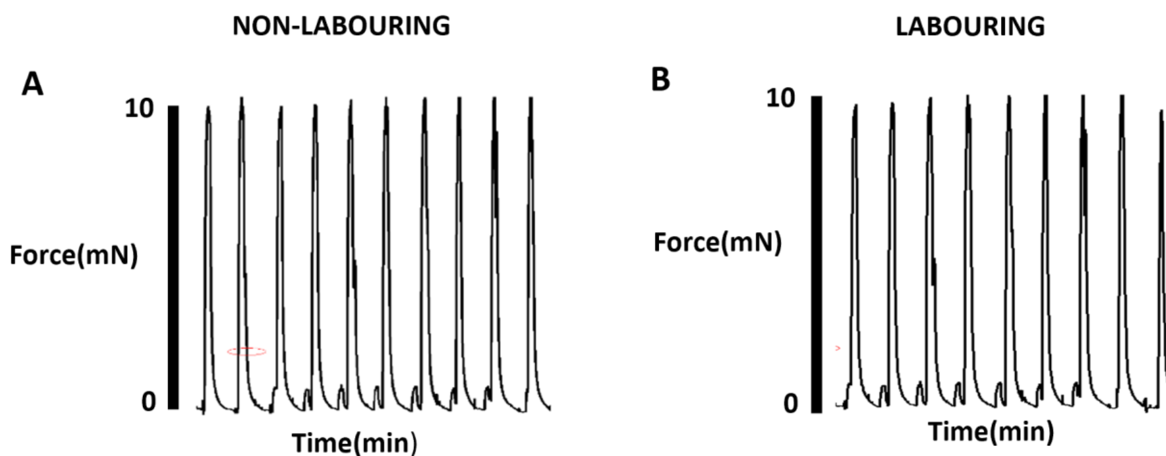


Figure 4.15 Spontaneous contractions in non-labouring(A) and labouring(B) uterine tissues. Representative traces of Spontaneous myometrial contractions No significant difference was found between the two groups.

The mean amplitude, frequency, and area under the curve (AUC) were calculated and compared for 6 non-labouring and 6 labouring tissues for a period of 20 minutes.

a) Force amplitude

The mean amplitude for spontaneous contractions in non-labouring tissues was 10.5 ± 1mN while in labouring tissues the mean force was 10.4 ± 1mN. There was no significant difference between myometrial spontaneous contraction force amplitudes in non-labouring and labouring tissues ($p > 0.05$).

b) Frequency

The number of myometrial contractions in a selected period of 20 minutes was measured. In non-labouring tissues, the mean frequency was 7.5 ± 0.5 while in labouring tissues the mean frequency was 7.2 ± 0.8. It was revealed that there was not a significant difference between non-labouring and labouring human tissues, ($p > 0.05$).

c) The area under the curve (AUC)

The mean integral force of contraction was analysed in non-labouring (8.2 ± 2) and in labouring (8.4 ± 2) ($p > 0.05$). There was no significant difference between myometrial spontaneous contractions in non-labouring tissue and spontaneous contractions in labouring tissue.

In this study, non-labouring myometrium refers to the smooth muscle tissue of the uterus when it is not actively contracting during pregnancy. In contrast, labouring myometrium refers to the same smooth muscle tissue when it is undergoing contractions during the process of childbirth.

The main difference between non-labouring and labouring myometrium lies in the level of hormones and biochemical signals present in the tissue. Non-labouring myometrium is characterized by low levels of oxytocin, a hormone that stimulates uterine contractions, and high levels of progesterone, a hormone that maintains uterine quiescence during pregnancy. In contrast, labouring myometrium experiences an increase in oxytocin release, which triggers contractions, and a decrease in progesterone levels, allowing the uterus to contract. This hormonal shift is crucial for the initiation and progression of labour.

Physiologically, there are several key differences between non-labouring and labouring myometrium. Non-labouring myometrium is typically quiescent and relaxed. Its muscle fibres are relatively elongated and well-organized, allowing for the accommodation of the growing foetus. In the absence of contractions, blood flow to the uterus is also generally low in non-labouring myometrium.

In contrast, labouring myometrium undergoes a series of coordinated contractions that facilitate cervical dilation and the eventual expulsion of the fetus. During labour, the myometrium experiences increased tension and contraction, resulting in the shortening and thickening of the muscle fibres. Blood flow to the uterus also increases significantly during labour to ensure an adequate oxygen supply to the contracting muscle tissue.

It is worth mentioning that in this study, an in vitro approach was used. Even though the contractility measurement technic used is an efficient method, this means the myometrial tissue is separated from endocrinal and neuronal modulatory action. I compared contractile activities between the two groups to avoid bias in case there is difference with the parameters measured.

4.2.2.1.6 Analysis

As described in chapter 2, section 2.2.8.

4.2.3 Results

4.2.3.1 Investigating the effect of zero glucose solution.

The objective of this experiment was to investigate the effect of glucose depletion on uterine contractions and compare the results between non-labouring and labouring tissue in vitro. 12 non-labouring and 7 labouring tissues were used in this set of experiments.

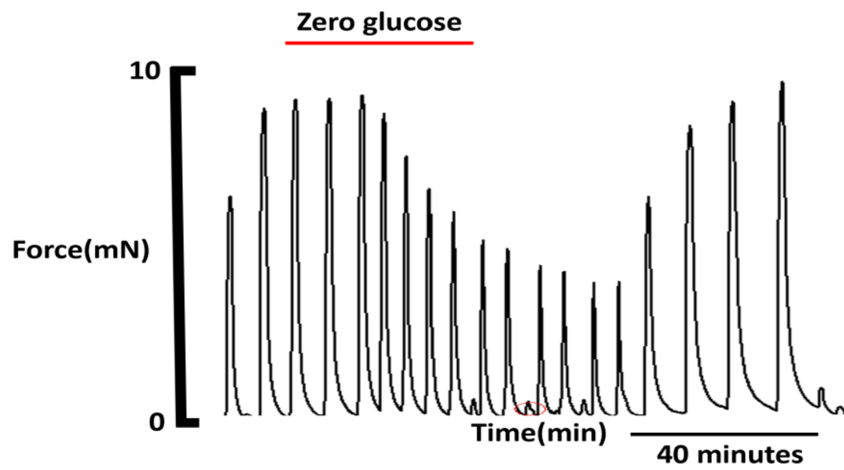
Zero glucose solution had a gradual inhibition effect on uterine contractility in both non-labouring and labouring tissues (Figure 4.16).

One-sample *t*-test zero glucose solution yielded a significant decrease in contractility in all parameters: amplitude down to 82 ± 6% ($p < 0.0001$), frequency (72 ± 1%, $p < 0.0001$) and AUC of 58 ± 7% ($p < 0.0001$) in non-labouring group ($n = 12$). (Figure 4.17)

In the labouring group, the force amplitude, and the area under the curve declined significantly and respectively down to 87 ± 2% ($n = 7$, $p < 0.0001$) and 79 ± 3 ($n = 7$, $p < 0.0001$) of control values but no significant difference with the frequency (107 ± 5, $n = 7$, $p = 0.3677$) (Figure 4.18).

An unpaired test revealed that the decline in amplitude was greater in non-labouring than in labouring myometria, but with no significant difference, with *p*-values of 0.05448. The decline in frequency was greater in non-labouring tissues than in labouring tissues, with significant difference ($p < 0.0001$) (Figure 4.18). In AUC, the difference in decline was significant as well ($p = 0.0419$). The recovery of contractions was maximal after the restoration of glucose.

NON-LABOURING



LABOURING

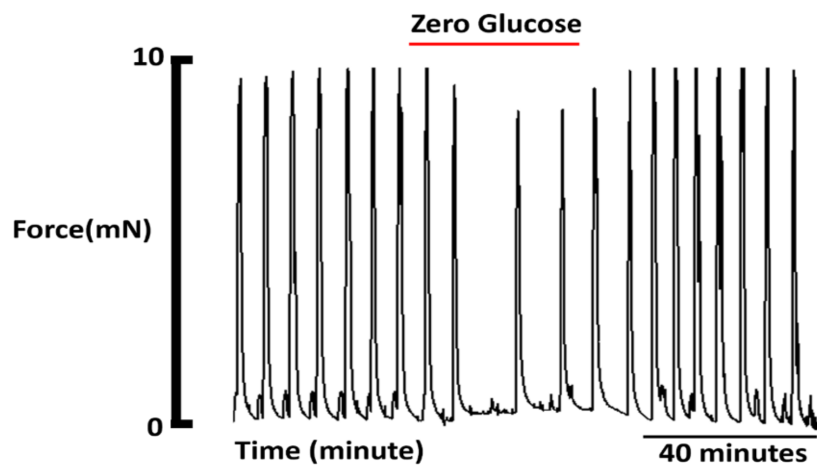


Figure 4.16 Inhibitory effect of zero glucose on uterine contractility in non-labouring and labouring myometria. Representative recording of the zero-glucose effect. Zero glucose solution had an inhibitory effect on uterine contractility in non-labouring and labouring myometria.

Zero glucose effect on non-labouring myometria

NON-LABOURING

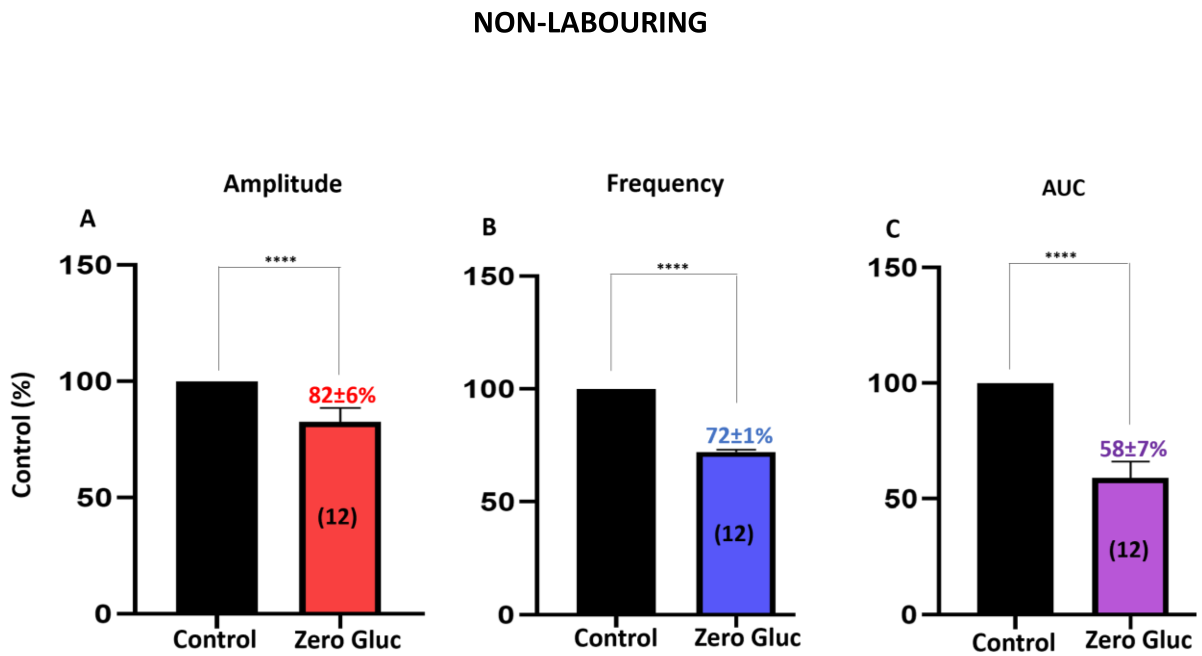


Figure 4.17 The effect of zero glucose on uterine contractility in non-labouring myometria. Zero glucose solution had a significant inhibitory effect on amplitude (A), frequency (B), and AUC (C). (n=12)

LABOURING

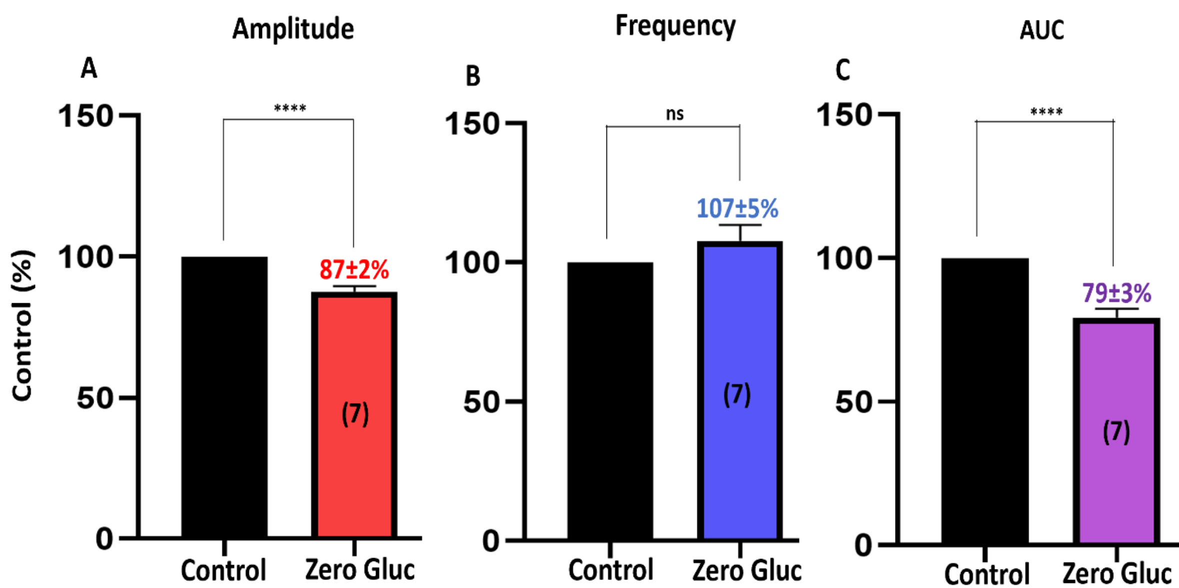


Figure 4.18 The effect of zero glucose on uterine contractility in labouring myometrium. Zero glucose solution had a significant inhibitory effect on amplitude (A) and AUC (C), but not on frequency (B)(n=7)

4.2.3.2 Zero glucose results summary

Non-labouring group	Zero glucose
Amplitude	82 ± 6% ($p < 0.0001$, n = 12) ****
Frequency	72 ± 1% ($p = 0.0014$, n = 12) **
AUC	58 ± 7% ($p = 0.0002$, n = 12) ****

P value of <0.05 was regarded as significant (*, **, *** and ns denote P<0.05, P<0.01, P<0.001 and non-significant).

Table 4.7 Summary of the effect of zero glucose on the non-labouring group. *P*-values result from one sample *t*- test, comparing each treatment with its own internal control period.

Labouring group	Zero glucose
Amplitude	87 ± 2% ($p < 0.0001$, n = 7) ****
Frequency	107 ± 5% ($p = 0.3677$, n = 7) ns
AUC	79 ± 3% ($p < 0.0001$, n = 7) ****

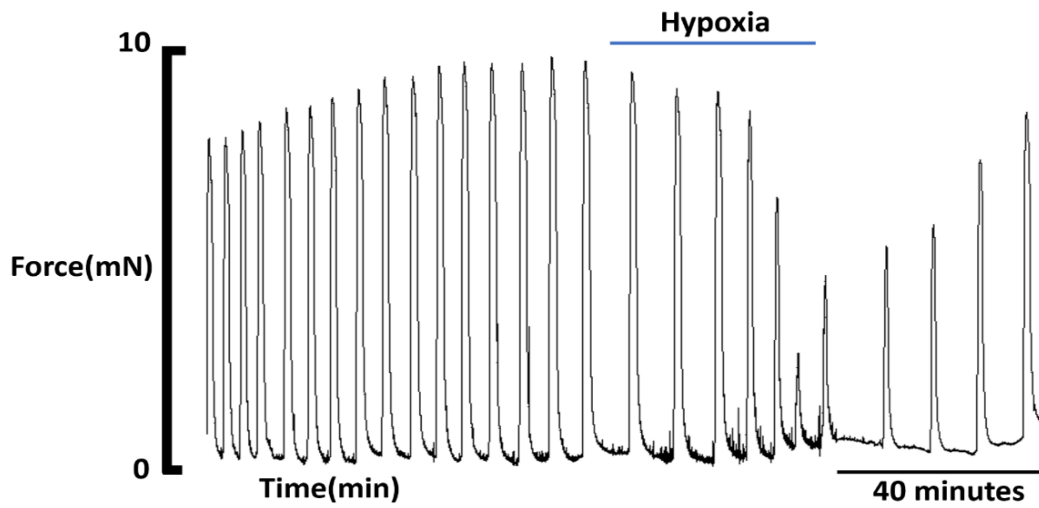
P value of <0.05 was regarded as significant (*, **, *** and ns denote P<0.05, P<0.01, P<0.001 and non-significant).

Table 4.8 The summary of the effect of zero glucose in the labouring group. *P*-values result from one sample *t*- test, comparing each treatment with its own internal control period. The difference in frequency was not significant.

4.2.3.3 Investigating the effect of hypoxia on uterine contractility.

The objective of this test was to explore the effect of hypoxia on uterine contractility and to compare the effects between non-labouring and labouring tissues. This experiment was useful because of the transient ischemia occurring during labour at each uterine contraction, due to the compression of vessels, thus inducing hypoxia. This decrease in blood supply is eventually accompanied by a decrease in glucose supply. Having information on the effect of hypoxia alone was important, before studying the combined effect of hypoxia and glucose depletion explained in the next set of experiments. It has been already demonstrated that hypoxia has an inhibitory effect on uterine contractions in humans (Alotaibi, 2015). 9 non-labouring and 6 labouring tissues were used. This study shows again that hypoxia had a gradual inhibition effect on uterine contractility in both non-labouring and labouring tissues (Figure 4.19). Tissues that were exposed only to hypoxia revealed a significant decline in contractile amplitude, which fell to 48 ± 7% (p = 0.0010, n = 9); in frequency, which fell to 52 ± 9 % (p = 0.0013); and in AUC, which fell to 47 ± 7% (p = 0.0007) of control values in the non-labouring group (Figure 4.20). A significant decline was observed in the labouring group as well: amplitude 51 ± 4% (p = 0.0008, n = 6), frequency 52 ± 5% (p = 0.0010), and AUC 44 ± 4% (p = 0.0015) (Figure 4.21). Under hypoxia, labouring, and non-labouring myometria were equally affected. Uterine contractions were fully restored under normoxic conditions.

NON-LABOURING



LABOURING

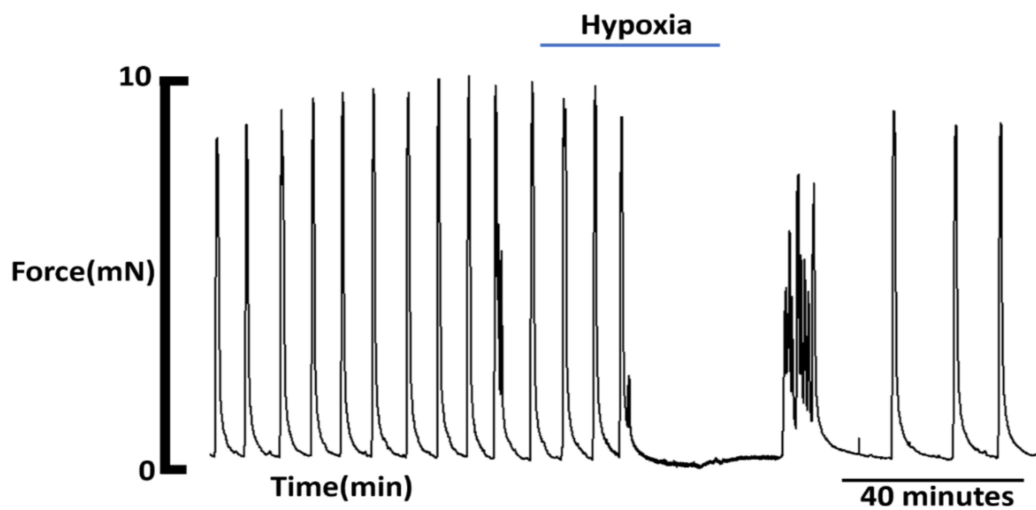


Figure 4.19 Inhibitory effect of hypoxia on uterine contractility in non-labouring and labouring myometrial strips. Representative traces illustrating contractile inhibition. Hypoxia had an inhibitory effect on uterine contractility.

NON-LABOURING

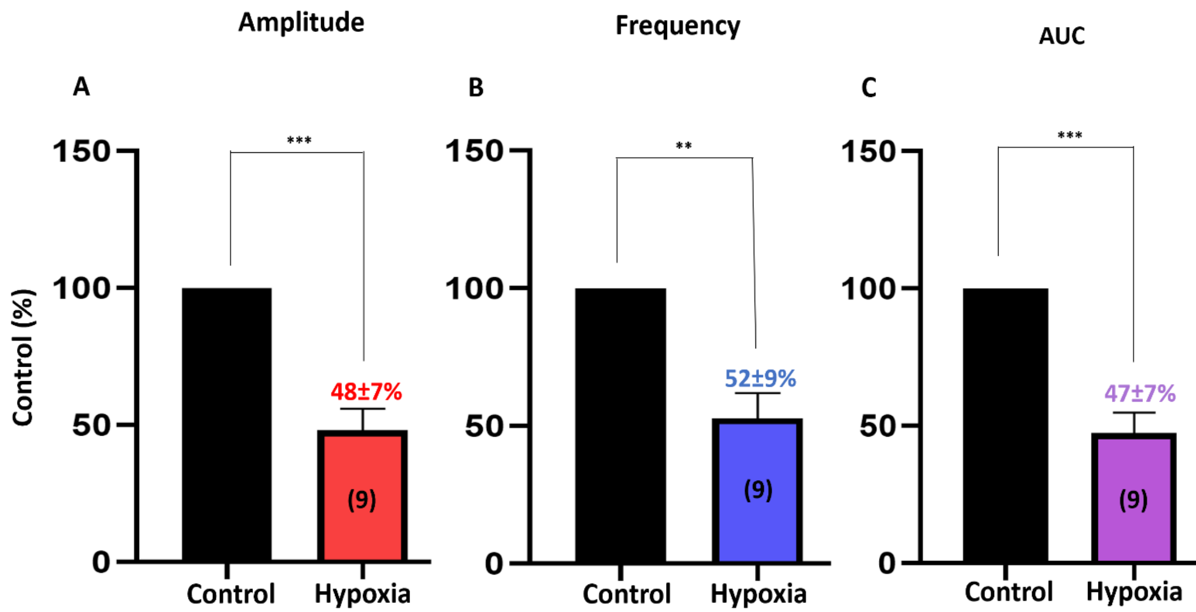


Figure 4.20 The Effect of hypoxia on uterine contractility in non-labouring myometria. Hypoxia had a significant inhibitory effect on all the parameters. (n=9)

LABOURING

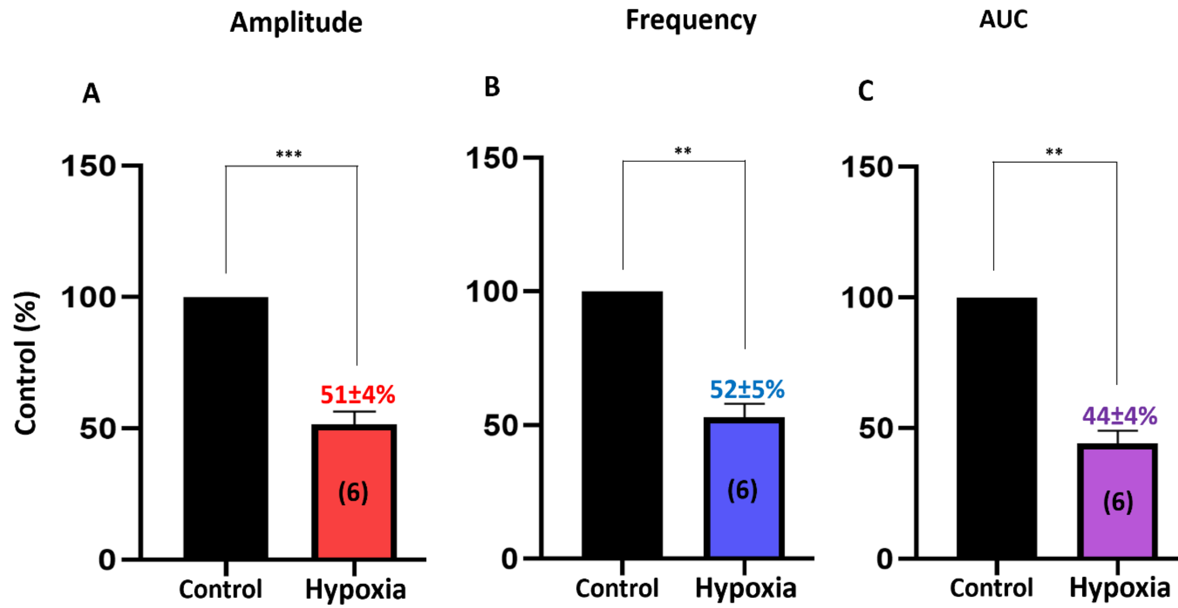


Figure 4.21 Effect of hypoxia on uterine contractility in labouring myometria. Hypoxia had a significant inhibition effect on amplitude (A), frequency (B) and AUC (C). (n=6)

4.2.3.4 Summary of hypoxia results

Non- labouring group	Hypoxia
Amplitude	48 ± 7% (p = 0.0010) *** n = 9
Frequency	52 ± 9% (p = 0.0013) ** n = 9
AUC	47 ± 7% (p = 0.0007) *** n = 9

P value of <0.05 was regarded as significant (*, **, *** and ns denote P<0.05, P<0.01, P<0.001 and non-significant).

