

# **The effect of obesity and dyslipidaemia on myometrial contractility**

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requirements of the University of Liverpool for the  
degree of Doctor in Philosophy

By

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## **Declaration**

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award. This thesis is being submitted in partial fulfilment of the requirements for the degree of PhD. This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by explicit references. The views expressed are my own. I hereby give consent for my thesis, if accepted, to be available for photocopying and for interlibrary loan, and for the title and summary to be made available to outside organisations.

**Seham Alsaif (Candidate)**

31 July 2019

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I remain forever in your debt

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May God bless your soul in heaven

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Thank you for being my rock

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You are the invaluable

# Publications and presentations

## Publications

**A short review of adipokines, smooth muscle and uterine contractility.**

Seham AlSaif, Sadaf Mumtaz, Susan Wray

**Inhibitory effect of visfatin and leptin on human and rat myometrial contractility.** Sadaf Mumtaz, Seham AlSaif, Susan Wray, Karen Noble

## Oral presentation

**The effects of obesity on myometrium.** The federation of European physiological societies conference, Kaunas, Lithuania, 2015

**The effects of obesity on myometrial contractility.** The 3<sup>rd</sup> annual preterm labour birth conference, Leeds, UK, 2017

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**The effects of obesity on myometrium.** Golf obesity congress, Abu Dhabi, UAE, 2018

## Poster presentations

**Inhibitory effect of visfatin and leptin on human and rat.** The 8<sup>th</sup> Saudi students' conference. London, UK, 2015

**The effects of obesity on myometrial contractility.** The federation of European physiological societies conference, Kaunas, Lithuania, 2015

**The effects of obesity on uterine contractility.** The 9<sup>th</sup> Saudi students' conference, Birmingham, UK, 2016

**Modulation of myometrial contractility from basic physiology to applied intervention.** ITM research day, 2017

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# Table of Contents

<b>Declaration</b> .....	<b>ii</b>
<b>Acknowledgment</b> .....	<b>iii</b>
<b>Publications and presentations</b> .....	<b>v</b>
<b>Table of Contents</b> .....	<b>vii</b>
<b>List of Abbreviations</b> .....	<b>xiv</b>
<b>List of Figures</b> .....	<b>xviii</b>
<b>List of Tables</b> .....	<b>xxii</b>
<b>List of appendices</b> .....	<b>xxiv</b>
<b>Thesis Abstract</b> .....	<b>1</b>
<b>Chapter 1: Introduction</b> .....	<b>4</b>
<b>1.1 The uterus</b> .....	<b>4</b>
1.1.1 Anatomy of the human uterus.....	4
1.1.2 Anatomy of the mouse uterus .....	6
1.1.3 The myometrium.....	9
1.1.4 The uterine myocytes .....	12
<b>1.2 Gap junctions</b> .....	<b>13</b>
1.2.1 Introduction.....	13
1.2.2 Structure .....	14
1.2.3 Functions in reproduction and myometrium .....	14
1.2.4 Connexins.....	15
1.2.5 Regulation of gap junctions .....	16
<b>1.3 Plasma membrane lipid rafts and caveolae</b> .....	<b>18</b>
<b>1.4 The physiology of uterine contractility</b> .....	<b>21</b>
1.4.1 An overview .....	21
1.4.2 Uterine contractility in non-pregnant and pregnant myometrium	
.....	21

1.4.3 The myometrial membrane potential .....	21
1.4.4 Ion channels .....	23
1.4.5 Excitation - contraction coupling in the myometrium.....	25
<b>1.5 The regulation of intracellular <math>[Ca^{2+}]_i</math> (<math>[Ca^{2+}]_i</math>) in myometrial contraction .....</b>	<b>28</b>
1.5.1 Calcium influx .....	28
1.5.2 Calcium efflux .....	29
1.5.3 Regulation of $[Ca^{2+}]_i$ by the sarcoplasmic reticulum (SR) .....	29
<b>1.6 The physiology of human pregnancy - a brief overview .....</b>	<b>31</b>
<b>1.7 The process of parturition and labour .....</b>	<b>31</b>
1.7.1 First stage of labour .....	34
1.7.2 Second stage of labour .....	34
1.7.3 Third stage of labour.....	34
<b>1.8 Induction of labour .....</b>	<b>35</b>
<b>1.9 Prolonged/dysfunctional labour .....</b>	<b>36</b>
1.9.1 An overview .....	36
1.9.2 Pathophysiology of uterine contractions in dysfunctional prolonged labour .....	37
<b>1.10 Mouse pregnancy and parturition .....</b>	<b>37</b>
<b>1.11 Maternal obesity.....</b>	<b>38</b>
1.11.1 Prevalence.....	38
1.11.2 Definitions and measurements .....	39
1.11.3 Gestational weight gain .....	41
1.11.4 Mouse obesity.....	43
1.11.5 Obesity and reproduction.....	44
1.11.6 Obesity and labour.....	47
1.11.7 Dyslipidaemia, labour and myometrial contractility .....	51
1.11.8 Adipokines, labour and myometrial contractility .....	52
<b>1.12 Clinical contribution and physiological justification .....</b>	<b>61</b>
<b>1.13 Aims of thesis .....</b>	<b>62</b>
<b>Chapter 2: Methodology .....</b>	<b>64</b>
<b>2.1 Contractility studies .....</b>	<b>64</b>
2.1.1 Tissue collection, preparation and dissection .....	64

2.1.2 Organ bath experiments .....	66
2.1.3 Force calibration .....	67
2.1.4 Drugs and solutions .....	67
2.1.5 Contractility Data Analysis .....	69
<b>2.2 Enzyme-linked immunosorbent assay (ELISA) .....</b>	<b>72</b>
2.2.1 Collection of maternal blood and analysis of adipokines .....	72
2.2.2 Enzyme-linked immunosorbent assay (ELISA).....	72
2.2.3 Advantages of an automated ELISA machine: .....	75
<b>2.3 Immunohistochemistry .....</b>	<b>77</b>
2.3.1 Tissue preparation and fixation:.....	77
2.3.2 Tissue processing, embedding and sectioning: .....	77
2.3.3 Cx43 and Cx26 IHC staining procedure .....	78
2.3.4 Imaging of myometrial sections .....	82
<b>Chapter 3: Examining myometrial contractility and responses to visfatin in wild-type and ApoE<sup>-/-</sup> mouse.....</b>	<b>84</b>
<b>3.1 Introduction .....</b>	<b>84</b>
3.1.1 Maternal obesity and reproduction.....	84
3.1.2 Dyslipidaemia and myometrial contractility .....	84
3.1.3 Visfatin and myometrial contractility.....	85
3.1.4 Apolipoprotein E knockout (ApoE <sup>-/-</sup> ) mouse as a model of dyslipidaemia.....	86
3.1.5 Aims of this study.....	87
.....	<b>90</b>
<b>3.2 Methods .....</b>	<b>91</b>
3.2.1 Tissue collection .....	91
3.2.2 Contractility measurements .....	91
3.2.3 Drugs and solutions .....	91
3.2.4 Data and statistical analysis .....	92
<b>3.3 Results .....</b>	<b>93</b>
3.3.1 Establishment of spontaneous contractions and control mouse myometrial traces recording <i>in vitro</i> .....	93
3.3.2 Comparing myometrial contractility between non-pregnant WT and ApoE <sup>-/-</sup> mice.....	93

3.3.3 Examining the effect of visfatin on non-pregnant WT and ApoE <sup>-/-</sup> mouse myometrial contractility. ....	105
3.3.4 Examining the effect of visfatin on term pregnant WT and ApoE <sup>-/-</sup> mouse myometrial contractility .....	115
3.3.5 Exploring the mechanism of action of visfatin on pregnant WT mouse myometrial contractility - NAD <sup>+</sup> pathway.....	126
<b>1- FK866</b> .....	<b>127</b>
<b>2- Nicotinamide Adenine Dinucleotide (NAD<sup>+</sup>)</b> .....	<b>131</b>
<b>2- Nicotinic Acid</b> .....	<b>133</b>
<b>3.4 Discussion</b> .....	<b>135</b>
<b>3.5 Conclusion</b> .....	<b>140</b>
<b>3.6 Limitations of the study</b> .....	<b>140</b>
<b>Chapter 4: Examining the effect of maternal BMI on pregnant human myometrial contractility <i>in vitro</i></b> .....	<b>142</b>
<b>4.1 Introduction</b> .....	<b>142</b>
<b>4.2 Methods</b> .....	<b>144</b>
4.2.1 Tissue collection and preparation .....	144
4.2.2 Contractility measurements .....	145
4.2.3 Data and statistical analysis .....	145
<b>4.3 Results</b> .....	<b>146</b>
4.3.1 Demographic data .....	146
4.3.2 Comparing the time to commencement of spontaneous activity .....	150
4.3.3 Comparing spontaneous contractile activity between different BMI categories.....	152
4.3.4 Comparing oxytocin-induced contractile activity between different BMI categories .....	156
.....	<b>158</b>
<b>4.4 Discussion</b> .....	<b>159</b>
<b>4.5 Conclusion</b> .....	<b>161</b>
<b>4.6 Limitation of the study</b> .....	<b>161</b>

<b>Chapter 5: Investigating the association between maternal adipokines dysregulation and prolonged labour on obese pregnant women – a cross-sectional study</b> .....	<b>163</b>
<b>5.1 Introduction:</b> .....	<b>163</b>
5.1.1 Maternal obesity and pregnancy adverse outcomes.....	163
5.1.2 Leptin and reproduction .....	164
5.1.3 Visfatin and reproduction .....	165
5.1.4 Maternal obesity and prolonged/dysfunctional labour.....	167
<b>5.2 Methods</b> .....	<b>168</b>
5.2.1 Study design and participants.....	168
5.2.2 Sampling strategy and variables included in the study .....	170
5.2.3 Ethical considerations:.....	172
5.2.4 Sample size determination .....	173
5.2.5 Analysis of plasma leptin and visfatin levels .....	174
5.2.6 Data management and statistical analysis.....	175
<b>5.3 Results:</b> .....	<b>176</b>
5.3.1 Study sample characteristics: .....	176
5.3.2 The relationship between maternal BMI and maternal demographic characteristics.....	180
5.3.3 The relationship between maternal BMI and pregnancy-related complications.....	184
5.3.4 The effect of maternal BMI on obstetric maternal interventions and maternal outcomes.....	184
5.3.5 The relationship between maternal BMI and neonatal characteristics .....	188
5.3.6 The relationship between maternal BMI and maternal plasma leptin levels at the start of labour.....	190
5.3.7 The relationship between maternal BMI and maternal plasma visfatin levels at the start of labour .....	192
5.3.8. The relationship between prolonged labour and maternal plasma leptin and visfatin levels at the start of labour in obese women.....	194
5.4.9. The relationship between maternal characteristics and maternal plasma leptin and visfatin levels during labour .....	194

5.3.10 The relationship between pregnancy-related complications and maternal plasma leptin and visfatin levels at the start of labour .....	196
5.3.11 The association between maternal outcomes and maternal plasma leptin and visfatin levels at the start of labour .....	196
5.3.12 The relationship between neonatal characteristics and maternal plasma leptin and visfatin levels at the start of labour .....	196
<b>5.4 Discussion</b> .....	<b>198</b>
<b>5.5 Conclusion:</b> .....	<b>202</b>
<b>5.6 Limitations of the study:</b> .....	<b>202</b>
<b>Chapter 6: Investigating the expression of gap junction proteins, Cx46 and Cx26, in the obese human myometrium</b> .....	<b>207</b>
<b>6.1 Introduction</b> .....	<b>207</b>
<b>6.2 Methods</b> .....	<b>209</b>
6.2.1 Tissue .....	209
6.2.2 IHC procedure .....	210
6.2.3 Scoring technique and statistical analysis .....	211
<b>6.3 Results</b> .....	<b>211</b>
6.3.1 Cx43 antibody optimisation .....	211
6.3.2 Comparing the myometrial expression of Cx43 in normal weight, overweight and obese pregnant women: .....	214
6.3.3 Cx26 Antibody optimisation .....	216
6.3.4 Comparing the myometrial expression of Cx26 in normal weight, overweight and obese pregnant women .....	218
<b>6.4 Discussion</b> .....	<b>220</b>
<b>6.5 Conclusion</b> .....	<b>221</b>
<b>6.6 Limitations of the study</b> .....	<b>222</b>
<b>Chapter 7: Discussion</b> .....	<b>224</b>
<b>7.1 Overview</b> .....	<b>224</b>
<b>7.2 Maternal obesity, dyslipidaemia and myometrial contractility</b>	<b>226</b>
<b>7.3 The effect of visfatin on mouse myometrial contractility</b> .....	<b>226</b>
<b>7.4 Obesity, adipokines and dysfunctional labour</b> .....	<b>228</b>
<b>7.5 Maternal obesity and gap junction proteins</b> .....	<b>229</b>
<b>7.6 Future directions</b> .....	<b>230</b>

.....	232
<b>References</b> .....	<b>233</b>
<b>Appendices</b> .....	<b>234</b>

## List of Abbreviations

hERG	human ether-a-go-go-related gene
[Ca <sup>2+</sup> ] <sub>i</sub>	Intracellular Ca <sup>2+</sup>
15-PGDH	15-hydroxy-PG dehydrogenase
a.u.	arbitrary units
ACTH	adrenocorticotropic hormone
ANOVA	Analysis of Variance
Apgar score	Appearance, Pulse, Grimace, Activity, and Respiration score
ApoE	Apolipoprotein E
ApoE <sup>-/-</sup> mouse	Apolipoprotein E knockout mouse
ATP	Adenosine triphosphate
BAT	brown adipose tissue
BK <sub>ca</sub>	large conductance calcium-activated potassium channels
BMI	body mass index
Ca <sup>2+</sup>	Calcium ion
CaCl <sub>2</sub>	calcium chloride
CaM	calmodulin
cAMP	cyclic adenosine-3,5-monophosphate
Cav-1	caveolin-1
CCE	capacitative calcium entry
cGMP	cyclic guanosine 3',5'-monophosphate
CICR	Ca <sup>2+</sup> -induced Ca <sup>2+</sup> release
Cl <sup>-</sup>	Chloride ion
ClCa	calcium-activated chloride channel
CIVR	volume-regulated chloride channel
CO <sub>2</sub>	Carbon dioxide
COX	cyclo-oxygenase
CRH	corticotropin-releasing hormone
C-section delivery	Caesarean section delivery
Cx26	connexin 26
Cx43	connexin 43
DAB	3,3'-Diaminobenzidine
DAG	Diacylglycerol

DMSO	Dimethyl sulfoxide
ECC	excitation - contraction coupling
ELISA	ELISA enzyme-linked ImmunoSorbent Assay
eNOS	endothelial nitric oxide synthase
EP receptors	extracellular protein receptors
EP2	prostaglandin E2 receptors
FFM	fat free mass
FM	fat mass
GDM	gestational diabetes mellitus
GDPR	general Data Protection Regulation
GJ $\alpha$ 1	Gap Junction $\alpha$ 1
GPCR	G-protein coupled receptor
GWG	gestational weight gain
H & E	haematoxylin and Eosin
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
hCG	human chorionic gonadotropin
HDL	high density lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HFHC diet	high fat high cholesterol diet
HIV	Human Immunodeficiency Virus
HRP	horseradish peroxidase
IDL	intermediate density lipoproteins
IHC	immunohistochemistry
IOL	induction of Labour
IOM	American Institute of Medicine
IP3	inositol triphosphate
IP3Rs	inositol triphosphate receptors
IR	insulin receptor
IUFD	Intrauterine Foetal Death
IUGR	Intrauterine growth retardation
K <sup>+</sup>	Potassium ion
KATP	ATP-sensitive potassium pump
KCL	Potassium chloride
KH <sub>2</sub> PO <sub>4</sub>	Monopotassium phosphate

K <sub>v</sub>	voltage-gated potassium channel
LBW	low Birth Weight
LDL	low density lipoproteins
LEPR	leptin receptor
LGA	large for gestational age
LMP	last menstrual period
MAP	mitogen-activated protein kinase
MC4R	melanocortin-4 receptor
MgSO <sub>4</sub>	magnesium sulfate
MLC	myosin light chain
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
mPGES-1	microsomal PGE synthase 1
MβCD	Methyl-β-cyclodextrin
NA	Nicotinic acid
Na <sup>+</sup>	Sodium ion
Na <sub>2</sub> HPO <sub>4</sub>	Disodium hydrogen phosphate
NaCl	Sodium chloride
NAD <sup>+</sup>	nicotinamide adenine dinucleotide
NALCN	Na <sup>+</sup> -leak channel
Nam	nicotinamide
Nampt	nicotinamide phosphoribosyltransferase
NaOH	sodium hydroxide
NBF	neutral buffered formalin
NCX	Na <sup>2+</sup> /Ca <sup>2+</sup> exchanger
NICE	The National Institute for Health and Care Excellence
NO	nitric oxide
O <sub>2</sub>	Oxygen
OD	optical density
P value	probability value
PBEF1	pre-B-cell colony-enhancing factor 1
PE	Preeclampsia
PGE <sub>2</sub>	prostaglandin E <sub>2</sub>
PIP <sub>2</sub>	phosphatidylinositol (4,5)-bisphosphate
PLC	phospholipase C

PMCA	plasma membrane Ca <sup>2+</sup> -ATPase
PSS	physiological saline solution
real-time PCR	real-time polymerase chain reaction
ROCCs	receptor-operated calcium channels
RyRs	ryanodine receptors
SERCA	sarco/endoplasmic reticulum Ca <sup>2+</sup> -ATPase
SFH	Symphysio-pubis fundal height
SGA	small for gestational age
SIRT1	silent information regulator 2-related protein
SOCCs	store-operated calcium channels
SOCE	store-operated calcium entry
SR	sarcoplasmic reticulum
t <sub>50</sub>	half-maximal amplitude
TG	triglycerides
TRP	transient receptor potential superfamily
VGSC	voltage-gated Na <sup>+</sup> channels
VLDL	very low-density lipoproteins
V <sub>m</sub>	resting membrane potential
WAT	white adipose tissue
WHO	World Health Organization
WT mouse	Wild type mouse

# List of Figures

## Chapter 1

<b>Figure 1.1</b> The structure of non-gravid human uterus.....	7
<b>Figure 1.2</b> Basic anatomy of mouse uterus.....	8
<b>Figure 1.3</b> Microanatomy of pregnant human myometrium.....	11
<b>Figure 1.4</b> Gap junction structure.....	17
<b>Figure 1.5</b> A schematic of the conventional biochemical structure of caveolae and lipid rafts.....	20
<b>Figure 1.6</b> A schematic of excitation-contraction coupling in the myometrium.....	27
<b>Figure 1.7</b> The molecular structure of visfain and leptin.....	58
<b>Figure 1.8</b> Proposed mechanisms of the action of visfatin effects on myometrial contractility.....	59

## Chapter 2

<b>Figure 2.1</b> Myometrial trace demonstrating the different contractile parameters measured for contractility data.....	71
<b>Figure 2.2</b> Sandwich ELISA principle.....	74
<b>Figure 2.3</b> ETI-MAX 3000 machine components.....	76
<b>Figure 2.4</b> Comparing Staining of a section of human myometrium (5uM thickness) with haematoxylin alone and H&E.....	81

## Chapter 3

<b>Figure 3.1</b> Suggested mechanism of action of visfatin on myometrium.....	89
<b>Figure 3.2</b> Pharmacological modulation of NAD <sup>+</sup> pathway.....	90
<b>Figure 3.3</b> Non-pregnant mouse myometrium: comparing the spontaneous myometrial contractility between Wild Type and ApoE <sup>-/-</sup> mouse.....	95
<b>Figure 3.4</b> Comparing spontaneous myometrial contractility between non-pregnant WT and ApoE <sup>-/-</sup> mouse.....	96

<b>Figure 3.5</b> Non-pregnant mouse myometrium: comparing the myometrial contractility between WT and ApoE <sup>-/-</sup> mouse in response to oxytocin.....	99
<b>Figure 3.6</b> Myometrial contractile activity of non-pregnant WT and ApoE <sup>-/-</sup> mouse in response to oxytocin.....	100
<b>Figure 3.7</b> Non-pregnant mouse myometrium: comparing the myometrial contractility between WT and ApoE <sup>-/-</sup> mouse in response to MβCD.....	103
<b>Figure 3.8</b> Myometrial contractile activity of non-pregnant WT and ApoE <sup>-/-</sup> mouse in response to MβCD.....	104
<b>Figure 3.9</b> Non-pregnant WT mouse myometrium: Effect of 10-minute application of 10mM visfatin on spontaneously contracting WT mouse myometrium.....	108
<b>Figure 3.10</b> Non-pregnant ApoE <sup>-/-</sup> mouse myometrium: Effect of 10-minute application of 10mM visfatin on spontaneously contracting ApoE <sup>-/-</sup> mouse myometrium.....	109
<b>Figure 3.11</b> Non-pregnant mouse myometrium: Effect of 10-minute application of 10mM visfatin on oxytocin-induced WT mouse myometrium.....	113
<b>Figure 3.12</b> Non-pregnant mouse myometrium: Effect of 10-minute application of 10mM visfatin on oxytocin-induced ApoE <sup>-/-</sup> mouse myometrium.....	114
<b>Figure 3.13</b> Term pregnant mouse myometrium: Effect of 10-minute application of visfatin on spontaneously contracting WT mouse myometrium.....	118
<b>Figure 3.14</b> Term pregnant ApoE <sup>-/-</sup> mouse myometrium: Effect of 10-minute application of 10mM visfatin on spontaneously contracting ApoE <sup>-/-</sup> mouse myometrium.....	119
<b>Figure 3.15</b> Term pregnant mouse myometrium: Effect of 10-minute application of visfatin on oxytocin-induced WT mouse myometrium.....	123
<b>Figure 3.16</b> Term pregnant mouse myometrium: Effect of 10-minute application of 10mM visfatin on oxytocin-induced ApoE <sup>-/-</sup> mouse myometrium.....	124
<b>Figure 3.17</b> Summary of the effects of visfatin on non-pregnant and pregnant WT and ApoE <sup>-/-</sup> mouse myometrium.....	125
<b>Figure 3.18</b> The effect of FK866 on oxytocin-induced term pregnant WT mouse myometrium.....	128

<b>Figure 3.19</b> The effect of FK866 and visfatin on oxytocin-induced term pregnant WT mouse myometrium.....	129
<b>Figure 3.20</b> The contractile profiles for the effect of FK866 (10µM) and visfatin (10nM) on oxytocin-induced term pregnant WT mouse myometrium.....	130
<b>Figure 3.21</b> The effect of NAD <sup>+</sup> on oxytocin-induced pregnant WT mouse myometrium.....	132
<b>Figure 3.22</b> The effect of nicotinic acid on oxytocin-induced pregnant WT mouse myometrium.....	134
<b>Chapter 4</b>	
<b>Figure 4.1</b> Correlation between maternal BMI (kg/m <sup>2</sup> ) and commencement of spontaneous activity.....	151
<b>Figure 4.2</b> Spontaneous and oxytocin-induced myometrial contractility in all BMI categories.....	153
<b>Figure 4.3</b> Comparing spontaneous myometrial contractile activity in different BMI categories.....	155
<b>Figure 4.4</b> Comparing oxytocin-induced myometrial contractile activity in different BMI categories.....	158
<b>Chapter 5</b>	
<b>Figure 5.1</b> Flow chart of the included and excluded women in the study.....	169
<b>Figure 5.2</b> Study population distribution according to maternal BMI.....	178
<b>Figure 5.3</b> The correlation between maternal BMI and maternal age.....	181
<b>Figure 5.4</b> The correlation between maternal BMI and gestational age.....	182
<b>Figure 5.5</b> The correlation between maternal BMI and parity.....	183
<b>Figure 5.6</b> The mode of delivery in relation to maternal BMI.....	186
<b>Figure 5.7</b> The relationship between maternal BMI and neonatal birthweight.....	189
<b>Figure 5.8</b> Maternal Leptin levels at the start of labor at different maternal BMI.....	191

**Figure 5.9** Maternal visfatin levels at the start of labour at different maternal BMI.....193

**Figure 5.10** The association between induction of labour and maternal plasma visfatin levels.....197

## **Chapter 6**

**Figure 6.1** Optimisation of the rat anti-Cx43 primary antibody concentration for IHC of human myometrial sections.....213

**Figure 6.2** CX-43 antibody positive and negative controls.....215

**Figure 6.3** Expression of Cx43 in the human myometrium.....215

**Figure 6.4** Optimisation of the rat anti-Cx26 primary antibody concentration for IHC of human myometrial sections.....217

**Figure 6.5** CX-26 antibody positive and negative controls..... 219

**Figure 6.6** Expression of Cx26 in the human myometrium.....219

## **Chapter 7**

**Figure 7.1** Summary of the proposed mechanisms examined in this thesis which might be contributed to poor myometrial contractility associated with maternal obesity.....225

# List of Tables

## Chapter 1

<b>Table 1.1</b> Phases of parturition.....	33
<b>Table 1.2</b> WHO classification of BMI.....	40
<b>Table 1.3</b> American Institute of Medicine guidelines (IOM) for total GWG for women with singleton pregnancy.....	42
<b>Table 1.4</b> Summary of the studies which examined the association between maternal BMI and the progression of labour.....	48
<b>Table 1.5</b> Summary of the effects of the <i>in vitro</i> studies which examined the effects of adipokines on myometrial contractility.....	54

## Chapter 3

<b>Table 3.1</b> Difference in the spontaneous responses to visfatin (10nM) between non-pregnant WT and ApoE <sup>-/-</sup> mouse myometrium.....	107
<b>Table 3.2</b> Difference in the response to visfatin (10nM) between non-pregnant WT and ApoE <sup>-/-</sup> oxytocin-induced mouse myometrium.....	112
<b>Table 3.3</b> Difference in the effect of visfatin (10nM) between spontaneously contracted term pregnant WT and ApoE <sup>-/-</sup> mouse myometrium.....	117
<b>Table 3.4</b> Difference in the response to visfatin (10nM) between term pregnant WT and ApoE <sup>-/-</sup> oxytocin-induced mouse myometrium.....	122

## Chapter 4

<b>Table 4.1</b> Summary of demographics characteristics for my study sample.....	147
<b>Table 4.2</b> Demographics for each BMI category.....	148
<b>Table 4.3</b> Difference in the contractile indices of spontaneous myometrial contractions in different BMI categories.....	154
<b>Table 4.4</b> Difference in the contractile indices of oxytocin-induced myometrial contractions in different maternal BMI category.....	157

## Chapter 5

<b>Table 5.1</b> Summary table of the diagnostic criteria for pregnancy-related complications.....	171
<b>Table 5.2</b> Summary table of study sample characteristics after application of exclusion criteria.....	179
<b>Table 5.3</b> The relationship between the mode of delivery and maternal BMI.....	187
<b>Table 5.4</b> Maternal characteristics in relation to maternal plasma leptin and visfatin levels during labour.....	195

## List of appendices

<b>Appendix 1</b> Publications.....	270
<b>Appendix 2</b> A copy of the ethics approval for the investigation of pathological and physiological effect of different agents, novel substances and biomarkers on human myometrial contractility.....	271
<b>Appendix 3</b> Patient information sheet and consent form for the collection of human uterine biopsy.....	272
<b>Appendix 4</b> A copy of the ethics approval for human immunohistochemistry testing of human tissue samples.....	273
<b>Appendix 5</b> ApoE <sup>-/-</sup> mouse data sheet (plasma cholesterol levels).....	274
<b>Appendix 6</b> A copy of the ethics approval for the cross-sectional study from King Fahad Hospital of the University Research Ethics committee.....	275
<b>Appendix 7</b> A copy of the ethics approval for the cross-sectional study from Liverpool Research Ethics committee.....	276
<b>Appendix 8</b> Patient information sheet and consent form for the cross-sectional study.....	277
<b>Appendix 9</b> ELISA Standard curves.....	278

# Thesis Abstract

## The effect of obesity and dyslipidaemia on myometrial contractility

Obesity is a growing health problem worldwide influencing women's health and childbearing. There is a close relationship between obesity and pregnancy adverse outcomes. Maternal obesity is associated with increased rates of dysfunctional labour, induction of labour and unplanned emergency C-section delivery. These complications suggest that obesity has an inhibitory effect on myometrial function leading to poor myometrial contractility. Little is known; however, of the underlying contractile dysfunction within the myometrium of obese women, preventing its excitability and hence labour initiation and progress. It has been suggested that obesity-related dyslipidaemia is also associated with adverse pregnancy outcomes. To date, no studies have examined the *in vitro* contractility in a dyslipidaemic model, nor examined the mechanism of action of adipokines, which a review of the literature shows are predominantly inhibitory on myometrial activity.

The aim of the first part of my thesis was therefore to determine if chronic dyslipidaemia results in altered myometrial contractility. ApoE<sup>-/-</sup> mice lack the *ApoE* gene and have high cholesterol and changes in adipokines. Using organ bath methods and measuring the parameters of contractility, it was found that there are no differences in contractility between non-pregnant wild-type and ApoE<sup>-/-</sup> mouse myometrium.

The second part of my thesis examined human obesity and its effects on myometrial contractility. Maternal obesity was not found to effect on the time to commencement of spontaneous contractions, nor on the parameters of spontaneous or oxytocin-induced pregnant human myometrium. These findings are discussed with respect to other previous work. It is suggested that maternal obesity is probably too broad category (unless very large numbers, preferably thousands, are studied, which is not practical for laboratory-based work) and should be refined, into for example, high adipokine levels.