Table 4.9 Summary of the effect of hypoxia on the non-labouring group. *P*-values result from one sample t-test comparing each treatment with its own internal control period.

Labouring group	Hypoxia
Amplitude	51 ± 4% (p = 0.0008) *** n = 6
Frequency	52 ± 5% (p = 0.0010) ** n = 6
AUC	44 ± 4% (p = 0.0015) ** n = 6

P value of <0.05 was regarded as significant (*, **, *** and ns denote P<0.05, P<0.01, P<0.001 and non-significant).

Table 4.10 Summary of the effect of hypoxia on the labouring group. *P*-values result from one sample t-test comparing each treatment with its own internal control period.

4.2.3.5 Investigating the effect of zero glucose under hypoxic conditions.

The objective of this set of experiments was to examine the effect of glucose depletion under hypoxic conditions. As hypoxia has an inhibitory contractile effect on myometrium, as mentioned above, I expected the combined effect of zero glucose and hypoxia to have a greater contractile inhibition.

7 non-labouring and 6 labouring myometrial tissues were used for this purpose.

Uterine contractile decline was pronounced with the combined action of zero glucose and hypoxia, with contractions frequently stopping completely. This was true for both the non-labouring and labouring group (Figure 4.22).

In the non-labouring group, a one-sample *t*-test revealed that amplitude significantly dropped to 48 ± 9% (p = 0.0015, n = 7), frequency to 53 ± 8% (p = 0.0039), and AUC declined to 33 ± 3% (p = 0.0006, n = 7) (Figure 4.23).

In the labouring group, amplitude fell to 35 ± 7% (p = 0.0059, n = 6); frequency fell to 52 ± 13% (p = 0.0117); and AUC fell to 30 ± 5% of control values (p = 0.0024) (Figure 4.24).

Zero glucose on the response to hypoxia had significant inhibitory effects on labouring and non-labouring tissue. Both types were equally affected. The decline in contractility was sharp, and full contractile recovery was observed after glucose and oxygen were restored.

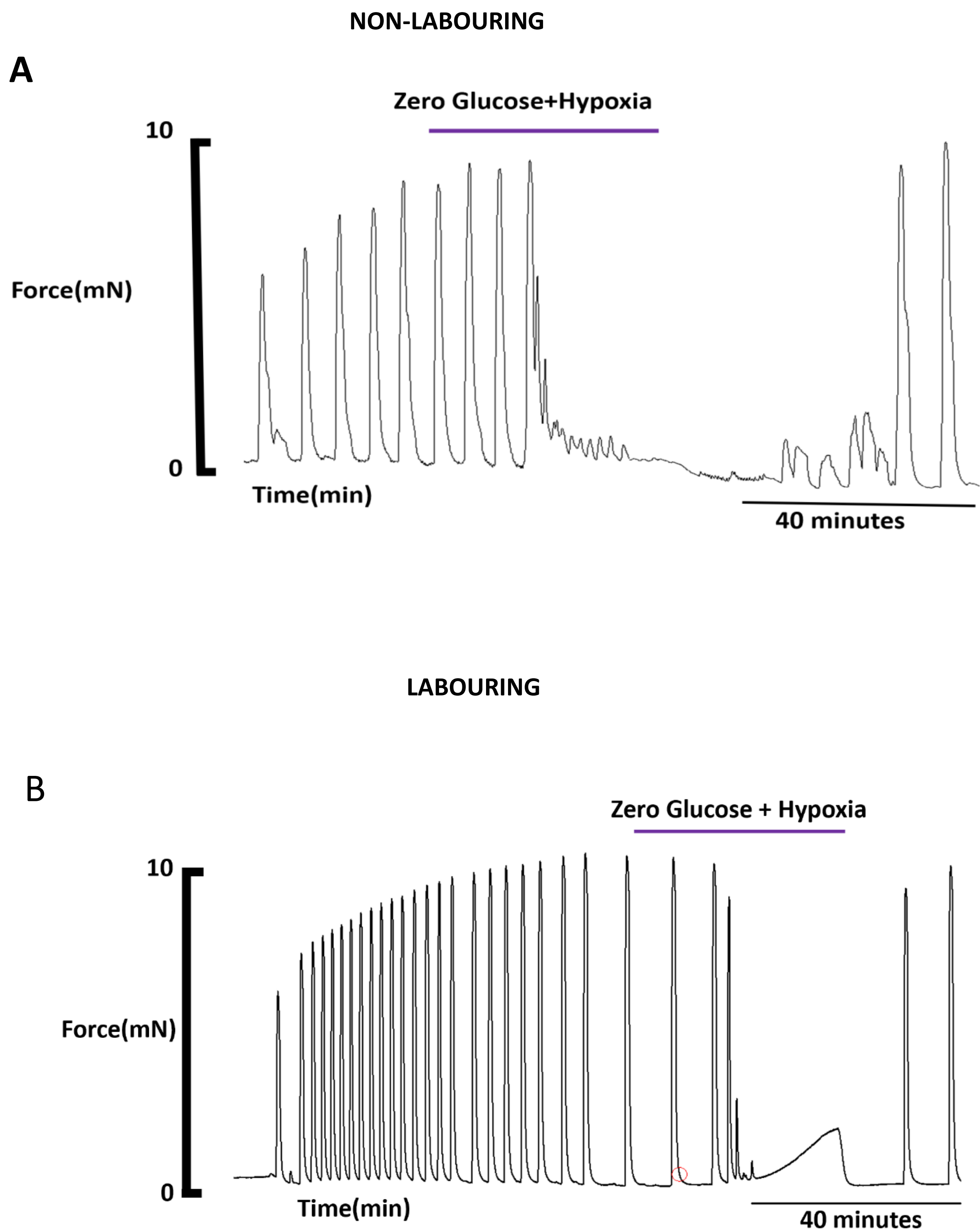


Figure 4.22 The effect of zero glucose on the response to hypoxia on uterine contractility in non-labouring and labouring myometrial tissues. The decline in contractility was most pronounced with the combined action of zero glucose and hypoxia, with contractions frequently stopping altogether (Representatives A&B)

NON-LABOURING

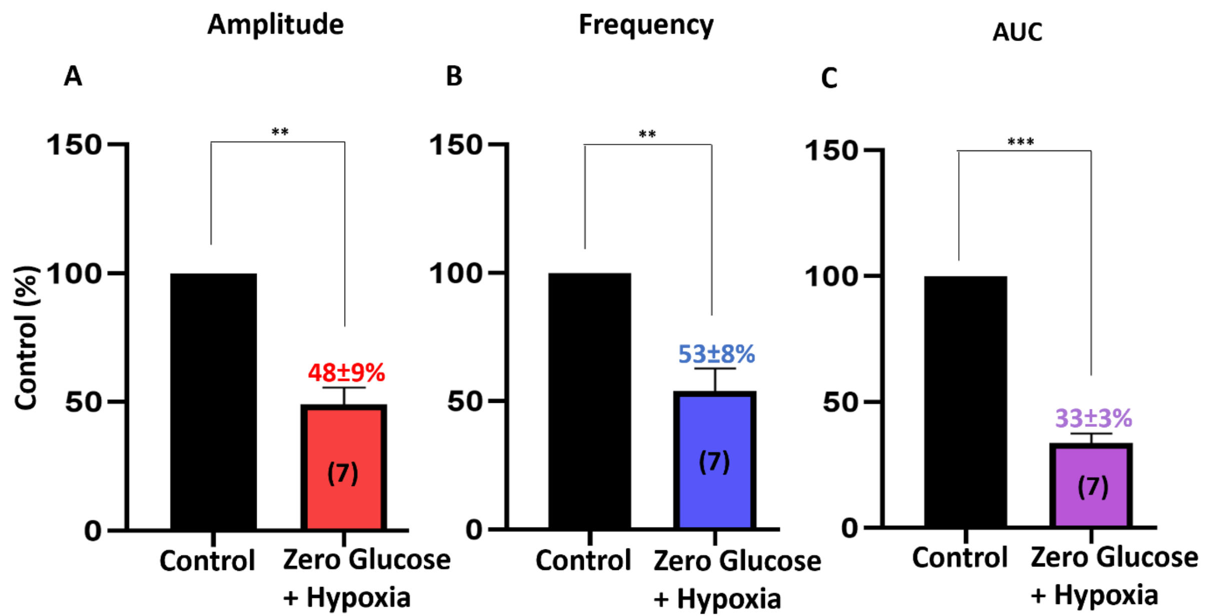


Figure 4.23 The combined effect of zero glucose and hypoxia on uterine contractility in non-labouring myometria. Under zero glucose and hypoxia, contractile amplitude, frequency, and AUC were significantly affected(n=7)

LABOURING

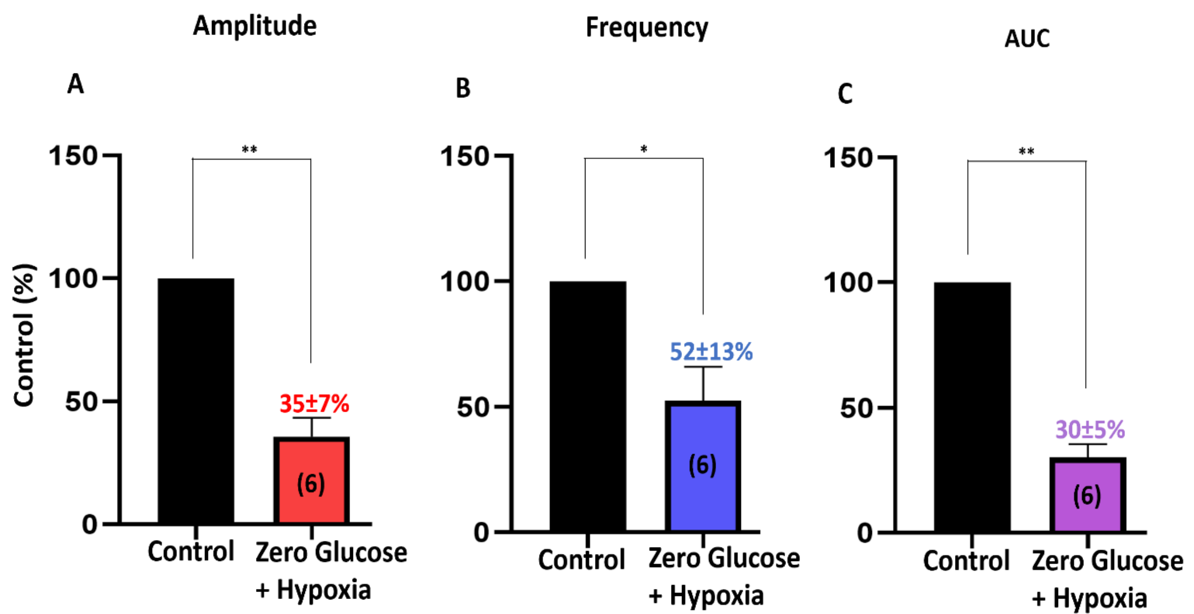


Figure 4.24 Combined effect of zero glucose and Hypoxia on uterine contractility in labouring myometrium. Under zero glucose and hypoxia, contractile amplitude, frequency, and AUC declined significantly. (n=6)

4.2.3.6 Results summary of the combined effect of zero glucose and hypoxia

Non- labouring group	Zero glucose + hypoxia
Amplitude	48 ± 9% (p = 0.0015) ** n = 7
Frequency	53 ± 8% (p = 0.0039) ** n = 7
AUC	33 ± 3% (p = 0.0006) *** n = 7

P value of <0.05 was regarded as significant (*, **, *** and ns denote P<0.05, P<0.01, P<0.001 and non-significant).

Table 4.11: Summary of the combined effect of zero glucose and hypoxia in the non-labouring group. *P*-values result from one sample t- test comparing each treatment with its own internal control period.

Labouring group	Zero glucose + hypoxia
Amplitude	35 ± 7% (*p* = 0.0059) ** n = 6
Frequency	52 ± 13% (*p* = 0.0117) * n = 6
AUC	30 ± 5% (*p* = 0.0024) ** n = 6

P value of <0.05 was regarded as significant (*, **, *** and ns denote P<0.05, P<0.01, P<0.001 and non-significant).

Table 4.12: Summary of the combined effect of zero glucose and hypoxia in the labouring group. *P*-values result from one sample t- test comparing each treatment with its own internal control period.

4.2.3.7 Investigating the oxytocin response under zero glucose conditions.

The purpose of this set of experiments was to explore how effective oxytocin is under zero glucose conditions.

For the experiment in humans, spontaneous uterine contractions under zero glucose conditions were taken as control. Under normal conditions, oxytocin requires energy in the form of adenosine triphosphate to initiate or to increase uterine contractile force in smooth muscles (Arrowsmith et al.2012). The objective of this protocol was to investigate and show the response to OT, expecting this response to be reduced by using a glucose-free solution. 7 non-labouring and 5 labouring myometrial tissues were used.

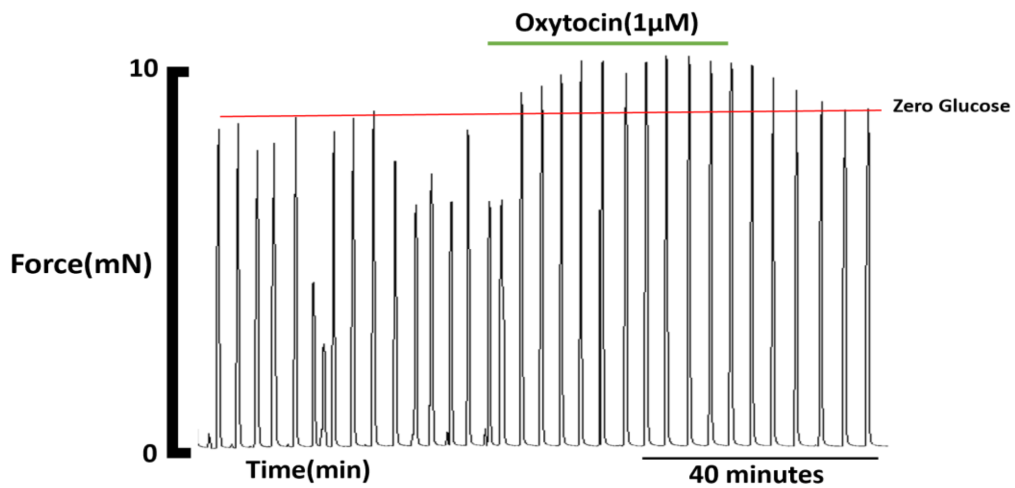
1µM oxytocin increased uterine contractility in non-labouring and labouring tissues under zero glucose conditions. (Figure 4.25). A one-sample *t*- test zero glucose physiologic solution and oxytocin yielded a non-significant increase in contractility amplitude to 107 ± 3% (p = 0.2001), frequency 100 ± 6% (p = 0.9477) and AUC 99 ± 4% (p = 0.9662) in the non-labouring group (n = 7). (Figure 4.26), compared to the internal control.

In labouring tissue, the results followed the same trend. The myometrial amplitude, frequency and AUC amounted to 104 ± 2% (p = 0.2119), frequency 93 ± 5% (p = 0.4841) and AUC 105 ± 5% (p = 0.1022) (n = 5). (Figure 4.27)

Arrowsmith et al. revealed that in human, oxytocin (10nM) in PSS (with glucose) produced +89 ± 2% increase in myometrial force amplitude and + 342 ± 47% (n = 28) in area under the curve compared to control in both non-labouring and labouring myometrial tissues together at term (Arrowsmith et al., 2012).Comparing my results to Arrowsmith's findings, it transpires that under zero glucose, the increase in force amplitude and integral force is extremely low either in non-labouring or labouring myometrial tissues. Non-labouring and labouring tissues are equally affected.

NON-LABOURING

A



LABOURING

B

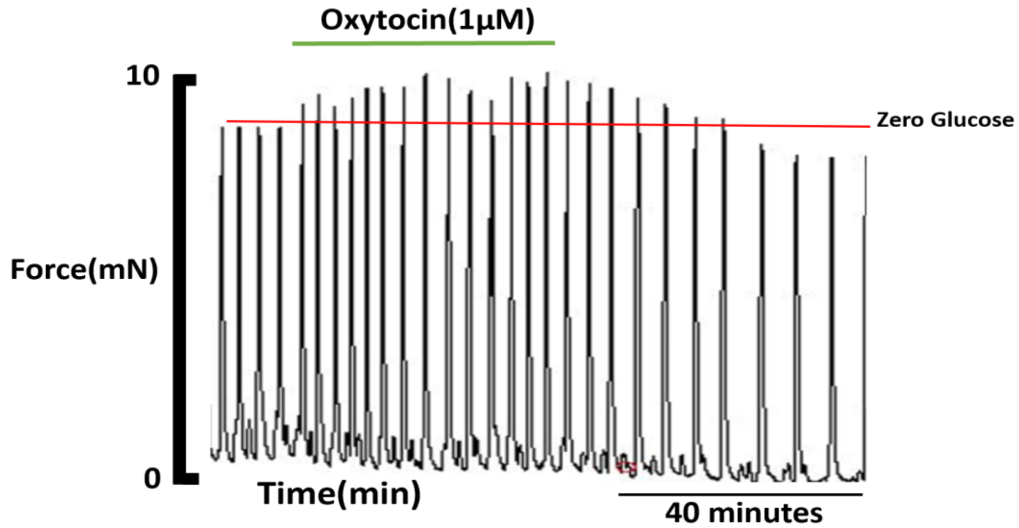


Figure 4.25 The increasing effect of oxytocin on uterine contractility in non-labouring and labouring myometrial tissues. Representative recordings showing that oxytocin has a minimal increasing contractile effect.

NON-LABOURING

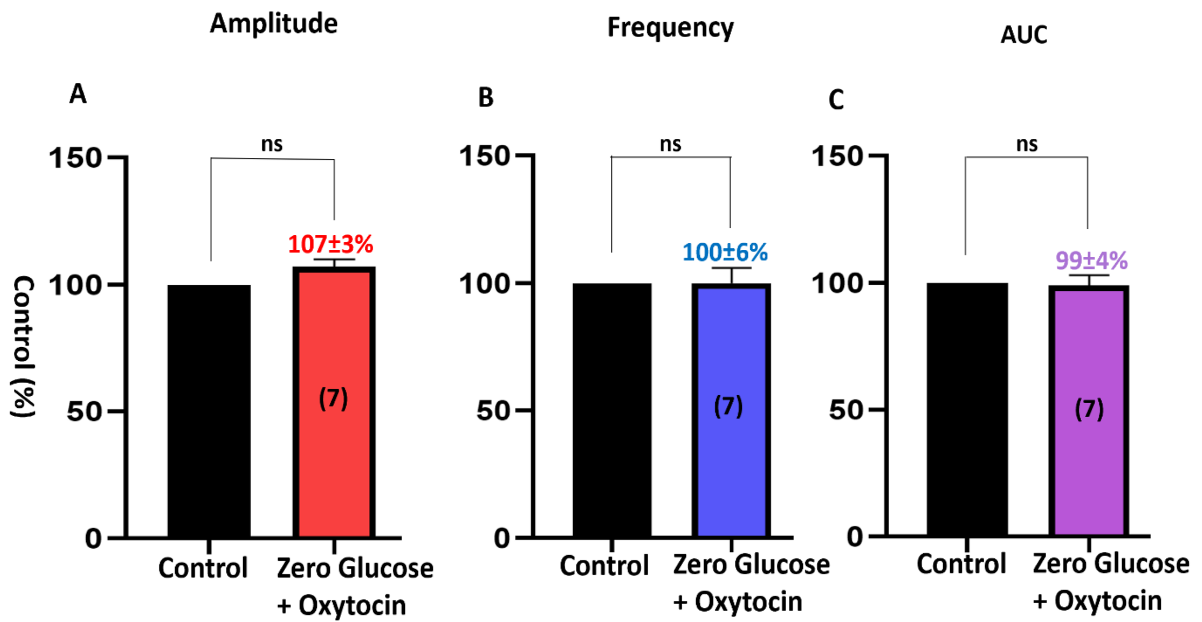


Figure 4.26 Effect of zero glucose on the response to oxytocin in non-labouring myometria. No significant increase was found in amplitude (A), frequency (B), and AUC (C). (n=7)

LABOURING

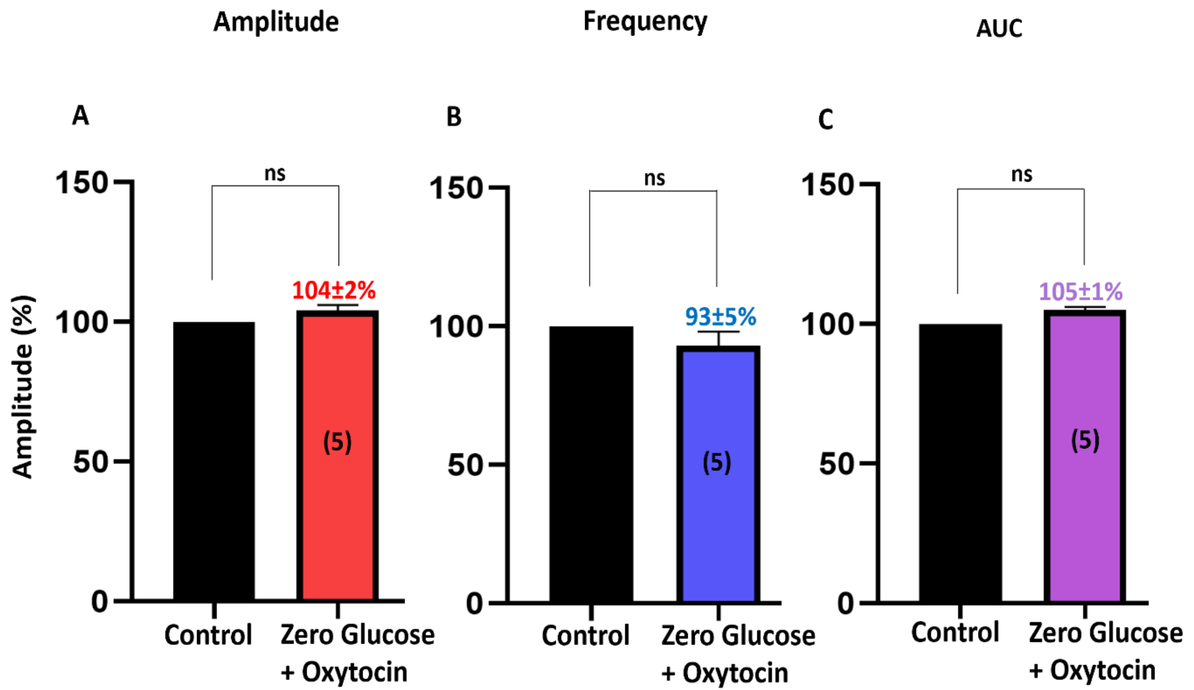


Figure 4.27 The effect of zero glucose on the response to oxytocin in labouring myometria. No significant increase was found in amplitude (A), frequency (B), and AUC (C). (n=5)

4.2.3.8 Results summary of the effect of zero glucose on the response to oxytocin

Non- labouring group	Oxytocin
Amplitude	107 ± 3% (p = 0.2001) ns, n = 7
Frequency	100 ± 6% (p = 0.9477) ns, n = 7
AUC	99 ± 4% (p = 0.9662) ns, n = 7

ns denotes non-significant.

Table 4.13 The summary of the effect of zero glucose on the response to oxytocin in the non- labouring group. *P*-values result from one sample t- test comparing each treatment with its own internal control period.

Labouring group	Oxytocin
Amplitude	104 ± 2% (p = 0.2119) ns, n = 5
Frequency	93 ± 5% (p = 0.4841) ns, n = 5
AUC	105 ± 1% (p = 0.1022) ns, n = 5

ns denotes non-significant.

Table 4.14 The summary of the effect of zero glucose the response to oxytocin in the labouring group. *P*-values result from one sample t- test comparing each treatment with its own internal control period.

4.2.3.9 Investigating the effect of zero glucose on the response to high concentration of potassium salt solution (high K^+)

The objective was to compare the results between high K^+ and high K^+ zero glucose on uterine contractility and between non-labouring and labouring tissue. Although, exposing a tissue to high K^+ solution is an experimental procedure to test the integrity of a muscle, under normal conditions, high K^+ depolarising effect on the myometrial cell provokes an influx of calcium into the cell and strong contractions ensue. I compared the results expecting the action of high K^+ to be reduced under glucose depletion conditions. Myometrial strips were exposed to a high concentration potassium salt solution (40mM) for 40 minutes before they were returned to the physiologic solution. In both non-labouring and labouring groups, the response to high K^+ solution comprised an initial increase in amplitude (peak), before decreasing and reaching a stabilised plateau. 10 non-labouring and 4 labouring uterine tissues were used for this set of experiments. (Figures 4.29 &4.31)

The unpaired *t*-test performed on the non-labouring group revealed that a significant difference was found between high K^+ and high K^+ zero glucose. The peak contractile amplitude from the high K^+ solution was significantly higher (141 ± 2%, n = 10) than the high K^+ zero glucose solution (126 ± 5%, n = 10) (p= 0.0122). The plateau amplitude was significantly higher with high K^+ (100 ± 2%, n = 10) than high K^+ zero glucose (74 ± 4%, n = 10) ($p < 0.0001$) (Figure 4.28).The unpaired *t*-test performed on the labouring group showed that the peak amplitude from high K^+ solution was the same (123 ± 4%, n = 4) as for high K^+ zero glucose solution (124 ± 4%, n = 4), with no significant difference (p = 0.9156) .The plateau amplitude was 79 ± 1% (n = 4) with high K^+, and 82 ± 5% (n = 4) with high K^+ zero glucose, but with no significant difference (p = 0.8533)(Figure 4.30).

In comparing the non-labouring and labouring tissues, a significant difference was obvious with peak amplitude on the response to high K^+ under normal conditions. The peak and plateau amplitudes were higher in non-labouring than in labouring tissues. The *p*-values were respectively 0.0007 and more pronounced in plateau amplitude ($p < 0.0001$). In contrast, no significant difference was found in these parameters on the response to high K^+ zero glucose, where the *p*-values were respectively 0.8167 and 0.3188 (Figure 4.32)

NON-LABOURING

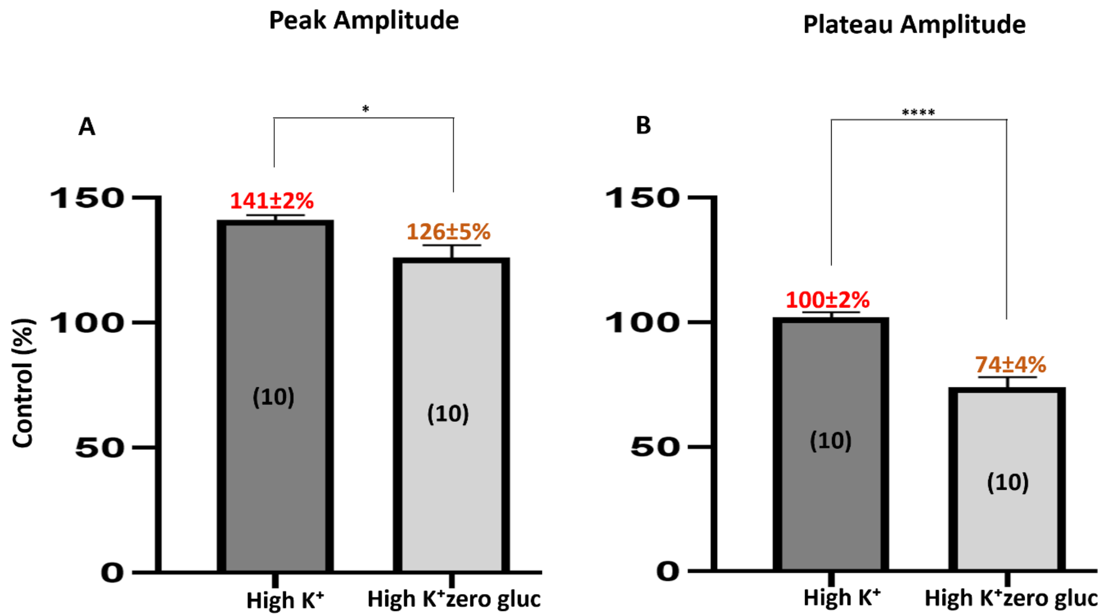


Figure 4.28 The effect of zero glucose on the response to high K^+ in non-labouring myometria. There was a significant difference in peak and plateau amplitudes under zero glucose conditions in non-labouring tissue. **High K^+(n=10), High K^+ 0 Gluc (n=10)**

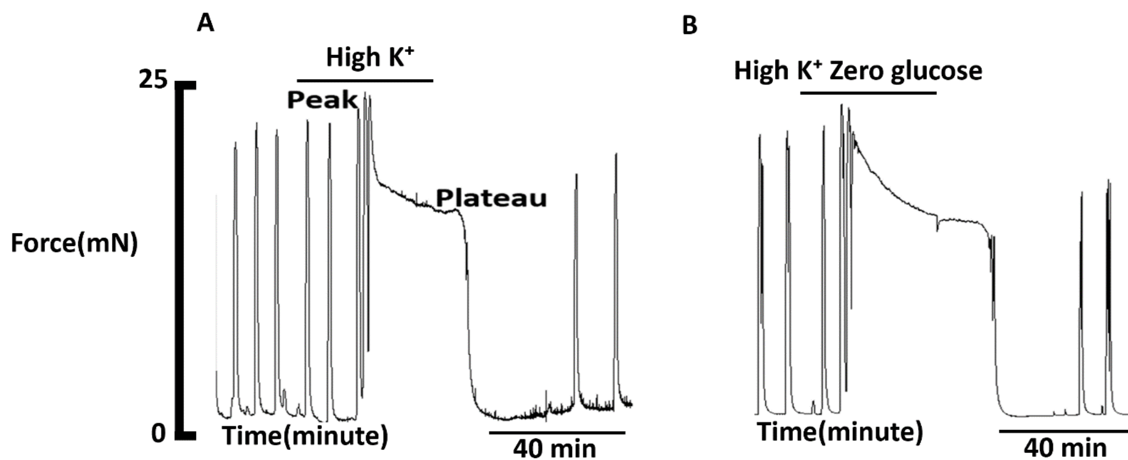


Figure 4.29 Traces of high concentration of potassium (high K^+) in non- labouring tissue from glucose and zero glucose (A&B). Representative traces of high concentration of potassium (high K^+) in non- labouring tissue under glucose (A) and zero glucose (B) conditions.

LABOURING

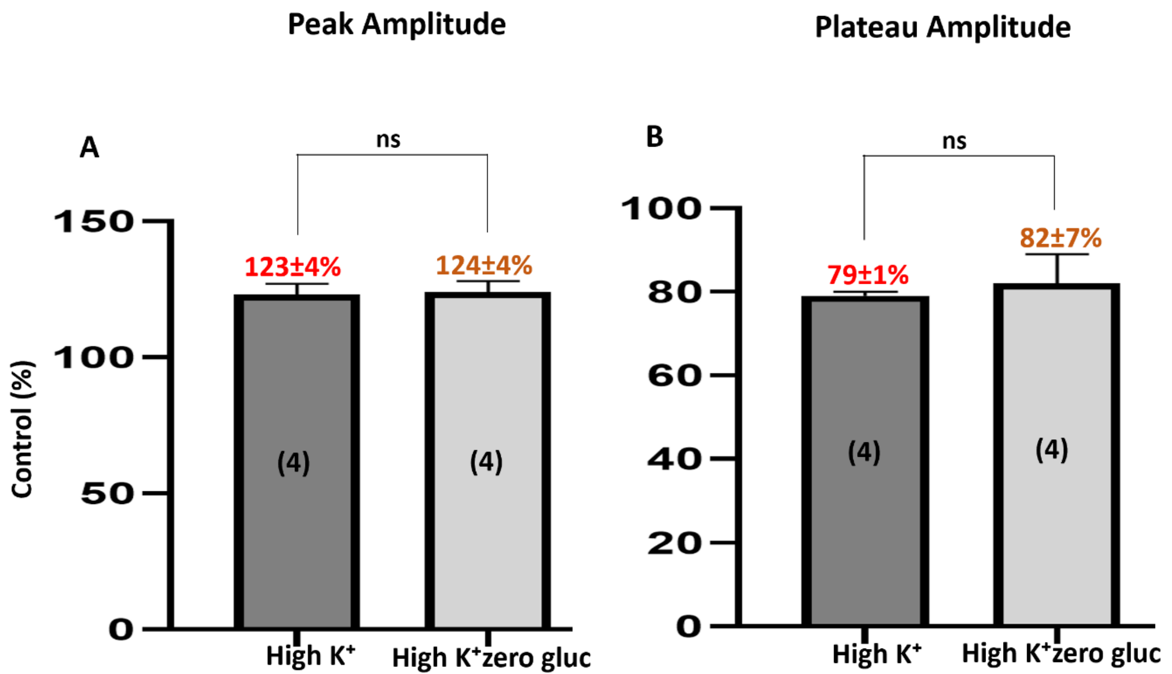


Figure 4.30 The effect of zero glucose on the response to high K^+ in labouring myometrium. There was no significant difference found in peak and plateau amplitude under zero glucose conditions in labouring tissue. **High K^+(n=4), High K^+ 0 Gluc (n=4)**

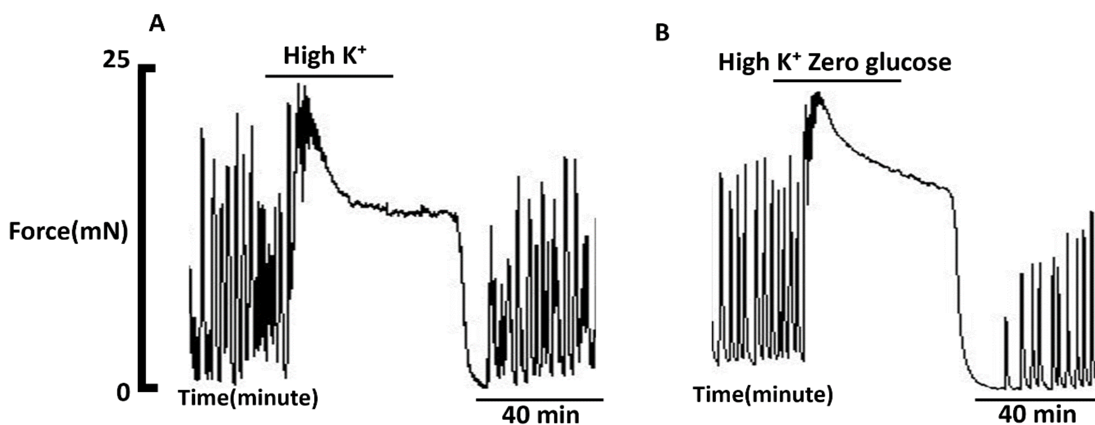


Figure 4.31 Traces of high concentration of potassium (high K^+) in labouring groups from glucose and zero glucose (A&B). Representative isometric traces of high concentration of potassium (high K^+) in non- labouring tissue under glucose (A) and zero glucose (B) conditions.

4.2.3.10 Comparison of high K^+ and high K^+ zero glucose response between non-labouring and labouring myometrial tissues

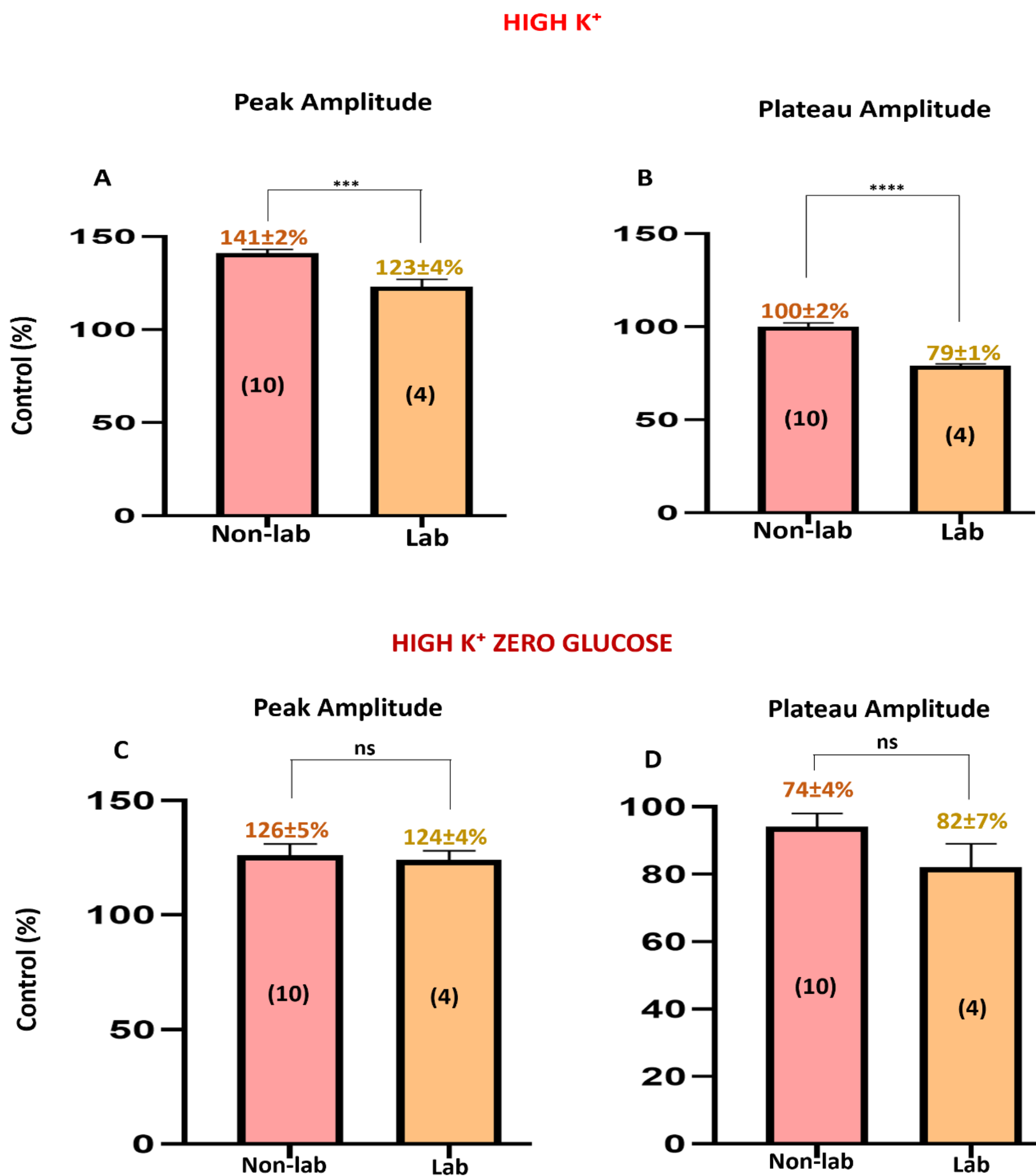


Figure 4.32 Graph comparing the effect of zero glucose on the response to high K^+ from non-labouring and labouring myometrial tissues. There was a significant difference in peak and plateau amplitudes with high K^+, but no difference with high K^+ zero glucose.

4.2.3.11 Results summary of the effect of zero glucose on the response to high K^+

Non-labouring	High K^+	High K^+ zero glucose	*P*-value
Peak amplitude	141 ± 2% (n = 10)	126 ± 5% (n = 10)	0.0122 *
Plateau amplitude	100 ± 2% (n = 10)	74 ± 4% (n = 10)	< 0.0001 ***

P value of <0.05 was regarded as significant (*, **, *** and ns denote P<0.05, P<0.01, P<0.001 and non-significant).

Table 4.15 The effect of zero glucose on peak and plateau amplitude on the high K^+ response in non-labouring myometria. The *p*-values result from unpaired *t*-tests comparing each treatment between high K^+ and high K^+ zero glucose.

Labouring	High K^+	High K^+ zero glucose	*P*-value
Peak amplitude	123 ± 4% (n = 4)	124 ± 4% (n = 4)	0.9156 (ns)
Plateau amplitude	79 ± 1%, (n = 4)	82 ± 5%, (n = 4)	0.8533 (ns)

P value of <0.05 was regarded as significant (*, **, *** and ns denote P<0.05, P<0.01, P<0.001 and non-significant).

Table 4.16 The effect of zero glucose on the peak and plateau amplitude of the high K^+ response in labouring myometria. *P*-values result from unpaired *t*-tests comparing each treatment between high K^+ and high K^+ zero glucose.

4.2.4 Discussion

In this chapter I determine the influence of glucose depletion on uterine contractility in humans. For human tissue, my findings demonstrate that zero glucose has an inhibition effect on uterine contractility. Except for the frequency, the contractile amplitude and the integral force significantly decreased compared to control values for both non-labouring and labouring tissues. The results demonstrate the difference between the two groups in enduring energy depletion. Non-labouring tissue seemed to be more affected than labouring. This is a difficult statement to believe since both tissues were ready for parturition and would have built similar glycogen stores. Besides labouring would have used some of stores but question may remain on the level of myometrial glycogen of the non-labouring samples used for contractility studies. The storage could probably be low. More myometrial samples could help confirm the statement. Literature on the impact of a glucose-free medium on uterine contractility in human is scarce. The study of Chiswick and colleagues on glycogen storage and contractility mentions the inhibitory effect of a glucose-free medium on uterine contractility

in humans (n = 8); in this study, the mean contractile amplitude and the AUC declined (to 83 ± 6% and 57 ± 9% respectively) compared to the control (100%) (Chiswick et. al., 2016). This observation was upheld by the preliminary results we published on the effect of zero glucose on uterine contractility in humans (Kaleta & Wray, 2019). The effect of glucose depletion on uterine contractility in humans did not differ from its effect on mice. The AUC in non-pregnant and pregnant mice yielded respectively 59 ± 3% (n = 8) and 54 ± 7% (n = 27). In humans the AUC declined respectively to 58 ± 7 (n = 12) in non-labouring and 79 ± 3% (7) in labouring tissue. Either in humans or mice, zero glucose has an inhibitory effect on uterine contractility.

Under hypoxic conditions, the decline in contractility was considerable in force amplitude and integral force. Both non-labouring and labouring tissues were affected the same way, indicating that both groups depend on oxidative metabolism. These data are in agreement with Monir-Bishty, who showed that oxygen depletion had a significant decline in contractility after exposure of human myometrial tissues to hypoxic conditions (Monir-Bishty et al., 2003). Additionally, these results were confirmed by Bugg et al. in a study on non-pregnant and pregnant women, where the exposure of myometrial tissues to hypoxia resulted in a significant decline in spontaneous contractility. As explained earlier, induced hypoxia results in the production of lactate, leading to a fall in pH. The ensuing acidification interferes with the calcium entry within the cell and consequently inhibits uterine contractions.
My studies tested the effect of zero glucose on the response to hypoxia in humans. Temporary period of hypoxia occurs in the myometrium as a result of uterine vessel compression at each contraction during labour. As blood supply to the myometrium decreases due to vessel compression, so does glucose supply. Therefore, it was of great importance to determine the combined effect of glucose depletion and hypoxia. I reported that the inhibition of uterine contractions was exacerbated by the joint effect of zero glucose and hypoxia, to the point of causing the uterine contractions to stop. The pronounced fall of amplitude and AUC was evident, and non-labouring and labouring groups were equally affected. Publications on the influence of glucose depletion on uterine contractile activity under hypoxic conditions in human is still scarce. Nevertheless, Sega et al. (2014) explored contractility response in human saphenous veins ex-vivo and stated that in the presence of oxygen and well-loaded glycogen conditions, the full contractile response to serotonin (5-HT)-induced contractility was 145 ±

3%. Glycogen depletion resulted in a decrease in serotonin maximal contractility down to 37 ± 1%. Insertion of 5.5 mM of exogenous D-glucose restored contractility to 125 ± 6%.

These findings were coherent with the results obtained by Pyla and colleagues while inducing contractility in the human saphenous vein rings with KCl. The exposure of this smooth tissue to glucose-free and hypoxic conditions for 30 minutes led to a considerable reduction of endogenous glycogen from ~1.78 µmol/gr in normoxic conditions to 0.36 in hypoxic conditions followed by a suppression of serotonin-induced contractility by ~68% from control values. Exogenous D-glucose restored the contractile response (Pyla et al., 2015). These results indicate that glucose depletion combined with hypoxic conditions may suppress smooth muscle contractility. The combined effect of glucose deprivation and hypoxia on myometrial contractility in human and mice was the same. The AUC declined to 10 ± 4% (n = 6) in non-pregnant and 19 ± 1% (n = 9) in pregnant mice. In humans the AUC fell to 33 ± 3(n = 7) in non-labouring and 30 ± 5% (n = 6) in labouring tissue. In both mice and human, the inhibitory effect of zero glucose together with hypoxia on uterine contractility was huge. However, mice were more affected than human.

As mentioned earlier on, during pregnancy, the myometrium undergoes significant changes in preparation for labour and delivery. One of these changes includes the storage of glycogen, which serves as an energy source for muscular contractions. Glycogen is a complex carbohydrate that is stored in various tissues as an energy reserve. In the myometrium, glycogen is stored to provide a readily available source of energy for the strong contractions that occur during labour. These contractions require a significant amount of energy, and glycogen serves as a fuel to power these contractions.

Additionally, the myometrial hypoxia hypothesis suggests that during labour, the myometrium experiences a decrease in oxygen availability, which is a normal physiological response. Glycogen storage in the myometrium helps in dealing with this hypoxia by providing an alternative energy source that can be used during periods of limited oxygen availability (Wray et al.,2021)

Intermittent myometrial hypoxia is a normal feature of labour, as the powerful contractions compress blood vessels. Alotaibi found that, as this cycle of contraction and relaxation repeats in labour, the hypoxia also engenders mechanisms that increase force; hypoxia-induced force increase (HIFI). This brief but repetitive hypoxic episodes stimulated the contractile activity, HIFI. In other words, cycles of brief hypoxia initiate and maintain the progressive augmentation of contractility needed for labour. The underlying mechanism involves adenosine and prostaglandin and a rise in intracellular Ca^{2+}. HIFI phenomenon is present in animal and human uterus during parturition. The author speculates that aberrations or deficiency in the HIFI mechanism could result in poor contractions and dysfunctional labour (Alotaibi 2015). In my study, the HIFI mechanism was not replicated but this could be helpful to test the HIFI under zero glucose conditions and compare the results and find out if zero glucose inhibits HIFI.

In conclusion, the myometrium does store more glycogen during pregnancy to cope with myometrial hypoxia during labour. This glycogen serves as an energy source for the strong contractions that occur during labour and helps the myometrium continue contracting even when oxygen availability is decreased. The storage of glycogen in the myometrium is a vital adaptation that ensures effective uterine contractions during labour and facilitates a successful delivery.

Considering the exploration of zero glucose on the response to oxytocin, my studies revealed that OT-induced contractility was significantly affected by zero glucose in the myometrium. In non-labouring tissue, the percentage increase in amplitude, frequency, and integral force under zero glucose conditions was reduced. The increase in amplitude and the area under the curve amounted to 7% and nil for frequency and AUC (n = 7) in my findings, compared to the normal OT-induced contractility values measured by Arrowsmith, where the increase in amplitude and AUC were respectively 76 ± 9% and 205 ± 46% (n = 23) compared to the control (100%) (Arrowsmith et al., 2012). In labouring tissues, the results followed the same trend. My studies did not reveal significant increases in all the parameters (n = 4) under zero glucose OT-induced contractility, while the increase was pronounced under normal OT conditions, where the increase in amplitude and AUC amounted to 57 ± 12% and 156 ± 26% (n = 35). This would indicate that, instead of compensating for the inhibitory effect caused by glucose depletion, the OT response was not strong enough to increase uterine contractions in the presence of zero glucose.

Bugg and colleagues went on to demonstrate that even the repeated administration of an agonist (OT) during hypoxia could not improve uterine contractility but contributed more to the decrease in contractile amplitude and integral force. The authors attributed this decrease to a desensitisation of myocytes induced by OT under hypoxic conditions following OT repetitive stimulations (Buggs et al.,2006; Robinson et al., 2003).
As mentioned earlier on, oxytocin is a hormone that is naturally produced by the body and plays a crucial role in the process of childbirth. It helps to stimulate contractions in the uterus, which can help to speed up labour and delivery. However, sometimes oxytocin infusion can fail to produce the desired effect. There are several reasons why this might happen.

One of the most common reasons for oxytocin infusion failure is that the woman's body is not ready for labour. This can happen if the cervix is not yet fully dilated or if the baby is not yet in the correct position for delivery. In these cases, it may be necessary to wait for a while before trying again. Another reason why oxytocin infusion might fail is that the dosage is incorrect. If the dosage is too low, it may not be effective in stimulating contractions. Conversely, if the dosage is too high, it can cause hyperstimulation of the uterus, which can put both the mother and foetus at risk (Hidalgo-Lopezosa et al.,2015)
Other factors that can contribute to oxytocin infusion failure include maternal obesity, gestational diabetes, and preeclampsia. Furthermore, contractility is impaired in diabetic women. It is worth noting that these women have a reduced oxytocin response. If the diabetic environment has already reduced contractility, for example through glycosylation of proteins and fibre damage, resulting in a slow progression of labour in these women, then oxytocin is likely to be required. Thus, decreased responsiveness to oxytocin would imply decreased efficacy, which may contribute to the increased rate of C-sections in this group of women (Al-Qahtani et al., 2012). Additionally, there is evidence that insulin *per se* is detrimental to contractility, possibly by causing hyperpolarisation (Tack, C.J.J et al., 1996; Ferrannini et al., 1999).