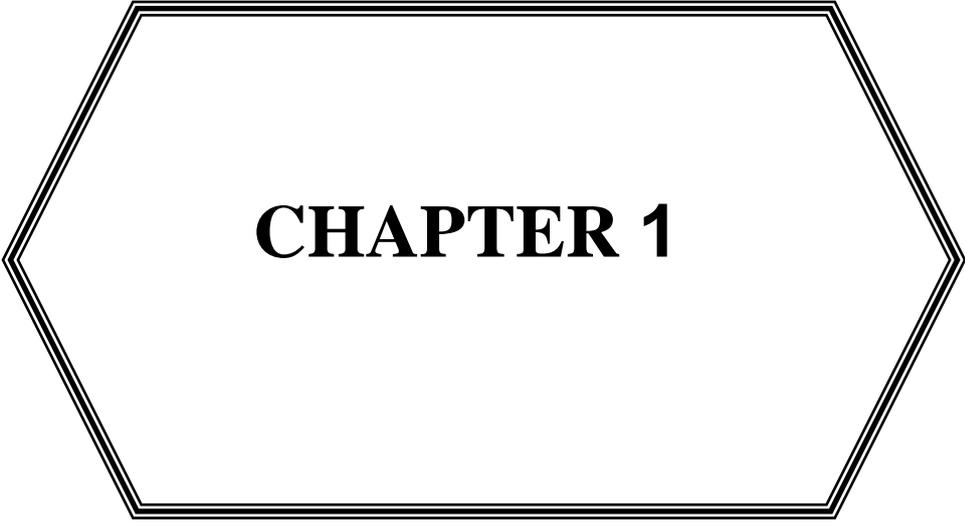
Visfatin is a novel adipokine which is increased with obesity. Its levels were found to be altered during pregnancy. My next aim was therefore to investigate the effects of visfatin on uterine contractility. One of my major findings in this thesis is that visfatin inhibited oxytocin-induced non-pregnant and pregnant mouse myometrial contractions *in vitro*. Then, the mechanism and pathway by which it was

inhibiting contractility was next investigated. Using organ bath experiments, it was found that NAD<sup>+</sup> pathway, was involved. My work is the first to show this in the uterus. This might have implications in the prevention and management of pregnancy-related complications in obese women, if visfatin levels or its targets can be decreased.

Having made these findings concerning visfatin, I next wanted to translate these findings to a clinical setting. Specifically, the hypothesis that high adipokine levels will be associated with poorer obstetric outcomes, and be higher in obese compared to normal weight women was tested. Then, the effect of plasma visfatin and leptin on maternal and neonatal outcomes of obese women was investigated, in a cross-sectional study. Plasma levels of visfatin and leptin were analysed by ELISA in pregnant women at the start of labour, with different BMI categories. It was found that maternal plasma leptin levels were positively correlated with increasing maternal BMI. Plasma visfatin levels were positively correlated with an increased risk of induction of labour. These findings further support the involvement of adipokines in modulation of myometrial contractility.

The final aim of my thesis was to test my idea that a contributory mechanism decreasing contractility in obesity is decreased gap junction expression. Gap junctions are essential to coordinated uterine contractions and successful parturition. Furthermore, the expression of myometrial gap junction proteins has been found to be altered with obesity in animals. To investigate this in human myometrium, the expression of gap junction proteins, Cx43 and Cx26, during pregnancy was examined in obese women and compared to normal weight women. Immunohistochemical analysis revealed that there is no association between maternal obesity and the expression of Cx43 and Cx26 in the myometrium. It is unclear why human and animal studies should produce different results.

My data indicates that there is likely to be a larger picture of alternative mechanisms that explain the clinically observed increased risk of pregnancy and delivery-related complications observed in obese women.



# **CHAPTER 1**

# Chapter 1: Introduction

This chapter introduces the adverse effects of obesity on the uterus in pregnant women, with a particular focus on adverse labour outcomes. The key anatomical and physiological features of the uterus and the myometrium, which are central to this thesis, will be described. This introduction will also highlight the emerging epidemic of maternal obesity, which poses a socioeconomic burden on a national scale. Dysfunctional labour as an outcome of obesity in pregnant women will be discussed, with regard to its pathophysiology with a focus on contractility. Part of this introduction was previously published as a review (AlSaif et al., 2015).

## 1.1 The uterus

### 1.1.1 Anatomy of the human uterus

The uterus is a major secondary sex organ of the reproductive system in humans and most other mammals. The human uterus is an inverted thick-walled, hollow, pear-shaped muscular organ, located in the middle of the pelvic cavity, between the urinary bladder anteriorly and the rectum posteriorly (Gartner and Hiatt, 2009). It weighs around 30-40g in non-pregnant women and increases up to 1 kg at term pregnancy. The normal adult uterus in nulliparous women measures approximately 7.0 – 9.0 cm long, 4.5 – 6.0 cm wide, and 1.5 cm thick (Umar et al., 2017). The uterine position can be anteroverted (most common) or retroverted and this can change after pregnancy (Gossman et al., 2019). The uterus is stabilised by several ligaments, including the broad ligament, round ligament, utero-ovarian ligament, cardinal ligament, and uterosacral ligaments (Kaniewska et al., 2018). These ligaments support the uterus and limit its movement within the pelvic cavity.

Macroscopically, the human uterus is composed of two main regions: the uterine body and the cervix (**Figure 1.1**) (Gartner and Hiatt, 2009). The body occupies the upper two thirds of the uterus and consists of three portions: the fundus, the corpus and the isthmus. The fundus is the broad rounded uppermost part of the uterus that leads into the fallopian tubes, terminating at the ovaries.

The corpus is the main largest region of the uterus which is connected to the cervix by the isthmus. The cervix is the lower barrel-shaped part of the uterus that connects it to the vagina. At the cervix, the muscular fibres are sparser. Instead, the cervix contains more connective tissue and mucus-producing glands. It becomes thinner during ovulation to allow the sperm to smoothly pass into the uterus. During delivery, the cervix dilates to allow the foetus to pass through the birth canal. This is brought about by contractions of the uterus.

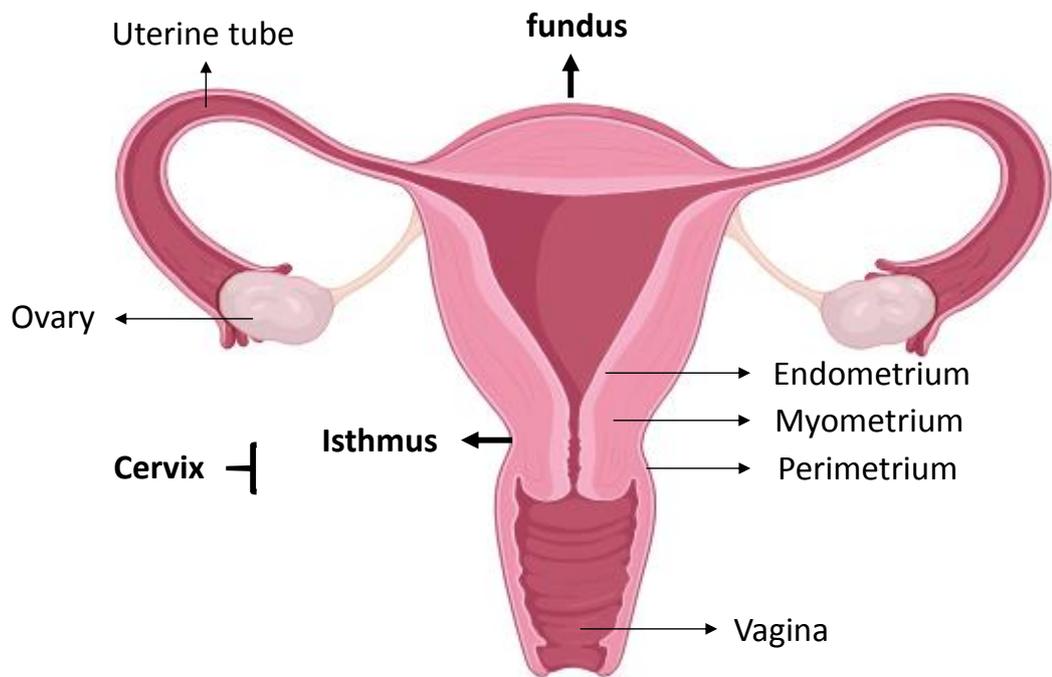
The uterus is composed of three basic layers: the innermost thin layer (endometrium), the thick muscular layer (myometrium) and the outer serous visceral layer (peritoneum) (Aguilar and Mitchell, 2010). The myometrium mostly consists of smooth muscle cells, where the excitation and contraction processes take place (Wray, 1993). The myometrium is the specific region of interest in this thesis (section 1.1.3). The endometrium is composed of an epithelial layer along with a mucus-secreting membrane which responds to reproductive hormones by changing its thickness during each menstrual cycle (Gartner and Hiatt, 2009). It is a non-excitabile layer, being a source of many cytokine hormones and metabolites, which both directly and indirectly can affect myometrial contractility.

The uterine blood supply is received mainly from the uterine artery, which is a branch of the internal iliac and hypogastric arteries on each side (Wray and Prendergast, 2019). It also supplied by the ovarian arteries which arise from the abdominal aorta. The myometrium is supplied by arcuate arteries deeply penetrating from the main uterine blood supply. These arteries disperse into further branches to the radial arteries, which terminate in the spiral arteries to supply the endometrium, decidua and the placenta during pregnancy. The uterine vasculature undergoes hypertrophy (an increase in the size of the existing cells) and hyperplasia (an increase in cell numbers) during pregnancy (Cipolla and Osol, 1994). This is due to the dramatic increase in the metabolic requirements of the pregnant uterus and the developing foetus. Classically, the uterine venous drainage matches the arterial blood supply. The uterine nerve supply is autonomic; sympathetic nerves from the inferior hypogastric plexus (T10–L1) supply the uterus and cervix and parasympathetic nerves mainly from the pudendal nerve (S2,3,4) supply the vagina and pelvic outlet (Morizaki et al., 1989).

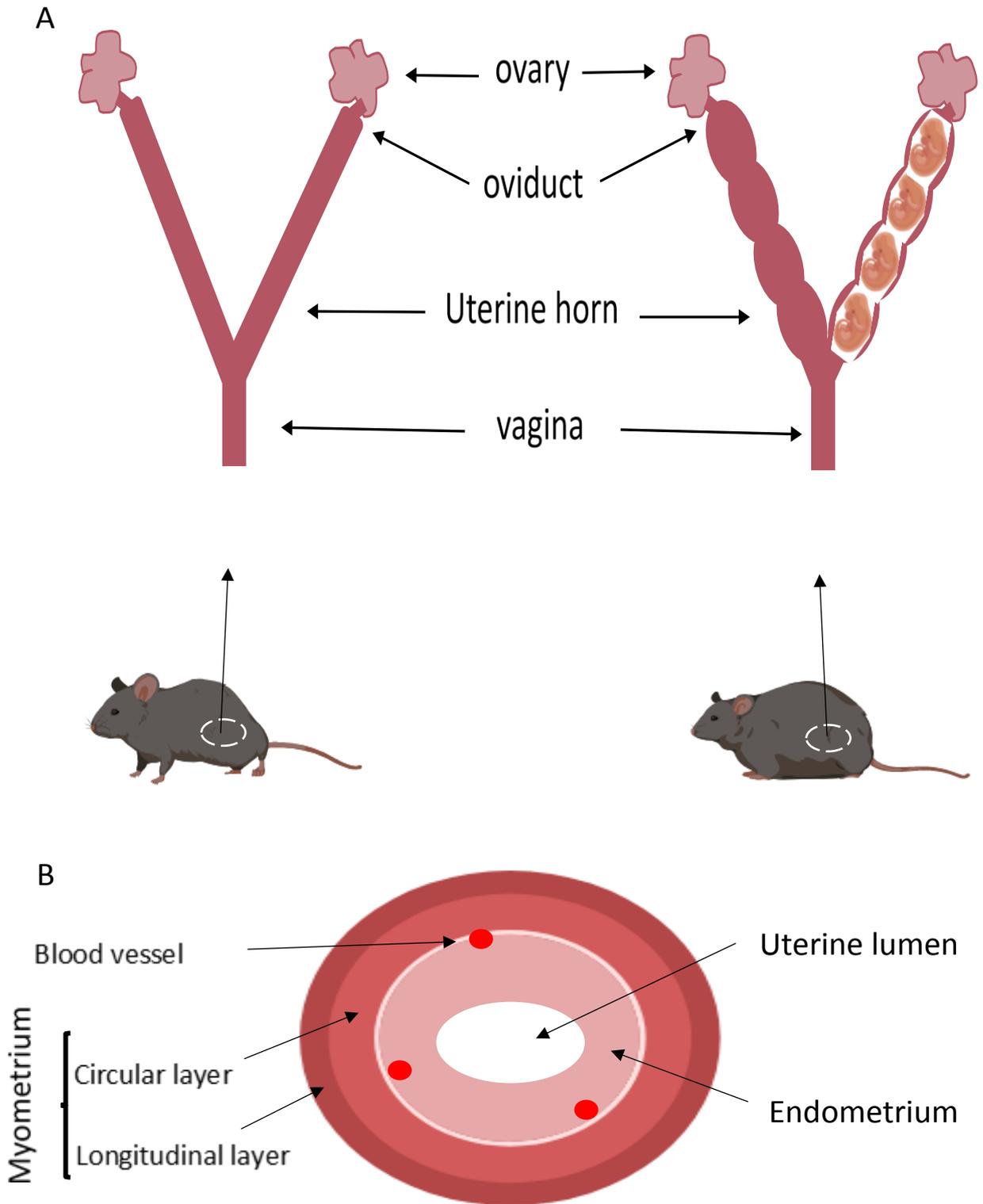
The uterus is a myogenic organ, which means that it is able to contract spontaneously without nervous or hormonal stimulation (Wray, 1993). It is also hormone-sensitive and its myometrial cells undergo both hypertrophy and hyperplasia during pregnancy, gradually returning to its original size within weeks after delivery (involution) (Monga and Sanborn, 2004).

### **1.1.2 Anatomy of the mouse uterus**

Mammalian species have different uterine morphological characteristics, which are reflected in functional differences, including reproductive and evolutionary adaptation. Unlike the human uterus, which is usually a single-chambered simplex structure, the mouse uterus is a double-chambered duplex organ which thereby allows multiple offspring (Croy et al., 2014) (**Figure 1.2**). It consists of two “horns”, which lead superiorly into the ovaries and inferiorly into the cervix. The uterine layers of the mouse are similar to those in human uterus, including the myometrium, which is composed of longitudinal and circular layers separated by the stratum vascularis (containing blood vessels).



**Figure 1.1 The structure of non-gravid human uterus.** Created by Biobender.com (an image library).



**Figure 1.2 Basic anatomy of mouse uterus.**

A) Gross anatomy of the mouse uterus, non-pregnant and pregnant.

B) Cross section of the non-pregnant mouse uterus.

### 1.1.3 The myometrium

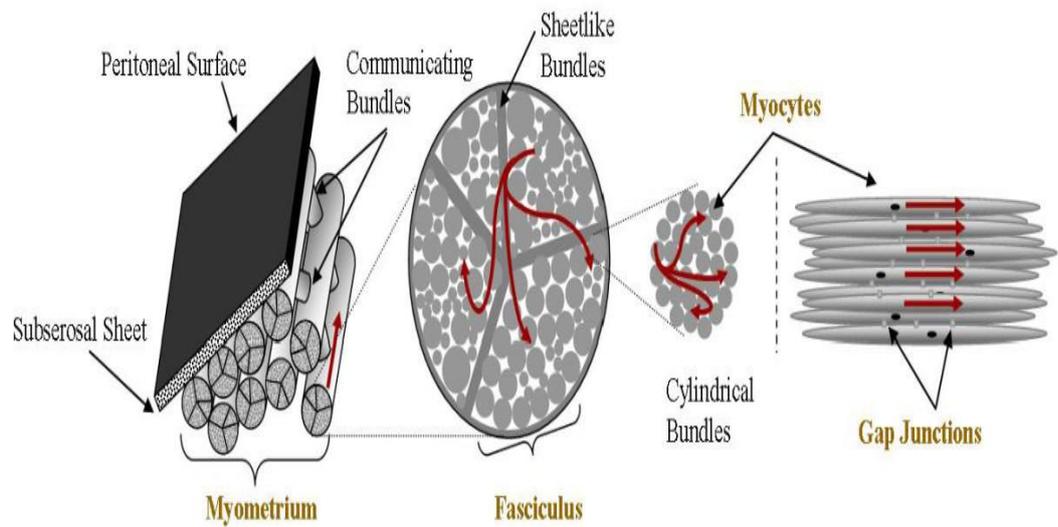
The myometrium is the middle thick layer of the uterine wall, consisting of uterine smooth muscle cells (myocytes), supporting stroma and vascular tissue (Young, 2007). It represents 90% of the uterine tissue mass and the majority of the uterine wall, with spontaneously active smooth muscle, which contract involuntarily (myogenic). Its main physiological function is to induce uterine contractions during labour.

The human myometrium can be subdivided into three poorly defined layers, each of which is arranged in a different direction: inner circular fibres, middle criss-crossing muscle fibres and outer longitudinal smooth muscles (Rudmann and Foley, 2013). The fibres of the outer layer are mostly longitudinal, while those of the inner layer are mostly circular. The middle layer contains large blood vessels - the stratum vasculare - which undergo marked thickening during pregnancy. The myometrial muscular fibres are arranged in spirals of different lengths and directions so as to increase their volume and capacity without causing pressure on the developing foetus (Henríquez Pino, 2017, Huszar and Naftolin, 1984, Young, 2007). The main layers are arranged into well-defined circular and longitudinal layers in the mouse and other non-primate species (Young, 2007, Huszar and Naftolin, 1984)

The largest macroscopically distinctive structures of the human myometrium are the fasciculi, which are collections of muscle bundles that control the contractile forces of the myocytes they contain (**Figure 1.3**) (Young and Hession, 1999). They are 1-2 mm in diameter and are supported by connective tissue comprised of fibrin, collagen and elastin. Fasciculi run parallel with the serosal surface of the uterus, obliquely down the anterior and posterior walls of the uterus, and transversely across both the fundus of the uterus and the lower uterine segment. Each fasciculus is composed of bundles of myocytes arranged into cylindrical, sheet-like fibres and communicating bundles.

During normal labour, the myometrium contracts, causing shortening of the uterus and reduction in the size of the uterine cavity (Garfield and Maner, 2007). As the myometrium contracts, it shows cycles of intermittent discrete contractions of varying amplitude, duration and frequency. It is these parameters of myometrial contractile activity that are coordinated to keep its main

physiological functions; they must, therefore, be fine-tuned if problems such as dysfunctional labour or preterm delivery are to be prevented.



**Figure 1.3 Microanatomy of pregnant human myometrium.** Red lines represent the spread of the contraction within the contracting myometrium. Taken from (Young and Hession, 1999).

### **1.1.4 The uterine myocytes**

Myocytes are the functional cells of the myometrium and are primarily responsible for the generation of contraction forces, propagation of action potentials and control of uterine contractility. They are also the dominant cell type within the uterus (Young, 2007). Their structure is typical of other smooth muscle cells, being elongated spindle-shaped cells tapered at the ends with a centrally located oval nucleus (Dawson and Wray, 1985). Their length varies from 30-50µm in non-pregnant myometrium to up to 500-600µm in pregnant myometrium (Broderick and Broderick, 1990, Blackburn, 2013). The nucleus is located in the centre of the cell and takes a cigar-like shape during muscle contraction. During pregnancy, the myocytes increase in size reaching a peak at term with a 10-fold increase in volume compared to their size at implantation (Blackburn, 2013). Myocytes are capable of acting as either pacemaker or pace-follower cells (Kao, 1959). Myocytes are connected to each other via gap junctions, which are discussed in section **1.1.4.2**

The myocyte contains thick myosin and thin actin myofilaments, which are found in long bundles spreading throughout the cell (Young, 2007, Wray, 1993). This random arrangement allows the myocytes to produce multidirectional contractions during parturition, irrespective of the position of the foetus. The plasma membrane of the myocyte is like any smooth muscle cell, it contains receptors, transporters, ion channels and structural proteins - providing means of transmitting signals. Smooth muscle cell also contains three main calcium regulatory proteins: calmodulin, caldesmon, and calponin. Calmodulin is the most important protein for contractility, as it is a calcium-binding protein that helps initiate the activation of contraction (Aguilar and Mitchell, 2010). Caldesmon and calponin have been connected to the reregulation of contraction (Carmichael et al., 1994, Yilmaz et al., 2013, Graceffa et al., 1996). The contractile machinery occupies the majority of the total myometrial cell volume; however the cells also contain several organelles related to contractility such as the sarcoplasmic reticulum and the mitochondria, which will be briefly discussed in the following sections (Broderick and Broderick, 1990).

#### **1.1.4.1 The mitochondrion**

The mitochondrion is a double-membrane-bound intracellular organelle located either scattered throughout the cytoplasm or near the nuclear pole (Broderick and Broderick, 1990). It occupies 3 to 9% of smooth muscle cell volume. In smooth muscle, mitochondria are proposed to play a role in regulating intracellular calcium concentrations  $[Ca^{2+}]_i$  during normal contraction (Babich et al., 2016, Gam et al., 2018, Gam et al., 2015, Gravina et al., 2011). They have the ability to take up, store and release  $Ca^{2+}$  and they are the main site for oxidative phosphorylation metabolism in all cells, including the myometrium (Gravina et al., 2010).

#### **1.1.4.2 The sarcoplasmic reticulum (SR)**

The smooth muscle contains a well-developed sarcoplasmic reticulum organelle (SR) that occupies around 2 to 7.5 % of the total smooth muscle volume (Somlyo, 1985). Like other smooth muscle, the myometrium possesses a SR that extends throughout the myocyte in a close association with the myofilaments and the nucleus (central SR) and plasma membrane near caveolae (peripheral SR) (Shmygol and Wray, 2004, Horowitz et al., 1996). The functional roles of the SR are to synthesise proteins (rough SR, which increases in volume during pregnancy) and to store and release calcium (smooth SR). Both were found to be present in the uterus (Ross and Klebanoff, 1971).

### **1.2 Gap junctions**

#### **1.2.1 Introduction**

Gap junctions, also known as connexons, are well-defined intercellular pores located between cells. They allow direct intercellular passage of inorganic ions, such as  $Ca^{2+}$ , in addition to small organic and signalling molecules (less than 1000 kDa in size), including cGMP, cAMP, and  $IP_3$  (Harris, 2007, Yeager and Harris, 2007). Furthermore, they allow the passage of electrical impulses between the cytoplasm of neighbouring cells (Alberts, 2008). They are known to play a role in the reproductive physiology of females. Gap junction alterations have been observed in obese high fat high cholesterol (HFHC) fed pregnant rats

at term pregnancy and during labour (Muir et al., 2016, Elmes et al., 2011). As described later, it was hypothesized in this thesis that one way obesity may affect uterine function, is by altering gap junction expression, hence it will be described in some detail what is known about them in the myometrium.

### **1.2.2 Structure**

Gap junctions are composed of connexins. Connexins are integral membrane proteins that form hexameric hemichannels containing N- and C-terminal cytoplasmic domains connected by four transmembrane domains, an intracellular loop and two extracellular loops (Figure 1.4) (Gerald and Elke, 2015). Each gap junction consists of two hemichannels, with each of them in turn composed of six connexin proteins. They are a type of intercellular channels composed of connexin proteins, also called gap junction proteins, that are distinct in their structure, function and tissue distribution (Sakai et al., 1992). Hundreds of gap junctions assemble along the cell membrane to form the gap junction plaque. Heterotypic channel is referred to a gap junction channel with different connexins, It was found that gap junctions can co-express more than one connexin isoform, giving rise to heteromeric gap junction (Nielsen et al., 2012).

### **1.2.3 Functions in reproduction and myometrium**

Multiple molecules, including glucose and amino acids, pass through gap junctions into the growing oocyte and between myocytes. Furthermore, gap junctions are involved in the embryo implantation (Edry et al., 2006, Winterhager and Kidder, 2015). A notable characteristic of gap junctions is that they have a relatively short half-life which found to be essential for the delicately regulated myometrial contractions (Laird, 2006, Berthoud et al., 2004). Most of the knowledge concerning gap junctions in the myometrium comes from the work of Garfield (Garfield et al., 1977)

Gap junctions allow the uterus to work as a functional syncytium, by providing low resistance regions between the myocytes to allow the spread of excitability and passage of ions and small molecules (Wray, 1993). Gap junction protein expression and size increase towards term pregnancy, reaching a peak at delivery (Xu et al., 2015, Sheldon et al., 2014), indicating their role in activation of the myometrium during labour. Indeed, there is a statistically significant

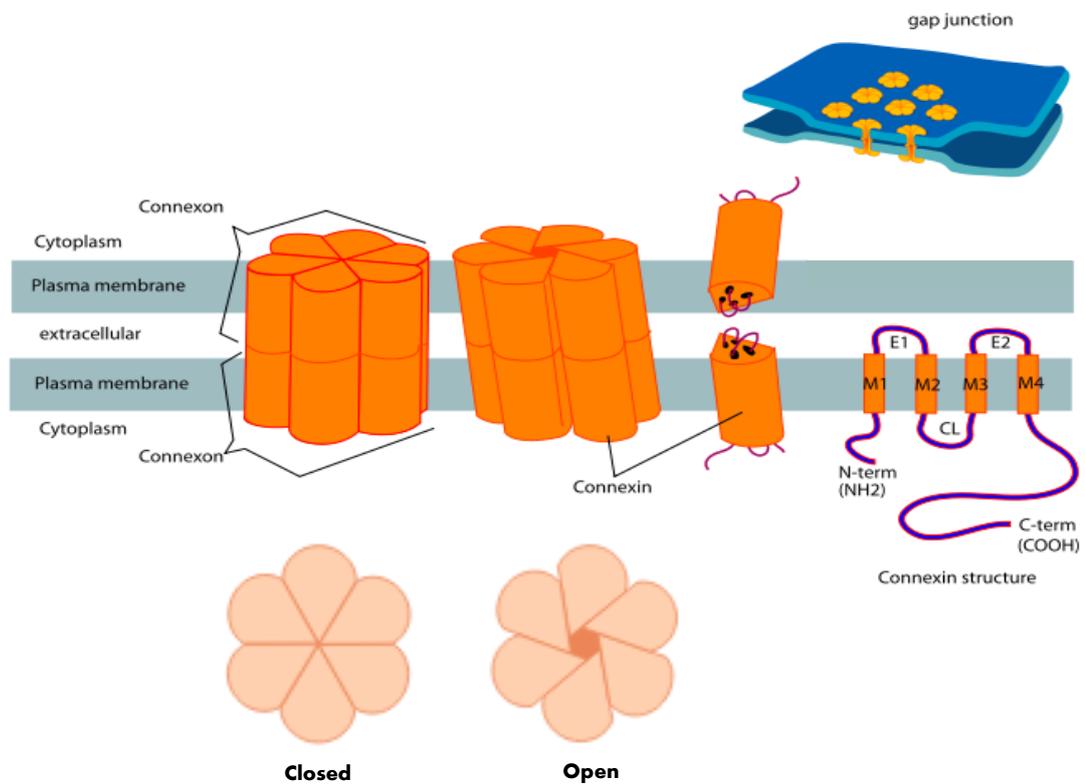
association between increased cervical dilation, increased frequency of uterine contractions and increased expression of myometrial gap junctions (Garfield and Hayashi, 1981). The majority of these gap junctions are degraded and disappeared within 24 hours post-delivery. Myometrial cells are electrically coupled by gap junctions, providing areas of low electrical resistance between cells, and, thereby, forming a gateway for efficient conduction of action potentials. Several studies have shown that when the gap junctions change in number, there is a concurrent change in metabolic and electrical coupling (Xu et al., 2015). Indeed, both gap-mediated intracellular communication and the myometrial ability to initiate action potentials are enhanced during parturition (Sakai et al., 1992, Miyoshi et al., 1996, Sheldon et al., 2014).

#### **1.2.4 Connexins**

There are 21 human gap junctions connexins (Willecke et al., 2002). The nomenclature of connexin subtypes is based on their relative molecular masses (Laird, 2006). Connexins can be subdivided into five groups ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , or  $\epsilon$ ) with respect to their length of the cytoplasmic loop and the extent of sequence identity (Nielsen et al., 2012). Several connexions are expressed in the human uterus including Cx43, Cx26, Cx40 and Cx45 (Elmes et al., 2011). Myometrial connexins are expressed differently during gestation. Cx43 is expressed in non-pregnant and early pregnant myometrium and increases significantly towards the end of pregnancy (Ou et al., 1997). Cx43 is the major component of gap junctions in the myometrium (Young, 2007). Cx26 is highly expressed during late pregnancy and decreases before labour onset. (Orsino et al., 1996). Cx40, on the other hand, is expressed in non-pregnant and early pregnant myometrium but is downregulated thereafter (Albrecht et al., 1996), while Cx45 is also expressed in the human myometrium; however, its role in contractility remains uncertain (Kilarski et al., 1998). There is evidence suggesting that different myometrial connexin subtypes are present within the same gap-junctional plaque, which has been suggested to be evolutionarily designed for complex modulation of intracellular communications during labour (Kilarski et al., 2001).

### **1.2.5 Regulation of gap junctions**

The uterine gap junction proteins are regulated mainly through endocrine mechanisms. Sex steroid hormones play a significant role in the regulation of gap junction proteins formation (Garfield et al., 1980). Maternal plasma changes in the ratio of progesterone and oestrogen hormones can influence the expression and the protein density of myometrial contractile associated proteins (MacKenzie and Garfield, 1985). Administration of exogenous progesterone at term was found to inhibit the expression of myometrial contractile associated proteins and myometrial contractility in ewe (Lye and Porter, 1978). In contrast, systemic withdrawal of progesterone in the myometrium induced contractile associated proteins' expression and stimulates myometrial contractility (Elmes et al., 2011). Steroid hormones may operate by modulation of prostaglandin synthesis or may directly affect gap junction protein formation (Garfield et al., 1977). Moreover, prostaglandins were found to have a role in the development of myometrial gap junction proteins (Garfield et al., 1977, Garfield et al., 1979). Garfield et al found that PGH<sub>2</sub> and arachidonic acid reverse the inhibitory effect of indomethacin on gap junction proteins formation in pregnant rats. On the other hand, PGE<sub>1</sub>, PGE<sub>2</sub> and PGF<sub>2a</sub> had no such effect (Garfield et al., 1980). Oestrogen, on the other hand, was found to greatly enhance the effect of myometrial stretch on gap junction formation (Wathes and Porter, 1982). The gap junction proteins' permeability was found to be upregulated by oxytocin, and downregulated by agonists that elevate intracellular cAMP e.g. prostaglandin E<sub>2</sub>, relaxin, isoproterenol and carbacyclin (Laird, 2010, Nielsen et al., 2012). Gap junction permeability is also regulated by protons which might have a link to dysfunctional labour (Swietach et al., 2007).