These conditions can interfere with the body's ability to produce and respond to oxytocin. In some cases, oxytocin infusion failure may be due to problems with the equipment used to administer the drug. For example, if the IV line becomes blocked or dislodged, it can prevent the drug from being delivered properly. (Gu P et al.,2021; Hautakangas et al.,2022)

Finally, it's worth noting that every woman's body is different, and what works for one woman may not work for another. In some cases, oxytocin infusion simply may not be effective for a particular woman (Dali et al.,2020)

In dysfunctional labour, oxytocin has been used clinically to improve uterine contraction force, but it has been reported that a significant fraction of women does not respond to exogenous OT during labour (Arrowsmith et al.,2010; Bugg et al.,2013; Lukasse et al.,2020) We believe that glucose depletion could probably contribute to OT failure in the management of labour dysfunction.

The depletion of glucose on the response to high K^+ was assessed in both groups (non-labouring and labouring). As mentioned earlier, high K^+ depolarises the cell membrane and causes the voltage-dependant L-type Ca^{2+}channel to open and allow the entry of a large amount of calcium into the cell. After treatment the contractile amplitude went back to normal spontaneous contractions. From our experiment, all the traces looked similar between the myometrial exposure to high K^+ and high K^+ in zero glucose and in both groups (non-labouring and labouring). In non-labouring tissues, the peak and plateau contractile amplitudes from high K^+ under glucose conditions were higher than the peak and plateau amplitudes from high K^+ under zero glucose conditions. The unpaired *t*-test found significant difference between them, but no difference in labouring myometrium. Additionally, no significant difference was found in the two parameters between non-labouring and labouring groups from high K^+ under zero glucose conditions. This experiment indicates that although no change in contractility was noticed between high K^+ and high K^+ zero glucose in the labouring group, the evidence of an inhibition effect in contractility was proved in the non-labouring group. The metabolic changes in labouring may possibly explain different results between these two groups. No such protocols have been previously done, either in animals or humans, that could help to compare with my findings. Potassium experiment has no clinical significance. It is a lab experiment to test the integrity of a muscle. In our study, I compared myometrial contractile activity between high K^+ and high K^+ zero glucose expecting to see inhibition in high K^+ zero glucose.

In conclusion, glucose deficiency significantly inhibits uterine contractions regardless of how they are produced. The high K^+ response was not diminished. This is most likely due to a steady state of calcium levels in the cell after calcium influx caused by cell membrane depolarisation. The decline in contractility was most pronounced with the combined action

of zero glucose and hypoxia, with contractions frequently stopping altogether. The findings showed that labouring myometrium is better able to withstand the effects of zero glucose than non-labouring myometrium. This is difficult to conclude due to a small sample size from labouring group since both groups are well prepared for labour. Besides, it is understood that labouring myometrium may have using up more of glycogen than non-labouring. Furthermore, glycogen storage is expected to decline during parturition process as mentioned in chapter 3. In this case, the level of glycogen storage from the two group was not determined before labour and the duration of labour might be a crucial factor in evaluating the depletion of glycogen. Nevertheless, depletion of glucose *in vivo* is likely *to* be a contributing factor to the pathway underlying contractility-related disorders. Exploring the role of glycogen during labour would be helpful.

4.2.5 Limitations in this study

Difficulty in obtaining human tissue in general and labouring in particular was a major limitation. Besides, consenting women in the middle of a difficult labour about to go into surgery was a challenge as well. Mouse tissue is used immediately, and human tissue stored overnight until use. it is because unlike with mouse tissue, when you can decide what time to collect the tissue, with human, the tissue arrive anytime during day and night and experiments take a long time (6-7 hours) compared to experiments with mouse myometrium (2hours). Likewise human labouring myometrial tissues, obtaining myometrial mouse labouring tissues was challenging as well. Sometimes human samples are stored in fridge until the morning while glycogen level in the tissue is deteriorating. Using human myometrial tissues, immediately after C/Section could be helpful. Although there were physiological similarities between mouse and human myometrial tissues, guinea pig myometrium was found to be more similar to human compared to mouse and could be used if possible.

CHAPTER FIVE

The effect of flavopiridol hydrochloride hydrate on uterine contractility

Chapter 5.

The effect of flavopiridol hydrochloride hydrate on uterine contractility

5.1 Introduction

As described in chapter 2 section 2.2.6.2**,** glycogen phosphorylase (GP) is the vital enzyme regulating glycogen metabolism. This enzyme is responsible for the breakdown of glycogen into glucose-1-phosphate (G1P), which is used in muscles to produce energy through the glycolysis pathway; in the liver G1P is usually transformed into G6P and then G6P into glucose as output for the use of different tissues (Newgard et al., 1989). The liver is not the main source of glucose for the uterus directly. During pregnancy, glucose is supply to the uterus and other tissues through the blood stream. The liver however plays a crucial role in maintaining blood glucose levels by storing glycogen and releasing glucose when needed. It acts as a reservoir for glucose, ensuring a steady supply to all organs and tissues,including the uterus during pregnancy.

There are two known types of interconvertible glycogen phosphorylase: the Ser^{14} phosphorylated GPa, known for its high activity along with high substrate affinity; and the dephosphorylated form, GPb, which possesses a minimal substrate affinity coupled with slight activity (Barford et al., 1991).

Glycogen phosphorylase contains approximately six regulatory sites. These include 1) Ser^{14}-phosphate recognition;2) the catalytic ;3) the site binding glycogen granules;4) G1P ;5) glucose; 6) glucose equivalents. In addition, GP contains an allosteric site which binds 1) G6P; 2) adenosine monophosphate (AMP);3) inosine monophosphate (IMP);4) adenosine triphosphate (ATP);5) the glycogen storage site;6)the inhibitor site that binds both caffeine along with correlated complexes;7) a novel allosteric inhibitor site that binds the potential antidiabetic agent (Oikonomakos et al., 2000).

Flavopiridol is a flavonoid that proved to have a strong and competitive inhibitory effect on Cyclin-dependent kinases (CDKs). It has an IC_{50} value ranging from 0.2 to 0.4 µM (Losiewicz et al., 1994). This product has been shown to possess cytotoxic and antiproliferative effects both *in vitro and in vivo* in some tumour lines and has reached stage II clinical trials for untreated metastatic malignant melanoma and chronic lymphocytic leukaemia (Czech et al., 1995; Senderowicz et al., 1998; Burdette et al., 2004; Byrd et al., 2007). Cyclin dependent kinases actions and detection of regulatory proteins are controlled by autophosphorylation. Crystallographic experiments have demonstrated that flavopiridol is a large-specificity CDK inhibitor and binds to the adenosine triphosphate site of CDK, causing unexpected changes of structure that submerge the inhibitor (Baumli et al., 2007; Kim et al., 1996; De Azevedo et al., 1996). This process leads to the development of new CDK modulators that can be used as agents for the management of malignancy (Hoessel et al., 1999; Sausville et al., 1999).
Experiments revealed that flavopiridol inhibited glycogen phosphorylase b (GPb) activity in the rabbit muscle with an IC_{50} at 1µM and glycogen phosphorylase a (GPa) with IC_{50} equal to 2.5µM (Kaiser et al., 2001). The flavopiridol inhibitory effect was poor with AMP activated GPa. In addition, flavopiridol treatment of lung carcinoma cells (A549 non-small cell) led to a considerable accumulation of glycogen. These results raised the possibility that the antitumor properties of this agent can also interfere with glucose (Oikonomakos et al.2000).
The stereochemical justification for the flavopiridol inhibition effect on glycogen phosphorylase was provided by Oikonomakos et al. through a crystallographic method after co-crystallising glycogen phosphorylase-b with flavopiridol and glycogen phosphorylase-a together with flavopiridol and glucose to determine the composition of these compounds. The findings revealed that flavopiridol bound at the inhibitor site. This is the zone caffeine binds to as well (Oikonomakos et al., 2000).

5.2 Objective

This chapter describes how the inhibition action of flavopiridol on glycogen phosphorylase was exploited to explore in vitro its impact on contractility from non-pregnant and pregnant uterine mice myometrium and labouring and non-labouring term pregnant human myometrium. This set of experiments was performed in the attempt to confirm the inhibition effect of zero glucose on uterine contractility as found in chapters 4 and 5. Blocking the action of glycogen phosphorylase with flavopiridol results in the prevention of breaking down of

glycogen into glucose (Glycogenolysis) and lead to glucose depletion in the myometrium. It was assumed that this process would eventually result in the inhibition of uterine contractility. As in chapters 4 and 5, the difference in uterine contractile effect between non-pregnant and pregnant mice was sought because of metabolic changes associated with pregnancy. The difference in contractility between non-labouring and labour was explored due to the metabolic changes linked with labour.

5.3 Methods

5.3.1 The effect of the DMSO solution on uterine contractility in pregnant and non-pregnant mice

Kinetic information in the presence of different concentrations of flavopiridol in zero glucose has an IC_{50} equal to 15.5 ± 0.3 μM (Oikonomakos et al., 2000). 12 μM was chosen in our laboratory to produce a maximal inhibition effect without damaging tissues. The flavopiridol hydrochloride hydrate stock solution was prepared in DMSO as described in Chapter 2 and subsequently diluted to a working concentration of 12μM in PSS. This 12μM flavopiridol hydrochloride solution contained 0.1% DMSO. Initial control experiments were conducted to evaluate whether a 0.1% DMSO solution alone had any effect on uterine contractility in spontaneously contracting pregnant and non-pregnant myometrial strips.

0.1% DMSO has been shown to be safe for the majority of cells, and 0.5% DMSO has been commonly used as a final concentration for cell culture without any toxicity. In some experiments, 5% DMSO was successfully used for some cells (DMSO usage in cell culture, Lifetein, 2021). No changes in contractions were observed on the myometrium from a rat when 0.17% of DMSO was added in an organ bath (Einer-Jensen,1971)

5.3.2 Exploring the effect of flavopiridol hydrochloride hydrate on uterine contractility in non-pregnant and pregnant mice.

Non-pregnant and pregnant uterine strips were prepared and mounted in an organ bath under zero glucose solution. Once stable contractions were achieved, myometrial tissues were treated with 12μM flavopiridol hydrochloride hydrate for 20 minutes. The analysis was conducted as previously described in Chapter 2, with the last 5 minutes of the treatment period analysed and compared to a 5-minute internal control period.

5.3.3 Exploring the effect of flavopiridol hydrochloride hydrate on uterine contractility in labouring and non-labouring myometria.

Labouring and non-labouring uterine strips were prepared and mounted in an organ bath as described in Chapter 2. Once stable contractions were achieved, myometrial tissues were treated with 12µM flavopiridol hydrochloride hydrate for 40 minutes.

Analysis was conducted as previously described in Chapter 2, with the last 20 minutes of the treatment period analysed and compared to a 20-minute control period. After stable and regular spontaneous contractions were established, a specific period of time immediately before starting an experiment was selected as the control period and assigned the value of 100%. Flavopiridol response was expressed comparatively to this control period. When the same treatment was employed to more than one uterine strip from animal or human, the results were averaged to produce a single value. Data capture and analysis were processed by LabScribe / DataTrax software. The contractile parameters measured included the amplitude, the frequency, and the area under the curve (AUC)**.** For statistical analysis, the flavopiridol effect was presented as a percentage in comparison to its internal control parameters. Microsoft Excel 365 and Origin Pro 2018 software were used for the analysis of data. Data was expressed as mean ± SEM, where N symbolised the number of mice or human in each group. One-sample *t*-tests were carried out to establish the significance between the internal control period and the treatment. The unpaired tests were conducted to compare one treatment to another using Graph Pad Prism 8 software. A *p*- value of < 0.05 was regarded as significant (*, **, and *** denoted $p < 0.05$, $p < 0.01$, and $p < 0.001$).

5.4 Results

5.4.1 Exploring the effect of flavopiridol hydrochloride hydrate on uterine contractility in non-pregnant and pregnant mice.

A 0.1% DMSO solution had no effect on spontaneous uterine contractions in non-pregnant or pregnant mice (Figures 5.1 and 5.3).

In non-pregnant mice, 12µM flavopiridol produced a clear decrease in contractility. Contractile amplitude, frequency and AUC decreased respectively down to 55 ± 4% (p = 0.0016, n = 6), 89 ± 8% (p = 0.0034, n = 6) and 48 ± 1% ($p < 0.0001$, n = 6). (Figure 5.2). Uterine contractility fully recovered gradually when the drug was removed.

In term pregnant mice, contractile parameters followed the same trend upon treatment with flavopiridol. 12µM flavopiridol produced a clear decrease in contractility. Contractile amplitude and AUC significantly declined down to 44 ± 2% of control ($p < 0.0001$, n = 8) and 49 ± 3% of control ($p < 0.0.0001$, n = 8), respectively. The decrease in frequency (94 ± 2%), but was not significant ($p = 0.3533$, n = 8) (Figure 5.4).

These results demonstrate that inhibition of glycogen phosphorylase with flavopiridol has an equivalent inhibitory effect in both groups. However, no significant difference was found between them in frequency and AUC, but a significant difference was observed in amplitude (unpaired *t*-test, non-pregnant vs pregnant; amplitude $p = 0.0434$, frequency $p = 0.5558$, AUC $p = 0.7943$) (Figure 5.5). Again, progressively there was a full recovery of contractility immediately after the removal of flavopiridol.

Non-Pregnant

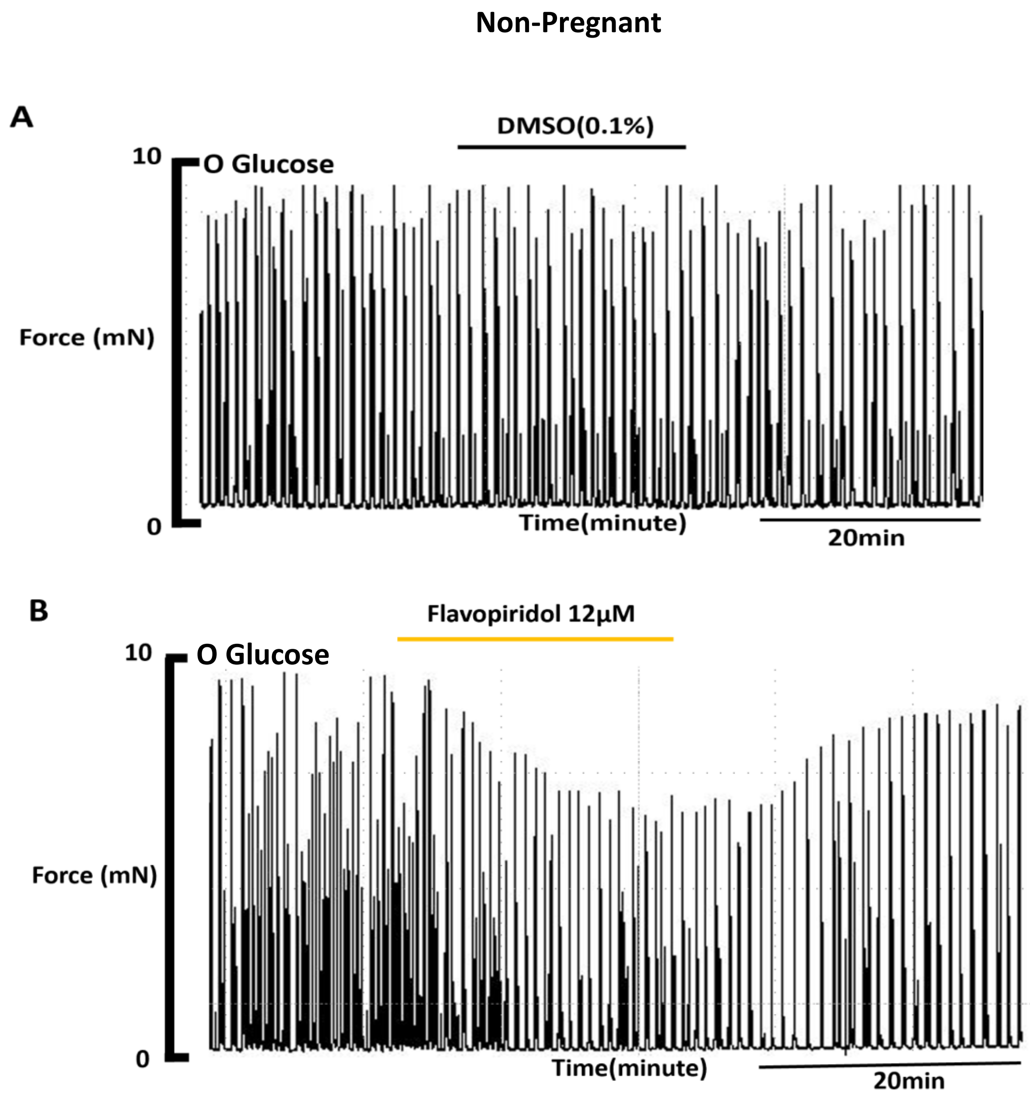


Figure 5.1 The effect of DMSO and flavopiridol on uterine contractility in non-pregnant tissue. DMSO had no impact on uterine contractions(A). Flavopiridol had an inhibitory effect(B) (Representative traces)

5.4.1.1 Flavopiridol influence on uterine contractility in non-pregnant mouse.

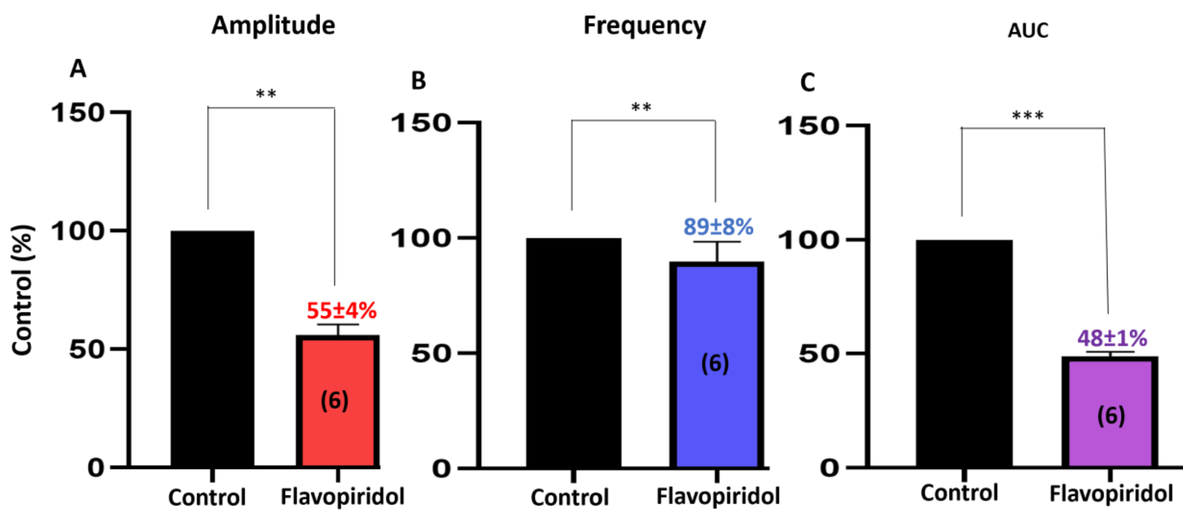
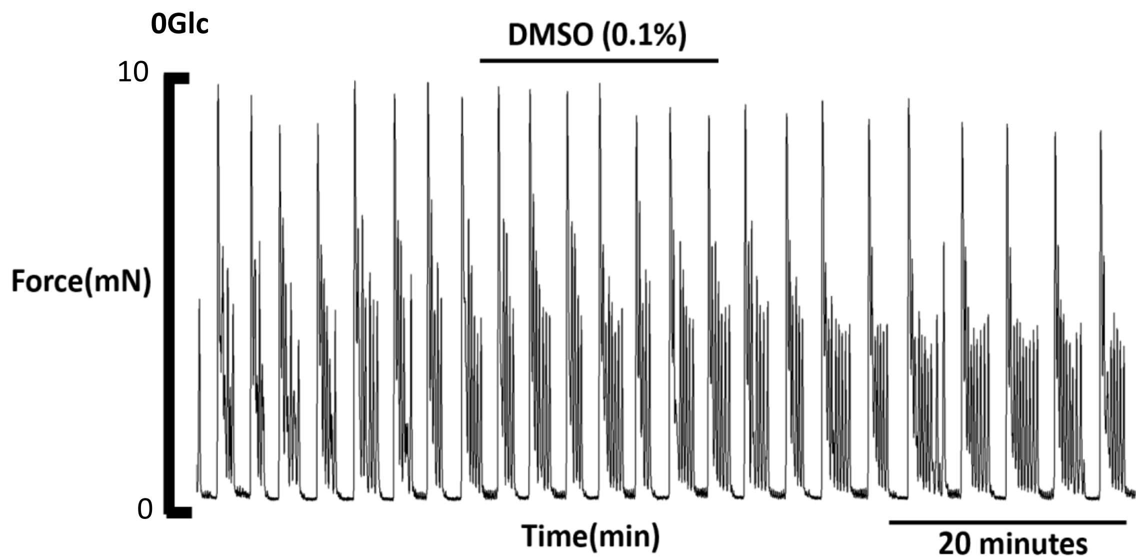


Figure 5.2 The effects of flavopiridol on contractile parameters on uterine contractility in non-pregnant tissue. Under flavopiridol all the contractile parameters were significantly affected in non-pregnant mice(n=6). The force integral was reduced by half of control under flavopiridol in non-pregnant mice.

18-DAY PREGNANT

A



B

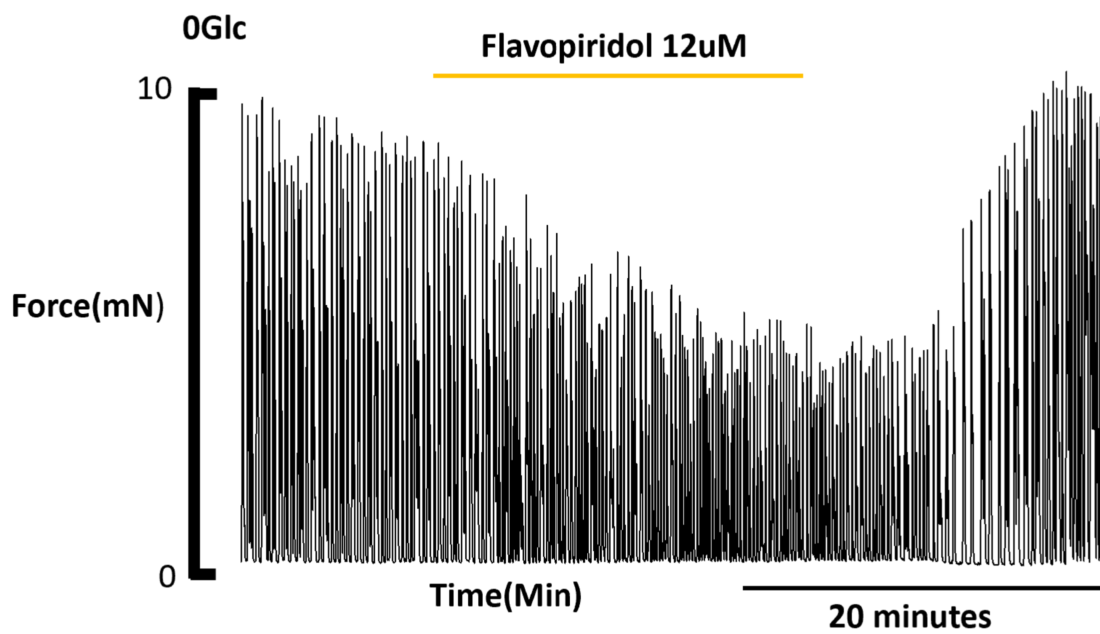


Figure 5.3 The effect of DMSO on myometrial contractility in pregnant mice. 0.1% DMSO had no effect on myometrial contractility(A). Flavopiridol displayed an inhibition impact on uterine contractility in pregnant tissues (B).