**Figure 1.4 Gap junction structure.** Each connexon consists of six connexin proteins and two hemichannels combine to form a gap junction. The pore formed by the connexin protein assembly which allows the transfer of ions and metabolites between cells. Adapted from molecular biology of the cell (Alberts, 2008).

### 1.3 Plasma membrane lipid rafts and caveolae

As the plasma membrane plays a key role in myocyte excitability and signalling, and is largely composed of lipids, its role or composition may be expected to change in obesity, for example with changes in expression of lipid transporters or cholesterol content of the membrane. The mammalian plasma membrane consists of three distinct structural classes of lipids: phospholipids, glycolipids and cholesterol. Phospholipids are the most predominant class and play a major role in forming the membrane lipid bilayer. Cholesterol is also a basic component of the plasma membrane and accounts for ~25% of lipids (van Meer et al., 2008). Cholesterol plays an essential role in cell signalling via both its effect on membrane fluidity and its association with lipid rafts and caveolae (Noble et al., 2006, Quest et al., 2004). Apolipoprotein E is an essential protein of the cholesterol transport system which promotes the clearance of lipoprotein remnants from the circulation, mainly very low density lipoproteins (VLDL), high density lipoproteins (HDL) and chylomicrons (Phillips, 2014). It protects from atherosclerosis and contributes to lipid homeostasis. A mouse model lacking the *ApoE* gene (*ApoE*<sup>-/-</sup>) in this thesis was used to study myometrial contractility in a hypercholesterolemic / dyslipidaemic model.

Lipid rafts, also called sphingolipid-cholesterol rafts, are localised subdomains of the plasma membrane that contain high concentrations of cholesterol and glycosphingolipids (Pike and Linda, 2003, Róg and Vattulainen, 2014). Cholesterol generally increases stiffness of the membrane bilayer and in regions where this is high, the decreased fluidity gives rise to the “lipid rafts” that float throughout the plasma membrane (Simons and Ikonen, 2000). Lipid raft mobility is found to be proportional to the cholesterol content (Pralle et al., 2000). Reductions in plasma membrane fluidity can influence the function of multiple membrane components in the vascular smooth muscle cells, such as Ca<sup>2+</sup> influx during the contraction–relaxation coupling (Tulenko et al., 1990). Lipid rafts have been implicated in cellular signalling transduction and excitation-contraction coupling within the smooth muscle tissue including the myometrium

Caveolae are cholesterol-rich microdomains that are classed as a type of lipid rafts forming structural omega-shaped invaginations of the cell membrane. These are driven by polymerisation of the protein, caveolin, instead of the lipid

raft GPI-anchored proteins (Quest et al., 2004). Caveolae also contain more free cholesterol in proportion to sphingolipids compared to lipid rafts (Pike et al., 2002). They are found to be abundant in smooth muscle cells (Gherghiceanu and Popescu, 2006, Taggart, 2001) and are the most stable lipid raft in the cell membrane (Thomsen et al., 2002). They significantly increase the cell membrane surface area by ~70% (Broderick and Broderick, 1990). Mechanistically, lipid rafts, including caveolae, express a wide range of ion channels, pumps, receptors and exchangers, which all modulate signal transduction, membrane trafficking and contractility (Head et al., 2014, Smith et al., 2005, Turi et al., 2001).

Caveolin is a 21-22 kDa tyrosine-phosphorylated substrate scaffolding protein expressed in both caveolae and trans-Golgi-derived vesicles (Rothberg et al., 1992). Three caveolin types have been identified (Caveolin 1-3); Caveolin-1 (Cav-1) is the most abundant caveolin in mammalian cells and is essential for the generation of caveolae (Vogel et al., 1998). It is a cholesterol-binding protein and an essential protein for caveolae formation (Murata et al., 1995). Cav-1 is expressed in several tissues, such as smooth-muscle cells, adipocytes, fibroblasts, endothelial cells and some epithelial cells (Williams and Lisanti, 2004). It was found to decrease at the onset of labour in rat myometrium (Muir et al., 2016). There are two isoforms of caveolin-1; caveolin-1 $\alpha$  and caveolin-1 $\beta$ . Cav-2 is closely co-expressed with Cav-1, which it requires for proper functioning; in contrast, Cav-3 is expressed abundantly in striated muscle cells (Song et al., 1996, Williams and Lisanti, 2004). Cav-1 and Cav-3 have been found to regulate Ca<sup>2+</sup> homeostasis in another smooth muscle (cerebral artery) (Kamishima et al., 2007). The strong binding of caveolins to cholesterol and sphingolipids in caveolae along with caveolin associations with the membrane cytoskeleton, results in the typical omega-shaped structure of caveolae (**Figure 1.5**).



## **1.4 The physiology of uterine contractility**

### **1.4.1 An overview**

It is essential to understand the normal uterine physiology governing its function during pregnancy, parturition and birth, to gain novel insight into methods of treating myometrium-related complications. When approaching the end of pregnancy, the uterus becomes capable of shifting from a quiescent state to an active contractile state that produces the powerful contractions needed for successful parturition. *In vitro*, the myometrium is capable of contracting spontaneously for days without hormonal or neuronal stimulation (Gullam et al., 2009, Wray, 1993). Myometrial contractility, which is a dynamic phasic phenomenon, is controlled by several membrane pumps, ion channels, transmitters and chemicals, and can be modulated by agonists and hormones (Noble et al., 2009). Multiple ions are involved in the maintenance and changes of the myocyte membrane potential especially  $K^+$ ,  $Cl^-$  and, crucially  $Ca^{2+}$ .

### **1.4.2 Uterine contractility in non-pregnant and pregnant myometrium**

There are functional and anatomical differences between the non-pregnant and pregnant human myometrium. The non-pregnant uterus is not a quiescent inert organ as it is able to initiate contractions to aid the sloughing of the inner endometrial lining of the uterus during menstruation, as well as to facilitate the passage of spermatozoa to the fallopian tubes. The uterine contractile patterns, however, differ between non-pregnant and pregnant myometrium. The former produces more focal and sporadic contractions (Togashi, 2007), giving rise to rhythmic and wave-like activity during the menstrual cycle (van Gestel et al., 2003). In contrast, during early pregnancy, the uterine contractions develop an irregular pattern of weak intensity to maintain the growing foetus; these then strengthen and become regular in pattern during labour to mediate delivery of the foetus and placenta

### **1.4.3 The myometrial membrane potential**

The myometrium is an excitable tissue characterised with a membrane potential that is determined by the gradient of ions across the plasma membrane,

and largely contributed to by  $K^+$  (Pehlivanoğlu et al., 2013). The action potential is characterized by cyclic depolarisation and repolarisation of the plasma membrane. The variations in the concentration and the membrane permeability of the ions on either side of the plasma membrane determine the resting membrane potential (RMP). At RMP, the ionic gradient is kept at a relative high intracellular  $K^+$  concentration and high extracellular  $Na^+$ ,  $Ca^{2+}$ ,  $Cl^-$  concentrations. The resting membrane potential measured in human uterus decreases towards term from approximately  $-70$  mV to approximately  $-45$  mV (Parkington et al., 1999, Nakajima, 1971).  $Ca^{2+}$  has the largest electrochemical gradient, which at resting state is  $10^4$  higher in the extracellular space than in the cell cytosol (Sanborn, 2000). The presence of intracellular ions with a net negative charge builds a potential difference, with the net negative intracellular potential. These dynamics in ionic movement across the plasma membrane regulate both spontaneous and agonist-induced contractions of myocytes in the non-pregnant and pregnant myometrium (Sanborn, 1995, Parkington and Coleman, 1990). The presence of multiple membrane proteins, such as pumps, channels and exchangers, also facilitates the ionic balance on both sides of the membrane. Ion channels open and close in response to certain changes in their local environment and can be modulated by alterations in membrane potential/voltage or binding of a specific agonist. Voltage-sensitive channels are largely involved in the RMP. The RMP recorded from the myometrium notably varies according to reproductive status, the species and the region of the myometrium from which electrical activity is recorded (Parkington and Coleman, 1988, Parkington et al., 1999).

Spontaneous membrane depolarisation was thought to be generated by pacemaker cells found in the myometrium (Shmygol et al., 2007a); however, the relative contribution of these cells to contraction within the myometrium remains uncertain. Upon depolarisation, voltage-gated  $Ca^{2+}$  channels open at the activation threshold of  $-60$  to  $-30$  mV (Triggle, 1996, Perez-Reyes, 2003). Thereafter, the influx of  $Ca^{2+}$ , perhaps with  $Na^+$ , occurs along the electro-chemical gradient. The increasing intracellular concentration of  $Ca^{2+}$  is the major component of action potential generation and subsequent myometrial contractions. Cell membrane repolarisation is then driven by inactivation of voltage-gated  $Ca^{2+}$  channels and  $K^+$  efflux occurs via activation of voltage-

sensitive potassium channels. (Wray, 1993, Wray et al., 2003, Parkington and Coleman, 2001).

## **1.4.4 Ion channels**

### **1.4.4.1 Voltage-gated calcium channels**

Voltage-gated calcium channels are a group of voltage-dependent ion channels expressed in the plasma membrane of excitable cells, with major permeability to  $\text{Ca}^{2+}$  ions (Yamakage and Namiki, 2002). In general, there are three types of voltage-gated calcium channels, two of which are found in the myometrium: L-type and T-type channels (Wray et al., 2003, Young et al., 1993). These are composed of a complex structure of four different subunits: an ion-conducting pore subunit,  $\alpha_1$ , and three other regulatory subunits;  $\alpha_2\delta$ ,  $\beta_{1-4}$ , and  $\gamma$  (Dolphin, 2006). The  $\alpha$  subunit confers the functional and pharmacological properties of the  $\text{Ca}^{2+}$  channel, in terms of ion permeability, voltage sensing and drug binding. The other subunits are classified as 'auxiliary'.

The main voltage-gated calcium channel subtype in the myometrium is the L-type and it is essential for excitation-contraction coupling (Lipscombe et al., 2004). So produces high voltage-activated and long-lasting currents; therefore, they account for the majority of the calcium current recorded in the myometrium. L-type calcium channel expression varies with pregnancy, suggesting a key role during labour (Collins et al., 2000, Tezuka et al., 1995, Mershon et al., 1994), and their function is regulated by sex hormones (Helguera et al., 2002, Batra, 1987, Okabe et al., 1999). Pharmacological inhibition of L-type  $\text{Ca}^{2+}$  channels abrogates myometrial action potentials, spontaneous myometrial contractions and  $\text{Ca}^{2+}$  transients (Coleman et al., 2000).

T-type calcium channels are low-voltage-gated and, thus, require a relatively small depolarisation to be activated, as well as being slowly deactivated (Catterall, 2011). They are composed of three major  $\alpha$  subunits (Cav3.1- Cav3.3) (Blanks et al., 2007). T-type calcium currents have been identified in the human myometrium and may be involved in the initiation of the action potential (Young and Zhang, 2005, Young et al., 1993), the regulation of spontaneous phasic contractions and the generation of  $\text{Ca}^{2+}$  transients (Lee et al., 2009). However it is open to debate about how much they contribute *in vivo*, as they are likely to be

inactivated at smooth muscle unlike neuronal resting membrane potential (Perez-Reyes, 2004).

#### **1.4.4.2 Potassium channels**

As mentioned earlier, activation of outward currents carried by potassium ions occurs during membrane repolarisation following an action potential. The primary function of potassium channels is to maintain the resting membrane potential close to the reversal potential of  $K^+$  ions ( $\sim -84\text{mV}$ ). It causes hyperpolarisation (or repolarisation), thereby terminating action potential generation and ultimately making contraction and cellular excitability less likely to happen (Khan et al., 2001). Multiple types of potassium channels have been identified in the myometrium, including the voltage-gated channel ( $K_v$ ), the ATP-sensitive potassium pump ( $K_{ATP}$ ), the calcium-activated potassium channels (BK, IK and SK) and at least one inward rectifier (Smith et al., 2007). Of these channels, BK channels are the most abundant potassium channel in both the pregnant and non-pregnant human myometrium (Perez et al., 1993, Khan et al., 1997); however, they have little functional role in the myometrium (Aaronson et al., 2006, Noble et al., 2010).  $K_v$  and  $K_{ATP}$  channels have been suggested to be involved in maintaining uterine quiescence and in the initiation of myometrial contractions (Knock et al., 1999, Brainard et al., 2007).

#### **1.4.4.3 Chloride channels**

$Cl^-$  channels are integral membrane proteins that specifically allow the movement of  $Cl^-$  ions across the cell membrane. Several studies have reported the existence of  $Cl^-$  channels in the myometrium (Adaikan and Adebisi, 2005, Arnaudeau et al., 1994, Yarar et al., 2001). Two chloride channels have been identified in smooth muscle cells; the calcium-activated chloride channel ( $Cl_{Ca}$ ) and a volume-regulated chloride channel (CIVR) (Nelson et al., 1997, Shi et al., 2007). These chloride channels can be activated by different mechanisms including changes in cAMP,  $[Ca^{2+}]_i$ , pH, extracellular ligands and cell swelling. Smooth muscle cells unusually have high  $Cl^-$  concentrations, thus if  $Cl^-$  channels open,  $Cl^-$  ions will move with its concentration gradient and cause membrane depolarisation.  $Cl_{Ca}$  is activated by  $Ca^{2+}$  entry through L-type  $Ca^{2+}$  channels (Jones et al., 2004); they are normally closed at resting free  $[Ca^{2+}]_i$  at  $\sim 100\text{nM/L}$

and are generally excitatory, providing triggers for signal transduction (Leblanc et al., 2005). CIVR, on the other hand, is activated by cell swelling during normal metabolism and is involved in depolarisation and the maintenance of contractile force (Mohammed Fahad Alotaibi, 2012).

#### **1.4.4.4 Sodium channels**

Fast Na<sup>+</sup> currents have been measured in pregnant rat and human myometrium. The cells possessing these channels found to increase Na<sup>+</sup> channel receptors close to parturition (Inoue and Sperelakis, 1991). Voltage-gated Na<sup>+</sup> channels (VGSC) were found to be able to mediate phasic contractions maintained over long periods of time in non-pregnant rats myometrium (Seda et al., 2007). The Na<sup>+</sup>-leak channel (NALCN) was found to produce the Na<sup>+</sup>-dependent leak current in myometrial cells (Reinl et al., 2015).

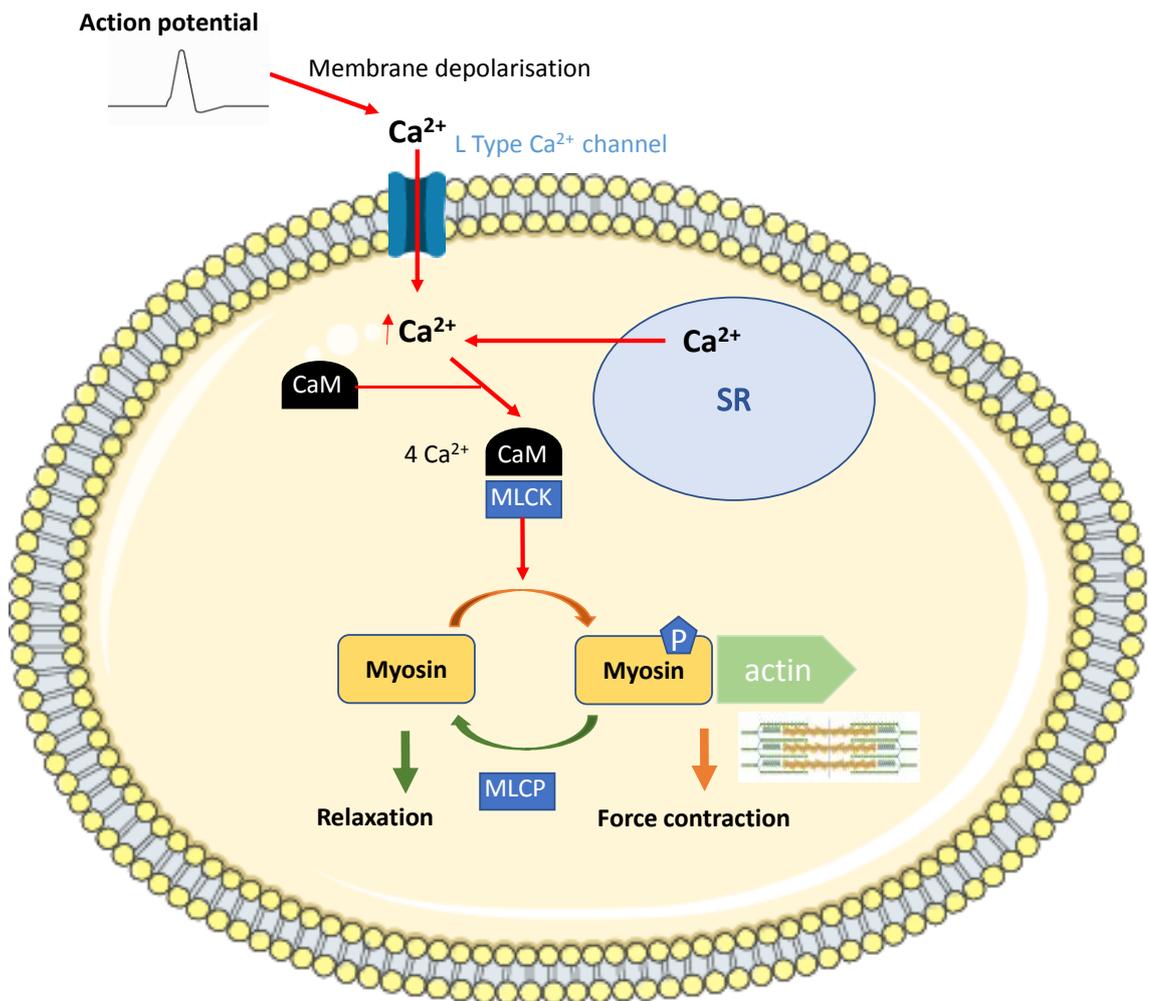
#### **1.4.5 Excitation - contraction coupling in the myometrium**

Excitation - contraction coupling (ECC) is the sequence of events, between the generation of an action potential and initiation of muscle contraction, and is predominantly regulated by [Ca<sup>2+</sup>]<sub>i</sub> (Pehlivanoğlu et al., 2013). The primary drive in the rise of [Ca<sup>2+</sup>]<sub>i</sub> is the depolarisation of the plasma membrane. Increased [Ca<sup>2+</sup>]<sub>i</sub> is crucial for myometrial contraction to occur. This is achieved by entry of extracellular calcium ions via voltage-gated L-type Ca<sup>2+</sup> channels and also their release from intracellular SR stores (Wray, 1993, Wray, 2007). Physiologically, the regulation of [Ca<sup>2+</sup>]<sub>i</sub> can be divided into the following phases: maintenance of basal concentrations to maintain the resting tone of the myometrium; a spike in [Ca<sup>2+</sup>]<sub>i</sub> that following agonist stimulation and resulting in contraction; and the restoration of [Ca<sup>2+</sup>]<sub>i</sub> to the resting state, following stimulation (Aguilar and Mitchell, 2010). These processes are basically regulated by instant alterations in ion channel permeability and pump and exchanger mechanisms.

Opening L-type calcium channels causes a significant calcium influx into the myocyte. It is the major source of calcium for contraction and if the channels are inhibited e.g. by nifedipine, there is no rise of calcium and no contraction in the uterus. As mentioned earlier, calcium binds to calmodulin (CaM) – a calcium-binding protein that binds four calcium ions. The calcium-CaM complex then activates myosin light chain kinase (MLCK), which is the main enzyme driving

contractility (Longbottom et al., 2000). When activated, MLCK phosphorylates serine 19 on the regulatory light chain of myosin (MLC), triggering the interaction between actin and myosin myofilaments and subsequent actin-myosin cross-bridge cycling. This is followed by hydrolysis of Mg-ATP and the initiation of contraction (Taggart et al., 1997) (**Figure 1.6**). Contraction is then abolished by reversing the above steps, starting with dephosphorylation of MLC by myosin light chain phosphatase (MLCP), deactivation of myosin, and subsequent inhibition of cross-bridge cycling and consequent cessation of contraction (i.e. relaxation). In addition, the L-type calcium channels inactivated and  $\text{Ca}^{2+}$  entry is terminated, leading to dissociation of  $\text{Ca}^{+2}$  from calmodulin.

Agonists can inhibit or augment ECC via multiple intracellular pathways that either increase or reduce  $\text{Ca}^{2+}$ . The sensitivity of the cellular myofilament to  $\text{Ca}^{2+}$  can be modulated in a process known as calcium sensitisation (Somlyo and Somlyo, 1998). MLCK has been shown to be phosphorylated by many kinases, leading to reduced activity and ultimately desensitisation of the contractile process. Phosphorylation of MLCP diminishes its activity and subsequently inhibits the inhibitor of MLC cross-bridge cycling, thereby sensitising the contractile machinery (Somlyo and Somlyo, 2003). MLCP is more involved in calcium sensitization. There is, unlike the case of vascular smooth muscle, no direct evidence for calcium sensitization in the uterus, thus when it has been sought and  $[\text{Ca}^{2+}]_i$  measured, there is no change in the relation between  $\text{Ca}^{2+}$  and force (Kupittayanant et al., 2001). Using indirect observations, others have suggested that oxytocin, promotes calcium sensitisation (McKillen et al., 1999)



**Figure 1.6 A schematic of excitation-contraction coupling in the myometrium.** The process starts with changes in the ionic permeability of the membrane by membrane depolarisation, triggering the initiation of action potential, opening of L-type  $\text{Ca}^{2+}$  channels and subsequent  $\text{Ca}^{2+}$  influx. Calcium binds to calmodulin (CaM) to form the calcium-CaM complex, which then activates myosin light chain kinase (MLCK) and leads to phosphorylation of the light chain of myosin (P). Phosphorylated myosin interacts with actin and stimulates cross-bridge cycling, thereby promoting uterine contraction. Relaxation, on the other hand, is mediated by dephosphorylation of light chain of myosin by myosin light chain

## **1.5 The regulation of intracellular $[Ca^{2+}]_i$ ( $[Ca^{2+}]_i$ ) in myometrial contraction**

A transient elevation in  $[Ca^{2+}]_i$  is the major trigger for smooth muscle contraction, as mentioned earlier (Shmygol et al., 2007a) and the key mechanism of calcium entry to produce spontaneous contractions is via L-type calcium channels in the myometrium. In addition, calcium can also enter the myocyte through receptor-operated calcium channels (ROCCs) and store-operated calcium channels (capacitative calcium entry) (Putney and Ribeiro, 2000). Furthermore, calcium can be removed from the cytoplasm by sequestration into the SR via the sarco/endoplasmic reticulum  $Ca^{2+}$ -ATPase (SERCA) or out of the cell by the plasma membrane  $Ca^{2+}$ -ATPase (PMCA) pump and the  $Na^{2+}/Ca^{2+}$  exchanger (NCX) (Matthew et al., 2004).

### **1.5.1 Calcium influx**

Calcium enters the cell predominately via L-type calcium channels, as detailed earlier. It can also enter the myocytes independently of membrane depolarisation via nonspecific cation- and receptor-operated channels. These channels are activated following binding of specific ligands/agonists. Some channels, when activated, promote myometrial contraction; agonists for these channels include oxytocin, prostaglandin FP and thromboxane. Other receptors, such as the  $\beta_2$ -adrenoceptor and prostaglandin EP2 promote relaxation via activation of cyclic AMP (Pehlivanoğlu et al., 2013).

Store-operated calcium channels (SOCCs) and  $Cl_{Ca}$  channels have also been implicated in  $Ca^{2+}$  influx. SOCC's are activated by depletion of the SR calcium, which results in capacitative calcium entry (CCE) or store-operated calcium entry (SOCE) (Putney and Ribeiro, 2000). This increase in calcium concentration contributes to myometrial contractility (Tribe et al., 2000). SOCE was found to occur in both the rat and human myometrium (Noble et al., 2009). These channels also belong to the transient receptor potential superfamily (TRP) (Petersen et al., 1995). Many types of channels have been found in the human myometrium, including TrpC1, TrpC3, TrpC4 and TrpC6 (Ku et al., 2006); they are functionally upregulated towards term and during labour (Noble et al., 2009, Dalrymple et al., 2004). As described earlier,  $Cl_{Ca}$  channels contribute to the

excitability in uterine myocytes, by triggering depolarisation via their opening, which can then lead to the opening of L-type calcium channels (Jones et al., 2004).

### **1.5.2 Calcium efflux**

As discussed,  $\text{Ca}^{2+}$  is removed from the myocyte by two mechanisms: the plasma membrane  $\text{Ca}^{2+}$ -ATPase pump (PMCA) and the  $\text{Na}^{2+}/\text{Ca}^{2+}$  exchanger (NCX) (Shmigol et al., 1998). While  $\text{Ca}^{2+}$  entry occurs down the electrochemical gradient,  $\text{Ca}^{2+}$  extrusion occurs against the concentration and electrical gradients, which is why it needs energy in the form of ATP, directly in the case of PMCA, and indirectly for the NCX. The recovery of calcium to resting levels was shown to be abolished if these mechanisms were inhibited (Shmigol et al., 1999). These are the two major mechanisms restoring  $\text{Ca}^{2+}$  to resting values following excitation and maintaining low resting concentrations. In the myometrium, the predominant mechanism responsible for calcium extrusion is PMCA; hence 65%-70% of the calcium efflux is attributed to it and the remaining 30%-35% is governed by NCX (Shmigol et al., 1998, Taggart et al., 1997). Both PMCA and NCX utilises the  $\text{Na}^{+}$  gradient created by  $\text{Na}/\text{K}$ -ATPase to operate the cellular extrusion of calcium. PMCA is also calmodulin-dependent with affinity to calmodulin depending on the isoform. The PMCA has a lower capacity and variable affinity (according to the isoform), while the NCX has a lower affinity for  $\text{Ca}^{2+}$  but holds a higher capacity of ions (Bradley et al., 2002).

### **1.5.3 Regulation of $[\text{Ca}^{2+}]_i$ by the sarcoplasmic reticulum (SR)**

#### **1.5.3.1 $\text{Ca}^{2+}$ release from the SR**

The myometrial SR contains two types of  $\text{Ca}^{2+}$  releasing channels; the inositol 1, 4, 5-triphosphate receptors (IP<sub>3</sub>Rs) and ryanodine receptors (RyRs). Conversely, the sarcoplasmic reticulum calcium-ATPase (SERCA) pump on the SR membrane sequesters  $[\text{Ca}^{2+}]_i$  into the SR lumen.

The myometrium can be activated both by changes in  $[\text{Ca}^{2+}]_i$  and also by agonists. Agonist-stimulation of the myometrium occurs via activation of specific receptors coupled to G-protein activates phospholipase C (PLC) and subsequently two second messengers, Inositol triphosphate (IP<sub>3</sub>) and

diacylglycerol (DAG), from hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PIP<sub>2</sub>). IP<sub>3</sub> binds to its receptors, IP<sub>3</sub>Rs, on the SR and activates the release of calcium from the SR into the cytoplasm, contributing to increasing [Ca<sup>2+</sup>]<sub>i</sub>. All IP<sub>3</sub>R isoforms (type1-3) have been observed in the pregnant and non-pregnant myometrium (Morgan et al., 1996), with variable sensitivities to both IP<sub>3</sub> and Ca<sup>2+</sup> (Mikoshiba, 2007).

The RyRs, as the name indicates, are sensitive to ryanodine and are primarily activated by local [Ca<sup>2+</sup>]<sub>i</sub> increase through Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR) (Curtis et al., 2008). All three isoforms of RyRs (RyR1-RyR3) have been identified in the uterus and their expression in the myometrium changes with pregnancy (Martin et al., 1999). Some interactions between IP<sub>3</sub>Rs and RyRs have been reported in smooth muscles (McGeown, 2004). Although Ca<sup>2+</sup> release from the SR through RyRs has been reported in cultured myometrial cells (Holda et al., 1996), based on the [Ca<sup>2+</sup>]<sub>i</sub> and molecular studies, as well as pharmacology and force measurements it has been shown that RyRs play no role in myometrium (Taggart and Wray, 1998, Kupittayanant et al., 2002, Matsuki et al., 2017, Dabertrand et al., 2007, Mironneau et al., 2002). Consistent with this, there are no Ca<sup>2+</sup> sparks or spontaneous transient outward currents in myometrium (Burdyga et al., 2007).

### **1.5.3.2 Ca<sup>2+</sup> uptake into the SR**

Cytosolic [Ca<sup>2+</sup>]<sub>i</sub> is always maintained at relatively lower levels compared to the extracellular and SR-luminal concentrations. Therefore, active transport via SERCA is required for the uptake of Ca<sup>2+</sup> into the SR, against its electrochemical gradient (Moller et al., 1996, Marin et al., 1999). This utilises ATP with a counter transport of protons (Levy et al., 1990). Three isoforms of the SERCA pump have been identified: SERCA 1, SERCA 2 and SERCA 3 (Wray and Burdyga, 2010). SERCA isoforms 2a and 2b (housekeeping SERCA) and 3 have been identified in both the animal and human myometrium, including the labouring myometrium (Tribe et al., 2000). It was found that blocking SERCA with cyclopiazonic acid and thapsigargin enhances myometrial contractions (Burdyga and Wray, 1999, Tribe et al., 2000). Although the SR has a large capacity for taking up and storing calcium, inhibition of other calcium extrusion mechanisms (such as PMCA and NCX) in single cells, shows that SERCA was unable to replace their function

(Shmigol et al., 1999). The activity of PMCA and NCX, but not SERCA, has been shown to be influenced by changes in sarcolemmal cholesterol levels (Ortega and Masoliva, 1984, Kutryk and Pierce, 1988).

## **1.6 The physiology of human pregnancy - a brief overview**

The uterus is a vital reproductive organ that enables successful pregnancy and parturition to take place. It regularly accepts the fertilised ovum and provides a fortified environment for the process of endometrial implantation, after which it undergoes periods of hypertrophy and hyperplasia to compensate for the increasing metabolic requirements of the growing foetus. The uterus provides nourishment, blood supply and mechanical support to the foetus throughout the gestational period. As pregnancy approaches term, the uterus undertakes multiple preparatory processes for the onset of labour contractions.

The average length of a human pregnancy is 40 weeks (280 days) from the first day of the woman's last menstrual period (LMP). Nevertheless, only about 4% of women actually give birth on their expected due date and the majority deliver between 37-42 weeks. Whilst preterm delivery is typically classified as delivery prior to 37 weeks, and post-term delivery is defined as delivery after 42 weeks, term has been traditionally defined as delivery occurring between 37 and 42 weeks. Although LMP and symphysio-pubis fundal height (SFH) are common methods of estimating the gestational age, foetal biometry using ultrasound in early pregnancy is the most accurate method (Whitworth et al., 2015, Taipale and Hiilesmaa, 2001) and is an integral part of the obstetric assessment (March et al., 2012). Normal uncomplicated pregnancy is defined as a singleton gestation without maternal or foetal risk factors.

## **1.7 The process of parturition and labour**

Normal parturition is divided into four phases: myometrial quiescence (prelude to labour), activation (preparation for labour), stimulation (the process of labour) and recovery (involution). Physiological changes of each parturition phase are summarised in **Table 1.1**. These phasic interactions include maternal and foetal hypothalamic-pituitary-adrenal axis activation, inflammation and myometrial stretch (Kota et al., 2013). Labour is the physiological process of delivering the baby, the placenta, the umbilical cord and the accompanying

membranes (the products of conception) from the uterus via the birth canal. Normal labour is defined to be spontaneous delivery at term, natural in onset, remaining low-risk from commencement to delivery, with persistent vertex presentation of the baby. Both maternal and neonatal health should also be good during pregnancy and following childbirth (WHO, 2011a).

Several measurable parameters of labour are commonly used to assess both the physiology and progress of labour, including uterine contractions, cervical dilation, descent of the presenting part, maternal and foetal observational recordings (partogram) (Gould, 2000). The labour process is continuous, although it comprises three main stages – known as the first, second and third stages of labour. These phases primarily correspond to the myometrial and cervical physiological changes during delivery as a result of mechanical, biochemical and hormonal changes (Norwitz et al., 1999, Irani and Foster, 2015). The active stage of labour refers to the first and the second stages collectively. It is clinically considered that there is a physiological variation in the length and progression of labour between mothers, especially in early labour (Kominiarek et al., 2011, Zhang et al., 2010b, Zhang and Duan, 2018). Parturition in literature frequently refers to the process of labour.

**Table 1.1 Phases of parturition.** CRH; corticotropin-releasing hormone, ACTH; adrenocorticotrophic hormone, COX2; cyclooxygenase-II enzyme.

Phase	Phase 1 Quiescence	Phase 2 Activation	Phase 3 Stimulation	Phase 4 Involution
<b>Function</b>	Prelude to labour	Preparation of labour	Process of labour	Parturient recovery
<b>Duration</b>	Most the time of pregnancy (95% of gestation)	Over weeks in the late 3rd trimester	From start of labour to delivery	Lasts until restored fertility
<b>Clinical changes</b>	Contractile unresponsive Phase and cervical softening	Increased myometrial responsiveness to contractile stimuli associated with cervical ripening	Uterine contractions, cervical effacement and Dilatation associated with Foetal and placental delivery.	Uterine involution, breast feeding associated with cervical repair and healing
<b>Hormonal changes</b>	Myometrial suppressing hormones (Progesterone, relaxin, prostacyclin).	Oestriol, prostaglandines, placental CRH, foetal ACTH, foetal cortisol and COX 2.	Prostaglandins and oxytocin	Mainly oxytocin (vasoconstriction) and Inflammatory pathways initiated.

### **1.7.1 First stage of labour**

The first stage of labour commences from the onset of uterine contractions to full cervical dilatation. It commonly lasts 8-12 hours in primiparous and 3-8 hours in multiparous women. First stage of labour is classically divided into two phases; the latent and the active phases. The latent phase precedes the active phase, whereby the cervix softens, is partially effaced and dilates to 3-4 cm with irregular uterine contractions. This is followed by the active phase, whereby the painful uterine contractions become regular, with a substantial degree of cervical effacement, and this lasts until the full dilatation of the cervix. The latent phase is longer and can last several hours, while the active phase is shorter and lasts 2-3.5 hours. The normal rate of cervical dilatation during the active phase is 1cm/hour (Friedman, 1955); however, this varies significantly according to the population, parity, foetal weight and head circumference, whether the labour is spontaneous or induced and the use of epidural anaesthesia (Juhasova et al., 2018). A prolonged latent phase of labour occurs in 5-6.5% of women. Prolongation of this stage is highly associated with adverse maternal and foetal outcomes.

### **1.7.2 Second stage of labour**

The second stage of labour, also known as the pushing stage, usually begins from full dilatation of the cervix and lasts until the delivery of the baby. The duration of this stage is variable; it can last 1-2 hours in primiparous and 0.5-1 hours in multiparous women. This stage can be further subdivided into two phases: the first is an early passive phase, which starts from the full dilatation of the cervix until the passive descent of the foetal head through the maternal pelvis. This stage is followed by the active (expulsive) phase, which begins when contractions become expulsive and the pushing actively starts. A prolonged second stage of labour in nulliparous women is defined lasting around 2-3 hours with epidural analgesia (American College of Obstetrics and Gynecology Committee on Practice Bulletins-Obstetrics, 2003).

### **1.7.3 Third stage of labour**

This is the last stage of labour, commencing at the time of complete neonatal delivery and lasting until the complete delivery of the placenta and

membranes. It commonly lasts up to an hour, if spontaneous, and 5-15 minutes if manually managed. Delivery of the placenta is often associated with strong contractions, vaginal bleeding during placental separation and umbilical cord lengthening. The basic physiology of the third stage of labour is still not well understood. The most common complication occurring during this stage is postpartum haemorrhage, which can be prevented or controlled by active management of the third stage of labour – this can involve proper clamping and cutting of the umbilical cord and administration of uterotonic agents, including oxytocin (Güngördük et al., 2018).

## **1.8 Induction of labour**

Induction of labour (IOL) is a process in which the uterus is artificially promoted to initiate labour for the purposes of improving the quality of the delivery outcomes and reducing maternal and neonatal morbidity and mortality (Organization, 2011). IOL is recommended when the risks of waiting for the onset of spontaneous labour outweigh the risks associated with shortening the duration of pregnancy by induction. The most common indications of IOL is post-term pregnancy, which is at the top of the list, followed by maternal hypertension and suspected foetal compromise, as well as premature rupture of the membranes (Alhazmi et al., 2018, MacKenzie, 2006). IOL for postdates, maternal hypertension, GDM and LGA were found to be associated with an increasing risk of C-section delivery (Mei-Dan et al., 2017).

There is a strong evidence that maternal obesity is contributing to the rising rates of induction of labour in pregnant women (O'Dwyer et al., 2013, Farah et al., 2009, Ellis et al., 2019). It was recently suggested that a high BMI at is a risk factor for IOL during a late-term pregnancy (Ferrazzi et al., 2019). Two distinctive methods of labour induction are commonly used, frequently in combination: pharmacological agents and mechanical stimulation to stimulate cervical effacement, dilatation and eventually uterine contractions (MacKenzie, 2006). Many pharmacological agents are used for IOL; however, oxytocin and prostaglandins are the most common used agents in IOL (WHO, 2011b).

## 1.9 Prolonged/dysfunctional labour

### 1.9.1 An overview

The exact definition of prolonged labour (or dysfunctional labour) is controversial. The term usually refers to a prolonged duration of the active phase of labour and was defined by WHO as the failure of the presenting part of the foetus to progress into the birth canal, despite strong uterine contractions (Hofmeyr, 2004). The National Institute for Health and Care Excellence (NICE), on the other hand, defined prolonged labour as cervical dilatation of less than 2 cm in 4 hours in first-time labour. The American Pregnancy Association define prolonged labour as lasting  $\geq 20$  hours in nulliparous women and  $\geq 14$  hours in multiparous women (Albers, 1999). It was also defined previously as incurrence of cervical dilatation in 2 hours or a dilatation rate less than 1 cm/hour in nulliparous women; however, this definition has led to over-diagnosis of prolonged labour (Neal et al., 2010). Prolonged duration of labour is commonly described as failure of progress, dystocia, as well as dysfunctional or obstructive labour. It is also the most common cause of emergency C-section deliveries.

It is estimated that almost 8% of all women giving birth will experience prolonged labour during delivery and the majority of these will be first-time labours; however, this estimation varies between the populations (Nystedt and Hildingsson, 2014, Wray, 2007). Prolonged labour is mainly depend on subjective information on the timing and strength of uterine contractions self-reported by pregnant women. However, it can be diagnosed using a partogram (Lavender et al., 2013). A partograph is a graphical record of key maternal and foetal changes during labour, which are plotted against time. The frequency of prolonged or augmented labour can also significantly reduced by using a partogram (Javed et al., 2007).

Harper *et al.* demonstrated that prolonged labour is associated with a higher risk of maternal fever, shoulder dystocia, and other adverse neonatal outcomes (Harper et al., 2014). Consistent with Harper *et al.* findings, Sandstrom *et al.* found that prolonged labour and pushing are associated with higher risk of adverse neonatal outcomes (Sandström et al., 2017). In contrary, Cheng *et al.* indicated that nulliparous women with prolonged active labour had higher rates of C-section deliveries with no observed adverse neonatal outcomes (Cheng et

al., 2010). It was also found that a prolonged second stage of labour correlates with successful vaginal delivery rates, although with a minor increase in maternal and neonatal morbidity and perinatal mortality (Laughon et al., 2014). Many factors were recognised to influence the length of labour, such as parity, maternal age, foetal weight and position, pelvic size and shape, duration of pregnancy, pre-pregnancy weight and gestational weight gain (Piper et al., 1991, Nesheim, 1988, Greenberg et al., 2007, Allen et al., 2009, Senecal et al., 2005).

### **1.9.2 Pathophysiology of uterine contractions in dysfunctional prolonged labour**

In dysfunctional labours not complicated by foetal or maternal anatomical complications, the cause must reside with the myometrial contractions. Quenby *et al.* were the first to show that in myometrial capillary blood there was an acidosis, increase in lactate and reduction in O<sub>2</sub> saturation, without systemic changes, in labouring women (Quenby et al., 2004). The pathophysiology of uterine contractions in prolonged labour was thoroughly explained previously (Neilson et al., 2003). The metabolic requirements can be compensated in a healthy mother, if however a labour is prolonged, the uterus will steadily deplete its metabolic reserves of glycogen and ATP, which ultimately builds an acidic environment due to ATP depletion, anaerobic metabolism and systemic ketoacidosis. Myometrial lactic acidification was proven to reduce, and/or further abolish, rat and human uterine contractility (Hanley et al., 2015, Taggart and Wray, 1995, Taggart and Wray, 1993, Parratt et al., 1995) along with changes in intracellular Ca<sup>2+</sup> underlie these changes in contractility (Pierce et al., 2003). Indeed, the pathological mechanism underlying obstructed labour in parous women has proven relatively difficult to elucidate; hence, they developed adaptive reactions to the effects of acidification. Thus bicarbonate, by neutralising the acid in the myometrium, was found to reduce threatened dysfunctional labour and lactate in the amniotic fluid is now a marker for dysfunctional labours (Wiberg-Iltzel et al., 2018, Wiberg-Iltzel et al., 2014, Sterpu et al., 2018).

### **1.10 Mouse pregnancy and parturition**

The average mouse life-span is approximately 24 months; however, its reproductive life span is appreciably shorter, at 7-8 months (Wilkinson et al.,

2012). Many different factors can influence mouse reproduction, including age, strain, breeding environment, diet, general health and pheromones (Baker et al., 1932, Dominic, 1966, Franks and Payne, 1970, Keveme, 1983). Mice of both sex frequently reach sexual maturity at 4-7 weeks of age (Nelson et al., 1990). The mouse oestrous cycle is short (4-5 days) and ovulation usually occurs 8-12 hours after the onset of the cycle. If fertilisation occurs, developing embryos can be palpated by the 14<sup>th</sup> day of gestation.

Mouse pregnancy usually lasts for a couple of days. The gestation period for the pregnant mouse varies from 18 to 22 days but varies between mouse strains and within a strain as well (Atchley, 1991). The C57BL/6J mouse strain which has an 18.5-day gestation period was used in this thesis. Multiple factors can affect the duration of pregnancy in mice. As with humans, it has been established that there is an inverse relationship between the gestation period and the number of litters in mouse (Dewar, 1968, Biggers et al., 1963). Unlike human pregnancy, where the average gain in weight is approximately 20% of the pre-pregnancy body weight, the total weight gained during pregnancy in mice is altered according to the number of mice in the litter – ranging from an average of 5.5 g (30%) for litters of one mouse up to an average of 19.5 g (70%) for litters of ten mice (Fekete, 1954).

Inbred females have a tendency to have longer pregnancies than non-inbred females; hence non-inbred females tend to produce larger litters and pups. The average pup number is 4-12. The gestation period can be greatly prolonged when the pregnant mother continues to nurse a previous litter. When mice are maintained under a standard light-dark cycle, mating typically occurs at night and birth occurs frequently between the hours of midnight and 4:00 A.M; nevertheless, it can also occur anytime of the day or night. Parturition usually last 1-3 hours in mouse and the mouse becomes sexually receptive 24 hours after parturition.

## **1.11 Maternal obesity**

### **1.11.1 Prevalence**

Maternal obesity has become a concern due to the noticeably high risk of obesity-related complications in the pregnant mother and her child. The

prevalence of maternal obesity is increasing at an alarming rate worldwide. It is estimated that 13% of the world's population is obese and this prevalence has almost tripled in adults over the last 40 years, with the highest rate observed in women of child-bearing age (WHO, 2018). According to the 2019 NHS statistics, 30% of women are obese and 29% of these have increased health risks associated with obesity (National Health Service, 2019). Around one in five women booking for antenatal care in the UK are clinically obese (Kanagalingam et al., 2005, National Health Service, 2016). The healthcare cost of obesity poses a considerable burden on Western economies (Specchia et al., 2014, Morgan et al., 2014), especially in case of morbid obesity (Grieve et al., 2013). Indeed, it was estimated that this cost is increased five-fold in obese women compared to women who are non-obese (Schneid-Kofman et al., 2005).

### **1.11.2 Definitions and measurements**

According to the WHO, obesity is defined as excessive or abnormal fat accumulation that presents a risk to health (WHO, 2000). There are many ways in which a person's health in relation to their weight can be classified, including body mass index (BMI), waist circumference, waist-to-hip ratio and skinfold thickness. BMI is a crude population measure of obesity and is widely used in clinical research. It is calculated by dividing the individual's weight in kilograms by the square of his or her height in meters ( $\text{kg}/\text{m}^2$ ). BMI is inexpensive and easy to measure however, it is not a perfect measure as it does not accurately assess body fat. It does not distinguish between body fat and lean body mass. Bones and muscles are denser than fat, so a muscular person or an athlete may have a high BMI, yet not have too much fat. However, as most people are not athletes, for most of the population BMI is a very good representative of obesity. Particularly useful for my studies is that women have had their height and weight measured at their first antenatal visit, worldwide for many decades. This means information about obesity can be viewed and assessed with a degree of confidence from international sources. In addition, unlike other obesity measures, BMI has standardised cut-off points for being overweight vs obese (**Table 1.2**). Thus, BMI is more useful in clinical studies as an indicator of health problems related to increased body fat. Maternal obesity during pregnancy is defined as a  $\text{BMI} \geq 30 \text{ kg}/\text{m}^2$  at the first trimester of pregnancy (Modder, 2010).

**Table 1.2 WHO classification of BMI (kg/m<sup>2</sup>)**

<b>BMI Class</b>	<b>BMI (kg/m<sup>2</sup>)</b>
Underweight	<18.5
Normal weight	18.5-24.9
Overweight	25-29.9
Obesity class I	30-34.9
Obesity class II	35-39.9
morbid obesity	≥40 Obesity class III

### 1.11.3 Gestational weight gain

Gestational Weight Gain (GWG) is defined as the total amount of weight gained during pregnancy. The extent of weight gained during pregnancy can influence the short and long-term health of both the pregnant woman and her child. Optimal weight gain during pregnancy is physiologically essential for a healthy pregnancy to compensate for the growing foetal requirements. It usually consists of approximately 8 kg of water, representing increased amniotic fluid and plasma volume, 1 kg of maternal, placental, and foetal protein lean mass and additional variable amounts of adipose tissue (1–6 kg) (Institute of Medicine and National Research Council Committee to Reexamine, 2009).

Insufficient GWG, regardless of pre-pregnancy BMI, was found to be associated with preterm birth, low birth weight, small for gestational age (SGA) babies and infant death (Wen and Lv, 2015, Davis-Moss and Hofferth, 2018, Ikenoue et al., 2018, Blomberg, 2011a), whilst excessive weight gain was found to be associated with large for gestational age (LGA) infants, postpartum weight retention and preeclampsia (Scifres et al., 2014, Devlieger et al., 2016, Cedergren, 2006, Siega-Riz et al., 2009).

In 2009, new guidelines on recommended GWG were published by the American Institute of Medicine (IOM); these, based on pre-pregnancy BMI, indicate that the GWG should be lower with increasing BMI (**Table 1.3**) (Rasmussen et al., 2009). Excess GWG beyond American Institute of Medicine guidelines in obese women will result in increased adiposity rather than lean body mass weight (Berggren et al., 2016). However, Bodnar *et al.* observed that women with severe pre-pregnancy obesity would benefit from lower GWG than recommended by the IOM (Bodnar et al., 2010).

Maternal obesity was shown to influence pregnancy outcomes more than excessive increase in GWG (Nohr et al., 2008, Nelson et al., 2010); however, the predicted risk for most adverse pregnancy outcomes in obese pregnant women is exacerbated by profound GWG (Kapadia et al., 2015). Based on population studies, weight gain during pregnancy has an inverse relationship with maternal pre-pregnancy BMI, with an increased risk in young nulliparous women (Chu et al., 2009). Low GWG has been shown to improve pregnancy outcomes by decreasing the risk of preeclampsia, LGA and SGA neonates and C-section

delivery (Institute of Medicine and National Research Council Committee to Reexamine, 2009).

**Table 1.3 American Institute of Medicine guidelines (IOM) for total GWG for women with singleton pregnancy.**

<b>Pre-pregnancy BMI class</b>	<b>Recommended GWG (kg)</b>
Underweight	12.5-18
Normal weight	11.5-16
Overweight	7-11.5
Obese	5-9

#### 1.11.4 Mouse obesity

Unlike for humans, there is no defined BMI for mice. Obesity is usually based on increased adiposity – i.e. the ratio of fat mass (FM) per fat-free mass (FFM). There are several models of obesity currently used for research purposes (Lutz and Woods, 2012) and most of them have monogenic mutations in the leptin pathway (gene or receptor). Circulating leptin levels are increased in obese humans and also in obese mouse models (Frederich et al., 1995). Diet- induced models are also frequently used in research. These mice are often used as preclinical experimental models for obesity research. Sever obesity mouse models are frequently produced by mutation of leptin, *LEPR* (leptin receptor) and *MC4R* (melanocortin-4 receptor) genes (Fairbrother et al., 2018). The C57BL/6J mouse is the most frequently studied model of polygenic obesity (Chu et al., 2017, Scroyen et al., 2013).

Apolipoprotein E (ApoE) is an arginine-rich apolipoprotein with a molecular mass of 34 000 Da (Shore et al., 1973). The Apolipoprotein gene family (*Apo*) is highly implicated in lipid metabolism. There are five *Apo* genes: *ApoA*, *ApoB*, *ApoC*, *ApoD* and *ApoE*. Obesity was found to be pertinent to ApoE, which is found in all lipoproteins (except for low-density lipoprotein (LDL)) and is involved in the clearance of circulating cholesterol and triglycerides (TG) (Ferreira et al., 2011, Kashyap et al., 1995). Mice lacking *ApoE* gene (*ApoE*<sup>-/-</sup> mouse) develop hypercholesterolemia, followed by obesity; thus, they are widely used as a model of hyperlipidaemia and atherosclerosis (Pendse et al., 2009, Kypreos et al., 2009, Huang et al., 2006, Prendergast et al., 2014). The mice are healthy with normal reproductive performance, but have a remarkably high plasma (cholesterol-rich) very low-density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) (Hakamata et al., 1998). Though extremely rare, humans lacking *ApoE* gene are reported to have elevated plasma cholesterol levels in the form of VLDL and IDL (Schaefer et al., 1986). Although it was reported that *ApoE*<sup>-/-</sup> mice can be leaner than wild-type mice (Schreyer et al., 2002, Hofmann et al., 2008, Chiba et al., 2003), a study in experimental mouse models have provided a link between *ApoE*<sup>-/-</sup> mouse and obesity (Kypreos et al., 2009).

### **1.11.5 Obesity and reproduction**

It is acknowledged that obesity is detrimental to several functions of the female reproductive system and underlies numerous metabolic disorders. These effects of obesity on pregnant and non-pregnant women and childbirth are still not mechanistically understood, making this subject an increasing focus of research (OECD, 2018). Obese women are also shown to experience more difficulties in getting pregnant and are more prone to subfecundity and infertility (Killick et al., 2009). It has been shown that weight loss increases the chances of obese women conceiving (Dağ and Dilbaz, 2015, Silvestris et al., 2018).

Maternal obesity is closely related to several pregnancy-related complications, which can adversely affect both the mother and the foetus (Leddy et al., 2008). Maternal complications include spontaneous abortions, stillbirth, gestational diabetes mellitus, hypertensive disorders, preterm labour, obstructive labour, C-section delivery, post-partum haemorrhage, sepsis and increased risks of respiratory and cardiovascular morbidity (Baeten et al., 2001, Cedergren, 2004, Vesco et al., 2009, Magann et al., 2013, Wang et al., 2002, Schneider et al., 2019, Boots and Stephenson, 2011, Blomberg, 2011b, Chu et al., 2007b, Lisonkova et al., 2017).

A recent study has found that obese pregnant women have a six times higher risk of developing gestational hypertension than women of a normal weight (Stang and Huffman, 2016). The risk of mild and severe preeclampsia in obese pregnant women is increased by 3- to 8-fold, which significantly increases the risk of perinatal morbidity (Roberts et al., 2011, Bodnar et al., 2007, Cedergren, 2004, Norman and Reynolds, 2011). Metabolic changes and systemic inflammation, resulting from a high BMI, are also suggested to be predispose obese women to preeclampsia (Wolf et al., 2001).

Meta-analyses of several cohort observational studies have concluded that maternal obesity is closely associated with increased risk of gestational diabetes mellitus (Chu et al., 2007a, Najafi et al., 2019, Torloni et al., 2009). Moreover, Muller and Nirmala have demonstrated that pre-pregnancy BMI is the most significant risk factor of GDM (Muller and Nirmala, 2018). In addition, central obesity in early pregnancy was found to be an independent high-risk phenotype for GDM (Zhu et al., 2019). Women who become pregnant with a high BMI have

a six-fold likelihood of developing GDM, compared to normal weight women (Stang and Huffman, 2016). Hedderson et al. studied women's weight change five years before pregnancy and observed that women who gained excess weight at a rate of 1.1 to 2.2 kg/year had a small increase in risk of GDM compared to women who gained weight at a higher rate of 2.3 to 10.0 kg/year (these had a 2.5-fold increased risk of developing GDM) (Hedderson et al., 2008).

The likelihood of spontaneous labour and non-operative vaginal delivery reduces with increasing BMI (Scott-Pillai et al., 2013, Athukorala et al., 2010, Chu et al., 2007c, Frolova et al., 2018b). Obesity was found to increase the risk of elective and emergency C-section delivery independent of its effects on pregnancy outcomes (Gilead et al., 2012, Neumann et al., 2017, Poobalan et al., 2009, Weiss et al., 2004, Barau et al., 2006, Rowe et al., 2018). Obese women are 2-3 times more likely to have emergency C-section delivery compared to women of normal weight (Carlson et al., 2015). A dose-response relationship was observed between increasing maternal BMI and increasing risk of emergency C-section delivery; this is principally attributed to labour abnormalities categorised as failure to progress and/or cephalopelvic disproportion (Carpenter, 2016, Young and Woodmansee, 2002). The risk of C-section delivery was found to be elevated by 10% if maternal BMI increased only by 1 kg/m<sup>2</sup> (Hautakangas et al., 2018) There is a modest relationship between increased GWG and C-section delivery rates (Nelson et al., 2010).

It has been shown that oxytocin takes longer to reach optimal levels in the myometrium in obese women, compared to normal weight women (Nkoka et al., 2019). Moreover, the increased C-section delivery rates in obese women was shown to be caused by significant slowing of the first stage of labour and failure to progress (Zhang et al., 2007a, Cedergren, 2009). Obese women were reported to require higher rates of IOL than normal weight women and this correlated with higher rates of C-section delivery (Arrowsmith et al., 2011).

Higher maternal BMI in the first trimester is associated with increased risk of prolonged pregnancies (Denison et al., 2008, Olesen et al., 2006). Obese women have a two-fold increased tendency to develop prolonged pregnancy (Carlson et al., 2015). Maternal obesity is also associated with labour complications following IOL in postdates (Arrowsmith et al., 2011). Obese women are also at risk of spontaneous preterm delivery, preterm birth and extreme

prematurity – particularly in women with very high BMIs (Shaw et al., 2014, Ehrenberg et al., 2009, Hendler et al., 2005b, Cnattingius et al., 2013). Maternal obesity was also found to be a significant risk factor for postpartum haemorrhage (Fyfe et al., 2012, Blomberg, 2011b, Butwick et al., 2018). Nulliparous obese women displayed a two-fold increase in postpartum haemorrhage risk compared to normal BMI women, regardless of the mode of delivery (Fyfe et al., 2012). Zhang *et al.* have shown that the excessive vaginal blood loss observed in obese women is consistent with poor myometrial contractility (Zhang et al., 2007a).

Foetal complications observed in obese pregnant women include foetal macrosomia, stillbirth, late foetal death, prematurity, intrauterine growth restriction, neural tube defects and congenital birth malformations (Ehrenberg et al., 2004, Rasmussen et al., 2008, Watkins et al., 2003, Radulescu et al., 2013, Cnattingius et al., 1998, Yao et al., 2014, Rankin et al., 2010, Baeten et al., 2001, Gaudet et al., 2014). Risk of recurrent miscarriages, including euploid miscarriages (Boots et al., 2014), is also increased in obese pregnant women who conceive both spontaneously (Boots and Stephenson, 2011) or with assistance (Metwally et al., 2008). Maternal obesity has been found to increase the risk of third trimester stillbirth by two-folds and maternal GWG was reported as an independent risk factor for foetal macrosomia and neonatal adiposity (Sommer et al., 2015, Starling et al., 2015). Furthermore, there is strong evidence linking high maternal BMI with child obesity (Heslehurst et al., 2019).

Other additional concerns related to intrapartum, postpartum, operative and postoperative complications in obese patients have been reported in obese pregnant women (Van Eerden, 2011, Norman and Reynolds, 2011, Carlson et al., 2015). These postpartum complications include post-partum depression, obstructive sleep apnoea, deep venous thromboembolism, metabolic syndrome, osteoarthritis and malignancies (Ma et al., 2016). Maternal obesity has been recognised as a significant risk factor for anaesthesia related maternal mortality (Saravanakumar et al., 2006, Lisonkova et al., 2017, Biel et al., 2017). Post-operative complications are also commonly encountered in obese women, including postpartum haemorrhage, infection, postoperative endometritis and prolonged hospitalisation (Leth et al., 2011, Perlow and Morgan, 1994, Stamilio and Scifres, 2014).

### 1.11.6 Obesity and labour

Obesity has been recognised to be a predisposing factor for labour-related complications in nulliparous women (Thomson and Hanley, 1988). A summary of the studies that examined the association between maternal BMI and the progression of labour are shown in **Table 1.4**. Obese women display a unique clinical phenotype during parturition (Pevzner et al., 2009, Hill et al., 2015). Obesity is shown to influence most of the processes governing parturition, including: prostaglandin insensitivity, delayed cervical ripening, decreased myocyte action potential initiation and contractility, myometrial oxytocin receptor downregulation, altered myocyte gap junction formation and impaired myocyte neutralisation of reactive oxygen species (Carlson et al., 2015). Several studies have shown that obese women with prolonged labour usually experience a delayed first stage of labour (Carpenter, 2016, Chin et al., 2012, Samy et al., 2015, Vahratian et al., 2004, Maged et al., 2017, Carlhäll et al., 2013, Hilliard et al., 2012, Kominiarek et al., 2011, Verdiales et al., 2009, Cedergren, 2009, Bogaerts et al., 2013, Norman et al., 2012). Frolova *et al* found that obese women are associated with a longer second stage of labour (Frolova et al., 2018a). A recent study has shown that GWG positively correlated with the duration of both the first and second stage of labour in women with a normal weight maternal BMI (Zhou et al., 2019). In contrast, some studies have found that the total duration of active labour was not significantly affected by maternal BMI; instead there was an observed increased risk of C-section delivery in high-BMI nulliparous women (Ellekjaer et al., 2017, Shaban et al., 2014). Furthermore, Robinson *et al.* found no association between high maternal BMI and the length of the second stage of labour or C-section delivery risk (Robinson et al., 2011). Rodríguez-Mesa *et al.* have also agreed that maternal obesity has no significant effect on the duration of labour in nulliparous women (Rodríguez-Mesa et al., 2019). Interestingly, a recent study has found no association between maternal obesity and the third stage of labour in morbidly obese women (Cummings et al., 2018). Collectively, maternal obesity is most likely to have an effect on the first stage of labour, which was the stage of interest in this thesis.

**Table 1.4 Summary of the studies which examined the association between maternal BMI and the progression of labour.** ✓ Refers to the existence of the association. ✗ Refers to the absence of the association. The absence of a symbol means that the outcome was not tested in the study.