5.4.1.2 Flavopiridol influence on uterine contractility in pregnant mouse.

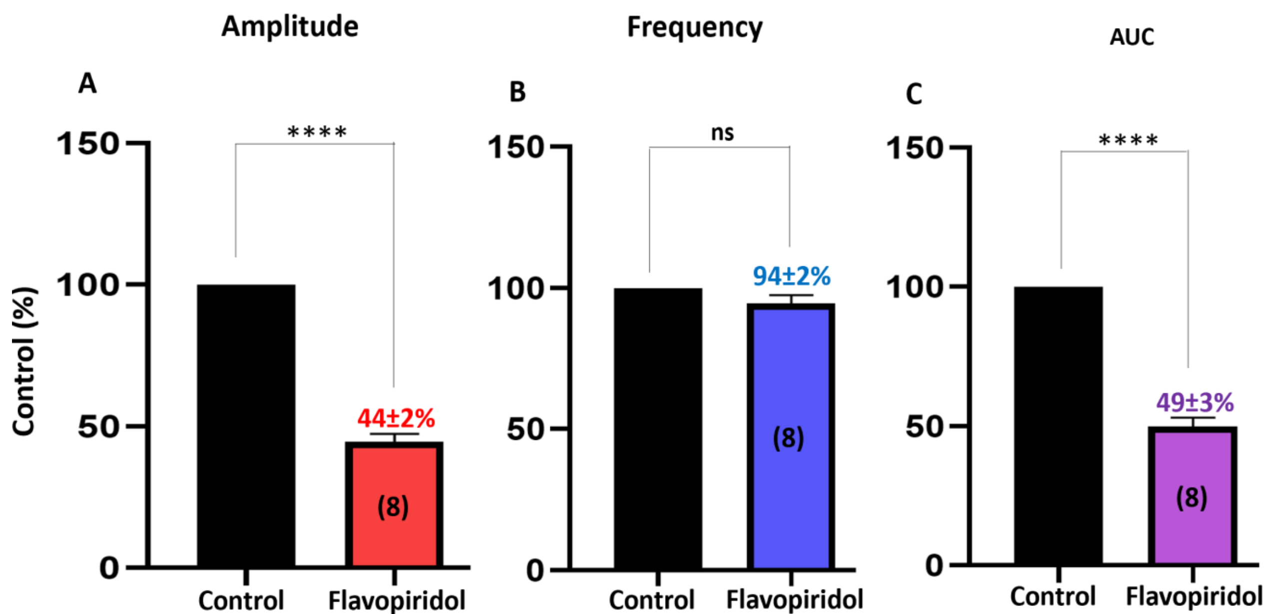


Figure 5.4 The effects of flavopiridol on contractile parameters on uterine contractility in pregnant mice. Flavopiridol had a considerable inhibitory effect on myometrial contractile amplitude in 18-day pregnant mice(n=8) (A). The effect of flavopiridol was minimal on myometrial contractile frequency on 18-day pregnant mice. No significant difference was found compared to the control group (B). The area under the curve was more affected in the flavopiridol group compared to the control group of 18-day pregnant mice (C).

5.4.1.3 Flavopiridol response difference between non-pregnant and pregnant uteri

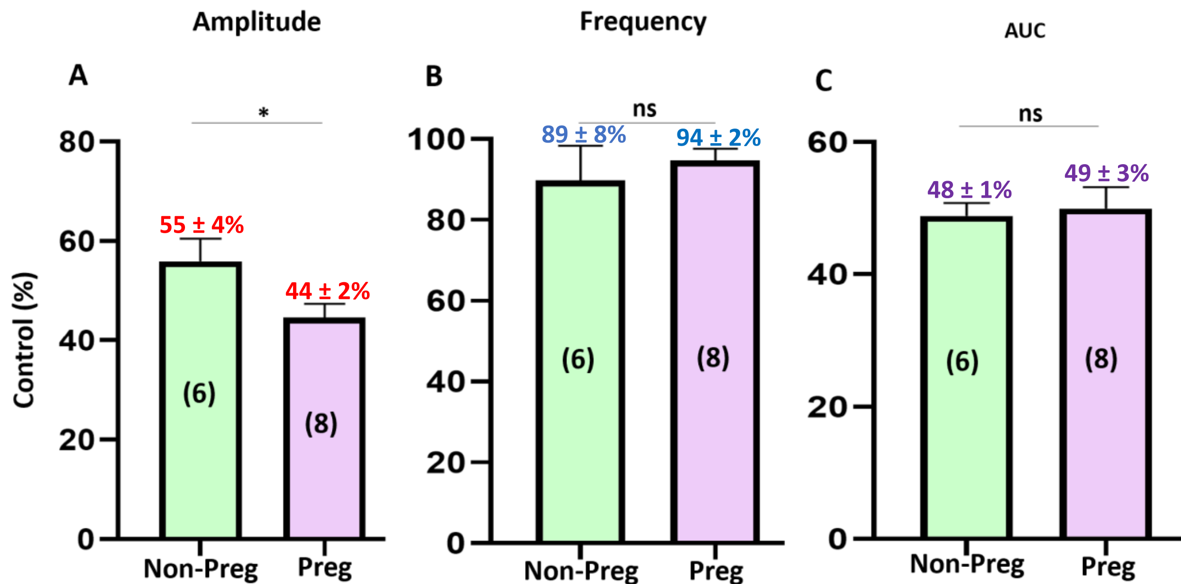


Figure 5.5 Evaluation of flavopiridol impact between non-pregnant and pregnant uteri. No significant difference was found between the responses of the two groups to flavopiridol for frequency and AUC (A&C). There was a significant difference for amplitude (A). Non-pregnant: (n=6). Pregnant(n=8)

5.4.2. Exploring the effect of flavopiridol hydrochloride hydrate on uterine contractility in humans.

A 0.1% DMSO solution had no effect on spontaneous uterine contractions in non-labouring myometrium (Figure 5.6A) and labouring myometrium (Figure 5.8A).

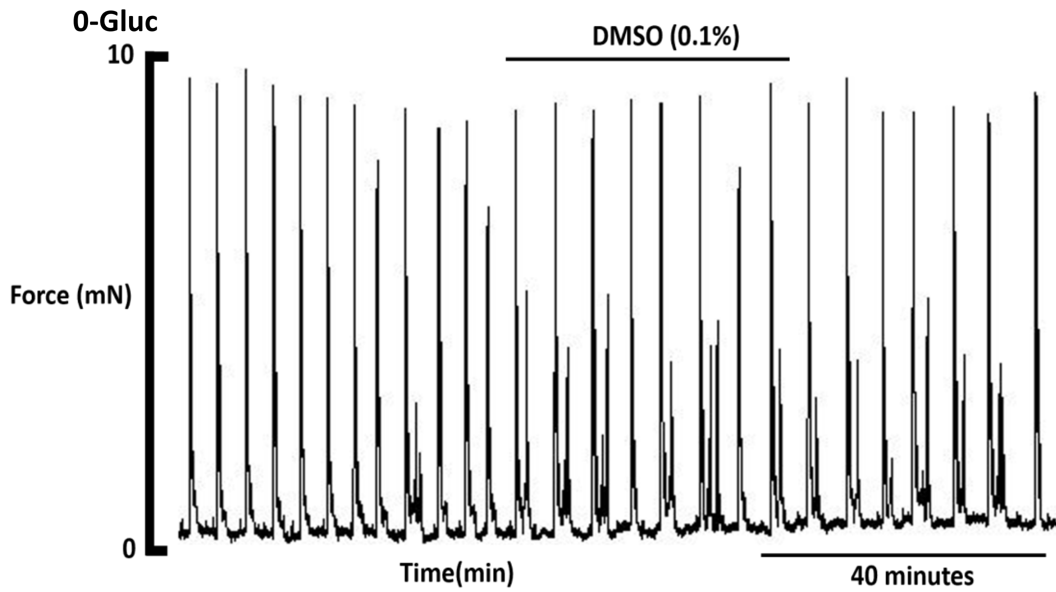
12µM flavopiridol had an inhibitory contractile effect in both non-labouring (Figure 6.6 B) and labouring myometrial tissues (Figure 5.8B).

In non-labouring term pregnant human myometrium, 12µM flavopiridol produced a clear decrease in contractility. Contractile amplitude, frequency and AUC significantly declined down to 64 ± 4% of control (p < 0.0001, n = 8), 88 ± 13% (p = 0.0014, n = 8) and 61 ± 5% (p < 0.0001, n = 8), respectively (Figure 5.7).

Similarly, for labouring human myometrium, contractile parameters followed the same downward trend in response to flavopiridol. Contractile amplitude, frequency and AUC decreased down to 39 ± 10% (p = 0.0175*, n = 5) 64 ± 4% (p < 0.0001, n = 5) and 31 ± 6% (p = 0.0067**, n = 5) correspondingly, compared to the control (Figure 5.9).

No significant difference was found between non- labouring and labouring myometrial tissues under flavopiridol on frequency (unpaired *t*-test p = 0.1857) (Figure 5.10B) while there was a significant difference on amplitude (unpaired *t*-test, p = 0.0206 *) and the AUC (p = 0.0030**) (Figure 5.10)

NON-LABOURING



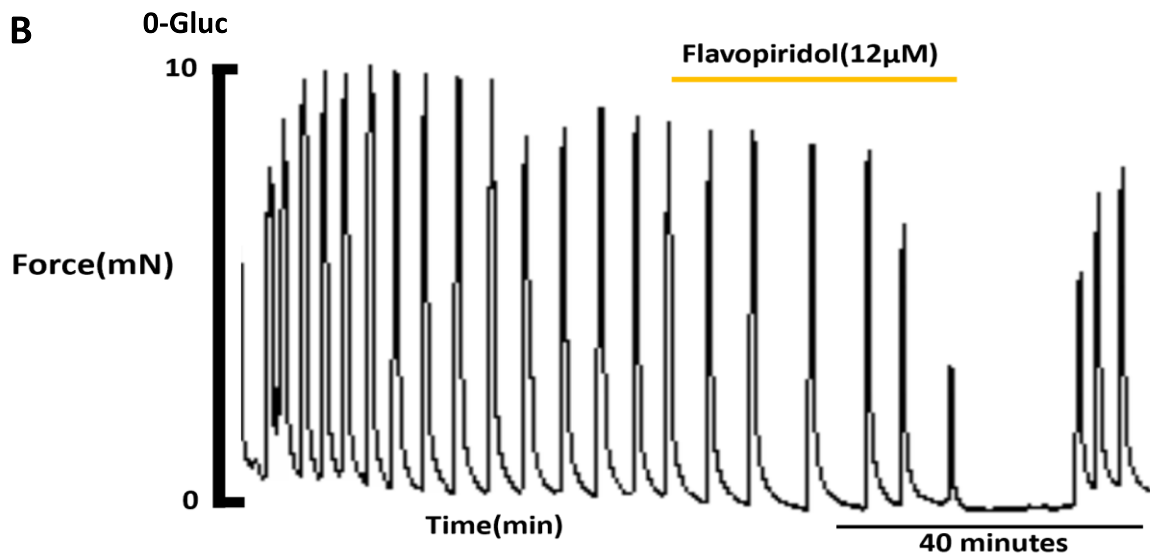


Figure 5.6 Flavopiridol effect on uterine contractility in a non-labouring uterus. 0.1% DMSO had no effect on contractility (A). Representative trace showing the inhibition impact of flavopiridol on contractility in a non-labouring uterus (B)

5.4.2.1 Flavopiridol influence on uterine contractility in non-labouring tissue.

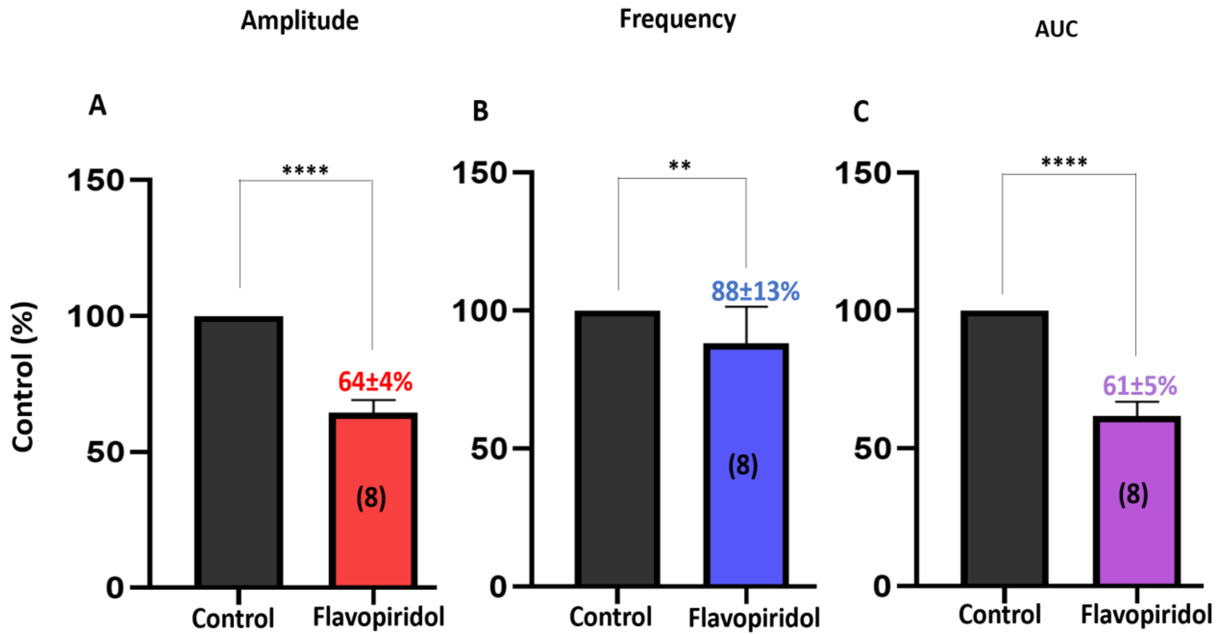


Figure 5.7 The effect of flavopiridol on uterine contractility in non-labouring myometrium. There was a remarkable difference found between in all the parameters and their control in non-labouring tissues under flavopiridol (n=8)

LABOURING

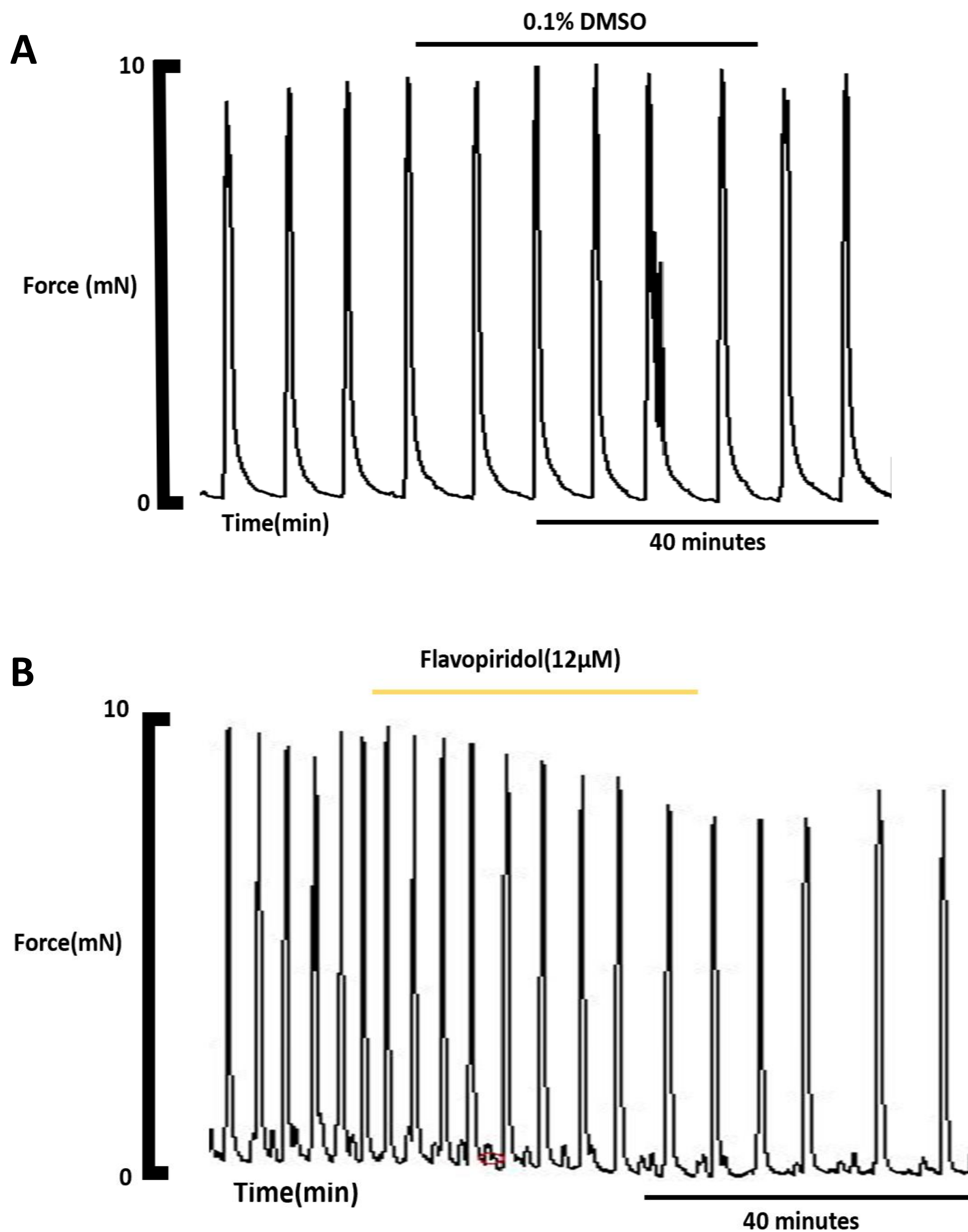


Figure 5.8 The effect of flavopiridol on uterine contractility in labouring myometrium. 0.1% DMSO had no effect on uterine contractility in labouring tissue(A). Inhibition effect of flavopiridol on uterine contractility in labouring uterus(B).

5.4.2.2 Flavopiridol influence on uterine contractility in labouring tissue.

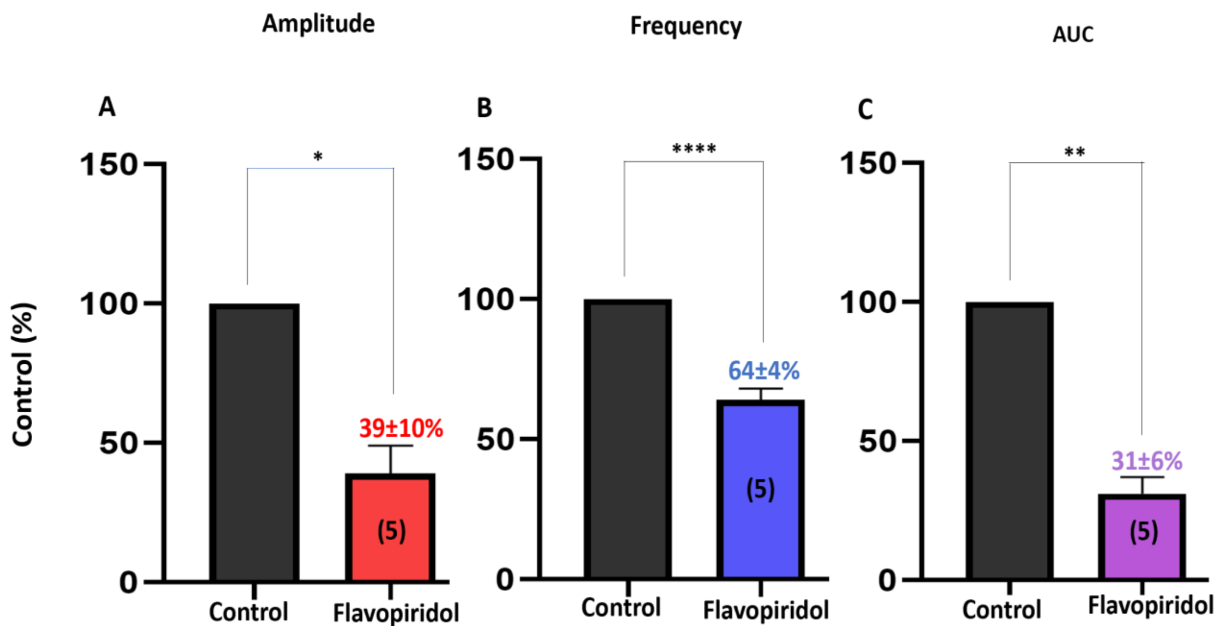


Figure 5.9 Effect of flavopiridol on uterine contractility in labouring myometrium There was significant difference between amplitude (A), frequency(B) and AUC (C) and their control in labouring tissues under flavopiridol (n=5)

5.4.2.3 Comparison of the effects of flavopiridol on non-labouring and labouring myometria

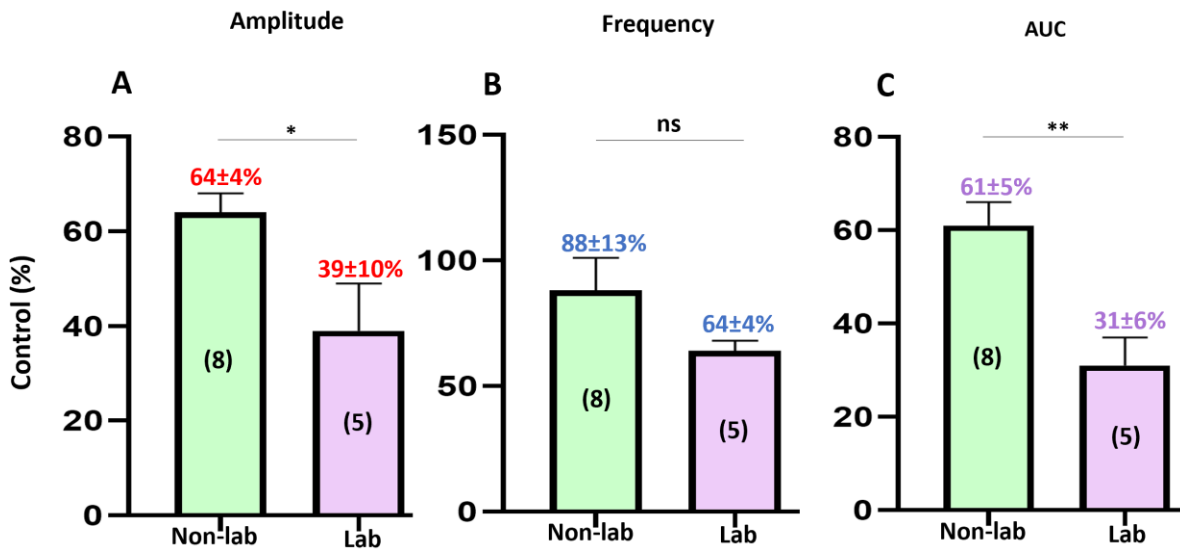


Figure 5.10 The comparison of the effect of flavopiridol between non-labouring and labouring myometria. No significant difference was found between non-labouring and labouring myometrial tissues under flavopiridol in frequency (B) while there a difference was found in amplitude and AUC. Labouring tissues(n=5) appeared to be more affected than non-labouring tissues(n=8).

5.4.2.4 Comparison of the effects between Zero glucose and Flavopiridol

MOUSE

NON-PREGNANT

Contractile parameters	Zero glucose	Flavopiridol
Amplitude	83 ± 3%	55 ± 4%
Frequency	82 ± 1%	89 ± 8%
AUC	59 ± 3%	48 ± 1%

Table 5.1 Comparison of the effect of zero glucose and Flavopiridol in non-pregnant mice. Similarities have been noticed between the inhibitive effect of zero glucose and flavopiridol on uterine contractility.

PREGNANT

Contractile parameters	Zero glucose	Flavopiridol
Amplitude	60 ± 4%	44 ± 2%
Frequency	91± 7%	94 ± 2%
AUC	54± 1%	49 ± 3%

Table 5.2 Comparison of the effect of zero glucose and Flavopiridol in pregnant mice. Similarities have been observed between the inhibitive effect of zero glucose and flavopiridol on uterine contractility.

HUMAN

NON-LABOURING

Contractile parameters	Zero glucose	Flavopiridol
Amplitude	82 ± 6%	64 ± 4%
Frequency	72± 1%	88 ± 1%
AUC	58 ± 7%	61 ± 5%

Table 5.3 Comparison of the effect of zero glucose and Flavopiridol in non-labouring. Similarities of the inhibitive effect of zero glucose and flavopiridol on uterine contractility were obvious in non-labouring myometrium.

LABOURING

Contractile parameters	Zero glucose	Flavopiridol
Amplitude	87 ± 2%	39 ± 1%
Frequency	102 ± 5%	64 ± 4%
AUC	79 ± 3%	31 ± 6%

Table 5.4 Comparison of the effect of zero glucose and Flavopiridol in labouring.
Resemblances of the inhibitive effect of zero glucose and flavopiridol on uterine contractility were evident in labouring myometrium.

5.4.3 Discussion

The inhibition action of flavopiridol on glycogen phosphorylase was used in this chapter to investigate in vitro its impact on uterine contractility in non-pregnant and pregnant mice, along with labouring and non-labouring myometrial tissues. The purpose of this set of experiments was to confirm the inhibition effect of zero glucose on uterine contractility described in chapters 4 and 5.

Experiments to find out if the flavopiridol inhibited glycogenolysis in myometrium have never been done before but previous studies demonstrated that flavopiridol inhibited glycogen phosphorylase activity in the rabbit muscle as mentioned in the introduction of this chapter (Kaiser et al., 2001). Furthermore, the management of lung carcinoma cells with flavopiridol resulted in a significant accumulation of glycogen. These findings raised the likelihood that the antitumor properties of flavopiridol could interfere with glucose as well (Oikonomakos et al.2000).

Stopping the action of glycogen phosphorylase with flavopiridol prevents glycogen from being broken down into glucose (glycogenolysis) and could result in glucose depletion in the myometrium as well. It was assumed that this process would eventually result in uterine contractility inhibition as demonstrated in chapter 4 with zero glucose.

As discussed in chapter 3 of this thesis, glycogen was found to be the principal source of energy during labour, an alternative source of energy in the myometrium could be fatty acid. Another way of exploring the inhibition of the myometrial contractile effect of zero glucose could be achieved by simultaneously neutralising the possible effect of fatty acid on uterine contractility.