The first author	The year	The study design	Number of participants	Parity	Prolonged first stage of labour	Prolonged second stage of labour	Increased risk of C-section delivery
Zhou <i>et al</i>	2019	retrospective cohort study	6,786	nulliparous	✓	✓	✓
Rodríguez-Mesa <i>et al</i>	2019	retrospective cohort study	710	nulliparous	✗	✗	✓
Maged <i>et al</i>	2017	prospective cohort study	600	nulliparous	✓	✗	✓
Ellekjaer <i>et al</i>	2017	retrospective cohort study	1,885	nulliparous	✗	✗	✓

<b>Samy <i>et al</i></b>	2015	cross-sectional study	80	nulliparous	✓	✗	✗
<b>Shaban <i>et al</i></b>	2014	cross-sectional study	574	nulliparous	✗	✗	✓
<b>Carlhäll <i>et al</i></b>	2013	prospective cohort study	63,829	nulliparous	✓		✓
<b>Norman <i>et al</i></b>	2012	retrospective cohort study	5,204	nulliparous or multiparous	✓		✗
<b>Chin <i>et al</i></b>	2012	retrospective cohort study	5,410	nulliparous	✓		✓
<b>Hilliard <i>et al</i></b>	2012	case-control study	375	Nulliparous	✓		✗
<b>Robinson <i>et al</i></b>	2011	retrospective cohort study	5,341	nulliparous		✗	✗

<b>Kominiarek <i>et al</i></b>	2011	retrospective cohort study	118,978	nulliparous or multiparas	✓	✗	✓
<b>Verdiales <i>et al</i></b>	2009	case-control study	105	nulliparous or multiparous	✓	✗	✗
<b>Cedergren</b>	2009	prospective cohort study	47,282	nulliparous	✓		✓
<b>Vahratian <i>et al</i></b>	2004	prospective cohort study	612	nulliparous	✓		

### **1.11.7 Dyslipidaemia, labour and myometrial contractility**

Lipid metabolism is naturally altered during gestation and results in physiological hyperlipidaemia, which is a normal maternal adaptation to pregnancy to provide free fatty acids to the developing foetus. This is mediated by increased maternal lipolytic activity to fulfil both maternal and foetal metabolic needs (Zeng et al., 2017, Lippi et al., 2007). This is partly attributed to maternal changes in oestrogen, progesterone and insulin (Montelongo et al., 1992, Desoye et al., 1987) and corresponds to significant increases in plasma VLDLs. Other lipoproteins, namely phospholipids and cholesterol, are less sensitive and do not increase as dramatically (Lippi et al., 2007, Mazurkiewicz et al., 1994, Montelongo et al., 1992). This increase in lipids have a role in preparing the myometrium for ischemic conditions during labour. Hyperlipidaemia that exceeds the physiological range and occurs due to obesity or gestational diabetes mellitus, on the other hand, can lead to multiple pregnancy-related adverse outcomes.

Obesity has been shown to be strongly associated with increased plasma cholesterol and LDL levels (Gostynski et al., 2004). Obese pregnant women were found to have raised plasma cholesterol levels (Ramsay et al., 2002) and exhibited changes in myometrial membrane cholesterol during pregnancy and labour (Pulkkinen et al., 1998). Dyslipidaemia was correlated with high pre-pregnancy BMI in women giving preterm birth and suffering with preeclampsia (Jiang et al., 2017, Sharami et al., 2012, Smith et al., 2018, Nascimento et al., 2016). Pathological hyperlipidaemia during pregnancy was also associated with preeclampsia and gestational diabetes mellitus (Nasioudis et al., 2019). In the first trimester, however, this condition was not associated with high risk of C-section deliveries (Fyfe et al., 2013); nevertheless, a high mid-pregnancy lipid profile was found to increase the risk of spontaneous preterm delivery (Mudd et al., 2012). This is inconsistent with several other studies, which found that preterm delivery is associated with low cholesterol levels (Welge et al., 2018, Moayeri et al., 2017, Oluwole et al., 2014, Shmygol et al., 2007b). Early pregnancy hypertriglyceridemia was observed to be associated with an increased risk of congenital anomalies and macrosomia in neonates (Nederlof et al., 2015, Jin et al., 2016). Noticeably, several studies were able to show that effects of hyperlipidaemia on adverse pregnancy outcomes were independent from the effects of pre-pregnancy maternal BMI (Jin et al., 2016, Chen and Scholl, 2008).

As mentioned, uterine caveolae fulfil an essential function in the cell membrane; all three isoforms of caveolin are expressed in the human myometrium (Taggart et al., 2000) and increase in number during pregnancy and towards term (Turi et al., 2001). In the myometrium, it was demonstrated that the oestrogen receptor is localised in caveolae and the formation of caveolae, in turn, is hormone-dependent (Turi et al., 2001). Moreover, another powerful uterotonic hormone, oxytocin, also appears to act through lipid rafts, as the activity of its receptor is diminished if lipid rafts are distorted and it is suggested that the receptor is shown to assume a high-affinity state only when localised to caveolae. (Klein et al., 1995).

Cholesterol was shown to inhibit both spontaneous and oxytocin-induced myometrial contractility *in vitro*, with the reverse effect exhibited when reducing cholesterol levels (Smith et al., 2005, Zhang et al., 2007a). It was suggested that cholesterol and lipid rafts may be involved in the activity of BK channels and large outward currents in uterine myocytes as  $Ca^{2+}$  signalling and cell excitability can be reduced by depleting cholesterol using methyl- $\beta$ -cyclodextrin (M $\beta$ CD) (Shmygol et al., 2007b, Dopico et al., 2012). M $\beta$ CD is a cholesterol chelator that is able to deplete myometrial cholesterol and eventually augment myometrial contractility. M $\beta$ CD has been shown to decrease cholesterol and disrupt caveolae formation in both the rat and human myometrium (Shmygol et al., 2007b, Smith et al., 2005, Zhang et al., 2007b). Moreover, hypercholesterolemia was recently found to disrupt oxytocin-induced uterine contractility in high cholesterol diet-fed pregnant mouse (Amol et al., 2017). These considerable effects of cholesterol on uterine contractility could explain why obese women with elevated cholesterol levels are likely to have prolonged pregnancies and C-section delivery, as well as why low plasma cholesterol levels are associated with preterm delivery (Edison et al., 2007, Oluwole et al., 2014). However, recent research has claimed another mechanism by which obesity may modulate myometrial contractility and other aspects of reproduction; by adipokines.

### **1.11.8 Adipokines, labour and myometrial contractility**

Adipokines are cell signalling mediators, secreted by adipose tissue (Fain et al., 2004). Adipose tissue is a metabolically dynamic organ that secretes a large spectrum of biologically active adipokines, which in turn are involved in the

regulation of metabolic homeostasis, energy and metabolism, inflammation and immunity (Mazaki-Tovi et al., 2013, Ouchi et al., 2011, Trayhurn and Wood, 2004, Coelho et al., 2013). A number of bioactive adipokines are secreted by adipose tissue including leptin, visfatin, resistin, ghrelin, tumour necrosis factor-alpha, omentin-1, chemerin, interleukin-6 and adiponectin (Xinwang et al., 2013). Adipose tissue has been shown to have endocrine, paracrine and autocrine functions – making it an interesting organ for research. Two types of adipose tissues have been identified; the dominant white adipose tissue (WAT), which stores energy, and brown adipose tissue (BAT), which provides potential for thermogenesis. BAT is considered as a therapeutic target to treat obesity (Trayhurn, 2018, Kim and Plutzky, 2016). BMI and percentage of body fat was found to have an inverse correlation with brown adipose tissue activity in adults (Cypess et al., 2009). In obesity, the white adipose tissue becomes dysfunctional, and as well as influencing glucose and lipid metabolism, it overproduces pro-inflammatory adipokines whilst also reducing the output of anti-inflammatory insulin-sensitising adipokines. Adipokines have been found to have a vital role in the development of obesity-related complications and inflammatory conditions (Leal and Mafra, 2013). The physiological roles of adipokines are evolving and we already know they can influence the activity of smooth muscle tissues, including the vascular, respiratory, gastrointestinal, urogenital, hepatic systems (AlSaif et al., 2015). When I reviewed the literature, there are four adipokines that have been studied for their effects on myometrial contractility: leptin, visfatin, ghrelin and apelin (Mumtaz et al., 2015, Moynihan et al., 2006, Hehir and Morrison, 2012, Dayangaç et al., 2010, Hehir et al., 2008, Mostafa and Samir, 2013). These studies are summarised in **Table 1.5** below. Following this review, I decided that visfatin was potentially the most interesting adipokine, relatively recently discovered, increased with obesity and pregnancy with several systemic effects, has some pro-inflammatory effects during pregnancy, associated with multiple pregnancy adverse effects and that it may contribute, along with leptin, to poor labours in obese women.

**Table 1.5 Summary of the effects of the *in vitro* studies which examined the effects of adipokines on myometrial contractility.**

<b>Adipokine</b>	<b>First author and year</b>	<b>Tissue type</b>	<b>Effect on myometrial contractility</b>	<b>Type of contraction</b>
<b>Visfatin</b>	(Mumtaz et al., 2015)	pregnant rat and human	inhibitory effect	spontaneous and oxytocin induced contractions
<b>Leptin</b>	(Mumtaz et al., 2015)	pregnant rat and human	inhibitory effect	spontaneous and oxytocin induced contractions
	(Moynihan et al., 2006)	pregnant human	inhibitory effect	spontaneous and oxytocin induced contractions
<b>Apelin</b>	(Hehir and Morrison, 2012)	pregnant human	inhibitory effect	spontaneous and oxytocin induced contractions
<b>Ghrelin</b>	(Dayangaç et al., 2010)	virgin rat	stimulatory effect	spontaneous contractions
	(Mostafa and Samir, 2013)	virgin rat	inhibitory effect	spontaneous and oxytocin induced contractions
	(Hehir et al., 2008)	pregnant human	inhibitory effect	spontaneous and oxytocin induced contractions

### 1.11.8.1 Visfatin:

Visfatin, is also known as nicotinamide phosphoribosyltransferase (Nampt) or pre-B-cell colony-enhancing factor 1 (PBEF1). It is a 52-KDa adipocyte-derived cytokine with multiple systemic effects, including pro-inflammatory and endocrine signalling, insulin-mimetic effects and influence on the lipid profile and insulin resistance (**Figure 1.7a**) (Stastny et al., 2012). Visfatin is also synthesised in the bone marrow, liver, lymphocytes and skeletal muscle (Sethi and Vidal-Puig, 2005). Initially called PBEF1, the term 'visfatin' evolved due to the cytokine's abundant expression in human visceral adipose tissue (Zhang et al., 2011). It was shown to be upregulated in some animal models of obesity (Bełtowski, 2006) and has attracted recent attention due to its essential functions. Studies suggest that plasma visfatin concentration is increased during the development of obesity (Bełtowski, 2006), with significantly higher expression in morbidly obese women (Terra et al., 2012). Both plasma visfatin and visceral adipose tissue visfatin mRNA levels show a significant relationship with obesity (Berndt et al., 2005). The role of visfatin during pregnancy has not yet been clarified, although its myometrial gene expression is increased in pregnant women at term (Morgan et al., 2008). In the rat, visfatin mRNA has been shown to be elevated in white fat at day 21 (close to term) of gestation (Josephs et al., 2007). The pattern of changes in plasma visfatin concentrations during gestation vary between normal and obese women (Mazaki-Tovi et al., 2009b).

The uterine visfatin receptor has not yet been identified. Visfatin has been reported to act through binding to insulin receptor (IR) in an allosteric site from insulin, although the specific binding site of visfatin in the insulin receptor has not been identified (Cheng et al., 2011, Brown et al., 2010). Furthermore, visfatin induces the same insulin receptor signal transduction pathway as insulin and it induces the phosphorylation of insulin receptor substrate 1 and 2 (IRS1 and IRS) (Xie et al., 2007). It was speculated that visfatin may act on another as yet unconfirmed receptor to exert its physiological functions (Kim et al., 2008, Dahl et al., 2012). Importantly, increased visfatin concentrations in early pregnancy can lead to insulin resistance and, ultimately, the development of gestational diabetes mellitus (Mastorakos et al., 2007).

Visfatin had no effect on the smooth muscles of the aorta, even in higher concentrations (50nM) (Wang et al., 2009). Nevertheless, several studies have agreed that visfatin has an inhibitory effect on vascular smooth muscle contractility (Saddi-Rosa et al., 2010, Yamawaki, 2011, Yamawaki et al., 2009). It was found that visfatin, at a relatively low dose (10nM), exerts a significant inhibitory effect on both spontaneous and oxytocin-induced contractions in pregnant rat and human myometrial tissue *in vitro* (Mumtaz et al., 2015). It produces both a reduction in the amplitude and the area under the curve (AUC) of spontaneous contractions, as well as a significant reduction in the AUC of oxytocin-induced contractions. These findings might explain the association between obesity and pregnancy-related complications.

The mechanism through which visfatin exerts its effects on the myometrium and other smooth muscles is unclear and requires further research. Several mechanisms of action have been postulated (mostly in smooth muscle) (**Figure 1.8**), including activation of endothelial nitric oxide synthase (eNOS), which leads to enhanced production of relaxant nitric oxide (Vallejo et al., 2011), stimulates the synthesis of cyclic guanosine 3',5'-monophosphate (cGMP) and, ultimately, prevents calcium release from intracellular stores (Moncada and Higgs, 2006, Lovren et al., 2009). Visfatin also activated endothelial mitogen-activated protein (MAP) kinase which was shown to attenuate endothelial nitric oxide synthase (eNOS) phosphorylation which ultimately increase nitric oxide production in mesenteric microvessels (Lovren et al., 2009, Vallejo et al., 2011). Nitric oxide is a potent smooth muscle relaxant in blood vessels and has a role in endothelial repair and regeneration; however, in intact tissue (rather than cultured cells), there is only a small, if any, role of nitric oxide in myometrium (Norman, 1996). It was also shown that visfatin may act via prostaglandin E2 (PGE2) in chondrocytes, which is one of the arachidonic acid-derived prostaglandin metabolites synthesized through cyclooxygenases (COX) catalysis, exhibits multiple physiological and pathological actions through different subtypes of extracellular protein receptors (EP receptors). Visfatin was found to stimulate the release of PGE2, by increased microsomal PGE synthase 1 (mPGES-1) synthesis and decreased NAD<sup>+</sup>-dependent 15-hydroxy-PG dehydrogenase (15-PGDH) biosynthesis, which are essential enzymes involved in the PGE2 biosynthesis and catabolism (Gosset et al., 2008). PGE2 was documented to

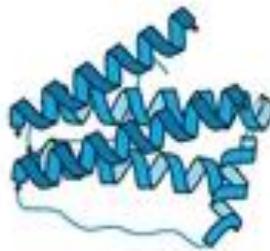
have a dose-dependent relaxant effect on spontaneous human myometrial contractions *in vitro* through the activation of prostaglandin E2 receptors (EP2) (Slater et al., 2006, Jana et al., 2010), therefore visfatin might produce its inhibitory effect on myometrial contractility through PGE2 pathway.

An alternative hypothesis is that visfatin plays a role in the NAD<sup>+</sup> pathway by acting as rate-limiting Nampt enzyme for conversion of nicotinamide to NAD<sup>+</sup> in the salvage pathway (Rongvaux et al., 2002) (**Figure 1.8**). NAD<sup>+</sup> is one of the active metabolite forms of Vitamin B3, which is essential to cellular energy metabolism and biosynthesis. The NAD<sup>+</sup> metabolic pathway is a vital biomarker of health (Yang and Sauve, 2016) and it has been shown that NAD<sup>+</sup> induces a reduction in spontaneous and oxytocin-induced myometrial contractility (Wang et al., 2006). Increasing levels of visfatin in obese women leads to increase the production of SIRT1 (silent information regulator 2-related protein), which is an NAD-dependent anti-inflammatory protein deacetylase. This, in turn, will prevent the pro-inflammatory cascades leading to spontaneous labour from taking place (Tsai et al., 2015). Although SIRT1 was found to be expressed in the myometrium; however, the SIRT1 related mechanism was not previously tested on the myometrium.

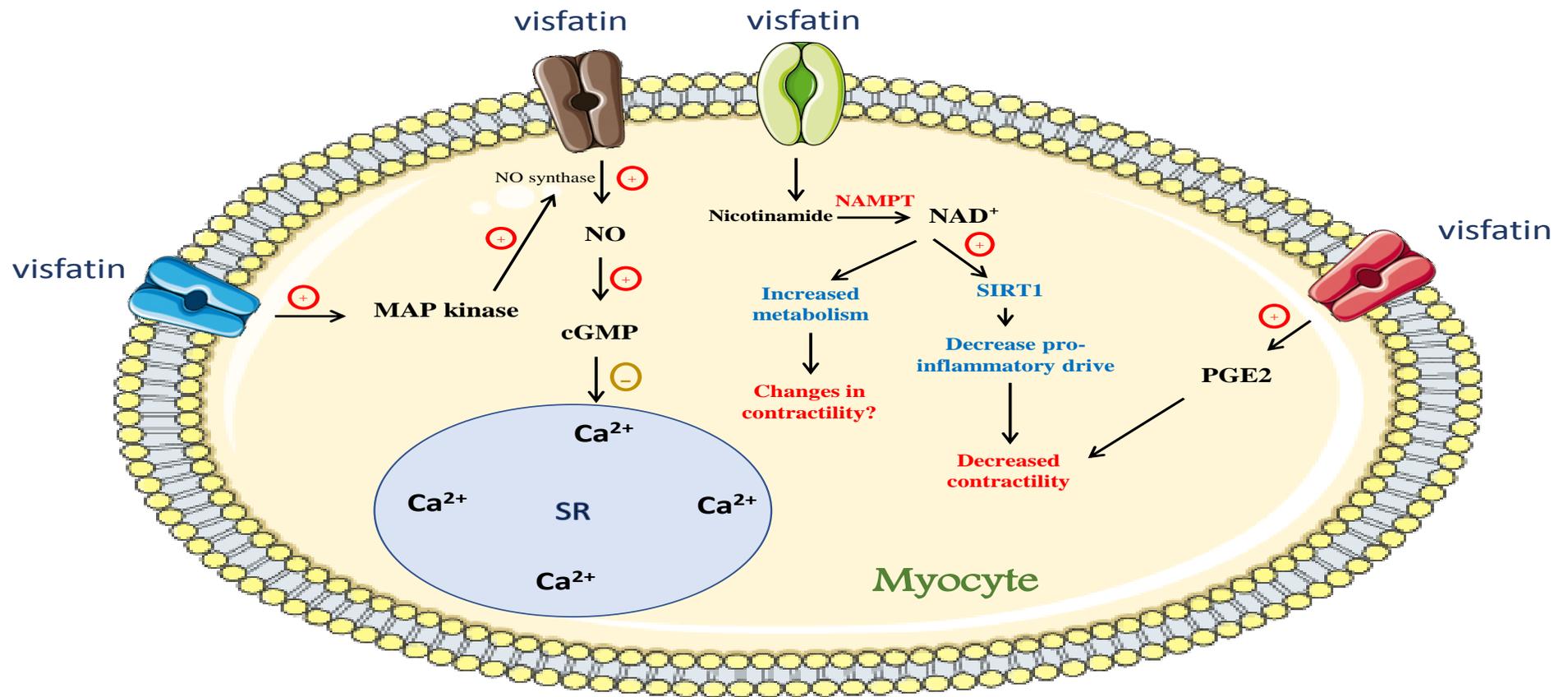
**A) Visfatin**



**B) Leptin**



**Figure 1.7 The molecular structure of visfain and leptin.** The visfatin molecule consists of 491 amino acids (A) whilst leptin is a 167-amino-acid protein (B). Taken from (Vrachnis et al., 2012)



**Figure 1.8 Proposed mechanisms of the action of visfatin effects on myometrial contractility.** a. Through mitogen-activated protein (MAP) kinase. b. Through activation of nitric oxide synthase (NO synthase) and nitric oxide (NO). c. Through activation NAD<sup>+</sup>. d. Through PGE2 activation. Nampt, nicotinamide phosphoribosyltransferase (visfatin); NAD<sup>+</sup>, nicotinamide adenine dinucleotide; SIRT1, silent information regulator 2-related protein; cGMP, Cyclic guanosine monophosphate; PGE2, prostaglandin E2.

### 1.11.8.2 Leptin

Leptin is a 167-amino acid peptide with a molecular weight of 16kD, primarily produced in and expressed by adipocytes (**Figure 1.7b**) (Münzberg and Morrison, 2015). It is synthesised from white fat and the leptin receptor is expressed in the human myometrium, umbilical cord, foetal membrane and placenta (Wendremaire et al., 2010, Akerman et al., 2002). Leptin's main function is energy homeostasis and regulation of food intake (Zhang et al., 2005, Margetic et al., 2002). It also has a role in glucose metabolism and insulin resistance in states of energy deficiency (Park and Ahima, 2015, Triantafyllou et al., 2016). In reproduction, leptin has been recognised as a regulator of ovarian function, embryonic development and implantation and its receptors have been identified at every stage of the hypothalamic-pituitary-gonadal axis (Cervero et al., 2006, Moschos et al., 2002, Lecke et al., 2011). Leptin levels were observed to be influenced by oestrogen, human chorionic gonadotropin (hCG) and glucocorticoids (Mathew et al., 2018). Serum leptin concentrations have been shown to increase throughout pregnancy, then rapidly fall in the post-partum period. (Considine et al., 1996, Chien et al., 1997, Sivan et al., 1998). Prenatal and perinatal plasma leptin concentrations have been shown to correlate with GWG, neonatal weight and post-partum weight retention (Sámano et al., 2017, Stein et al., 1998). The majority of studies reported a lack of association between plasma leptin levels and the outcomes of assisted reproductive therapies (Catteau et al., 2016)

Leptin plasma concentrations and leptin (*ob*) gene expression were found to be increased with obesity and, in turn, decreasing fat mass reduces adipose tissue production of leptin (Considine et al., 1996, Hamilton et al., 1995b). There is a strong relationship between plasma leptin levels and maternal-foetal outcomes in obese pregnant women (Vernini et al., 2016, Henson and Castracane, 2006, Plowden et al., 2015). Leptin studied at its physiological concentrations in both normal weight and obese pregnant women (Hendler et al., 2005a) and it has been reported to inhibit spontaneous and oxytocin-induced myometrial contractility in pregnant women (Moynihan et al., 2006). I have previously confirmed these findings in both pregnant rat and human term elective C-section deliveries (Mumtaz et al., 2015). It was demonstrated that leptin at 1µM produced a small but significant inhibitory effect on spontaneous and agonist-

induced human contractions, with significant reduction of the AUC observed. A similar but less potent inhibitory effect of leptin on rat myometrial contractility was also found. The mechanism by which leptin inhibits myometrial contractions is still ambiguous. One of the postulated mechanisms is activation of cGMP, which directly inhibits L-type  $\text{Ca}^{+2}$  channels and thus intracellular  $\text{Ca}^{+2}$  (Hu et al., 1997). Another postulated mechanism, derived from the observed action of leptin on vascular smooth muscle, is the reduction of intracellular  $\text{Ca}^{+2}$  release from the SR (AlSaif et al., 2015). A prospective cohort study conducted recently by Carlhäll *et al.* has concluded that there is no significant relationship between leptin levels and duration of the active phase of labour (Carlhäll et al., 2018).

## **1.12 Clinical contribution and physiological justification**

Maternal obesity, particularly when accompanied by dyslipidaemia, is linked to the pathophysiology of pregnancy and delivery-related complications, including commonly encountered dysfunctional labour. Fat accumulation in the birth canal may lead to development of birth resistance and reduced muscle contraction in pregnant women, as well as obstruction of labour and cephalopelvic disproportion (Zhou et al., 2019). However, the metabolic changes, not the anatomical reasons, are highly suggestive to explain obesity related complications. Alteration in serum levels of several adipokines, including leptin and visfatin, throughout human gestation have been reported. Pharmacological modulation of adipokines in the myometrium may become an alternative clinical treatment strategy. It has been suggested that obese women should be treated differently due to the antagonising action of leptin in labour by using syntocinon (oxytocin) early in the first and second stages of labour (Wuntakal et al., 2013).

To the best of my knowledge, this is the first study exploring the mechanism of action of the novel adipokine, visfatin, on pregnant mouse myometrial contractility. Findings from this study will provide insight for scientists and clinicians on the effect of obesity on gestation, with the potential of uncovering new therapeutic targets to manage obesity-related complications. Given that obesity was observed to reduce gap junction proteins in the mouse myometrium, it is useful to study the effect of maternal obesity on myometrial gap junction proteins to potentially uncover the mechanisms underlying poor uterine contractility in obese pregnant women. In addition, this thesis investigates the

effect of obesity on pregnancy and delivery outcomes in Saudi women; in particular, so that the association between adipokines dysregulation and pregnancy and delivery-related complications in different population can be understood.

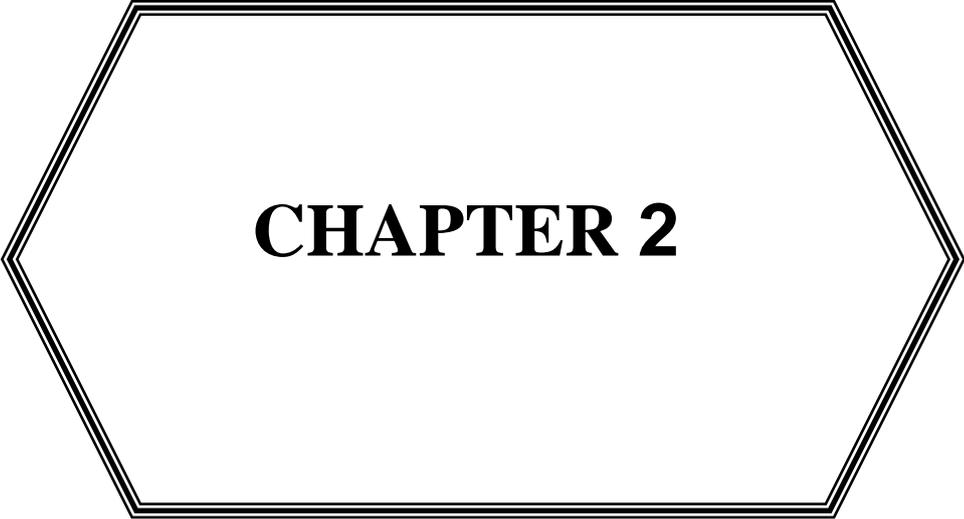
### **1.13 Aims of thesis**

The main objective of this thesis was to examine the effect of obesity on myometrial contractility in an attempt to explore part of the pathophysiological interactions linking maternal obesity with dysfunctional labour.

Objectives of this study:

- 1- Examine the differences in contractility between WT and ApoE<sup>-/-</sup> mouse myometrium to test the hypothesis that chronic dyslipidaemia disrupt myometrial contractility (Chapter 3)
- 2- Investigate the effect of visfatin on non-pregnant and pregnant WT and ApoE<sup>-/-</sup> mouse myometrial contractility to test the hypothesis that a dyslipidaemic environment has an impact on visfatin's effects (Chapter 3)
- 3- Compare the contractility between pregnant obese and non-obese human myometrium, to test the hypothesis that the myometrium of obese women contracts less well compared to normal weight women (Chapter 4)
- 4- Study the relationship between adipokines dysregulation and prolonged labour in obese pregnant women to test the hypothesis that obese women with high plasma visfatin and leptin are more prone to end with dysfunctional labour than normal weight women (Chapter 5)
- 5- Investigate the expression of gap junction proteins, to test the hypothesis that maternal obesity decreases the expression of the gap junction proteins, Cx46 and Cx26, during pregnancy (Chapter 6)

By using this range of different techniques, models and approaches a much more complete understanding of obesity, dyslipidaemia and uterine contractility will be obtained.



# **CHAPTER 2**

## Chapter 2: Methodology

### 2.1 Contractility studies

#### 2.1.1 Tissue collection, preparation and dissection

Access to fresh myometrial tissue samples, both mouse and human, is a basic requirement for this thesis. Using human myometrial tissue in the physiological studies of the myometrium has been shown to be safe with no effect on the morbidity and mortality of the donating women (McElvy et al., 2000). However, human myometrial samples are often difficult as obtaining them requires surgical intervention. Mouse tissue, by contrast, is more readily available than human tissue. Although human and mouse tissues may have similar responses to certain experimental interventions, it may not be the case in all responses. Nonetheless, the mouse model of pregnancy is still valuable as a research tool as it allows insights into aspects of reproductive physiology to be discovered. Notably, mouse research is of particular value in generating specific genetically modified models which aid in exploring basic mechanistic pathways. Therefore, while human tissue may be considered the 'primary standard' for *in vitro* studies, animal models, including mouse, should be used as a supportive tool alongside human studies.

##### 2.1.1.1 Animal tissue

Virgin wild type C57BL/6J and C57BL/6J Apolipoprotein E knockout (ApoE<sup>-/-</sup>) mice, aged 8 weeks and having a body weight of 180–240 g, were purchased and used in this study (Charles River, Kent, UK). The average number of pups for each mouse was from 3 to 6 pups. Wild type C57BL/6J black mouse type was the first to have its genome sequenced and is the most widely used inbred strain. They are susceptible to diet-induced obesity, atherosclerosis and type 2 diabetes. ApoE<sup>-/-</sup> are homozygous for the ApoE<sup>tm1Unc</sup> mutation showing a marked increase in plasma cholesterol and are usually used as a hypercholesterolemia model. The mouse was generated by injecting electroporated cells that had undergone successful recombination of wild-type

and plasmid-derived *ApoE* gene sequences into blastocysts of C57BL/6J mice. A breeding ApoE<sup>-/-</sup> colony was launched and maintained in-house.

All mice were maintained on a normal chow diet. Non-pregnant and 18-days pregnant mice were humanely killed by cervical dislocation under CO<sub>2</sub> anaesthesia in accordance with UK Home Office legislation, for Schedule 1 killing, that I was trained to perform. For the time mated mice, the male of the same genotype was placed in the cage with the female and left overnight. The gestational age of the rat was defined from day 0, when the male was placed in the cage to mate. Final body weight of the mouse was measured using standard laboratory scales.

After euthanasia of the pregnant mice, the pups were removed from the uterus and humanely killed by decapitation in accordance with UK Home Office legislation. The uterine horns were then dissected, cleaned, and placed in Krebs-Henseleit physiological saline solution (PSS) at pH 7.4 (solution content given below). Fresh PSS was made daily and all the experimental tissues were placed into it. All dissection procedures were made in an agar-filled petri dissection dish at room temperature. The uterine tissue was rinsed and thoroughly cleaned to remove any remaining placenta, foetal membranes, fat and any excess blood in preparation for myometrial dissection. A small section of the uterine horn, approximately 1cm long, was dissected. To do this, a longitudinal incision into the uterine tube was made and the uterus opened out. The underlying placental tissue was removed and discarded.

### **2.1.1.2 Human tissue**

Full-thickness biopsies, measuring 1cm x 1cm, were obtained from the middle of the upper edge, lower segment uterine incision at the time of C-section section delivery. Uterine biopsies were obtained from the upper lip of the lower uterine segment incision in the midline at C-section delivery, as previous work has shown that contractility at this region is also representative of the upper segment (Luckas and Wray, 2000). All biopsies were placed immediately into chilled Hanks Balanced Salt Solution (HBSS): (137mM NaCl; 5.1mM KCl; 0.44mM KH<sub>2</sub>PO<sub>4</sub>; 0.26 Na<sub>2</sub>HPO<sub>4</sub>; 5mM glucose, 10mM HEPES, pH 7.2) and thereafter transferred to our department. All biopsies were collected and handled using a protocol to prevent tissue degradation and to ensure all conditions for

experiments were the same. Biopsies were used either on the day of collection or the next day after storage at 4°C. Biopsies were used more within the 18 hours post-delivery.

Samples were transferred from HBSS into PSS where they were trimmed and cleaned of any accompanying blood vessels, endometrium or peritoneum. Using blunt dissection, an opening was made between muscle bundles to expose inner tissue not subjected to any external trauma. Small longitudinal strips of muscle were dissected from the biopsy, each approximately 1mm x 5mm and placed in PSS. A copy of the ethics approval for the investigation of pathological and physiological effect of different agents, novel substances and biomarkers on human myometrial contractility (Reference. 10/H1002/49, dated 26 August 2010) and the patient consent form are given in **Appendices 2 and 3**, respectively.

### **2.1.2 Organ bath experiments**

Longitudinal myometrial strips (~1 mm x 4 mm) were mounted for isometric recording under 2 g of tension in organ baths (5 ml) superfused with PSS at 2 ml/min for human and 4 ml/min for mouse tissue. The tissue baths contained 5 mL of PSS maintained at 37°C and were gassed continuously with a 100% oxygen at a flow rate of 4ml/min and at pH 7.4. Contractility data was recorded via a tension transducer (Labscribe 2; World Precision Instruments, Aston, UK) and the contractions recorded at a sampling rate of 10Hz, amplified and stored in a commercial data acquisition system (Labscribe 2; World Precision Instruments, Aston, UK). Myometrial strips were allowed to steady for a period of at least 30 minutes to obtain reproducible spontaneous contractions and enhanced by oxytocin for a minimum of 45 minutes to achieve a stable baseline activity. The drugs were then added to the tissue bath at specific intervals. 100% oxygen was chosen hence HEPES was used as a buffer instead of bicarbonate.

Control experiments were performed simultaneously. The integral of contractile performance was measured by calculating the area under the curve for a determined period for each drug concentration. The effect of any drug and the respective controls were analysed by calculation of the amplitude, duration, frequency and integrals of force (AUC) before and after incubation using OriginPro 9.0 software. In some experiments, more than one strip was used for the same drug application and the average value was calculated and fed as a

single value.

### 2.1.3 Force calibration

The overall concept of organ bath experiments is to produce mechanical signals to be amplified then converted into electrical signals which will be ultimately be transduced into digital signals using Axon software. To calibrate the digital signal to force in Newtons, it was compared with force recordings obtained from a previously known amount of force, where 1kg produces a force of 9.8N.

### 2.1.4 Drugs and solutions

All chemicals were purchased from Sigma Aldrich (Dorset, UK) unless otherwise stated.

#### 2.1.4.1 Physiological saline solution (PSS)

Physiological saline solution was freshly prepared at the day of the experimentation to the following specification:

Salt	Molarity	Weight
Sodium Chloride (NaCl)	154mM	9g/L
Potassium Chloride (KCl)	5.6mM	0.42g/L
Magnesium Sulphate (MgSO <sub>4</sub> )	1.2mM	0.29g/L
HEPES Buffer (HEPES)	10.9mM	2.6g/L
Glucose	8mM	1.44g/L
Calcium Chloride	2mM	2ml/L

The pH of the PSS was adjusted to 7.4. by addition of 4mM alkaline sodium hydroxide (NaOH). PSS was made on the day of experimentation at room temperature and was maintained at 37°C during experimentation. The volume required for each experimental day was calculated according to the number of tissue baths used, the expected length of the experiment, and the flow rate of the organ bath system.

#### 2.1.4.2 Oxytocin

1mM Oxytocin stock solution was prepared by dissolving the oxytocin

acetate salt hydrate lyophilised powder in deionised water. Then, a 10 $\mu$ M solution was prepared from the 1mM stock solution and stored at -20°C as stated in the manufacturer's instructions. Adequate oxytocin concentrations were used to augment the contraction frequency and force without causing tonic contractions. Concentrations of 1nM and 0.5nM were used for non-pregnant and pregnant mouse myometrial strips, respectively. A lower oxytocin concentration was used for the pregnant myometrium because it was more active and easier to be excitable.

#### **2.1.4.3 Visfatin**

Visfatin, murine recombinant, was purchased in lyophilised solid form and centrifuged prior to opening. 1 $\mu$ M stock solution was reconstituted in PBS and stored at - 20°C (can be stored up to 12 months). Then different dilutions were prepared from the stock solution to produce 10nM, 50nM, 100nM. The myometrial strips were incubated for control and test response for the allocated time at 37°C. Incubation experiments were used with visfatin to use the lowest amount of the drug because it is highly expensive.

#### **2.1.4.4 Methyl- $\beta$ -cyclodextrin (M $\beta$ CD)**

Methyl- $\beta$ -cyclodextrin was purchased in powder form and freshly prepared in PSS prior to application. A 2% working concentration was used.

#### **2.1.4.5 FK866**

FK866 hydrochloride hydrate was purchased in powder form and prepared in DMSO (10 mg/mL) to be stored at -20°C following the manufacturer's instructions. Typically, 10 $\mu$ M stock solution was prepared in PSS prior to application.

#### **2.1.4.6 NAD<sup>+</sup>**

$\beta$ -nicotinamide adenine dinucleotide sodium salt (NAD<sup>+</sup>) was purchased in powder form and reconstituted in PSS. A 100 $\mu$ M stock solution was made prior to experimentation.

#### **2.1.4.7 Nicotinic acid**

Nicotinic acid was purchased in powder form and prepared in NaOH. A 10 $\mu$ M stock solution was made prior to experimentation. Serial dilutions of 1 $\mu$ M, 100nM, 10nM and 1nM were prepared in PSS.

### **2.1.5 Contractility Data Analysis**

The contractility data was calculated from myometrial strips which had been observed to reach a steady state by producing stable spontaneous contractions, and then to be compared to internal control prior to the drug application which included contractions of regular frequency and equal amplitude. Matched vehicle effects were examined simultaneously. Data analysis was carried out in Origin Pro (Version 8.5) to analyse the four main contractility parameters: amplitude, duration, frequency and area under the curve. An illustration of the contractility parameters measured and statistically analysed for organ bath experiments is shown in **Figure 2.1**.

#### **Force amplitude**

The amplitude (mN) was measured by subtracting the value of the baseline from the peak of the contraction waves. It represents the height of the contraction. Contractions was calculated for the control period which was then compared to the average obtained from any treatment periods.

#### **Duration**

Duration (minutes) of the contraction is represented by how long a simple contraction lasts and was measured by calculating the time at the half-maximal amplitude ( $t_{50}$ ) point of the contraction.

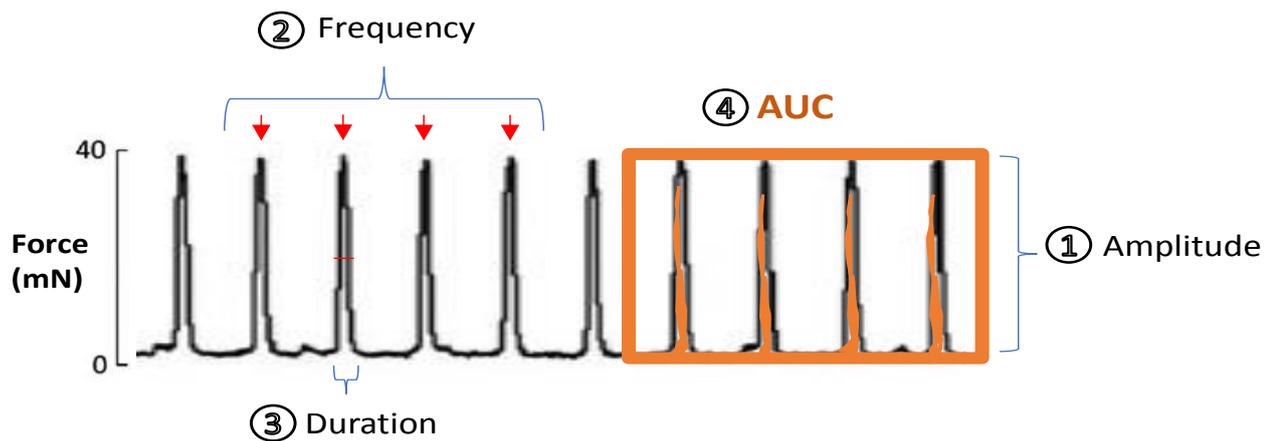
#### **Frequency**

Frequency is the number of contractions at a given time period and was calculated from the time period between the beginning of one contraction to the beginning of the next consecutive contraction. Contractions were analysed and the average time was calculated for both control and treatment periods.

#### **Area under the curve (AUC)**

The AUC (or Mean Integral of Force) is an indication of the overall contractility (frequency, duration and force amplitude combined effects).

Contractions for both control and treatment drug applications was selected and the area under each contraction per time was calculated and compared.



**Figure 2.1 Myometrial trace demonstrating the different contractile parameters measured for contractility data.**

(1) Amplitude of contraction (mN) represents the wave contraction from top to bottom (the height). (2) Frequency of contraction is the number of contractions in a defined period of time. (3) Duration of contraction corresponds to the time of a single contraction. (4) AUC represents the integral force of contraction which is the overall activity in defined period of time.

## **2.2 Enzyme-linked immunosorbent assay (ELISA)**

It was found that visfatin and leptin have an inhibitory effect on human myometrial contractility *in vitro*. It would be useful to test if there was a relationship between plasma levels of maternal adipokines, visfatin and leptin, and dysfunctional labour in obese women. A cross-sectional study was designed to determine the association between maternal adipokines and prolonged labour in obese women. ELISA was used to measure maternal plasma leptin and visfatin concentrations.

### **2.2.1 Collection of maternal blood and analysis of adipokines**

Maternal blood samples were collected during labour in vacutainer test tubes with a clot activator and gel for plasma separation and were centrifuged at 4 °C for 15 min at 1000g. The serum samples were stored at -80 °C until leptin and visfatin analyses were performed using enzyme-linked immunosorbent assay (ELISA).

### **2.2.2 Enzyme-linked immunosorbent assay (ELISA)**

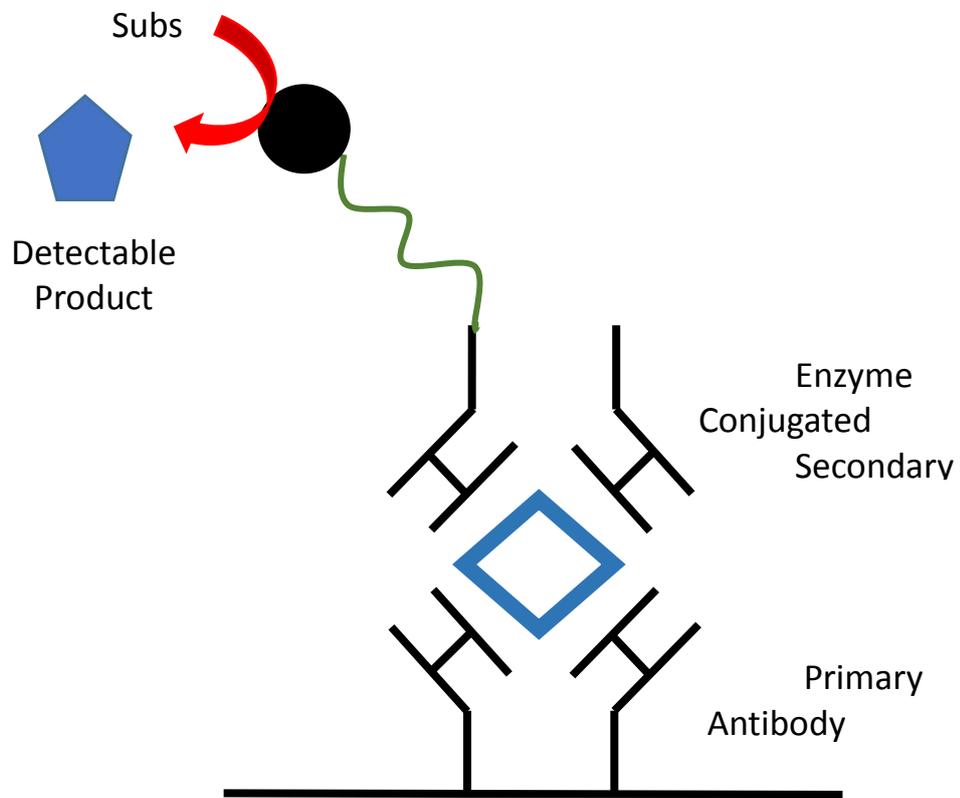
The hospital haematology lab automated ELISA machine (ETI-MAX 3000) was used to conduct the ELISA test. The ETI-MAX 3000 is a fully automated microliter plate analyser performing the complete sample processing (sample dilutions, sample and reagent dispensing, wash processes, incubations, plate transports) and the photometric measurement and sample evaluation. The ELISA testing was carried out under the supervision and help of a specialised hospital technician (Ali Alsadiq).

A quantitative sandwich enzyme immunoassay technique was performed to measure the maternal plasma leptin and visfatin during labour. Human PBEF/visfatin DuoSet ELISA kit, DuoSet Ancillary Reagent Kit 2 and a human leptin quantikine ELISA Kit were purchased from R&D Systems, Minneapolis, USA. The basic principle used was as follows (**Figure 2.2**).

All reagents were brought to room temperature before use. The Calibrator Diluent RD5P was used to dilute the standards and the samples and it was diluted in deionised water (1:5). The substrate solution was prepared by mixing colour reagents A and B in equal volumes within 15 minutes of use. The substrate

solution should be protected from light to prevent colour dissociation. 200µl of this mixture is required per well. The wash buffer was prepared by adding 20 mL of Wash Buffer Concentrate to deionised or distilled water to prepare 500 mL of wash buffer.

Monoclonal antibodies specific for human leptin and visfatin were pre-coated onto a microplate and 100µl of Assay Diluent RD1-19 was added to each well. Then, 100µl of standard, control, or sample was added per well and incubated for 2 hours at room temperature. Any leptin or visfatin present was bound by the immobilised antibody. The wells were washed with the wash buffer (400µl x 3) by the machine autowasher. After washing away any unbound substances, 200µl of conjugated enzyme-linked monoclonal antibodies specific for human leptin and visfatin were added to the wells to be incubated for 1 hour at room temperature. The wells were washed with the wash buffer (400µl x 3) to remove any unbound antibody-enzyme reagent. Then, 200µl of substrate solution was added to each well and incubated for 30 minutes at room temperature. The machine provides light protection. 50µl of Stop Solution was added to each well and colour developed in proportion to the amount of leptin and visfatin bound in the initial step. The enzymatic activity is stopped due to the acidification of the sample products by the stop solution. The colour in the wells changed from blue to yellow. The optical density (OD) of each well was measured within 30 minutes, using an automated microplate reader set to 450 nm. A standard curve was created by reducing the data using computer software capable of generating a log/log curve (concentration versus OD). A log curve was used to linearize the data generated.



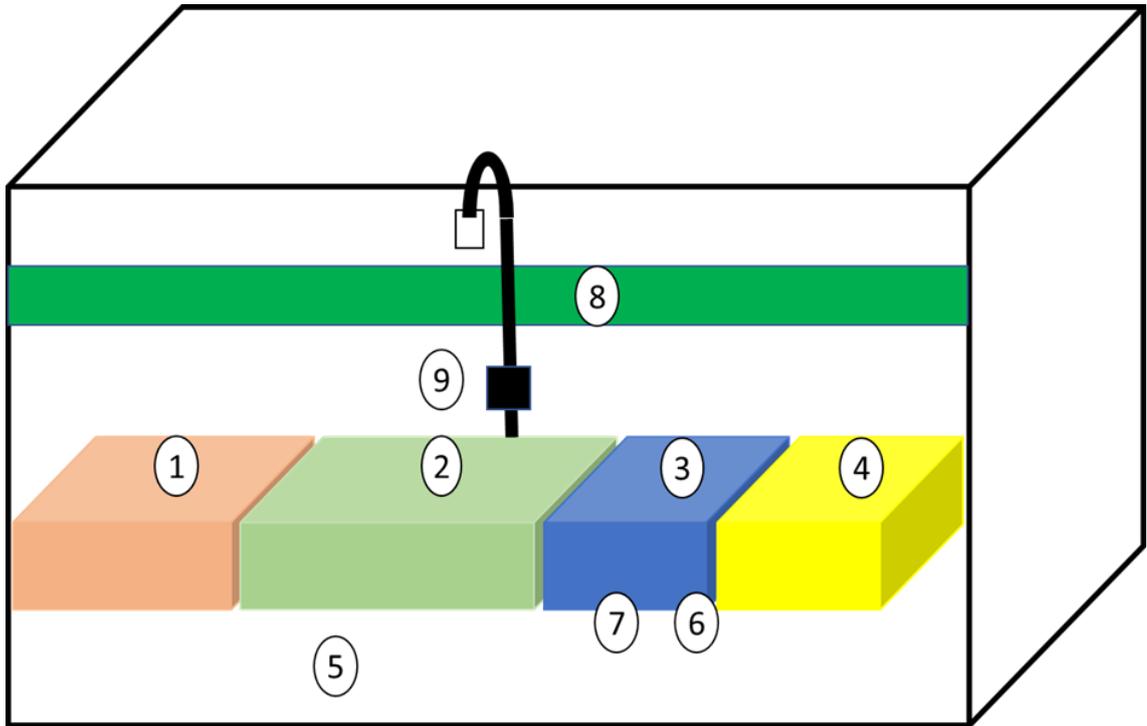
**Figure 2.2 Indirect ELISA principle.**

The wells were coated by primary antibodies (anti-leptin or anti-visfatin). When the required proteins bound to the wells, enzyme conjugated secondary antibodies are added. The secondary antibodies are specific for leptin or visfatin antigens. Finally, a substrate is added which is converted by the conjugated enzyme onto a detectable product.

### **2.2.3 Advantages of an automated ELISA machine:**

An automated ELISA machine (**Figure 2.3**) was used in this thesis that has several advantages:

- 1- Safe and reliable results: Barcoded samples and reagents reduce operator error. Full process control to guarantee safe results. No carry-over with disposable tips.
- 2- Random access and batch mode: single analyte per plate or multiple analytes on the same plate.
- 3- High through-put: four plates and up to seven.
- 4- Multitasking: Multiple SW functions and options available during the run.



**Figure 2.3 ETI-MAX 3000 machine components:**

- 1- Tray for tip racks and dilution tubes
- 2- Patient samples and reagent unit
- 3- Tip eject station, pipettor rinsing position, pipettor station
- 4- Test plate compartment, plate transport unit
- 5- Drawer with wash unit and photometer
- 6- Position of incubators, heated and for room temperature (dark)
- 7- Waste container for tips
- 8- Guide rail for pipettor (X and Y movement)
- 9- Pipettor (movement in Z-direction)

## **2.3 Immunohistochemistry**

Previous studies have suggested that obesity has an effect on myometrial gap junction protein expression in pregnant rat myometrium (Muir et al., 2016, Elmes et al., 2011). None of these studies has examined this effect in pregnant human myometrium. Immunohistochemistry was used in this study to test the expression of Cx43 and Cx26 in pregnant human myometrium of obese women and compare it to normal weight and obese women.

### **2.3.1 Tissue preparation and fixation:**

Biopsies of human myometrial tissues were taken at elective caesarean section, after informed consent, at Liverpool Women's Hospital, Liverpool, UK. The ethical standards for human experimentation established in the declaration of Helsinki were followed (WMA Declaration of Helsinki, 2013). A copy of the patient information sheet and consent form and the ethics approval for immunohistochemistry testing of human tissue samples (2<sup>nd</sup> amendment, Reference. 10/H1002/49 dated 12 July 2012) are given in **Appendices 3 and 4**, respectively.

Fixation is essential to preserve the cells and prevent their natural autolysis by the inactivation of the lysosomal enzymes and the inhibition of bacterial growth. The fixative used was chosen to provide adequate fixation with maximum cell morphology preservation. Small pieces of human myometrium, approximately 0.5mm x 0.5mm x 0.5mm were dissected and placed into neutral buffered formalin (NBF) containing 10 % formalin (4% formaldehyde) for a minimum of 24 hours at room temperature. The fixed samples were processed and embedded in paraffin wax, then mounted onto glass slides. For control tissues and antibody optimisation, animal tissue was also used. Heart and liver were dissected from 22-day pregnant Wistar rats. They were then placed in NBF, 10%.

### **2.3.2 Tissue processing, embedding and sectioning:**

After tissue fixation, the samples were removed from the fixative and prepared for tissue embedding. Automated processing of the samples was done by a Shandon Citadel 1000 processing machine (Thermoelectron Corporation,

UK). For tissue embedding, tissue infiltration with paraffin is required. As the tissue is water-based at this stage, it must be dehydrated before introducing paraffin. The sections were dipped in a series of ascending grades of ethanol baths from 60% (1 hour), 70% (1 hour), 90% (1 minute) and 100% (1 hour), 100% (1½ hours) and 100% (2 hours). The tissue then was cleared using xylene (the 1<sup>st</sup> process: 1 hour; the 2<sup>nd</sup> process: 2½ hours; the 3<sup>rd</sup> process: 2½ hours). The tissue was then ready for paraffin (VWR International) infiltration (the 1<sup>st</sup> process: 2½ hours; the 2<sup>nd</sup> process: 2½ hours). Tissue embedding was performed by Shandon Histocentre 3 (Thermoelectron Corporation, UK). The ethanol and the xylene were purchased from Chemistry solvent stores (University of Liverpool).

After tissue embedding, the samples were sectioned at a thickness of 5µM by a rotatory microtome (Microm HM335, MIROM UK Ltd, UK). The sections then transferred to a pre-heated water bath at 37°C and then fixed onto glass slides. The slides were left to dry overnight and stored in racks at room temperature until the day of the immunohistochemistry staining. Tissue preparation, fixation, processing, embedding and sectioning were undertaken by me with the help and the guidance from our lab technicians at the Liverpool Women's Hospital, Sarah Northy and Helen Cox.

### **2.3.3 Cx43 and Cx26 IHC staining procedure**

Immunohistochemistry (IHC) combines histological, biochemical and immunological techniques for the identification of specific tissue antigens by using a specific antigen/antibody reaction tagged with a visible label. Sections from human myometrium were chosen randomly and labelled before the staining begin. The slides were baked in section dryer (model E28.5, Thermo Scientific) for 60 minutes at 60°C prior to the IHC staining to dry them out and ensure that the tissue sections adhered to the slides. The sample ID, the antibody/ stain used, the concentration and the date of the experiment were clearly written on the slide. The slides were kept hydrated during the experiment to prevent unspecific staining. The slides were settled in an appropriate size staining metal slide rack (Thermo Scientific). The rack was submerged in a glass bath containing 100% Xylene (solvent-based agent) for 10 minutes twice to remove the paraffin wax from the section. By the end of this step, the majority of wax inside and outside the tissue had been removed. The rehydration step is essential to prepare the

tissue for the water-based antibodies and stains. The sections were then immersed in a series of descending grades of ethanol baths from 100% (twice, 5 minutes), 90% (1 minute) and 70% (1 minute) to slowly rehydrate the sample before being transferred into a bath containing distilled water for 5 minutes. The steps of dewaxing and rehydration were done inside a fume hood. The slides were then immersed into a bath of boiling 10mM Sodium Citrate buffer (VWR International Ltd.) pH=6.0 in a Tefal Clipso Easy 6L pressure cooker for 20 minutes. This acid-base antigen retrieval method is crucial to expose the antigen for the desired antibodies. The slides were then transferred to distilled water.

The cells have their own hydrogen peroxidases. Excess enzyme will cause overstaining and oversaturation. To prevent false positive results, H<sub>2</sub>O<sub>2</sub> (Sigma Aldrich) was used at 0.3% to remove nonspecific staining by blocking endogenous hydrogen peroxidase activity which is conjugated to the secondary antibody. The slides were incubated with H<sub>2</sub>O<sub>2</sub> for 10 minutes at room temperature. Each slide then was tapped off from excess water before marking a circle around the tissue section with DAKO hydrophobic barrier pen (Vector Labs) which creates sufficient space for the reagents and allows the reagents to remain localised to the tissue. Slides were then incubated for 20 minutes with nonspecific horse whole-serum block. Slides were left overnight incubation with the appropriate primary antibody in blocking solution at 4°C (rat connexion 43 and mouse connexion 26 monoclonal antibodies, Thermo Scientific). A 50µl of solution per tissue section was allowed. This amount is enough to cover the whole tissue section on the slide. After each step, the slides were washed with buffered saline solution (TBS, Sigma Aldrich) and all the incubations were made in a humidified chamber to keep the environment moist and to prevent tissue dry out which can cause nonspecific binding.

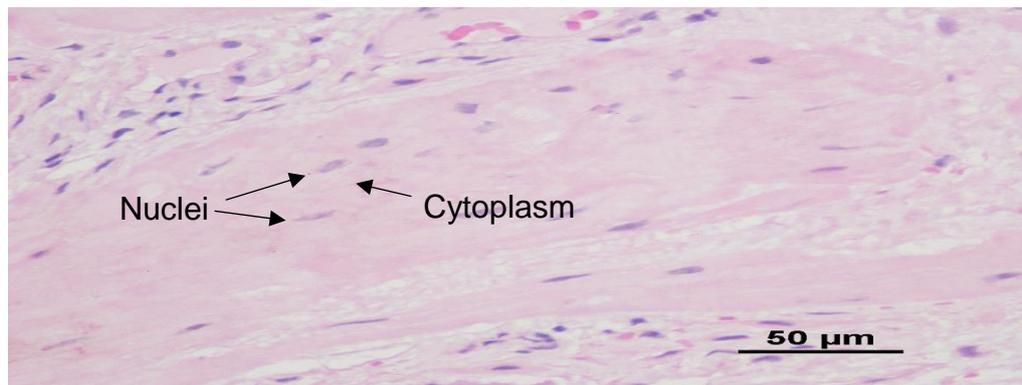
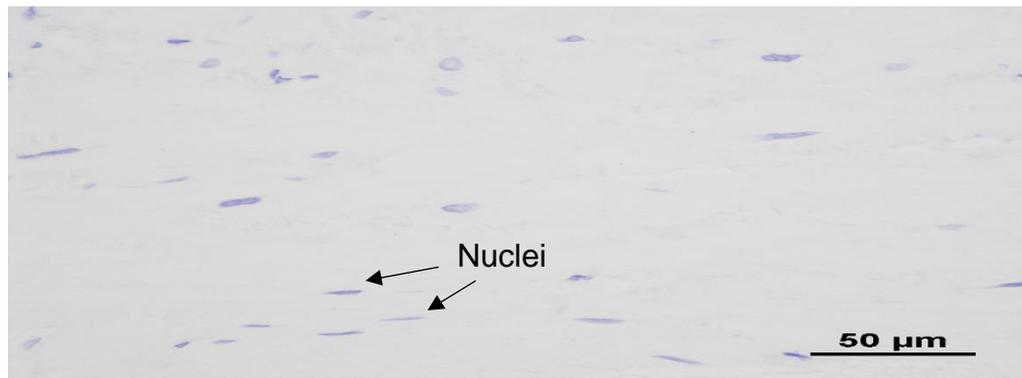
One myometrial section was incubated overnight in blocking solution without adding the primary antibody, to be used as a negative control. Specific tissue known to express the protein of interest was used as an external positive control. One sample was tested in each experimental set with the same antibody to be used as an internal positive control. The following day (after 16 hours incubation), the primary antibody was washed twice with TBS before the slides were incubated with the secondary antibody (labelled polymer-HRP) for 30 minutes at room temperature. The secondary antibody must be raised against

the immunoglobulin of the species which the primary antibody is made. Impress universal antibody horse serum for anti-rabbit Ig and goat serum anti-mouse Ig peroxidase polymer detection kit (Vector Labs) were chosen accordingly to match the primary antibodies used. The secondary antibody was then washed twice with TBS.

ImmPACT 3,3'-Diaminobenzidine (DAB, Vector Labs) was chosen as a stain because horseradish peroxidase (HRP) labelled secondary antibodies were used. DAB is most commonly used in immunohistochemical (IHC) staining as a chromogen. DAB is oxidized by hydrogen peroxide in a reaction classically catalysed by HRP. A brown precipitate (chromogen) is developed by the oxidised DAB at the location of the HRP, which can be visualised using light microscopy. DAB solution was added into each slide for 10 minutes at room temperature and then the reaction was stopped by adding distilled water. Once all slides had developed stain, some were counterstained with Haematoxylin (nuclear stain) by adding filtered Shandon Gill 2 Haematoxylin (Thermo Scientific) for 2 minutes followed by tap water washing until the water clears. The slides were then swiftly dipped in 1% acid alcohol solution to wash off any extra stain and immediately rinsed with tap water to maximise the intensity of the blue nuclear stain.

The slides were then dehydrated, to be prepared for mounting, by immersing them in a series of ethanol solutions from 70% (1 minute), 90% (1 minute), and 100% (twice, for 3 minutes), before being placed back into 100% xylene (5 minutes then 10 minutes). Cover-slips were then mounted with a xylene based mounting medium using DPX mountant (Thermo Scientific) and left to dry overnight before imaging. The steps of rewaxing and dehydration were performed in a fume hood.