Depleting a muscle tissue of fatty acids in vitro can be achieved by several methods. One common approach is to incubate the muscle tissue with a lipid oxidation inhibitor, such as etomoxir. Etomoxir specifically inhibits the enzyme carnitine palmitoyltransferase, which is involved in the transport of fatty acids into the mitochondria for oxidation. By inhibiting this enzyme, etomoxir prevents the utilization of fatty acids as a fuel source and depletes the muscle tissue of fatty acids. (Romer et al.,2021)

Additionally, the addition of a fatty acid binding protein inhibitor, such as BMS309403, can also prevent the uptake and utilisation of fatty acids by the muscle tissue. These methods can

be used alone or in combination to achieve effective depletion of muscle tissue fatty acid levels in vitro (Xu et al.,2022)

The comparison of the results between the above two groups of mice was pursued, as in chapters 4 and 5, due to the metabolic changes occurring in the myometrium during pregnancy. For humans, the metabolic changes arising during labour were the reasons for comparing the response between non-labouring and labouring tissues.

For the research on animals, non-pregnant (6) and pregnant (8) mice were used. For the research on humans, myometrial tissues from non-labouring (8) and labouring (5) women were used.

The effect of flavopiridol on amplitude is clear in all four models I have tested – pregnant and non-pregnant mouse and labouring and non-labouring human. It is the effect on amplitude that is having the largest contribution to AUC, especially in mice.

In mice, my study found that the exposure of myometrial strips to flavopiridol chloride hydrate resulted in inhibition in contractility. The contractile amplitude, frequency and the integral force significantly declined compared to control in both non-pregnant and pregnant mice and both groups of mice were equally affected. Some resistance was noticed on the frequency while in pregnant, the frequency was not affected. The effects on frequency are minor and biologically insignificant. The errors on frequency measurements in the mice are relatively large. This is because frequency is hard to measure because of lots of bursts of contractions and many not reaching full strength. Frequency, especially in pregnant mice is usually very difficult to accurately measure – unlike in human.

In mice there is no difference between non-pregnant and pregnant. There is a slightly greater fall in amplitude, but its significance only just reaches the 0.05 threshold.

This difference of response between non-pregnant and pregnant could be explained by physiological changes (Chapter 1, section 1.2.4.9) Some key differences include 1. Size and shape: In a non-pregnant mouse, the uterus is relatively small and has a tubular shape. However, in a pregnant mouse, the uterus undergoes remarkable enlargement and takes on a more globular shape to accommodate the developing embryos.2. Hormonal Activities:

During pregnancy, the levels of hormones such as estrogen and progesterone increase significantly. These hormones play a vital role in preparing the uterus for implantation of the fertilised eggs, maintaining pregnancy, and regulating various physiological changes in the reproductive system.3. Tissue structure: In a non-pregnant mouse, the uterine tissues consist

of thin structures. However, during pregnancy, the endometrium and myometrium undergo changes to form specialised structures which provide the necessary support and nourishment to the developing embryos.4. Blood Supply: During pregnancy, the uterus experiences increased blood flow to support the growing embryos. The blood vessels in the uterus expand and develop new branches, ensuring an adequate supply of oxygen and nutrients to the developing embryos.5. Ion Pathway and Glycogen Storage:

The non-pregnant uterus typically has low ion permeability and low glycogen storage. In contrast, the pregnant uterus undergoes changes in ion channel expression and increases glycogen storage. These changes are essential for uterine muscle contractions and provide a source of energy for the developing embryos.6. Fatty Acid Metabolism: During pregnancy, the uterine tissue increases its fat storage capacity to provide the necessary energy for embryo development. This includes the accumulation of fatty acids, which can be utilised as a fuel source during times of increased energy demand during labour.

Overall, these differences in the uterus of a non-pregnant mouse and a pregnant mouse reflect the adaptations necessary to support embryonic development, hormonal regulation, and metabolic changes associated with pregnancy.

For humans, all the parameters measured were affected as well. The contractile amplitude, frequency and area under the curve significantly declined. The frequency was equally affected in both groups although some resistance was noticed as well. The labouring group appeared to be more affected than non-labouring. The effect is more reduced in the labouring than non-labouring myometrium.

In human, amplitude significantly decreased in both, but more so in labouring. Frequency in non-labouring has a large error bar which can be attributed to natural variation between samples/women and although 8 is a respectable sample size but it is still not big in statistical terms. The frequency goes down in both human groups. The fact that it does not appear to be different between the groups. I may have probably got a difference with increased n numbers.

In human the important point for discussion is that the drug has more action in the labouring group on amplitude. This may well be because glycogen reserves and other biochemical fuels, such as free fatty acids, have already started to deplete as the women have been labouring.

Depending on the drug kd (drug affinity) in myometrium, it may be that when there is lots of glycogen in non-labouring the drug does not stop all phosphorylase activity, and contractions are better able to carry on albeit with decreased amplitude. In the labouring group, the drug prevents phosphorylase to the same extent, but there are less reserves around to support contractions.

Some of the reasons that may contribute to the difference in response to flavopiridol between non-labouring and labouring include1. Hormonal changes: During pregnancy, the levels of various hormones, such as oestrogen and progesterone, dramatically fluctuate to support fetal development and maintain pregnancy. However, as labour approaches, hormonal changes occur, leading to contractions and other physiological changes in the uterus. These changes can significantly impact the characteristics of the uterine tissue and its functioning, making it distinct from a pregnant uterus.2. Structural changes: As labour progresses, the cervix undergoes gradual dilation and effacement, allowing the baby to pass through the birth canal. This process involves changes in the uterine shape, size, and muscle tone. These structural alterations directly affect the biomechanics of the uterus and its contractile properties, which can influence the results of research studies conducted during labour.3. Inflammatory response: Labour is considered an inflammatory process as the body prepares for the birthing process. Inflammation is a complex biological response involving immune cells and mediators. It can lead to the release of various substances that may affect the uterine environment, such as prostaglandins and cytokines. Understanding the inflammatory response during labour is essential for managing complications and ensuring optimal reproductive health outcomes.4. Variations in uterine activity: Uterine contractions during labour have a distinct pattern and intensity compared to the regular, more rhythmic contractions seen during pregnancy. These differences are crucial for facilitating the progression of labour. As a result of these factors, the biological environment, and characteristics of a uterus in labour significantly differ from that of a pregnant uterus. This may imply that, besides known physiological difference between the two groups, it is logical to believe that labouring myometrium is in a position to use more glycogen than non-labour. The inhibition of contractile activity of zero glucose and flavopiridol shows similar responses however, either in mouse or human, more exploration is still needed to find out why contractile frequency oppose some resistance to zero glucose and flavopiridol. It is assumed

that these results show that by preventing glycogen breakdown, flavopiridol creates glucose depletion which is responsible for a myometrial inhibition response. These findings demonstrate the dependence of myometrial contraction on glucose. Glucose mainly comes from the breakdown of glycogen.

Flavopiridol has same effects in both – it significantly decreases amplitude in all 4 cases (Human and animal). Irrespective of species or pregnancy state or labour state, inhibiting supply of glucose from glycogen decreases contractility, via decreasing amplitude. Flavopiridol supports all my zero-glucose work.

It was challenging to compare the results of this work with other studies since this is the first time that this kind of research has been conducted.

CHAPTER SIX

Role of glycogen in the myometrium of women with dysfunctional labour

CHAPTER 6

Role of glycogen in the myometrium of women with dysfunctional labour

6.1 Introduction

The maintenance of muscle activity and its duration depend on the level of glycogen in the muscle; higher starting glycogen amounts in the muscle correlate with higher performance and durability (Bergstrom et al., 1967).Besides, Green and colleagues stated that endogenous glycogen is the principal source of energy for muscle contraction (Green et a.,1991), but there has been no evidence to prove the direct cause and effect between glucose depletion and muscle dysfunction (Bergstrom et al., 1967). It transpired that during persistent physical exercise, more energy is used, and the available ATP may not be sufficient to keep up with the demands of the muscle so, the mechanism of contractility in the muscle may thus be compromised (Bergstrom et al., 1967)

Many researchers have mentioned the importance of glycogen, and the endurance and performance of a muscle function during exercise; they have established that the amount of glycogen in a muscle before and during exercise may augment or decrease the endurance and performance of a muscle, as glycogen is the principal energy supplier for contraction. It has been suggested that the higher the starting amount of glycogen in the muscle, the better (Green et al., 1991). This statement links glucose depletion and muscle fatigue (Hultman et al., 1983) and has been corroborated by the results of the study conducted by Bergstrom et al. (1967). However, this mechanism is still to be clarified, since this fatigue occurs as a result of varied factors including energy exhaustion, metabolite build up, sarcoplasmic reticulum dysfunction, genetics, temperature, and the environment (Bergstrom et al.,1967). Moreover, muscle contractile force output depends on contractile processes. Malfunction at some of the challenging points of the cross-bridges may result in the development of muscle inability to produce and maintain force output**.** (Roberts et al.,1989; Sahlin et al.,1992)

The influence of glycogen reserve on the metabolism of smooth muscle is still debatable. To clarify glycogen's role as oxidative substrate, Allen et al. conducted a study on hog carotid arteries to measure the metabolism of glycogen, glucose, and acetate. These arteries contained glycogen levels ranging from 1 to 11 µmol/g and were left to contract in PSS containing acetate (1mM) and glucose(5mM) for 6 hours at 37°C. Concomitantly, glycogen, glucose and acetate oxidation were measured. It was found that 10% of the substrates oxidised in hog carotid tissue at high glycogen levels were from glycogen oxidation; so, when there was sufficient substrate availability, glycogen contributed very little to vascular smooth muscle's substrate oxidation (Allen et al.,2020). Allen et al. infer that regardless of the glycogen concentration, glucose predominates as an oxidative substrate in vascular smooth muscle. During uterine contractions, the myometrium may rely on the combination of available glucose and glucose deriving from glycogen breakdown. The preference of for one source over the other may depend on factors such as availability and rate of uptake of glucose, energy demands and hormonal influences (Hearris 2018)

In chapter 3 of this study, I discussed my structured literature review of myometrial glycogen storage. The review indicated that myometrial glycogen increases gradually during pregnancy and falls drastically after delivery; that glycogen is used and depleted as contractions occur during labour. I therefore suggested that for normal delivery it must be necessary to have enough glycogen in the myometrium to maintain strong uterine contractions and sustain the metabolic challenges occurring during parturition. In this chapter I seek to determine the role of glycogen in women with dysfunctional labour by measuring the glycogen content in their myometria, hypothesising that women with low glycogen are prone to develop dysfunctional labour compared to women labouring normally.

6.2 Materials and methods

6.2.1 Patients and tissue preparation

Five elective (that is non-labouring) women were recruited to measure the myometrial glycogen amount just before labour, to compare the level of glycogen within the group and to have information on the glycogen levels just before labour. Due to rapid degradation of human tissue, it was pertinent to conduct a degradation time course of glycogen in the myometrium in elective tissues. The objective of this measurement was to have to have an

insight on the amount of glycogen lost in the myometrium for the next 24 hours. Monitoring this loss was important for reliable results since the glycogen measurement process in labouring tissues started several hours after biopsy collection. Measuring glycogen in labouring tissues helped to compare the content of glycogen in normal and dysfunctional labour. expecting the glycogen to be low in dysfunctional myometrium.

The non-labouring women were recruited between November 2021 and July 2022, and the 15 labouring women were recruited between January 2019 and May 2022.The mean maternal age at the time of delivery was 32 years (± 1.7 years) in the non-labouring group. The mean gestational age was 39 weeks. The indications for elective C/S included previous C/S (4) and brittle asthma (1). The mean maternal age at the time of delivery in labouring was 30 years (± 1.8 years) and the mean gestational age was 37 weeks+3. The indications for C/S in labouring included presumed foetal compromise; failure to progress (delayed first or second stage); undiagnosed breech; cephalopelvic disproportion; planned C/S but started labour before the C/S booked date; premature rupture of membranes (PROM); Spontaneous rupture of membranes (SROM) and maternal request. Among the labouring women there were 4 with multiple pregnancies (MP), 2 with diabetes, 1 with chorio-amnionitis, and 1 with pre-eclampsia.

6.2.2 Glycogen measurement

The method used in measuring the level of glycogen in the myometrium is well elaborated in chapter 2 of this thesis. Collected biopsies were immediately plunged in the Hanks balanced salt solution to preserve tissues from rapid deterioration. The myometrial tissues were then frozen in liquid nitrogen within 24 hours and kept at -80°C. Homogenisation of tissues was performed after the freeze-drying process was completed. The homogenates were boiled then centrifuged before mixing the lysates from centrifugation with buffers and enzymes in the 96 well plate. Finally, the plate was transferred to fluostar at 570nm for reading.

For time course studies (elective C/S), biopsies were instantly frozen into liquid nitrogen at time 0. The rest of the tissues was kept in Hanks balanced salt solution at 4°C to preserve tissue from degradation, then frozen in pieces in liquid nitrogen progressively for 20, 40, 60, 120, 240, 360 and 1440 minutes. The frozen tissues were kept in the freezer at -80°C for glycogen measurement. For labouring tissues, biopsies were placed into Hanks balanced salt

solution at 4°C, then frozen into liquid nitrogen within 24 hours, then kept at -80°C for later glycogen measurement.

a. The freeze-drying processes.

The myometrial samples kept in the tubes were taken out of the freezer then placed in a Modulyo freeze-drier after removing the lids from the tubes. The lids were replaced with a pierced piece of foil to allow air exchange. I started by closing the pump, then cooled the freeze-drier to -40°C. Finally, I switched on the button to start the freeze-drying process. The tissues were left in the freeze-drier for 24 hours.

b. Tube preparation

Beads, tissues, and water added to each tube were weighed and recorded on a spreadsheet. Roughly 0.5ml beads were added to the homogenisation tube and then precooled on dry ice. Approximately 10mg tissue were cut in a weigh boat (over dry ice, to ensure the tissue remained frozen) and added to the homogenisation tube. The tube was stored in the -80°C freezer until homogenisation.

c. Homogenisation

1000 µl water were added to each of the tubes containing the tissue and reweighed. Each tube was homogenised at 4500 rpm for 3 minutes (in bursts of 90 seconds), then placed on crushed ice between each homogenisation. Each tube was boiled for 5 minutes to remove enzyme activity then placed back on ice. Each tube was centrifuged at 4°C for 5 minutes at 13000 x g to remove debris.

6.2.2.1 Glycogen assay

Hydrolysis and development buffers were prewarmed before use. Glycogen standards were prepared by mixing 10µL of 2 mg/mL with 90ul water (0.2mg/ml).

a. Preparation of glycogen standards

Standard	Volume of glycogen standard	Volume of water	Volume of diluted standard/ well	Volume of hydrolysis buffer	Concentration
S1	5µL	20µL	10µL	40µL	0.4µg/ml
S2	10µL	15µL	10µL	40µL	0.8µg/ml
S3	15µL	10µL	10µL	40µL	1.2µg/ml
S4	20µL	5µL	10µL	40µL	1.6µg/ml
S5	25µL	0µL	10µL	40µL	2µg/ml

Table 6.1 Glycogen standards preparation

b. Preparation of samples

Volume needed	Final dilution when hydrolysis buffer added
5µL neat sample per duplicate well. Adding 45µL hydrolysis buffer per well	1:10

Table 6.2 Sample preparation: dilution

The hydrolysis enzyme mix was reconstituted in 220 µL of hydrolysis buffer and mixed well by pipetting (not vortexed) and kept on ice while in use.

2 µL of the hydrolysis enzyme mix were added to all wells (except the blank) and incubated for 30 minutes at room temperature. The plate seal was added to reduce evaporation.

During the incubation the development enzyme mix was reconstituted in 220 µL of development buffer and mixed well by pipetting and kept on ice while in use.

c. Preparation of the master reaction mix

Reagent	Volume
Buffer	46 µL
Development enzyme mix	2 µL
Peroxidase substrate	2 µL

Table 6.3 Master reaction mix before plate reading.

50µl master reaction mix was added to each well, mixed on a shaker and incubated in the dark for 10-30 minutes. Finally, the plate was read on Fluostar at 570nm.

6.3 The results

In elective samples(term), a large variation in the level of glycogen between samples within the time zero group (standard deviation = 10, n = 4) was noted (Tab 6.5, "2nd Column). In the first 6 hours after the collection of biopsies, the level of glycogen in the myometrium decreased by ~ 22 % (from time 0 to time 360 minutes). After 24 hours (time 1440 minutes), almost 53 % of glycogen were lost (from 13 ± 4 to 6 ± 3 µg/mg, mean) (Tab.6.5)

In labouring tissue, the glycogen content was generally low. Almost all the samples had a glycogen level less than 1 µg/mg) (Tab 6.6). The indication for C/S determined the difference between normal labour and dysfunctional labour. The 1st and 2nd stage delays during labour were considered as dysfunctional (linked to the myometrium) (Tab.6.9) and the other indications for C/section were linked to foetal reasons. (Tab.6.7). Small sample size could not allow an appropriate statistical comparison of myometrial glycogen level between women with dysfunction labour(n=4) (Tab. 6.10) and women with normal labour(n=2) (Tab. 6.8) after excluding diabetes, pre-eclampsia, chorioamnionitis, prematurity, multiple pregnancies, and abnormal BMI. In addition, the duration of labour was not identical for each sample.

6.3.1 Demographic information and obstetric history in non-labouring women

Subject number	Age	Gestational age (weeks)	BMI	Parity	Gravidity	Indications for C/S
Subject 1	26	39	33.5	0	1	Brittle asthmatic
Subject 2	39	39	24.3	1	2	Previous C/S
Subject 3	30	39	25	1	2	Previous C/S
Subject 4	39	39 + 2	20.8	1	2	Previous C/S
Subject 5	28	39 + 6	20.6	1	2	Previous C/S

Table 6.4. Demographic information of samples from elective C/S. The patients were between 26 and 39 years old; the mean gestational age was 39 weeks. The gravidity, the parity and the indications for C/S were almost the same.

6.3.2 Glycogen time course (µg/mg)

Time (minutes)	T0	T20	T40	T60	T120	T240	T360	T1440
Subject 1	**15.132**	12.174	10.126	17.82	13.297	8.789	11.54	**4.606**
Subject 2	**2.15**	1.858	1.036	1.469	1.494	1.866	1.94	**0.602**
Subject 3	**9.62**	20.58	15.15	7.4	9.74	11.52	10.71	**2.58**
Subject 4	**27.47**	18.99	19.05	18.3	19.39	20.35	18.12	**17.42**
Average	**13.5930**	13.4005	11.3405	11.24725	10.98025	10.63125	10.5775	**6.3020**
Std. dev.	**10.67187**	8.515111	7.780454	8.233222	7.474811	7.64782	6.644288	**7.590117**
SEM	**4.356774**	3.476279	3.176357	3.361199	3.0511579	3.12221	2.712519	**3.098652**

Note: Std. dev: Standard deviation, SEM: Standard error of the mean

Table 6.5 Time course exhibiting the degradation of glycogen content in the myometrium in elective C/section samples over 24 hours. The glycogen content degraded progressively from the time of sample collection to third of value over 24 hours. One subject was excluded because of abnormal BMI.

6.3.3 Demographic information, obstetric history, and glycogen content in Labouring women

Subject number	Age	Gestational age (weeks)	BMI	Parity	Gravidity	Indications for C/S	Glycogen µg/mg
Subject 1	33	39 + 6	23.2	0	5	Maternal request	0.325
Subject 2	28	39 + 4	22.3	0	1	Delay 1st stage, chorioamnionitis	0.388
Subject 3	39	37 + 6	35.3	10	14	Delay 1st stage	0.586
Subject 4	34	37 + 6	23.3	4	5	Failure to progress, 1st stage (MP)	0.631
Subject 5	36	36 + 6	30	0	2	Maternal request (diabetic)	0.770
Subject 6	34	38 + 5	22.5	1	3	Delay in 2nd stage	0.745
Subject 7	36	37 + 1	32.4	2	9	Unstable lie(transverse)	0.902
Subject 8	41	28 + 5	31.8	0	1	Presumed foetal compromise (MP)	1.026
Subject 9	30	39 + 4	41.2	1	2	Presumed fatal compromise (diabetic)	0.123
Subject 10	32	37	-	0	1	Breech, pre-eclampsia (MP)	0.322
Subject 11	23	38	21.8	1	2	SROM	0.183
Subject 12	22	34 + 5	29.8	0	1	PROM (MP)	1.416
Subject 13	26	40	25.6	0	1	Delay 1st stage + foetal distress	0.752
Subject 14	29	40	24.2	0	1	Delay 1st stage	1.202
Subject 15	30	39 + 6	24.1	0	1	Delay 1st stage + foetal distress	0.679

Table 6.6 Glycogen content in labouring myometrium. The patients were between 22 and 41 years old; the mean gestational age was 37 weeks (+ 3) and the gravidity 1 - 14, the parity between 0 - 10.

6.3.4 Demographic information, obstetric history, and glycogen content in Normal labour

Subject number	Age	Gestational age (weeks)	BMI	Parity	Gravidity	Indications for C/S	Glycogen µg/mg
Subject 1	33	39 + 6	23.2	0	5	Maternal request	0.325
Subject 2	36	36 + 6	30	0	2	Maternal request (diabetic)	0.770
Subject 3	36	37 + 1	32.4	2	9	Unstable lie (transverse)	0.902
Subject 4	41	28 + 5	31.8	0	1	Presumed foetal compromise (MP)	1.026
Subject 5	30	39 + 4	41.2	1	2	Presumed foetal compromise (diabetic)	0.123
Subject 6	32	37	-	0	1	Breech, pre-eclampsia (MP)	0.322
Subject 7	23	38	21.8	1	2	SROM	0.183
Subject 8	22	34 + 5	29.8	0	1	PROM (MP)	1.416
Average							**0.633375**
Std. Dev.							0.464889
SEM							0.175711

Note: Std. dev: Standard deviation, SEM: Standard error of the mean

Table 6.7 Glycogen content in normal labouring myometrium. The patients were between 22 and 41 years old; the mean gestational age was 36 weeks (+ 3) and gravidity 1 - 9, parity between 0 - 2.

6.3.5 Demographic information, obstetric history, and glycogen content in Normal labour, excluding abnormal samples.

Subject number	Age	Gestational age (weeks)	BMI	Parity	Gravidity	Indications for C/S	Glycogen µg/mg
Subject 1	33	39 + 6	23.2	0	5	Maternal request	0.325
Subject 2	23	38	21.8	1	2	SROM	0.183

Table 6.8 Glycogen content in normal labouring myometrium excluding abnormal samples. The patients were between 23 and 33 years old; the mean gestational age was 38 weeks (+ 3) and gravidity 2 - 5, parity between 0 - 1. Diabetic subjects, pre-eclampsia, prematurity, multiple pregnancies, and abnormal BMI were excluded.

6.3.6 Demographic information, obstetric history, and glycogen content in Dysfunctional Labour

Subject Number	Age	Gestational Age (weeks)	BMI	Parity	Gravidity	Indications for C/S	Glycogen µg/mg
Subject 1	28	39 + 4	22.3	0	1	Delay 1st stage, Chorioamnionitis	0.388
Subject 2	39	37 + 6	35.3	10	14	Delay 1st stage	0.586
Subject 3	34	37 + 6	23.3	4	5	Failure to progress, 1st stage (MP)	0.631
Subject 4	34	38 + 5	22.5	1	3	Delay in 2nd stage	0.745
Subject 5	26	40	25.6	0	1	Delay 1st stage + Foetal distress	0.752
Subject 6	29	40	24.2	0	1	Delay 1st stage	1.202
Subject 7	30	39 + 6	24.1	0	1	Delay 1st stage + Foetal distress	0.679
Average							**0.711857**
Std. Dev.							0.248761
SEM							0.094023

Note: Std. dev: Standard deviation, SEM: Standard error of the mean

Table 6.9 Glycogen content in dysfunctional labouring myometrium. The patients were between 26 and 39 years old; the mean gestational age was 38 weeks (+ 3), gravidity 1-14, parity between 0-10.

6.3.7 Demographic information, obstetric history, and glycogen content in Dysfunctional Labour excluding abnormal samples.

Subject Number	Age	Gestational Age (weeks)	BMI	Parity	Gravidity	Indications for C/S	Glycogen µg/mg
Subject 1	34	38 + 5	22.5	1	3	Delay in 2nd stage	0.745
Subject 2	26	40	25.6	0	1	Delay 1st stage + Foetal distress	0.752
Subject 3	29	40	24.2	0	1	Delay 1st stage	1.202
Subject 4	30	39 + 6	24.1	0	1	Delay 1st stage + Foetal distress	0.679

Table 6.10 Glycogen content in dysfunctional labouring myometrium excluding abnormal samples. The patients were between 26 and 34 years old; the mean gestational age was 39 weeks (+ 3), gravidity 1-3, parity between 0-1. Chorio-amnionitis, multiple pregnancy and abnormal BMI were excluded.

6.3.8 Discussion

This thesis is the first study exploring in vitro the role of glycogen in the myometrium from parturient with dysfunctional labour, so there is very little previous data on this subject with which to compare my results. I started running the glycogen degradation time course by measuring the level of glycogen in the myometrium of women undergoing elective C/section at Liverpool Women s' Hospital. Firstly, I monitored the degradation of glycogen over 24 hours. A large variability of the amount of glycogen between samples was noted. Some samples had high level of glycogen and others had extremely low levels at time zero (using an instant collection of samples during C/sections) (Tab 6.5). The glycogen amount decreased by 22% in the first 6 hours but decreased drastically by half after 24 hours (time 1440). The positive aspect of measuring glycogen from pre-labouring women is that it helped me have an insight on the starting level of glycogen in the myometrium just before labour. As mentioned previously, the myometrium is well prepared to sustain the metabolic challenges occurring during labour and among these preparations is the increase level of glycogen. It is logical to believe that the higher level of glycogen in the myometrium, the better the sustainability of the force and endurance during labour. This corresponds to the study of Bergstrom et al. who found a correlation between higher starting amount of glycogen in the skeletal muscle and higher muscle performance / maintenance during exercise (Bergstrom et al., 1991). The findings were reinforced by Costill et al.; they stated that the initial glycogen amount in the muscle was directly associated with the ability to maintain exercise for a longer period. Athletes who had 140 -230 mmol per kg or over, were able to withstand exercise better than those having 70 to 110 mmol per kg (Costill et al., 1980).

The glycogen amount in the myometrium was measured both from women labouring normally and from women with dysfunctional labour. As previously mentioned in this chapter, as soon as the myometrial biopsy was taken, it was placed into HBSS to preserve tissues from degradation. The tissues were then frozen in liquid nitrogen within 18 hours from collection. This means for each sample taken during a C-section, a certain amount of glycogen was lost between collection and freezing. The level of glycogen in the two groups were generally low (< 1µg/mg). It was challenging to make a comparison between these two groups because the

time of collection of each sample and the time of freezing the sample in the liquid nitrogen for glycogen measurement was not the same. It was hard to determine with exactitude the time spent, and the amount of glycogen lost for each sample before measurement. Besides, the duration of the onset of labour and the time the C/section decision was made was not the same for all the women recruited. This means, the amount of glycogen lost in the myometrium was not the same between the start of parturition and the C/section. It could be helpful if the duration of labour (the time of the onset of labour and the time of C/section) and the sample freezing time in the liquid nitrogen were the same. A sample size of at least 6 for each group be statistically enough to provide a meaningful conclusion.

The sample size was too small to compare the glycogen level in the myometrium between normal labour and dysfunctional labour after excluding the factors that could influence the myometrial glycogen storage (Diabetes, multiple pregnancy, chorio-amnionitis, preeclampsia, prematurity, abnormal BMI). A sample size of at least 6 for each group be statistically enough to provide a meaningful conclusion.

What was obvious was the very low amount of glycogen found in the labouring tissues compared to the amount in elective myometrium, suggesting that glycogen is used as an energy substrate during labouring process. I therefore hypothesise that women with a low level of glycogen in the myometrium from the elective C-section group time zero, or starting time (time just before labour), are predisposed to develop myometrial dysfunction during labour because of energy exhaustion.

In this chapter, glycogen storage was determined from the myometrium of women with normal labour and women with dysfunctional labour. The comparison of results between these two groups was not conclusive. However, the disparity found in the level of glycogen between myometrial tissues from elective C-section (term pregnancy) or within this group, still upholds the hypothesis of this thesis. Low glycogen storage may contribute to labour dysfunction. The comparison of glycogen reserves between myometria from normal labour and dysfunctional labour still needs to be determined.

CHAPTER SEVEN

General discussion

Chapter 7

General discussion

This thesis explores the effect of altered metabolism on uterine contractility, with a particular interest in the effect of glucose deprivation. The main objective was to provide a better understanding of glucose depletion as a possible contributing factor to poor uterine contractility during labour. The main question in this study was: "Does glucose depletion contribute to labour dysfunction?" and I hypothesised that women with dysfunctional labour have low glycogen in the myometrium compared to women labouring normally. A structured review of myometrial glycogen storage was carried out, to bring evidence of studies already conducted on myometrial glycogen storage before parturition and immediately in post-partum to provide insight to the studies in this thesis. From the analysis it was noteworthy to find that in different species the results were similar. The myometrial level of glycogen increases progressively during gestation, then decreases immediately after labour. Glycogen storage in the myometrium is influenced by hormonal metabolism. The hormones involved are mainly oestrogen, progesterone, and relaxin. Oestrogen and relaxin promote an increase in glycogen, while progesterone promotes a decrease in glycogen. Glucose deprivation studies showed a striking level of myometrial glycogen depletion. When myometrial strips from rats were left to contract in glucose-free medium for 2 hours, the tissues lost almost 60% of their glycogen (West & Cervoni, 1955); in humans, a 30-minute-high K^+-stimulated contraction in zero glucose conditions led to an 80% depletion in glycogen (Rajkumar et al., 2015). From this literature, it can be understood that glycogen is the principal source of energy for myometrial smooth muscle, and that it plays a crucial role in the contractile activity of the uterus during parturition.

Glycogen needs to be maintained at an appropriate level to cope with the metabolic challenges occurring during labour and to produce enough contractions to achieve the expected outcome: that is, the delivery of the baby and the uterus contracting enough to ensure no haemorrhaging after delivery.

This structured review led me to investigate the effect of glucose depletion on uterine contractility in vitro in mouse and human tissues. This was because of the fact that at each contraction during labour, the blood supply to the myometrium is reduced due to the compression of uterine vessels, and consequently glucose supply is reduced as well. My study sought to discover the effect of this depletion; it determined that zero glucose had a significant inhibitory impact on spontaneous uterine contractility. The temporary ischemia created in the myometrium at each contraction results in brief hypoxia. In human and mouse, the combined effect of zero glucose and hypoxia was analysed, showing that the inhibitory impact was greater and causing contractions to stop. These findings are in line with published observations mentioned in chapters 4 and 5 (Shi et al., 2020). However, in humans, non-labouring tissues were more affected than labouring. However, my observation during contractility measurement revealed that some myometrial tissues from elective c/sections resisted the inhibition effect when treated with glucose-free medium. In mice, the same resistance was noticed in some non-pregnant tissues. These results suggest that labouring myometrium (human) is better able to withstand the effect of glucose depletion than the non-labouring myometrium, probably because of high reserve of glycogen in labouring tissue. It is logical to believe that both tissues (non-labouring and labouring) have built up enough glycogen stores but the content of glycogen stores in the non-labouring tissues used for contractility studies was not measured. This content might be low comparing to labouring. A large sample size is needed for an appropriate conclusion

The decline in contractility was more pronounced with the combined action of zero glucose and hypoxia, with uterine contractions frequently stopping entirely. This seems to be logical because of the inability of the myometrium to generate sufficient ATP due to glucose depletion resulting in inhibition of contractions. In addition, hypoxia decreases the pH. The lactate produced, inhibits calcium entry into myometrial cells. This double action affects uterine contraction more; the decline was sharp and relevant in mice as well as in humans. It is important to note that hypoxia is a normal process during labour. As mentioned earlier, at each contraction, uterine vessels are compressed, and blood supply is reduced along with oxygen and glucose levels. Hypoxia can be beneficial when it is brief and was even found to increase uterine force, a phenomenon called hypoxia-induced force increase (HIFI. Alotaibi & Wray, 2015). However, when it is prolonged, hypoxia becomes detrimental to the life of the foetus and can contribute to uterine dysfunction during parturition (Wray et al., 2021). During

gestation, the myometrium is being prepared to withstand hypoxia through the augmentation of the level of glycogen and lipid droplets (Milwidsky & Gutman, 1983). It is to be expected that if glycogen storage is not well built-up during pregnancy, the myometrium would probably be unable to withstand the recurrent bouts of transient hypoxia occurring during labour, which may result in uterine dysfunction.

Oxytocin (OT) is a natural hormone that acts during labour. It induces or augments uterine contractions and is clinically used to correct poor contractions. This thesis suggests that OT is not able to maintain its ability to increase uterine contractions under glucose - free conditions, as in my study. The action of OT was significantly inhibited in both mouse and human tissues. The capacity of OT to increase uterine contractile force has been published by many authors (Arrowsmith et al.,2012), however it has not been clear why there is still a good proportion of women who resist the action OT in augmenting labour. My data suggests that in some labouring myometrium, the development of glucose/glycogen depletion results in the failure of myometrial smooth muscle to respond strongly to OT stimulation.

The use of high K^+ in this study was an experimental procedure allowing the examination of glucose deprivation effect in the presence of large amount of calcium, since high K^+ induces a massive entry of calcium into the cell because of the depolarisation of membranes caused by the high K^+ solution. Again, some contractile inhibitions were observed. The effect of zero glucose on the response to high K^+ was different in mouse and human tissues. Significant differences were observed in the plateau amplitude between tissues treated with High K^+ (with glucose) and High K^+ without glucose. Zero glucose effectively inhibited the plateau amplitude in pregnant mice. Additionally, a significant difference was observed in the peak and plateau amplitudes in non-labouring tissues only in humans. This means that, by depolarising the cell membrane with a high concentration of potassium, the large influx of calcium into the cells managed to maintain myometrial tonicity whether in the presence or absence of glucose in non-pregnant mice and in human labouring tissue. This difference in high K^+ response may be attributed to anatomical and physiological differences between animal and human species and to the metabolic changes occurring during pregnancy and labour (Elucidated in chapter 1, section 1.2.4)

To confirm the above results, I explored the glycogenolysis pathway by blocking the action of glycogen phosphorylase (the main enzyme responsible for the breakdown of glycogen into glucose) with flavopiridol hydrochloride hydrate (a glycogen phosphorylase inhibitor;

flavopiridol is a new promising antitumor drug in phase II clinical trials (Senderowicz et al., 1998; Senderowicz, 1999). Blocking the effect of glycogen phosphorylase prevents the breaking down of glycogen into G-1-phosphate, and therefore glucose is not produced. My study revealed that flavopiridol had a significant inhibitory effect on spontaneous myometrial contractility in mouse and human. This is the first study that has sought to examine the effect of flavopiridol on myometrial contractility.

My last step was to explore the role of glycogen in women labouring dysfunctionally by measuring the amount of glycogen to establish a link between low glycogen and labour dysfunction.

Due to the degradation of human tissue, glycogen content is being lost accordingly. Running a glycogen degradation time course led me to discover that there was a difference in the amount of glycogen between myometrial tissues in elective samples (at time zero). Prior to parturition some women were found to have high levels of glycogen in the myometrium, and others very low levels, suggesting that this difference may lead to myometrial dysfunction during labour for those having low levels of glycogen. This is consistent with the research work of Costill and Bergstrom, who found a correlation between low glycogen and poor performance in skeletal muscle during exercise (Costill et al., 1980; Bergstrom et al., 1991).

Among labouring tissues recruited, samples considered as labouring normally were from C/sections performed for foetal reasons or on request while labour was progressing normally, while samples considered as from dysfunctional labour were from C-sections processed for uterine reasons including failure to progress (delay in the first or second stage of labour). Overall, levels of glycogen in labouring tissues were very low compared to non-labouring (at time zero) due to labouring activity; but the comparison of glycogen levels between tissue labouring normally and tissue labouring disfunctionally could not be conclusive because of the small sample size. Besides, the potential loss of glycogen between the time of collection of each sample and the glycogen measurement processing time could not be evaluated. Furthermore, the duration of labour was not the same samples. Uterine samples were meant to be frozen in liquid nitrogen immediately after biopsies were taken during C/sections for good preservation of myometrial glycogen instead of keeping the biopsy in HBSS. Another element to consider was the duration of labour which was not the same for the biopsies collected. This means the amount of glycogen lost in the myometrial tissues was not the same between the onset of labour and the time the C/section was decided.

Measuring glycogen levels in each elective C/S sample, coupled simultaneously with the measurement of contractions, could help establish the connection between low glycogen and contractile inhibition effect or vice versa. This is because of the inhibitory contractile resistance noticed in some myometrial tissues in both animal and human, implying that these tissues are well loaded with glycogen.

7.1 The study design.

The objectives of this study were reached and the research questions, partially answered. The methodology was efficiently designed to answer the research questions.

I conducted a structured literature review on myometrial glycogen content and provided evidence for glycogen storage before labour and immediately after parturition. I explored the effect of glucose depletion on uterine contractility and determined the changes that zero glucose produces on uterine contractility using animal and human model in vitro. To corroborate the findings of the previous objective, the effects of flavopiridol on uterine contractility were also explored and determined. As flavopiridol inactivates glycogenolysis, the results show that glucose was not produced, and this also had an inhibitive effect on contractions. This further illustrates the crucial role of glycogen in myometrial tissues.

I explored the role of myometrial glycogen during labour, seeking to establish the potential correlation between labour dysfunction and glycogen levels in women during the birthing process.

The study design used an approach that could allow me to achieve a suitable structural literature review which was conducted with appropriate and recommended searching engines and assisted by Cochrane library. The method used followed a systematic framework suggested by Khan (2003), the best strategical implementations recommended for literature review. Regarding contractility studies and glycogen measurement the ethical approach related to animal handling, participants recruitment for the study, use of substances or human tissues was conducted in accordance with UK regulations. Concerning data Collection, All the contractility data in animal and human were recorded via a world precision device called voltage transducer, a very sensitive and higher precision Instrument. The contractions were then enhanced by a trans-bridge amplifier connected to the computer using special software (Labscribe2). This is the actual and best-known way of recording myometrial contractility data in vitro. Glycogen levels were quantitatively measured using a standard

laboratory technique, such as an enzymatic assay (Glycogen essay kit), the latest technic used for determining the level of glycogen in a muscle. The diagnosis of normal labour and labour dysfunction was determined based on well-established criteria.

Statistical analysis was performed using appropriate methods. A comparison of means between control and depletion of glucose was conducted using one sample t-tests and unpaired t-tests for contractility studies (Prism 9). A comparison of means of myometrial glycogen levels between normal labour was to be determined using unpaired t-tests if the sample size was enough and the sample collection procedure followed appropriately. However, the level of glycogen was determined in the myometrium of women from term and elective C/section just before the onset of labour highlighting the variation of in the level of myometrial glycogen between women. Some having high and others having low glycogen storage. These results prompted me to still believe that women with a low myometrial storage are more likely to develop myometrial dysfunction during labour because of low source of energy. As mentioned earlier, it was proven that the performance and endurance of a skeletal muscle depends on the muscle glycogen storage. Though the hypothesis of this study is yet to be proven, the first objectives have illustrated the importance of glycogen in uterine contractility indicating that glucose depletion occurring in the myometrium during labour may contribute to labour dysfunction. This study could be improved and further researched in future to test the hypothesis with more comparable data obtained by mitigating the aforementioned limitations. The study design enabled a comprehensive examination of the relationship between glycogen levels and labour dysfunction in women.

The findings from this study contribute to our understanding of the underlying mechanisms associated with labour dysfunction and potentially guide future interventions for improved obstetric outcomes.

7.2 Limitations of the study

Because of cost, guinea pigs could not be used. Guinea pig was the recommended model for parturition studies because of similarities with human in the physiology of labour.

Unlike with mouse tissue, for which I could decide when to collect the samples, obtaining human tissue was a challenge, particularly labouring tissue. It could arrive at any time during the day and night. Besides, experiments with labouring tissues take longer.

With labouring tissue, there were difficulties when asking women for consent when they were in the middle of difficult labour and about to go into surgery.

The first limitations were the low number of samples available overall, and also of uterine tissue in women labouring normally (who underwent C/section procedures for reasons such as fetal distress) which were less (2) than uterine tissue of women experiencing dysfunctional labour (4). Another limitation concerning samples, was that myometrial tissue was extracted at unknown and potentially varying points of the duration of labour resulting in differing levels of glycogen as it is converted to glucose and depleted during labour for energy which led to glycogen measurements that were not directly comparable.

The third limitation concerning the uterine samples was the manner in which myometrial tissue was stored prior to collection by the researcher. These tissues were extracted by medical professionals from C/sections performed at different times during the night and stored in Hanks' Balanced Salt Solution (HBSS) to prevent degradation of the samples, rather than instant freezing in liquid nitrogen. However, my research has demonstrated that after 6 hours in HBSS, almost 22% of glycogen is lost from tissues, which increases to 53% after 24 hours. This possibly had the greatest adverse impact on the comparability on the glycogen levels measured within the two groups of samples used in this study, as they were collected after varying levels of degradation.

7.3 Future work

The results in this thesis offer potential avenues for further exploration.

For contractility experiment in animal, guinea pig was the recommended animal model for parturition studies because of similarities with human in the physiology of labour.

It could be useful to establish a correlation between the levels of glycogen in the myometrium with the samples that resist contractile inhibition under zero glucose conditions. This could be made practically possible by freezing a piece of each biopsy in liquid nitrogen immediately

after the extraction of the baby during C/section, either for non-labouring or labouring tissues for glycogen measurement before contractility studies are conducted. This would help determine glycogen levels in the samples resisting inhibition and the samples not resisting contractile inhibition under zero glucose.

Dysfunctional labour studies in relation with glucose depletion still need to be pursued. A large number of samples is still needed, and good procedure of sample collection and glycogen measurement need to be put in place to surmount limitations. This will provide more insight for better analysis of results and allow the researcher to compare glycogen levels between women labouring normally and women labouring dysfunctionally. The link between glycogen levels and obstetric demographic parameters such as age, age of pregnancy, gravidity, parity and body mass index, multiple pregnancy in women with dysfunctional labour could be explored further.

Building a good storage of glycogen is necessary to maintain uterine contractile function during labour. This is generally achieved by a good and equilibrated nutrition during pregnancy. However, we need to explore why some pregnant women are not able to build up enough glycogen in the myometrium prior to parturition in a society where nutritional facilities are accessible. Stimulating glycogenesis pathway during pregnancy may help to increase the level of myometrial glycogen. Some ways to achieve this include Proper nutrition: Consuming a balanced diet during pregnancy is essential. Including foods rich in complex carbohydrates (Haff 2000). Regular exercise: Engaging in regular physical activity during pregnancy can enhance glycogen storage in muscles, including the myometrium (Ivy 1991). Hormonal regulation: Hormones play a vital role in glycogen synthesis. Insulin promotes glycogenesis by facilitating glucose uptake into the cells. Maintaining healthy insulin levels through proper diet and regular meals can stimulate glycogen production in the myometrium (Bouskila 2008). Adequate rest and sleep: Getting sufficient rest and sleep (7-8 hours) during pregnancy is important to support overall physiological processes, including glycogen synthesis to optimize glycogen storage (Petit et al.,2015). Supplementation: In some cases, healthcare providers may recommend supplements to support glycogen synthesis during pregnancy. These may include nutrients like vitamin D, which is involved in glycogen metabolism (Asemi 2015, Alghannam et al.,2018) Managing stress: High stress levels can negatively impact glycogen synthesis. Engaging in stress-reducing activities like prenatal yoga,

or deep breathing exercises can help maintain optimal glycogen levels in the myometrium (Seal and Turner 2021)

Measuring glycogen myometrial storage in vivo in human before labour is still a challenge. Actually, glycogen content data are from biopsies collected during C/sections.

7.4 Conclusion

The mechanisms affecting the membrane potential and the E-C coupling of uterine smooth muscle are still an area of investigation. Numerous studies are exploring this issue to find a secure and efficient way that could be beneficial to human reproductive health. The potential clinical benefit of understanding the process of contraction is: Good and effective control of uterine contractions during labour to avoid uterine dysfunction.

This thesis discussed the effects of altered metabolism on uterine contractility, with a focus on glucose depletion in an in vitro mouse and human model, and the role of glycogen in dysfunctional labour, after a comprehensive and thorough review on myometrial glycogen storage. There is some evidence that zero glucose inhibits uterine contractility irrespective of how it is produced. There was a large variation in the amount of glycogen in women prior to parturition. Some have high glycogen, others have low. Those with low glycogen may be inclined to develop uterine dysfunction during labour.

It was challenging to compare the levels of myometrial glycogen between women labouring normally and women labouring dysfunctionally, because of the loss of glycogen during a long time spent between the time the sample was collected and the time the sample was processed for glycogen measurement. The sample size in the two groups was too small and the duration of labour was not established for each and every labouring sample.

Still, it is suggested that depletion of glucose in vivo is likely to be a contributing factor to the pathway underlying contractility-related disorders.

The findings of my research make a substantial contribution to the existing literature in a variety of ways. Firstly, my study expands the current understanding of the effects of altered metabolism on uterine contractility through providing novel insights and perspectives. I conducted an extensive review of the literature to identify any existing gaps and subsequently designed my research methodology to approach them. My findings address these understudied areas by offering some new empirical evidence and analysis, thus enriching the overall body of knowledge in the field. I critically evaluated and applied established theories

and frameworks in my study, and my findings adds to the ongoing discourse and helps to refine and improve current theories and concepts.

I not only conducted a theoretical analysis but also collected and analysed empirical data. As a result, my study provides practical insights that can be utilised by researchers within the relevant field, making it relevant and valuable for real-world scenarios and applications. In a trial, Wiberg-Itzel et al., (2018) observed that oral bicarbonate aided the reduction of acidity around the myometrium, which consequently improved contractile force and allowed many women with dysfunctional labour to proceed with normal labour and delivery. Unfortunately, their study does not illuminate the origins of the poor contractions and unlikely to clarify the causes of dysfunctional labour. Therefore, I have postulated GLYCOGEN as another important factor in myometrial biochemistry. By doing so, I hope to advance the field and stimulate further research and discussion to address the identified gaps and improve our collective understanding of the topic for proper management of labour dysfunction.

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Appendices

Appendix 1

Publications from this thesis

Publications

1. Kaleta, T. and Wray, S. (2019) The effect of zero-glucose on uterine contractility. ACTA PHYSIOLOGICA

2.S Wray and T Kaleta. The effect of zero glucose on human uterine contractility **Proc Physiol Soc 46** (2020) PC0107

Abstracts and conferences

1.Federation of European physiological society(FEPS) and the Italian Physiological Society(SIF) 10 Sept 2019 — **The effect of zero-glucose on uterine contractility**. **Kaleta** T, Wray S. Department of Molecular & Cellular Physiology, University of Liverpool.UK

2.European Physiological society.2021
https://www.physoc.org/abstracts/the-effect-of-zero-glucose-on-human-uterine-contractility/

Ashcroft FM, Rohm M, Clark A, Brereton MF. Is Type 2 Diabetes a Glycogen Storage Disease of Pancreatic β Cells? Cell Metab. 2017 Jul 5;26(1):17-23. doi: 10.1016/j.cmet.2017.05.014. PMID: 28683284; PMCID: PMC5890904.

Appendix 2

Ethics approval for the investigation of pathological and physiological effect of different agents on myometrial contractility

Appendix 3

Animal handling (PPL) certificate

Prospective Licensee Exam Results

MRes Biomed Sci Administrator <itmmres@liverpool.ac.uk>
Fri 01/12/2017 12:00

To: Kaleta, Tshikaya <T.Kaleta@liverpool.ac.uk>

Dear Tshikaya
Please find below the results from the Prospective Licensee exam

Module	Pass / Fail
L and E1	P
PIL A	P
PIL B	P
PIL C	N/A

If you have been successful in all modules - your certificate has been ordered and I will email you when it is ready for collection.

If you have been unsuccessful in a module – I can arrange a re-sit. Please let me know by Wednesday 6th December if you would like me to do this for you.
Kind regards.
Jo

ID - 201287759
Email - T.Kaleta@liverpool.ac.uk

Postgraduate Admissions & Student Office
Institute of Translational Medicine
University *of* Liverpool
Room LG40
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L69 3GE

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Appendix 4

Ethical approval for research at Liverpool Women Hospital
Research Ethics Committee

Liverpool Women's NHS
NHS Foundation Trust

PRIVATE AND CONFIDENTIAL

Crown Street
Liverpool
L8 7SS

Tel: 0151 708 9988
www.lwh.nhs.uk

7th May 2019

Dr Tshikaya Kaleta
Cellular and Molecular Physiology
Institute of Translational Medicine
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Liverpool Women's Hospital
Crown Street
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L8 7SS

Direct dial: 0151 702 4241
Direct fax: 0151 702 4299
Email: louise.hardman@lwh.nhs.uk

Dear Tshikaya,

Letter of Access for Research

Further to your request I can confirm your right of access to conduct research through Liverpool Women's NHS Foundation Trust (LWFT) for the purpose and on the terms and conditions set out below. This right of access commences on 7th May 2019 and ends on 31st October 2022 unless terminated earlier in accordance with the clauses set out below.

You have a right of access to conduct such research as confirmed in writing in the letter of permission for research from this NHS organisation. **Please note that you cannot start the research until the Principal Investigator for the research project has received permission to conduct the project.**

The information supplied about your role in research at Liverpool Women's NHS Foundation Trust has been reviewed and you do not require an honorary research contract with this NHS organisation. We are satisfied that such pre-engagement checks as we consider necessary have been carried out.

You are considered to be a legal visitor to Liverpool Women's NHS Foundation Trust premises. You are not entitled to any form of payment or access to other benefits provided by this NHS organisation to employees and this letter does not give rise to any other relationship between you and this NHS organisation, in particular that of an employee.

While undertaking research through Liverpool Women's NHS Foundation Trust, you will remain accountable to your employer, the University of Liverpool but you are required to follow the reasonable instructions of this organisation in relation to the terms of this right of access.

Where any third party claim is made, whether or not legal proceedings are issued, arising out of or in connection with your right of access, you are required to co-operate fully with any investigation by this NHS organisation in connection with any such claim and to give all such assistance as may reasonably be required regarding the conduct of any legal proceedings.