To check the cellular orientation within the myometrial tissue, haematoxylin and eosin (H&E) staining was used on some samples. Haematoxylin was used to stain the nuclei with dark blue stain and eosin stains the cytoplasm and the connective tissue fibres with pink stain (**figure 2.4**). The slides were placed in Eosin Y at room temperature after the haematoxylin was washed by acid alcohol. After the haematoxylin staining step, the process of dehydration was done as follows: 70% (1 minute), 95% (1 minute), Eosin Y (4 minutes), water rinse and incubation for 2 minutes and 100% (twice, for 1 minute), before being placed back into 100% xylene (5 minutes then 10 minutes).



**Figure 2.4 Comparing Staining of a section of human myometrium (5uM thickness) with haematoxylin alone and H&E.**

Only the nuclei of cells (nucleic acid, RNA and DNA) are stained with haematoxylin staining alone (blue - basic stain) (a). With H&E staining, in addition to nucleic haematoxylin staining, Eosin stains the cell cytoplasm and the extracellular structures including connective tissue fibres and collagen (Pink - acidic stain). H&E stain was used to check the cellular orientation within the myometrial tissue.

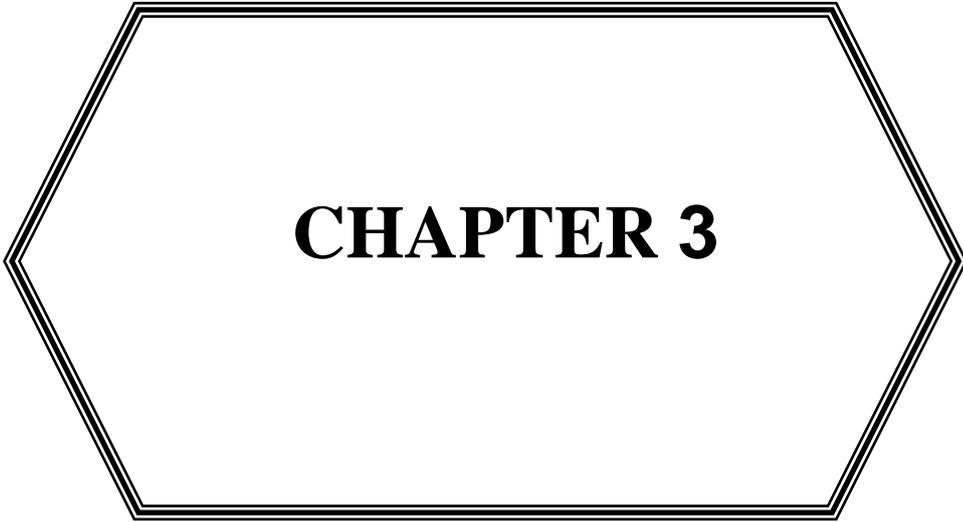
### **2.3.4 Imaging of myometrial sections**

The imaging apparatus used for IHC in this thesis was composed of the following:

1. Nikon Eclipse 50i Microscope, Nikon Corporation, Tokyo 100-8331, Japan
2. Nikon DS-Fi1 digital camera Head 5M pixel, Nikon Corporation, Tokyo 100-8331, Japan
3. Nikon Digital control unit DS-U2 USB, Nikon Corporation, Tokyo 100-8331, Japan
4. Nikon C-Mount TV adaptor, 0.63x, Nikon Corporation, Tokyo 100-8331, Japan
5. Personal computer (minimum specification 1GB RAM, 2.8GHz processor)
6. NIS-Elements-F software, developed for Nikon Instruments

After loading the NIS- Elements-F software (the image capture software), a slide was placed on the microscope stage and moved to an area that contained a section of myometrial tissue to be examined. The microscope was calibrated by using a scale slide for each objective 4x, 10x, 20x and 40x. The settings were adjusted to normal mode, 640x480 resolution, auto exposure and high colour contrast and sharpness. The whole tissue section was thoroughly visualised on 4x and 10x objectives to determine the most representative areas of the entire tissue for image capture. Spectral images were obtained using 4x, 20x and 40x objective magnifications and images were saved as TIFF files.

The negative control was examined first to make sure there was no staining, which is an indicator for a successful experiment. The internal control was compared after each experiment to ensure replicated results. To avoid biased results, all slides were blinded to the observer and visualised by two observers.



**CHAPTER 3**

# **Chapter 3: Examining myometrial contractility and responses to visfatin in wild-type and ApoE<sup>-/-</sup> mouse**

## **3.1 Introduction**

### **3.1.1 Maternal obesity and reproduction**

Obesity is a major problem worldwide, including the United Kingdom. Almost 61% of women in the United Kingdom are overweight (31%) or obese (30%) (National Health Service, 2019). This obesity is reflected in women of childbearing age and is linked to pregnancy and delivery-related complications (Baeten et al., 2001, Gilead et al., 2012). As well as obesity's known effects on major organs, it has a substantial influence on the contractile cellular physiology of many smooth muscles including vascular, airway, gastrointestinal, urinary bladder and interestingly, myometrium (AlSaif et al., 2015). A number of previous studies have suggested, by both laboratory and clinical observations, that obesity in humans is associated with impaired myometrial contractility and dyslipidaemia (Carlson et al., 2015, Wray, 2007, O'Brien et al., 2013).

### **3.1.2 Dyslipidaemia and myometrial contractility**

As discussed in chapter 1, adipose tissue is a multifunctional organ which secretes a large spectrum of adipokines, such as leptin, resistin, tumour necrosis factor-alpha, interleukin-6 and adiponectin (Axelsson et al., 2005), which have a critical role in the development of obesity complications (Hauner, 2005). It is physiologically normal to have hyperlipidaemia during pregnancy; this is a normal maternal adaptation to meet the increasing foetal metabolic needs, and increased steroid hormone synthesis, during pregnancy (Weissgerber and Wolfe, 2006). Hyperlipidaemia beyond the physiological range can, however, open the door to many obstetric complications. There is evidence that plasma cholesterol levels are higher in obese pregnant women compared to normal weight women (Ramsay et al., 2002) and dyslipidaemia associated with obesity might compromise the ability of the labouring uterus to contract efficiently (Azais et al., 2017, Hajagos-Tóth et al., 2017). Cholesterol was found to inhibit myometrial

contractility and calcium signalling *in vitro* indicating that dyslipidaemia together with changes in myometrial membrane cholesterol content might have a critical effect on obese women's myometrial contractility (Babiychuk et al., 2004, Shmygol et al., 2007b, Smith et al., 2005, Amol et al., 2017). In addition to dyslipidaemia, the metabolic, endocrine, and paracrine products of adipose tissue exert diverse biological effects on the smooth muscle of the myometrium (Mumtaz et al., 2015).

### 3.1.3 Visfatin and myometrial contractility

It has been suggested that adipokines dysregulation could be associated with pregnancy-related complications (Canverenler, 2015). Of these, visfatin appear to be particularly important in pregnancy (Marseglia et al., 2015); its plasma level being increased in preeclampsia (Shaheen et al., 2016, Zorba et al., 2012, Adali et al., 2009), gestational diabetes mellitus (Gok et al., 2011, Krzyzanowska et al., 2006, Mazaki-Tovi et al., 2009a) and intrauterine growth retardation (Jaquet et al., 2005, Malamitsi-Puchner et al., 2007). As discussed in detail in Chapter 1, visfatin is a visceral fat specific adipocytokine which has been reported to have many effects including pro-inflammatory and endocrine (Stastny et al., 2012). It is expressed in human myometrium (Esplin et al., 2005), human amniotic membranes (Ognjanovic and Bryant-Greenwood, 2002) as well as the placenta (Marvin et al., 2002). Visfatin gene expression was found to be upregulated in the myometrium and foetal membranes during parturition (Kendal and Bryant-Greenwood, 2007), resulting in an increase in its maternal plasma levels (Rosenblatt et al., 2007, Mazaki-Tovi et al., 2009b). Visfatin catalyses the first rate-limiting step in converting nicotinamide to nicotinamide adenine dinucleotide (NAD<sup>+</sup>), an essential coenzyme crucial for cellular metabolism and energy production (Dahl et al., 2012).

The mechanisms of visfatin's specific actions on myometrium are unknown. As discussed in Chapter 1, several mechanisms of action have been postulated from its actions on other smooth muscles and from the action of other adipokines on smooth muscles (**Figure 1.8**). In brief, these include, activation of endothelial nitric oxide synthase (eNOS) (Vallejo et al., 2011), PGE<sub>2</sub> release (Gosset et al., 2008), elevation of PKC (Sweeney, 2002, Nelson et al., 2008) and through the NAD<sup>+</sup> pathway (Azais et al., 2017, Bi et al., 2012). Unlike in vascular

tissue, nitric oxide is unlikely to be an important mechanism in myometrium (Mumtaz et al., 2015). The NAD<sup>+</sup> pathway mechanism might be applicable in myometrial smooth muscle. Visfatin has been found to have a critical role as the rate-limiting step in the synthesis of NAD<sup>+</sup> from nicotinamide (Wang et al., 2006). It is an essential co-enzyme for multiple metabolic processes including oxidative phosphorylation and glycolysis (Yang and Sauve, 2016). It is proposed to act as an intracellular ligand (receptor mediated). At the cellular level, it has been found that increased levels of visfatin in obese women increases the production of SIRT1 (a silent information regulator 2-related protein), which is an NAD-dependent anti-inflammatory protein deacetylase. This, in turn, will decrease the pro-inflammatory cascades leading to spontaneous labour (Tsai et al., 2015). SIRT1 was also found to promote endothelium-dependent vasodilation (Mattagajasingh et al., 2007). Thus there may be both negative and positive effects of visfatin on the myometrium.

### **3.1.4 Apolipoprotein E knockout (ApoE<sup>-/-</sup>) mouse as a model of dyslipidaemia**

As discussed in Chapter 1, obesity is associated with Apolipoprotein E (ApoE), which has an essential role in lipoprotein metabolism. It is required for the biological clearance of liver-derived VLDL remnants and diet-derived chylomicrons by the liver (Mahley, 1988). Therefore, mice lacking the functional *ApoE* gene, ApoE<sup>-/-</sup>, develop hypercholesterolemia followed by obesity and provide a widely used practical model of hyperlipidaemia and atherosclerosis (Pendse et al., 2009). The mice are healthy when they born, but have a markedly altered plasma lipid profile compared to normal mice, and rapidly develop atherosclerotic plaques. Their cholesterol plasma levels can reach as high as 680 mg/dl compared to 103 mg/dl in normal C57 black mice, with higher cholesterol levels in females (**Appendix 5**). They have a significantly elevated plasma cholesterol, very-low-density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) (Hakamata et al., 1998). Though extremely rare, some humans lack the *ApoE* gene, and have elevated plasma cholesterol levels in the form of VLDL and IDL (Schaefer et al., 1986). I therefore used ApoE<sup>-/-</sup> mouse to test if chronic dyslipidaemia affects myometrial contractility. Another reason for

choosing the ApoE model is that plasma visfatin levels were found to be 2.3 times higher in ApoE<sup>-/-</sup> mouse than C57BL/6J mouse (Zhou et al., 2013).

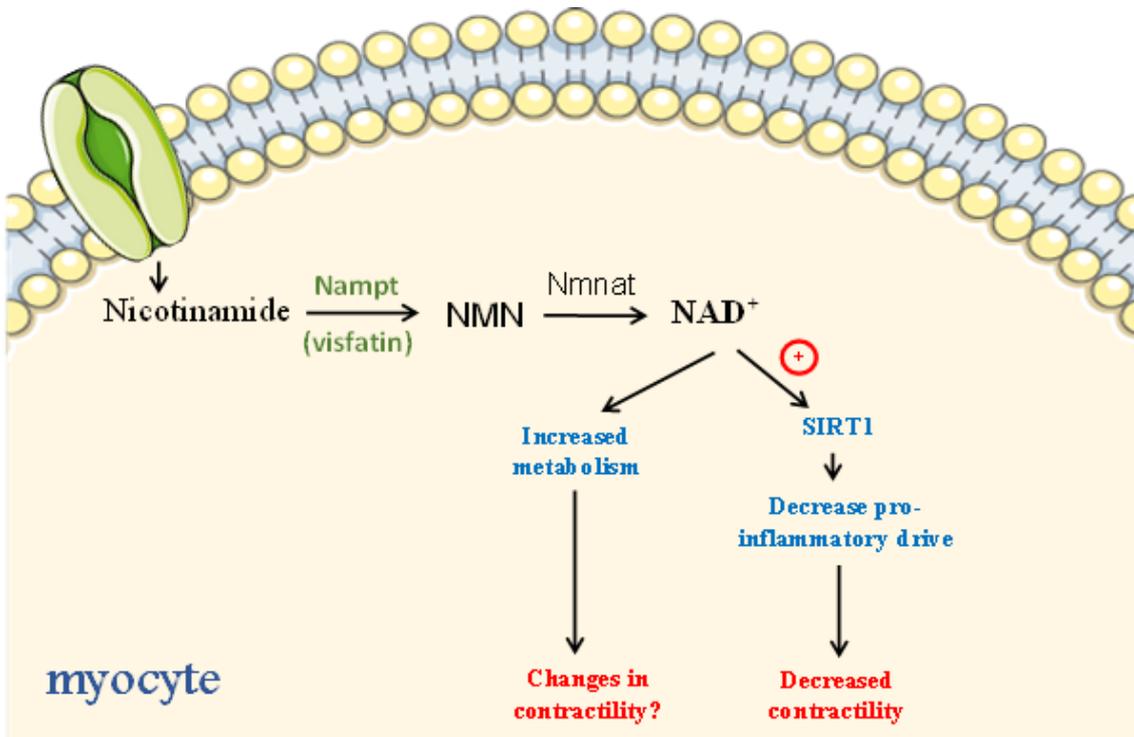
### 3.1.5 Aims of this study

Due to the increase in the serum levels, expression, and metabolic functions associated with visfatin during pregnancy and parturition, examining the effect of visfatin on myometrial contractility has become an area of interest. In research I previously conducted during my master's studies, it was found that visfatin has a relaxant effect on both human and rat myometrial contractility *in vitro* (Mumtaz et al., 2015). These results suggest that visfatin might have a role in impaired myometrial contractility associated with obesity.

A major aim of this chapter is to investigate the effects and the mechanism of action of visfatin on mouse myometrium *in vitro*. It was examined whether visfatin has effects on both non-pregnant and pregnant mouse myometrial contractility *in vitro*, and whether there were any gestational changes in the response to visfatin. Mouse species was chosen as this species allowed me to also study a genetically modified dyslipidaemic model (ApoE<sup>-/-</sup>). This chapter therefore seeks to determine if there are any differences in myometrial contractility between non-pregnant WT and ApoE<sup>-/-</sup> mouse. As discussed earlier, a novel mechanism proposed for visfatin effects is via the NAD<sup>+</sup> pathway. I therefore wanted to focus on examining if this NAD<sup>+</sup> pathway mechanism could be present in the myometrium. To do this, the NAD<sup>+</sup> pathway was modulated using pharmacological inhibition by FK866 and pharmacological activation by NAD<sup>+</sup> and NA (**See Figures 3.1 and 3.2**). FK866 is a highly specific noncompetitive inhibitor of NAMPT, an enzyme which catalyses the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide, an intermediate step in the biosynthesis of NAD<sup>+</sup> (Hasmann and Schemainda, 2003). FK866 was also used to inhibit visfatin in adipose tissue (Dimitriadis et al., 2019). Nicotinamide is the amide derivative of nicotinic acid. Both are essential for the generation of NAD<sup>+</sup>. Nicotinic acid is a better precursor, compared to nicotinamide (Nam), in elevating tissue NAD<sup>+</sup> levels (Hara et al., 2007). In general, it was hypothesised that chronic exposure to a hyperlipidemic environment during pregnancy would influence myometrial contractile mechanisms and eventually its ability to efficiently contract.

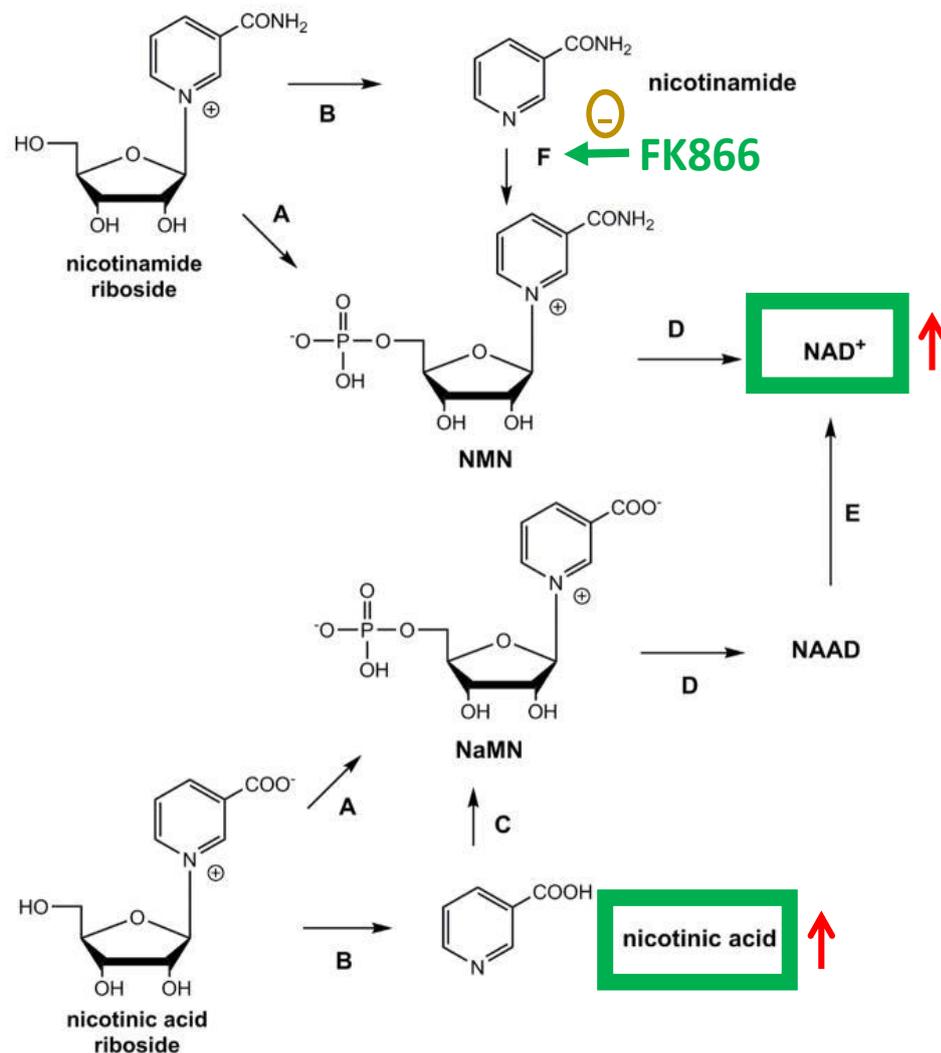
In summary, in this chapter I examined the following *in vitro*:

1. The differences between non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrial contractility in spontaneous and oxytocin-induced contractions.
2. The effect of visfatin on non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrial contractility.
3. The effect of visfatin on term pregnant WT and ApoE<sup>-/-</sup> mouse myometrial contractility.
4. The mechanism of action of visfatin on pregnant WT mouse myometrial contractility by modulation of NAD<sup>+</sup> salvage pathway.



**Figure 3.1 Suggested mechanism of action of visfatin on myometrium.**

NAMPT/visfatin catalyses the rate-limiting step in the biosynthesis of NAD<sup>+</sup> from nicotinamide which is essential for energy production and cellular metabolism. This might increase cellular metabolism or directly stimulate SIRT1. Both mechanisms can consequently lead to altered/decreased myometrial contractility. Nampt, nicotinamide phosphoribosyltransferase (**visfatin**); nicotinic acid/nicotinamide mononucleotide adenylyltransferase (Nmnat), NMN, Nicotinamide mononucleotide; NAD<sup>+</sup>, nicotinamide adenine dinucleotide; cGMP, SIRT1, silent information regulator 2-related protein.



**Figure 3.2 Pharmacological modulation of NAD<sup>+</sup> pathway.** To examine the NAD<sup>+</sup> pathway on oxytocin-induced term pregnant WT mouse, FK866 (10 $\mu$ M) which is a potent inhibitor of NAD<sup>+</sup> biosynthesis, NAD<sup>+</sup> (100 $\mu$ M) and Nicotinic acid (10 $\mu$ M) which are NAD<sup>+</sup> pathway enhancers, were applied. Enzymes are A) nicotinamide riboside kinase 1 and nicotinamide riboside kinase 2 (Nr1, Nr2) B) purine nucleoside phosphorylase C) nicotinic acid phosphoribosyltransferase D) nicotinic acid/nicotinamide mononucleotide adenylyltransferase (Nmnat) E) NAD<sup>+</sup> synthetase F) nicotinamide phosphoribosyltransferase (Namp1). NMN, Nicotinamide mononucleotide; NaMN, nicotinic acid mononucleotide; NAAD, nicotinic acid adenine dinucleotide; NAD, nicotinamide adenine dinucleotide. Adapted from (Yang and Sauve, 2016).

## 3.2 Methods

### 3.2.1 Tissue collection

Tissue collection was discussed in detail in Chapter 2. Briefly, the myometrial tissues used in this chapter were obtained from non-pregnant and term-pregnant wild type (WT) C57BL/6J and ApoE<sup>-/-</sup> mice (18-day gestation). ApoE<sup>-/-</sup> mice were created by targeted inactivation in embryonic stem cells, as explained in detail by van Ree et al (van Ree et al., 1994). Full thickness uterine tissue was collected from humanely killed mice using overdose of CO<sub>2</sub> anaesthesia and checked by cervical dislocation according to the UK Home Office legislative requirements. All experimental procedures were carried out in accordance with the UK Scientific Procedure Act 1986. Experimental tissues were placed in physiological saline solution (PSS) at pH 7.4 containing the following (mmol/L): NaCl 120.4, KCl 5.9, MgSO<sub>4</sub> 1.2, CaCl<sub>2</sub> 2.0, glucose 8, and HEPES 11 (Sigma Aldrich, UK). Tissues were stored at 4°C and used within 2 hours of collection. Further details of how the tissue obtained, prepared and dissected are described in **Chapter 2**.

### 3.2.2 Contractility measurements

Contractility experiments were performed as described in Chapter 2. Briefly, full thickness uterine tissues were dissected, cleaned and longitudinal strips (~1 mm × 4 mm) were further dissected and attached to a tension transducer under 10mN tension for mouse tissue in an organ bath filled with 5 ml of physiological saline superfused at a rate of at 4 ml/min and gassed with 100% O<sub>2</sub>. Experiments were performed at 37 °C and myometrial strips were allowed to equilibrate for at least 1 hour to obtain reproducible spontaneous phasic contractions. Contractility data were recorded via a tension transducer (World Precision Instruments, Aston, United Kingdom) and the signal amplified and stored in a commercial data acquisition system (Labscribe 2; World Precision Instruments, Aston, United Kingdom).

### 3.2.3 Drugs and solutions

All chemicals and solutions were prepared as described in chapter 2 and the drugs used are summarised in **Figure 3.2**. All the stock solutions were made

in PSS except for FK866 which was made in dimethyl sulfoxide (DMSO) and nicotinic acid which was made in NaOH. The drugs used are visfatin (10, 50,100,150nM) 2% M $\beta$ CD, FK866 (10 $\mu$ M) NAD<sup>+</sup> (100 $\mu$ M) and nicotinic acid (1 $\mu$ M, 100nM, 10nM and 1nM). In some experiments, Oxytocin was added to PSS at a final concentration of 1nM (non-pregnant mouse) and 0.5nM (pregnant mouse). Matched vehicle controls were performed accordingly. All the drugs were left to pass through the organ bath chambers except visfatin which was directly applied to the perfusate after stopping the flow and incubated for the designated time period. Spontaneous contractile control activity can be generated from paired myometrial strips allowed to contract spontaneously, or with the addition of the vehicle used for the specific drug solution applied. Myometrial strips were allowed to equilibrate for at least 30 minutes to obtain reproducible spontaneous phasic or oxytocin (1nM) induced contractions. Drugs were made in fresh saline solution on the day of experimentation. All chemicals were purchased from Sigma Aldrich, (UK), unless otherwise stated.

### **3.2.4 Data and statistical analysis**

The effect of the drugs and the internal controls before the application of the drugs were analysed and compared by measurements of the average of amplitude, duration, frequency and the integrals of force. Matched vehicle effects were examined simultaneously and representative traces were shown. Integrals of force is an index of the total activity by the tissue over a given time period. Each uterine strip was tested for a drug, and had a paired control response in their matched vehicle. In some experiments, more than one strip was used for the same drug application and the average value was calculated and used as a single value. The data was recorded with a data acquisition system and statistical analysis was performed using OriginPro 9.0 software (Microcal). Statistical analysis was carried out in prism GraphPad Prism version 5.01 (GraphPad Software, San Diego, USA).

Comparison of force records was performed using mean  $\pm$  se followed by a paired Student's *t*-test and one-way ANOVA with Bonferroni post hoc tests used to compare more than two groups. A probability value (P value) of <0.05 was taken as level of significance and (n) is the number of myometrial tissue strips examined from different animals. An asterisk (\*) denotes significant difference in

contractility compared to preceding control period  $p < 0.05$ , \*\*  $p < 0.005$  and \*\*\*  $p < 0.0005$ .

### **3.3 Results**

#### **3.3.1 Establishment of spontaneous contractions and control mouse myometrial traces recording *in vitro***

The body weight of wild type mouse was 180–220 g and of ApoE<sup>-/-</sup> mouse was 200–240 g. All tissues were superfused with physiological salt solution (PSS) at 37 °C, pH 7.4 until spontaneous contractile activity was established. In general, the commencement of contractions occurred within 30–60 minutes with continuous perfusion of PSS. Once contractions became steady, the control period was recorded and then the drugs to be tested were applied. These contractions are expected to be steady and regular and can last for many hours (throughout the experiment) when placed in appropriate physiological conditions, i.e. continually superfused with buffered PSS solution and bubbled with oxygen at 37°C and pH 7.4. Typical traces of spontaneous uterine contractions examined in this chapter are shown throughout the chapter results.

#### **3.3.2 Comparing myometrial contractility between non-pregnant WT and ApoE<sup>-/-</sup> mice**

In total, data was obtained from 7 different myometrial samples from both non-pregnant WT and ApoE<sup>-/-</sup> mouse. In order to compare the contractile activity between non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium, their spontaneous contractility was compared and their responses to both an agonist (oxytocin) and a cholesterol chelator drug (MβCD) were tested *in vitro*. Myometrial activity was compared directly to the original control contractions preceding the application of oxytocin and MβCD by two-tailed *t*-test.

##### **3.3.2.1 Comparing spontaneous myometrial contractility**

Spontaneous contractions from non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium were established, and the strip left to contract for at least 10 minutes producing steady stable contractions before the application of test solutions. Representative traces showing spontaneous myometrial contractions in non-

pregnant WT (A) and ApoE<sup>-/-</sup> (B) mouse are shown in **Figure 3.3**. The mean force amplitude, duration, frequency and the integrals of force (AUC) were compared for 7 non-pregnant WT and 7 non-pregnant ApoE<sup>-/-</sup> mouse (**Figure 3.4**).

#### **a) Force amplitude**

The mean force amplitude of spontaneous contractions calculated for WT mouse was  $10.3 \pm 1$  mN compared to ApoE<sup>-/-</sup> mouse of  $10.2 \pm 1$  mN. Contraction amplitude for WT mouse was shown to be not significantly different to ApoE<sup>-/-</sup> mouse myometrium,  $p > 0.05$  (**Figure 3.4a**).

#### **b) Duration**

The mean duration of spontaneous contraction for WT mouse was  $0.09 \pm 0.02$  minutes compared to  $0.13 \pm 0.01$  minutes calculated for ApoE<sup>-/-</sup> mouse. Duration of contraction for WT mouse myometrium was not significantly different compared to ApoE<sup>-/-</sup> mouse myometrium,  $p > 0.05$  (**Figure 3.4b**).