You must act in accordance with Liverpool Women's NHS Foundation Trust policies and procedures, which are available to you upon request, and the UK policy framework for health and social care.

Liverpool Women's

Liverpool Women's NHS
NHS Foundation Trust

You are required to co-operate with Liverpool Women's NHS Foundation Trust in discharging its duties under the Health and Safety at Work etc Act 1974 and other health and safety legislation and to take reasonable care for the health and safety of yourself and others while on Liverpool Women's NHS Foundation Trust premises. You must observe the same standards of care and propriety in dealing with patients, staff, visitors, equipment and premises as is expected of any other contract holder and you must act appropriately, responsibly and professionally at all times.

If you have a physical or mental health condition or disability which may affect your research role and which might require special adjustments to your role, if you have not already done so, you must notify your employer and the Trust prior to commencing your research role at the Trust.

You are required to ensure that all information regarding patients or staff remains secure and *strictly confidential* at all times. You must ensure that you understand and comply with the requirements of the NHS Confidentiality Code of Practice which can be found at https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/200146/Confidentiality_-_NHS_Code_of_Practice.pdf and the Data Protection Act 2018. Furthermore you should be aware that under the Act, unauthorised disclosure of information is an offence and such disclosures may lead to prosecution.

You should ensure that, where you are issued with an identity or security card, a bleep number, email or library account, keys or protective clothing, these are returned upon termination of this arrangement. Please also ensure that while on the premises you wear your ID badge at all times, or are able to prove your identity if challenged. Please note that this NHS organisation accepts no responsibility for damage to or loss of personal property.

We may terminate your right to attend at any time either by giving seven days' written notice to you or immediately without any notice if you are in breach of any of the terms or conditions described in this letter or if you commit any act that we reasonably consider to amount to serious misconduct or to be disruptive and/or prejudicial to the interests and/or business of this NHS organisation or if you are convicted of any criminal offence. You must not undertake regulated activity if you are barred from such work. If you are barred from working with adults or children this letter of access is immediately terminated. Your employer will immediately withdraw you from undertaking this or any other regulated activity and you MUST stop undertaking any regulated activity immediately.

Your substantive employer is responsible for your conduct during this research project and may in the circumstances described above instigate disciplinary action against you.

Liverpool Women's NHS Foundation Trust will not indemnify you against any liability incurred as a result of any breach of confidentiality or breach of the Data Protection Act 2018. Any breach of the Data Protection Act 2018 may result in legal action against you and/or your substantive employer.

If your current role or involvement in research changes, or any of the information provided in your Research Passport changes, you must inform your employer through their normal procedures. You must also inform your nominated manager in this NHS organisation.

Yours sincerely

Louise Hardman
R&D Manager

2

Liverpool Women's

Appendix 5

Patient information sheet and consent form for the collection of human uterine biopsy

UNIVERSITY OF LIVERPOOL

Liverpool Women's NHS
NHS Foundation Trust

Liverpool Women's Research Tissue Bank Consent Form

Collection and Storage of Samples for Research

If you agree to take part, please initial each statement and sign and date this form

I have read and understood the information leaflet (Version.......dated.................) on the above research project and have been given a copy to keep. I have had the opportunity to ask questions about the project and understand the benefits and risks of donating. ....

I agree to give samples of my tissue, blood or other biological sample from my current procedure/event. ....

I agree to give samples of my tissue, blood or other biological sample from any past procedure/event. ....

I understand how the samples will be collected, that giving a sample is voluntary and that I am free to withdraw my approval for use of the samples at any time without giving a reason and without my care or legal rights being affected. ....

I agree that Liverpool Women's Research Tissue Bank will become custodian of this tissue, blood and/or biological sample(s) for use in regulated research projects. ....

I agree that tissue bank staff can collect and store information on an ongoing basis for updating the tissue bank database from my health records for research that uses my samples and that this information may be viewed by regulatory authorities. I understand that some information may be held at different sources such as Disease Registries. I understand that information about me will be treated confidentially and stored securely. ....

I understand that any samples or information given to research groups will be anonymised and my identity will be protected. I understand that some of these projects may be carried out by international research collaborators. I agree to samples being sent to research groups based in the UK, within the European Union (EU), and outside of the EU. ....

I agree that it may be appropriate for genetic assessment of the samples to be carried out to determine whether genetic makeup has any influence on my condition. ....

I understand that I will not benefit financially if research using my samples leads to new treatments or medical tests. ....

Signed...................................Print Name...................................Date.....................

Clinical/Research Practitioner Statement I have explained the request for samples for research purposes and have answered such questions as the patient has asked.

Signed...................................Print Name...................................Date.....................
Please complete the following patient details:

Hospital Unit Number.......................................NHS Number.....................................

Copy to Liverpool Women's Research Tissue Bank; Copy to woman's clinical notes; Copy to woman

Liverpool Women's Research Tissue Bank, University Department, Liverpool Women's NHS Foundation Trust, Liverpool L8 7SS. Tel: 0151 702 4346

Version 2.0 IRAS ID: 268806 01.07.2019

Appendix 6

Patient information leaflet
Consent for storage and use of tissue for research from women

UNIVERSITY OF LIVERPOOL

Liverpool Women's NHS
NHS Foundation Trust

LWRTB
Liverpool Women's
Research Tissue Bank

Patient Information Leaflet
Collection and storage of tissue, blood and other biological samples for research from women

Consent for storage and use of tissue for research

Version 3.1: 27.09.2019

IRAS Project ID: 268806

Contact Information for LWRTB
Dr Dharani Hapangama
University of Liverpool
Liverpool Women's NHS Foundation Trust
Liverpool L8 7SS

Tel: 0151 702 4346

We would like to invite you to take part in clinical research. Before you decide whether to take part you need to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the research if you wish.

What is the Research Tissue Bank?

The Liverpool Women's Research Tissue Bank (LWRTB) collects and stores tissue and other samples taken at surgery or in clinic for use in medical and scientific research. By doing this the LWRTB is able to build up a valuable collection that will be used by research groups undertaking women's health research. There are many common conditions such as endometriosis, infertility, heavy periods and prolapse, malignant conditions such as endometrial cancer and pregnancy complications that our research teams are trying to find new treatments for. Therefore, it is hoped that the resulting knowledge from this research will help other women in the future.

Why have I been chosen?

Either, you are about to undergo a surgical operation or biopsy procedure, which may involve the removal of tissue as a part of your treatment. Or, you will be asked to give a biological sample during your routine appointment.

What will happen to me if I take part?

The tissue removed at your surgical operation is in most cases sent to the Department of Pathology to be tested by doctors and scientists to help in the diagnosis and treatment of your medical condition. In other cases, the tissue would simply be discarded. Often, it is not necessary to test the whole sample, which means that there will be tissue left over. If you agree to take part in this study, we are asking for your informed consent to store some of this tissue in the LWRTB for use in research projects. The tissue sample the LWRTB will store will be very small, usually no bigger than the size of a one pound coin.

If as part of your routine treatment, a doctor would normally take a blood sample, we may ask for your informed consent to take a small additional amount at the same time as this routine sample. This will usually be taken at the time of your surgery or in clinic and will be approximately 20ml, around 4 teaspoons worth.

In certain cases if it is relevant to your condition, we would also like your informed consent to take an additional biological sample (e.g. urine, amniotic fluid, myometrial biopsy, endometrial biopsy). We would ask you to provide a small sample of one or more of these, according to what is appropriate and we would store this together with the tissue and blood sample.

We would also like your consent to access your health records. These will be reviewed by LWRTB staff on an annual basis in order to update information on the Tissue Bank database. All information will be treated with the strictest confidence and held securely within the LWRTB. In addition, the LWRTB may seek to access information held by other sources such as NHS Trusts, Disease Registries (such as the North West Cancer Intelligence Service, NWCIS) and the UK Statistics Authority.

Appendix 7

Good clinical practice (GCP)
A practical guide to ethical and scientific quality standards in clinical research

NHS
National Institute for
Health Research

CERTIFICATE of ACHIEVEMENT

This is to certify that

Tshikaya Kaleta

has completed the course

Good Clinical Practice (GCP) Refresher: eLearning

29 March 2019

A practical guide to ethical and scientific quality standards in clinical research

Including EU Directives, Medicines for Human Use (Clinical Trials) Regulations & the Department of Health UK Policy Framework for Health & Social Care Research, as applied to the conduct of Clinical Trials & other studies conducted in the NHS

Modules completed:

Core
Team Roles
Eligibility
Safety Reporting
Electronic Studies and Source Text
Summary

This course is worth 3 CPD credits

Delivering research to make patients, and the NHS, better

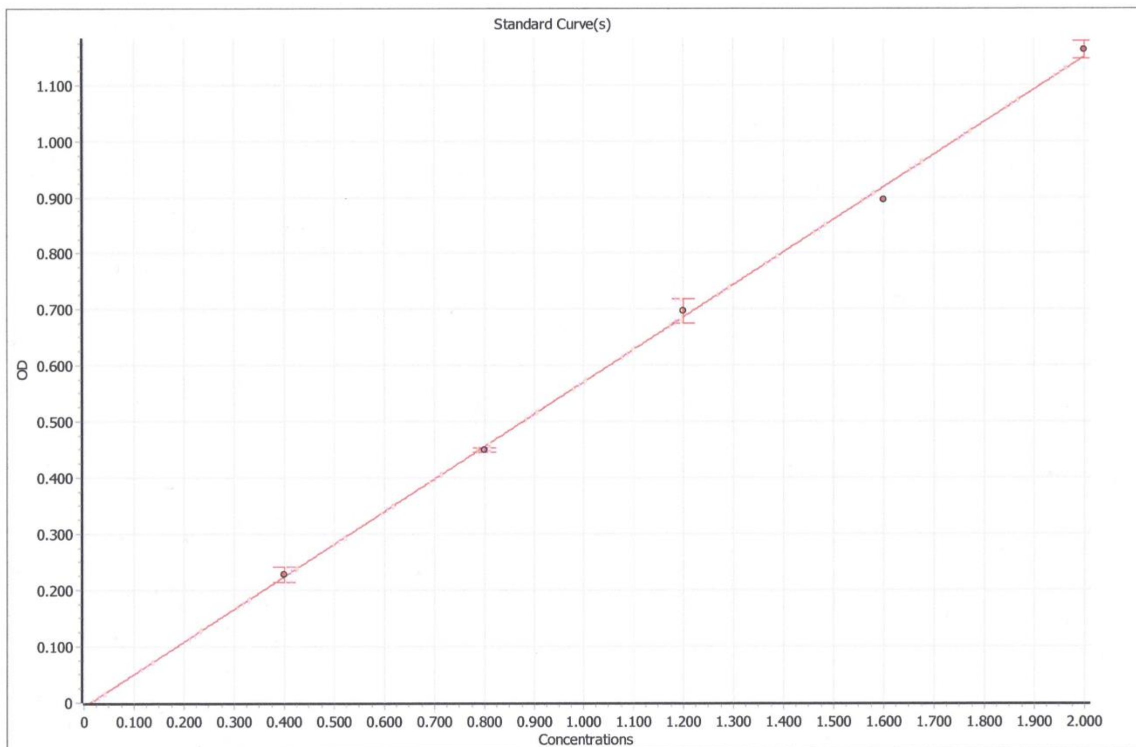
Appendix 8

Glycogen assay curves

User: USER
Path: C:\Program Files (x86)\BMG\Omega\User\Data
Test ID: 158
Test Name: Glycogen assay
Date: 09/05/2022
Time: 18:22:07
ID1: Glycogen time course one
Absorbance Absorbance values are displayed as OD
Grey fields contain deactivated wells: / Disabled by user: D02

Well	Content	Average over replicates based on Raw Data (570)	Standard Concentrations	Dilutions	Standard deviation over replicates based on Raw Data (570)	%CV over replicates based on Raw Data (570)	Standard error over replicates based on Raw Data (570)	Standard deviation over replicates based on Linear regression fit (570)	Linear regression fit based on Raw Data (570)
F01	Sample X1	0.576		5	0.02086	3.6	0.015	0.2	5.173
F02	Sample X1	0.576		5	0.02086	3.6	0.015	0.2	4.918
G03	Sample X10	0.848		5	0.04179	4.9	0.03	0.4	7.647
G04	Sample X10	0.848		5	0.04179	4.9	0.03	0.4	7.136
H03	Sample X11	1.108		5	0.018102	1.6	0.013	0.2	9.745
H04	Sample X11	1.108		5	0.018102	1.6	0.013	0.2	9.524
A05	Sample X12	1.084		5	0.012728	1.2	0.009	0.1	9.352
A06	Sample X12	1.084		5	0.012728	1.2	0.009	0.1	9.508
B05	Sample X13	1.054		5	0.074953	7.1	0.053	0.6	9.63
B06	Sample X13	1.054		5	0.074953	7.1	0.053	0.6	8.714
C05	Sample X14	0.922		5	0.024466	2.7	0.017	0.2	7.878
C06	Sample X14	0.922		5	0.024466	2.7	0.017	0.2	8.177
D05	Sample X15	1.152		5	0.040729	3.5	0.029	0.4	10.264
D06	Sample X15	1.152		5	0.040729	3.5	0.029	0.4	9.767
E05	Sample X16	1.275		5	0.032598	2.6	0.023	0.3	10.879
E06	Sample X16	1.275		5	0.032598	2.6	0.023	0.3	11.278
F05	Sample X17	1.624		5	0.083014	5.1	0.059	0.7	14.6
F06	Sample X17	1.624		5	0.083014	5.1	0.059	0.7	13.586
G05	Sample X18	1.025		5	0.062862	6.1	0.044	0.5	9.309
G06	Sample X18	1.025		5	0.062862	6.1	0.044	0.5	8.541

H05	Sample X19	1.147		5	0.042639	3.7	0.03	0.4	10.236
H06	Sample X19	1.147		5	0.042639	3.7	0.03	0.4	9.715
G01	Sample X2	1.207		5	0.03684	3.1	0.026	0.3	10.717
G02	Sample X2	1.207		5	0.03684	3.1	0.026	0.3	10.267
A07	Sample X20	1.097		5	0.047023	4.3	0.033	0.4	9.827
A08	Sample X20	1.097		5	0.047023	4.3	0.033	0.4	9.253
B07	Sample X21	1.148		5	0.034012	3	0.024	0.3	10.193
B08	Sample X21	1.148		5	0.034012	3	0.024	0.3	9.777
C07	Sample X22	1.173		5	0.023264	2	0.016	0.2	10.062
C08	Sample X22	1.173		5	0.023264	2	0.016	0.2	10.346
D07	Sample X23	1.066		5	0.1	12.8	0.096	1.2	10.111
D08	Sample X23	1.066		5	0.1	12.8	0.096	1.2	8.445
E07	Sample X24	1.009		5	0.048225	4.8	0.034	0.4	9.075
E08	Sample X24	1.009		5	0.048225	4.8	0.034	0.4	8.486
H01	Sample X3	0.729		5	0.026304	3.6	0.019	0.2	6.524
H02	Sample X3	0.729		5	0.026304	3.6	0.019	0.2	6.202
A03	Sample X4	0.606		5	0.03585	5.9	0.025	0.3	5.524
A04	Sample X4	0.606		5	0.03585	5.9	0.025	0.3	5.086
B03	Sample X5	0.562		5	0.033941	6	0.024	0.3	5.13
B04	Sample X5	0.562		5	0.033941	6	0.024	0.3	4.715
C03	Sample X6	0.663		5	0.001838	0.3	0.001	0.015879	5.786
C04	Sample X6	0.663		5	0.001838	0.3	0.001	0.015879	5.809
D03	Sample X7	0.631		5	0.011243	1.8	0.008	0.097107	5.59
D04	Sample X7	0.631		5	0.011243	1.8	0.008	0.097107	5.453
E03	Sample X8	0.137		5	0.01987	14.5	0.014	0.2	1.374
E04	Sample X8	0.137		5	0.01987	14.5	0.014	0.2	1.132
F03	Sample X9	1.377		5	0.010607	0.8	0.008	0.09161	12.023
F04	Sample X9	1.377		5	0.010607	0.8	0.008	0.09161	11.893
A01	Standard S1	0.227	0.4	1	0.014566	6.4	0.01	0.025162	0.389
A02	Standard S1	0.227	0.4	1	0.014566	6.4	0.01	0.025162	0.424
B01	Standard S2	0.45	0.8	1	0.004808	1.1	0.003	0.008306	0.798
B02	Standard S2	0.45	0.8	1	0.004808	1.1	0.003	0.008306	0.786
C01	Standard S3	0.698	1.2	1	0.02185	3.1	0.015	0.037743	1.245
C02	Standard S3	0.698	1.2	1	0.02185	3.1	0.015	0.037743	1.192
D01	Standard S4	0.896	1.6	1	0	0	0	0	1.561
D02	Standard S4	[illegible]	1.6	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
E01	Standard S5	1.162	2	1	0.015344	1.3	0.011	0.026506	2.041
E02	Standard S5	1.162	2	1	0.015344	1.3	0.011	0.026506	2.003


Standard Curve(s)
OD
1.100
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0
0 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800 0.900 1.000 1.100 1.200 1.300 1.400 1.500 1.600 1.700 1.800 1.900 2.000
Concentrations

User: USER
Path: C:\Program Files (x86)\BMG\Omega\User\Data
Test ID: 269
Test Name: Glycogen assay
Date: 03/11/2022
Time: 16:50:41
ID1: glycogen 15 min development 03.11.2022
Absorbance Absorbance values are displayed as OD

1. Average over replicates based on Raw Data (570)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.215	0.215	1.508	1.508	1.093	1.093	2.091	2.091	0.926	0.926	2.81	2.81
B	0.575	0.575	1.953	1.953	2.424	2.424	1.598	1.598	1.047	1.047		
C	0.669	0.669	2.266	2.266	1.545	1.545	1.065	1.065	1.141	1.141		
D	0.879	0.879	2.594	2.594	1.803	1.803	1.371	1.371	1.187	1.187		
E	1.109	1.109	1.256	1.256	0.44	0.44	0.633	0.633	0.456	0.456		
F	1.762	1.762	2.301	2.301	1.608	1.608	1.264	1.264	1.025	1.025		
G	2.346	2.346	1.038	1.038	1.331	1.331	1.108	1.108	2.336	2.336		
H	2.06	2.06	1.412	1.412	1.171	1.171	0.629	0.629	1.524	1.524		

2. Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	X4	X4	X12	X12	X20	X20	X28	X28	X36	X36
B	S2	S2	X5	X5	X13	X13	X21	X21	X29	X29		
C	S3	S3	X6	X6	X14	X14	X22	X22	X30	X30		
D	S4	S4	X7	X7	X15	X15	X23	X23	X31	X31		
E	S5	S5	X8	X8	X16	X16	X24	X24	X32	X32		
F	X1	X1	X9	X9	X17	X17	X25	X25	X33	X33		
G	X2	X2	X10	X10	X18	X18	X26	X26	X34	X34		
H	X3	X3	X11	X11	X19	X19	X27	X27	X35	X35		

Plate: BMG LABTECH 96

3. Standard Concentrations in ug

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.4	0.4										
B	0.8	0.8										
C	1.2	1.2										
D	1.6	1.6										
E	2	2										
F												
G												
H												

Plate: BMG LABTECH 96

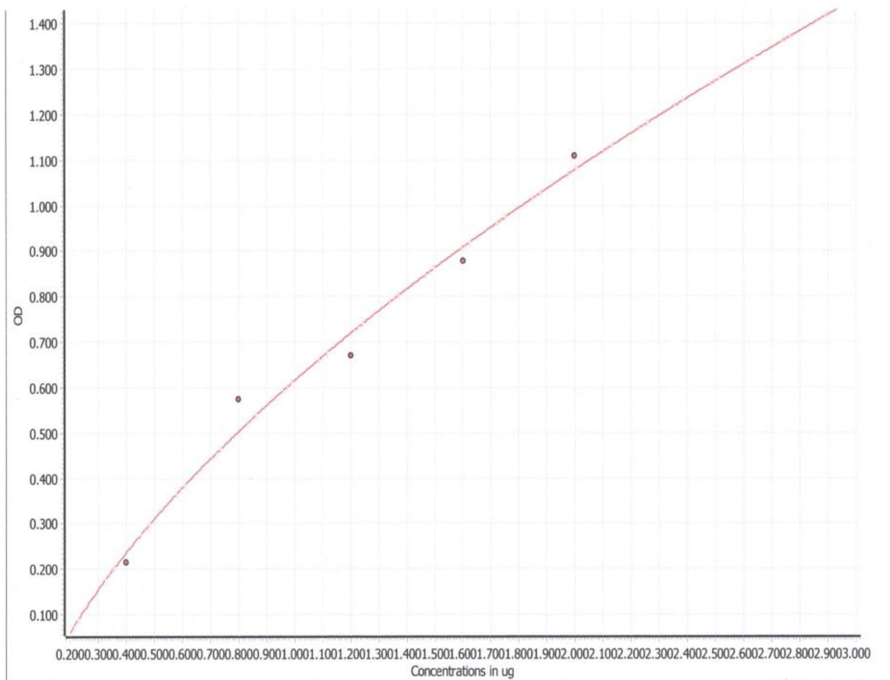
4. Raw Data (570)

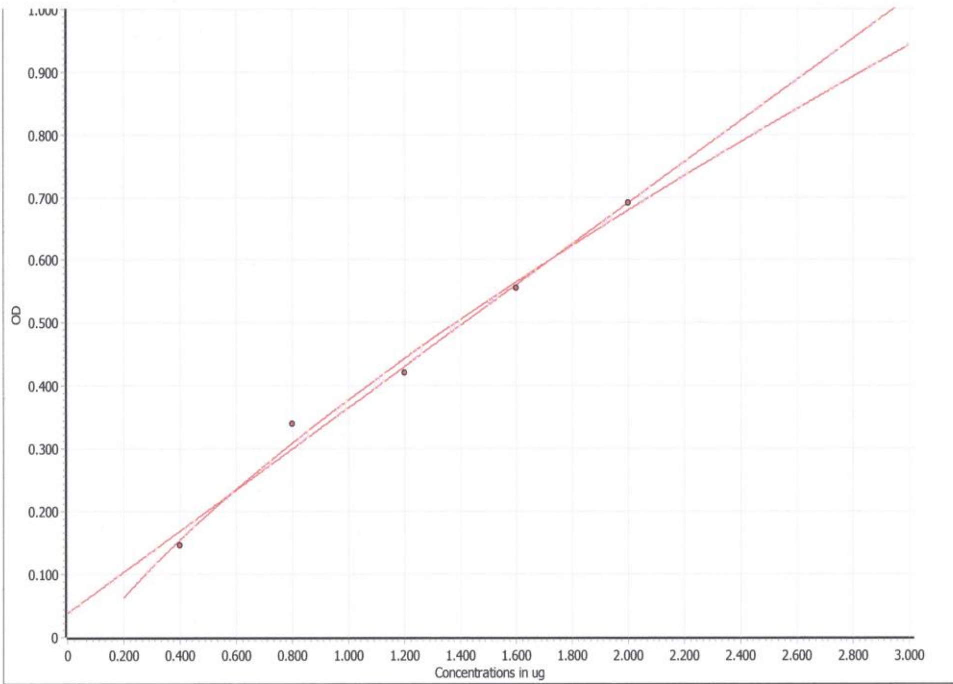
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.223	0.206	1.47	1.546	1.022	1.164	2.034	2.148	0.884	0.968	2.775	2.846
B	0.732	0.418	1.87	2.036	2.31	2.537	1.539	1.656	1.052	1.042		
C	0.647	0.691	2.313	2.218	1.525	1.565	0.998	1.132	1.114	1.169		
D	0.886	0.871	2.537	2.652	1.817	1.79	1.343	1.399	1.204	1.169		
E	1.087	1.131	2.104	0.409	0.432	0.448	0.612	0.654	0.437	0.476		
F	1.75	1.774	2.199	2.403	1.485	1.731	1.27	1.257	0.995	1.054		
G	2.218	2.475	0.957	1.118	1.282	1.38	1.112	1.104	2.287	2.384		
H	1.747	2.373	1.27	1.554	1.158	1.184	0.603	0.655	1.481	1.567		

5. 4-Parameter fit based on Raw Data in ug (570)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.384	0.362	> std range	> std range	9.309	11.056	> std range	> std range	1.545	1.736	> std range	> std range
B	1.224	0.66	> std range	> std range	> std range	> std range	> std range	> std range	1.935	1.91		
C	1.06	1.144	> std range	> std range	> std range	> std range	9.027	10.648	2.085	2.224		
D	1.551	1.518	> std range	> std range	> std range	> std range	13.427	14.211	2.316	2.225		
E	2.018	2.129	> std range	3.233	3.416	3.541	4.968	5.365	0.691	0.754		
F	> std range	> std range	> std range	> std range	> std range	> std range	2.489	2.453	1.798	1.939		
G	> std range	> std range	8.556	10.482	12.608	13.941	2.08	2.061	> std range	> std range		
H	> std range	> std range	12.436	> std range	10.981	11.309	0.977	1.074	> std range	> std range		

Standard curve





1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0
OD
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0.200
0.400
0.600
0.800
1.000
1.200
1.400
1.600
1.800
2.000
2.200
2.400
2.600
2.800
3.000
Concentrations in ug