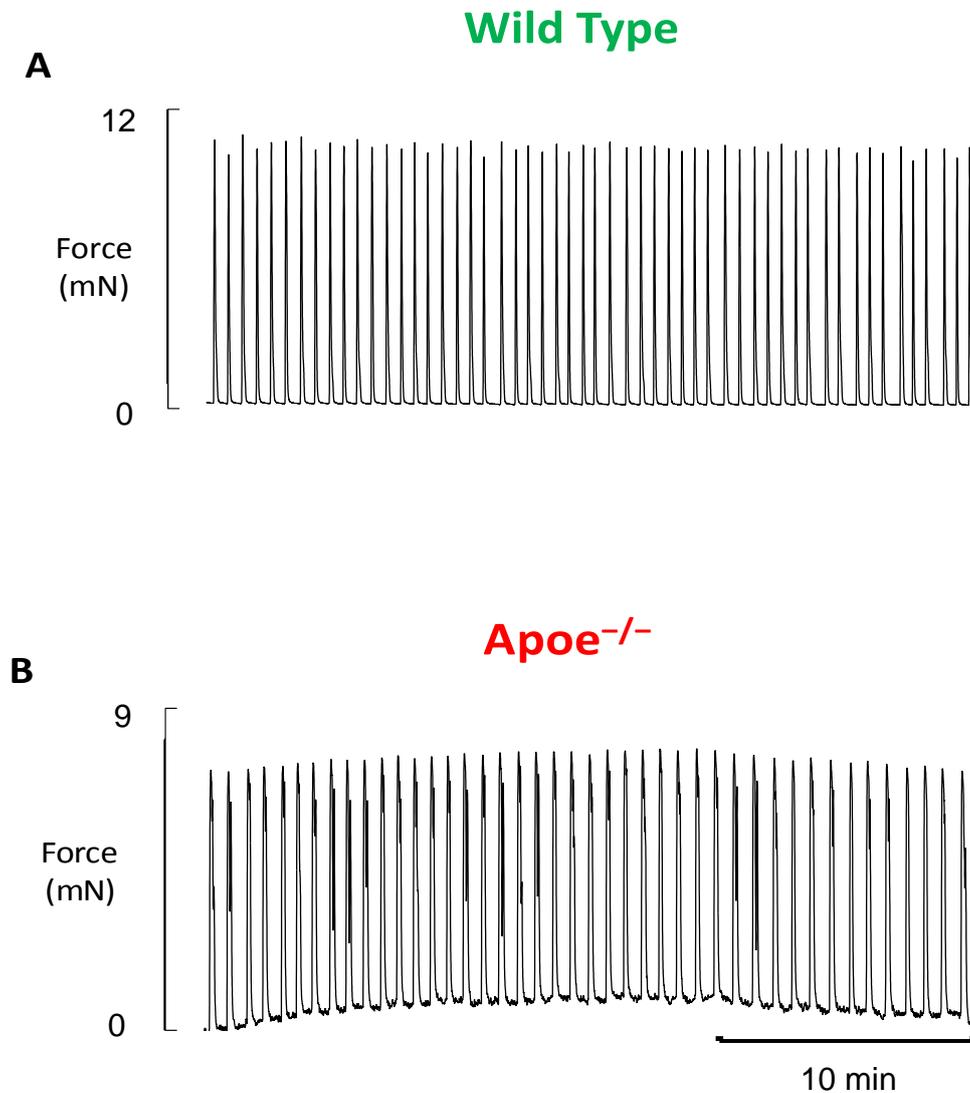
#### **c) Frequency**

Analysis of the number of contractions in a period of 10 minutes revealed that there was no significant difference between WT and ApoE<sup>-/-</sup> mouse,  $p > 0.05$ . The mean frequency for WT mouse was  $5.4 \pm 0.7$  contractions per 10 minutes compared to  $5.5 \pm 1$  contractions calculated for ApoE<sup>-/-</sup> mouse (**Figure 3.4c**).

#### **d) Integral force of contraction (AUC)**

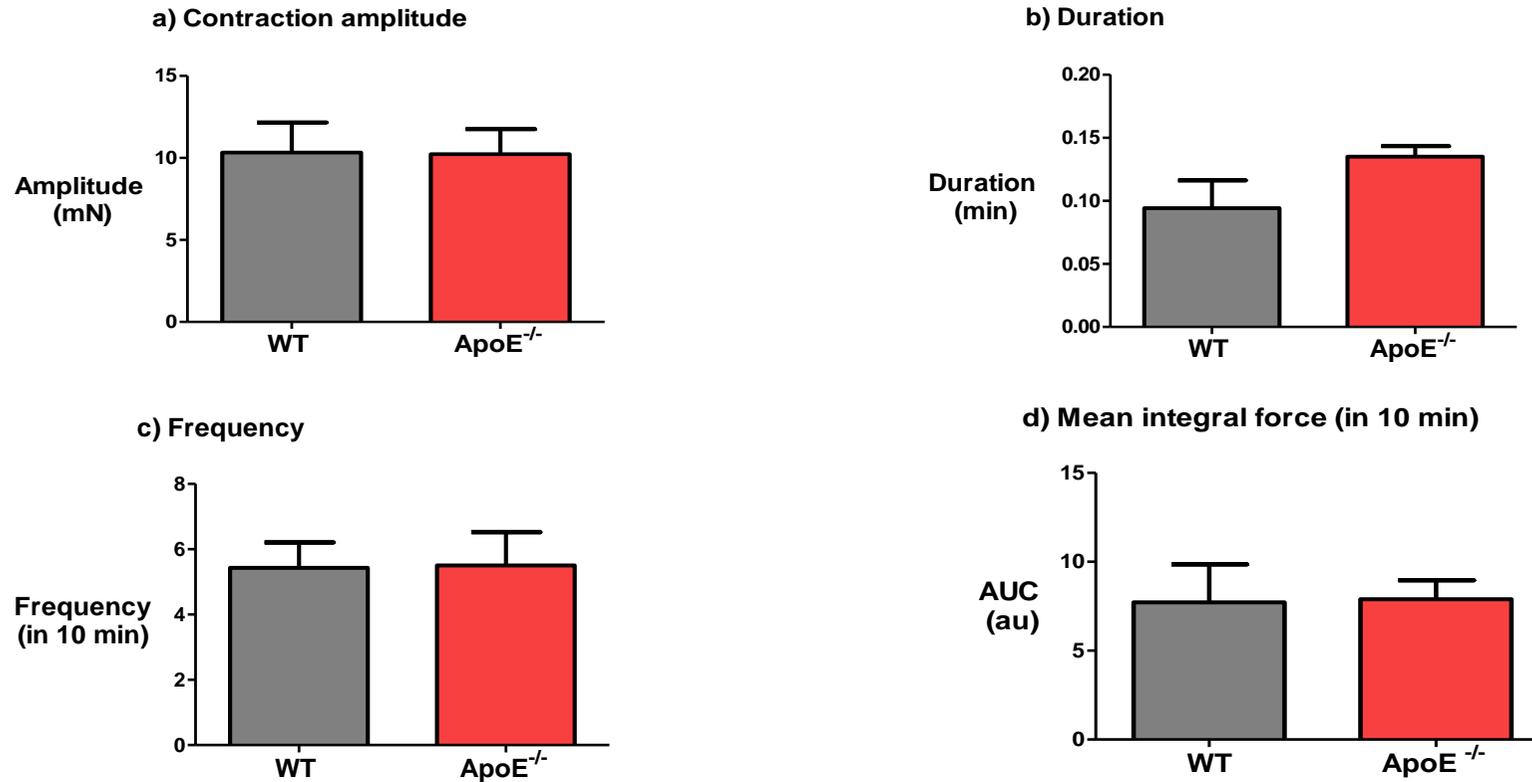
Analysis of AUC over a period of 10 minutes showed that there was no significant difference between WT and ApoE<sup>-/-</sup> mouse,  $p > 0.05$ . The mean AUC for WT mouse was  $7.7 \pm 2$  (arbitrary units, au) compared to  $7.8 \pm 1$  au calculated for ApoE<sup>-/-</sup> mouse (**Figure 3.4d**).

**Figure 3.3 Non-pregnant mouse myometrium: comparing the spontaneous myometrial contractility between Wild Type and ApoE<sup>-/-</sup> mouse.**



Typical traces of control spontaneous contractions obtained by dissecting myometrial strips from A) WT mouse B) ApoE<sup>-/-</sup> mouse. The data are paired. In this, and subsequent figures, unless noted otherwise, the strips were superfused with PSS, the pH was 7.4 and the perfusion rate of the solution was 4ml/min at 37 °C.

Figure 3.4 Comparing spontaneous myometrial contractility between non-pregnant WT and ApoE<sup>-/-</sup> mouse.



Bar charts showing the percent mean values for a) contraction amplitude (mN), b) duration (min), c) frequency (number of contractions per 10min) and d) integral of contractions (AUC) (au) for a period of 10 minutes. The contraction profiles shows that there is no significant difference between WT (n=7) and ApoE<sup>-/-</sup> (n=7) mouse spontaneous myometrial contractions (p>0.05).

### 3.3.2.2 Comparing the response to oxytocin

Contractions stimulated by oxytocin were compared between non-pregnant WT and ApoE<sup>-/-</sup> mouse *in vitro* (n=7). 1nM of oxytocin was added to PSS and applied to myometrial strips of both mouse types. The activity of the myometrium was examined during the application of oxytocin for 20 minutes. The tissue was able to fully recover after oxytocin application. **Figure 3.5** shows representative traces for the response of both non-pregnant WT (A) and ApoE<sup>-/-</sup> (B) mouse myometrium to oxytocin (1nM). Oxytocin has a significant stimulatory effect on both Wild type and ApoE<sup>-/-</sup> mouse myometrium. **Figure 3.6** illustrates bar charts showing the mean of each contractile parameter (amplitude, duration, frequency, and AUC) during the last 10 minutes of oxytocin application with any significant difference indicated by student *t*-test.

#### a) Force amplitude

The mean force amplitude of contractions in response to oxytocin calculated for WT mouse was  $12 \pm 2.2$ mN compared to ApoE<sup>-/-</sup> mouse of  $10 \pm 1.6$ mN. Contraction amplitude for WT mouse was shown to be not significantly different from ApoE<sup>-/-</sup> mouse myometrium,  $p > 0.05$  (**Figure 3.6a**).

#### b) Duration

The mean duration of contraction for WT mouse was  $0.1 \pm 0.02$  minute compared to  $0.1 \pm 0.01$  minute calculated for ApoE<sup>-/-</sup> mouse. Duration of contraction for WT mouse myometrium in response to oxytocin however was not shown to be significantly different compared to ApoE<sup>-/-</sup> mouse myometrium,  $p > 0.05$  (**Figure 3.6b**).

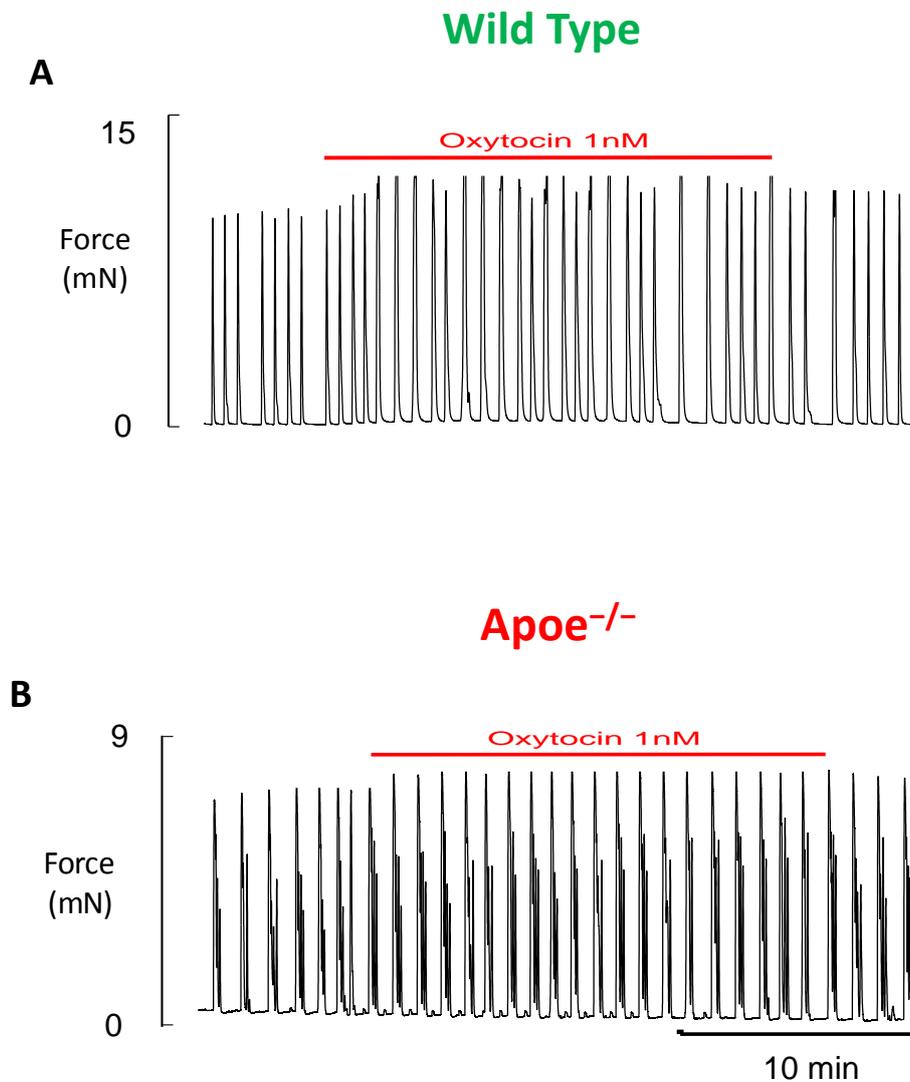
#### c) Frequency

The mean frequency in response to oxytocin calculated for WT mouse was  $0.6 \pm 0.06$  contractions per 10 minutes compared to  $1.4 \pm 0.8$  contractions per 10 minutes calculated for ApoE<sup>-/-</sup> mouse. Frequency of contractions for WT mouse was shown to be not significantly different to ApoE<sup>-/-</sup> mouse myometrium,  $p > 0.05$  (**Figure 3.6c**).

#### d) Integral force of contraction (AUC)

The AUC of contractions was calculated by measuring the area under the contraction curve in 10 minutes. The integral force of contraction for WT mouse myometrium was  $12.5 \pm 3.2$ , compared to  $10.1 \pm 1.2$  for ApoE<sup>-/-</sup> mouse. Contractile activity of WT mouse myometrium in response to oxytocin however was not significantly higher than non-pregnant ApoE<sup>-/-</sup> mouse myometrium,  $p > 0.05$  (**Figure 3.6d**).

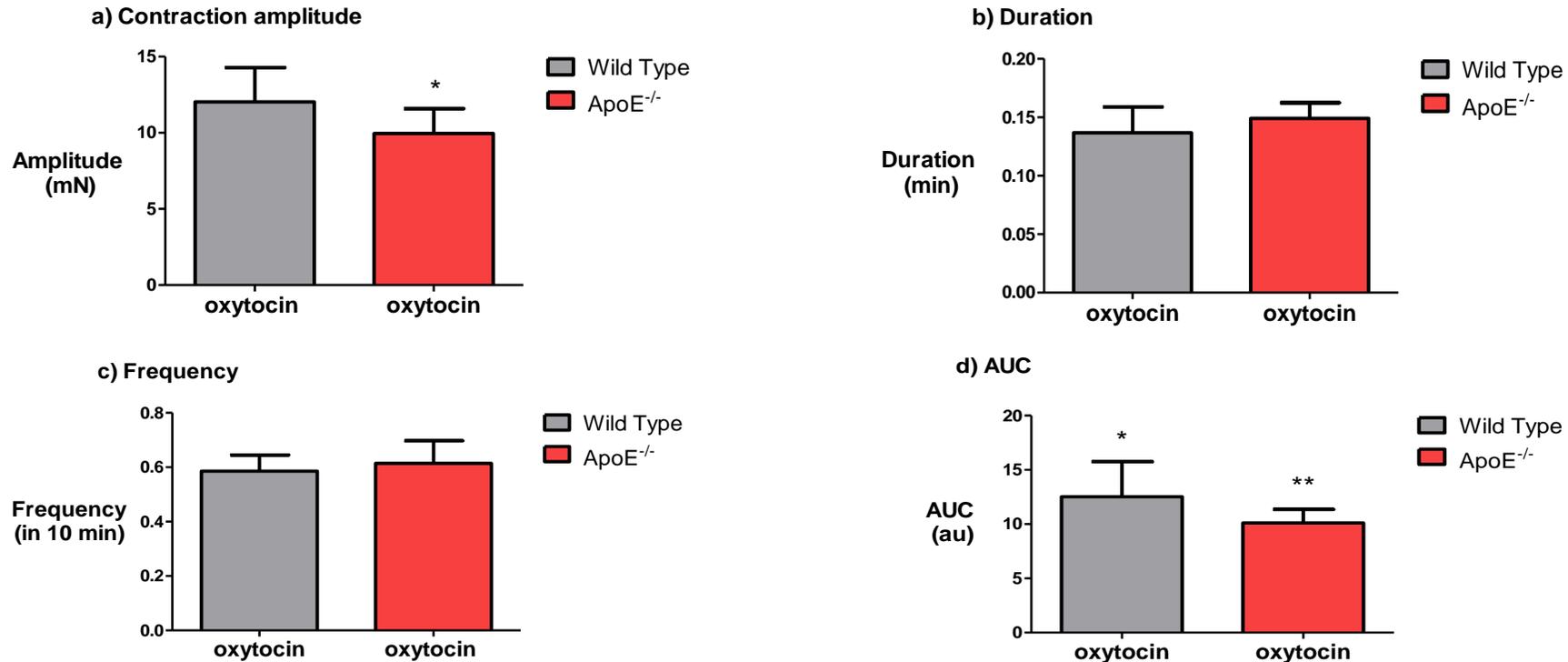
**Figure 3.5 Non-pregnant mouse myometrium: comparing the myometrial contractility between WT and ApoE<sup>-/-</sup> mouse in response to oxytocin.**



A representative trace for A) WT mouse B) ApoE<sup>-/-</sup> mouse. Oxytocin (1nM) had a stimulatory effect on both WT and ApoE<sup>-/-</sup> mouse myometrium.

**Figure 3.6 Myometrial contractile activity of non-pregnant WT and ApoE<sup>-/-</sup> mouse in response to oxytocin.**

100



Bar charts showing the mean values for a) contraction amplitude, b) duration, c) frequency and d) AUC for a period of 10 minutes. The AUC was significantly higher than control in WT mouse myometrium. Both the amplitude and the AUC were significantly higher than control in ApoE<sup>-/-</sup> mouse myometrium. By comparing all the contractile parameters using student *t test*, there was no significant difference between WT (n=7) and ApoE<sup>-/-</sup> (n=7) mouse myometrium in response to oxytocin ( $p>0.05$ ). \* Denotes significant difference in contractility compared to preceding control period  $p<0.05$  \*\* $p<0.005$  \*\*\* $p<0.0005$ .

### 3.3.2.3 Comparing the response to M $\beta$ CD

Knowing that myometrial cholesterol content in ApoE<sup>-/-</sup> mice is greater than WT mice and myometrial activity declines with cholesterol application (Zhang et al., 2007b), It was examined whether the depletion of cholesterol from contracting myometrial strips will produce different effects between non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium *in vitro* (n=5). Myometrial activity was observed during the application of M $\beta$ CD for 10 minutes. The parameters of contractions were calculated for the last 5 minutes. **Figure 3.7** also shows representative traces for the response of both non-pregnant WT (A) and ApoE<sup>-/-</sup> (B) mouse myometrium to 2% M $\beta$ CD application. Compared to the original control activity preceding M $\beta$ CD application, cholesterol extraction resulted in an insignificant stimulatory effect on both non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium by comparing the AUC. **Figure 3.8** shows the mean of each contractile parameter (amplitude, duration, frequency, and AUC) during the last 5 minutes of M $\beta$ CD application with any significant difference.

#### a) Force amplitude

The mean force amplitude of contractions in response to M $\beta$ CD recorded for WT mouse (10.9 $\pm$  2.5 mN) was not significantly different from ApoE<sup>-/-</sup> mouse (10.9 $\pm$  1.8 mN), p>0.05 (**Figure 3.8a**).

#### b) Duration

The mean duration of contraction for WT mouse was 0.1  $\pm$  0.02 minute compared to 0.1  $\pm$  0.01 minute calculated for ApoE<sup>-/-</sup> mouse for 10 minutes. Duration of contraction for WT mouse myometrium in response to oxytocin however was not shown to be significantly different from ApoE<sup>-/-</sup> mouse myometrium, p>0.05 (**Figure 3.8b**).

#### c) Frequency

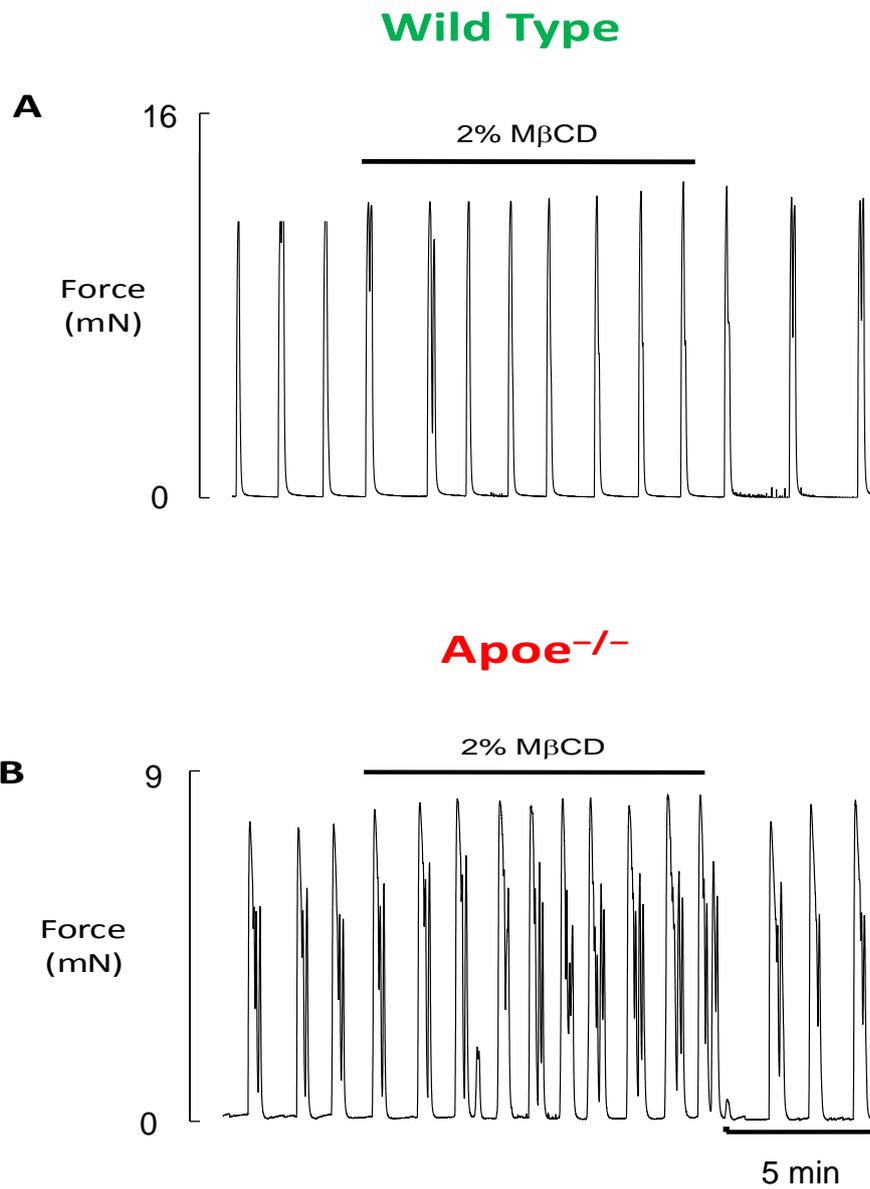
The mean frequency in response to M $\beta$ CD calculated for WT mouse was 1.1  $\pm$  0.5 contractions per 5 minutes compared to 0.6  $\pm$  0.1 contractions per 5 minutes calculated for ApoE<sup>-/-</sup> mouse. However, this difference is not statistically significant, p>0.05 (**Figure 3.8c**).

#### d) Integral force of contraction (AUC)

Analysis of AUC in a period of 10 minutes showed that the AUC of WT mouse was  $9.6 \pm 1.7$  compared to  $9.6 \pm 0.9$  recorded for ApoE<sup>-/-</sup> mouse. However, this difference is statistically not significant,  $p > 0.05$  (**Figure 3.8d**).

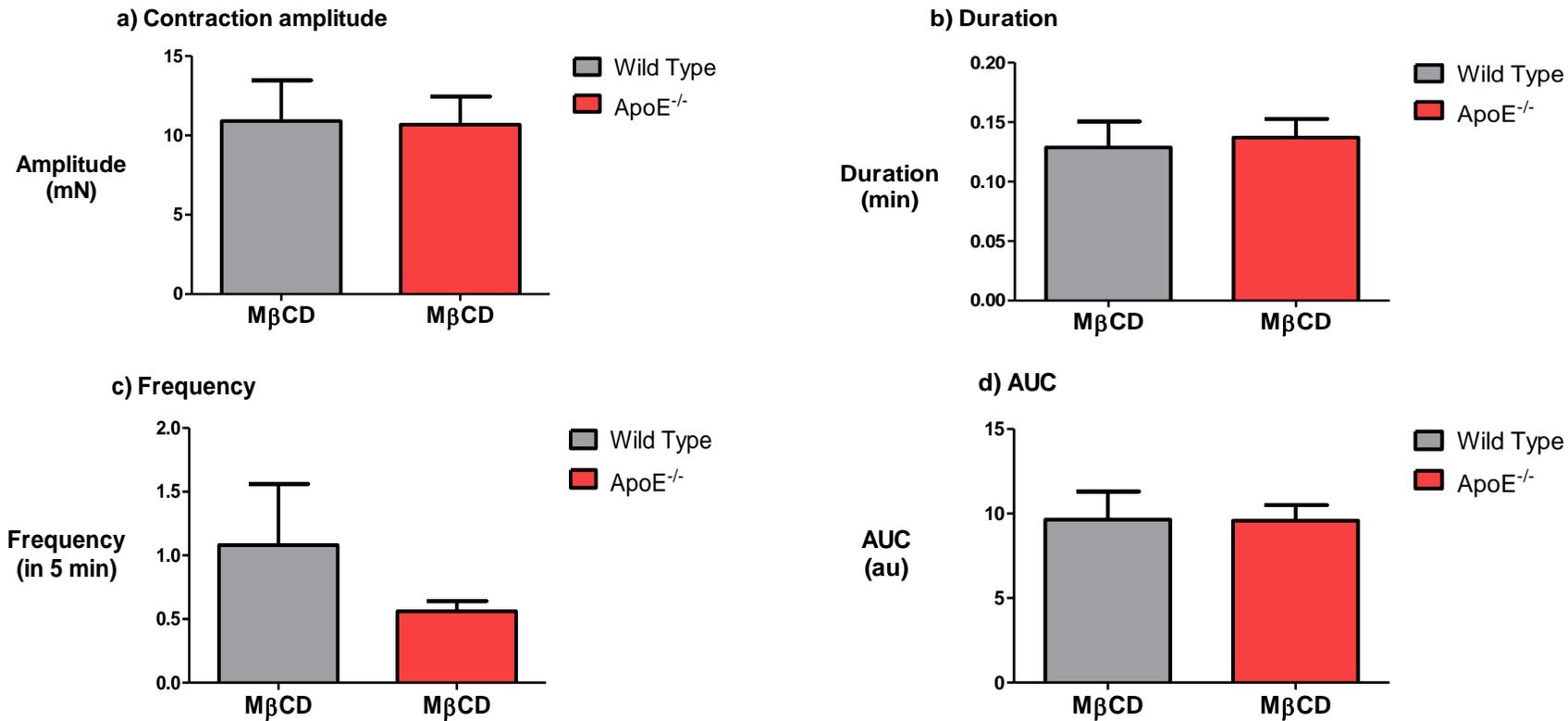
**Overall conclusion:** There is no difference in the contractility between WT and ApoE<sup>-/-</sup> mouse myometrium in both spontaneous contractions and the responses to oxytocin and M $\beta$ CD.

**Figure 3.7 Non-pregnant mouse myometrium: comparing the myometrial contractility between WT and ApoE<sup>-/-</sup> mouse in response to MβCD.**



A representative trace for A) WT mouse B) ApoE<sup>-/-</sup> mouse. MβCD (2%) had a stimulatory effect on both WT and ApoE<sup>-/-</sup> mouse myometrium.

Figure 3.8 Myometrial contractile activity of non-pregnant WT and ApoE<sup>-/-</sup> mouse in response to MβCD.



Bar charts showing the % mean values for a) contraction amplitude, b) duration, c) frequency and d) AUC for a period of 5 minutes. By comparing all the contractile parameters, there is no significant difference between WT (n=5) and ApoE<sup>-/-</sup> (n=5) mouse myometrium in response to MβCD (2%) (p>0.05).

### **3.3.3 Examining the effect of visfatin on non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrial contractility.**

Visfatin was tested on spontaneously contracted myometrium in the presence of, oxytocin. Myometrial activity was compared to the original control contractions preceding the application of visfatin by Student's *t*-test.

#### **3.3.3.1 The effect of visfatin on the spontaneous contractile activity of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium.**

After establishing steady state for spontaneous contractions, visfatin (10nM) was applied for a 10-minute period to spontaneously contracting non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium. Representative control traces from both spontaneously contracting WT and ApoE<sup>-/-</sup> mouse myometrium controls with the paired application of PBS are illustrated in **Figures 3.9A** and **3.10A**. As shown from the recordings presented in **Figures 3.9B** and **3.10B**, visfatin (10nM) had no effect on spontaneously contracted non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium. As seen in the original traces, no significant differences in non-pregnant contractility profiles were seen on the application of visfatin between WT and ApoE<sup>-/-</sup> mouse myometrium (**Table 3.1**).

##### **a) Force amplitude**

The mean amplitudes of WT and ApoE<sup>-/-</sup> mouse myometrium were not shown to be significantly different after the application of 10nM visfatin compared to the control ( $p>0.05$ ,  $n=8$ ). The mean force amplitudes of spontaneous contractions of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium recorded were  $13.2 \pm 2$  mN and  $9.7 \pm 1.4$  mN, respectively. Visfatin (10nM) had no effect on the amplitude of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium ( $p>0.05$ ,  $n=8$ ). There was no significant difference in the amplitude between WT and ApoE<sup>-/-</sup> mouse myometrium ( $p>0.05$ ,  $n=8$ ).

##### **b) Duration**

The mean duration of both WT and ApoE<sup>-/-</sup> mouse myometrium after the application of 10nM visfatin was not significantly different from the control, ( $p>0.05$ ,  $n=8$ ). The mean duration of spontaneous contractions of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium were  $0.7 \pm 0.1$  minutes and  $0.2 \pm 0.04$

minutes. Visfatin (10nM) had no effect on the duration of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium ( $p>0.05$ ,  $n=8$ ). There is no significant difference in the duration between WT and ApoE<sup>-/-</sup> mouse myometrium ( $p<0.05$ ,  $n=8$ ).

### **c) Frequency**

The mean frequency of both WT and ApoE<sup>-/-</sup> mouse myometrium after the application of 10nM visfatin was not significantly different from the control, ( $p>0.05$ ,  $n=8$ ). The mean frequency of spontaneous contractions of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium recorded after the application of visfatin 10nM were  $0.1 \pm 0.005$  and  $1.3 \pm 0.3$ . Visfatin (10nM) had no effect on the frequency of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium ( $p>0.05$ ,  $n=8$ ). Student *t test* showed this not to be significantly different from the control of each mouse and between WT and ApoE<sup>-/-</sup> mouse myometrium ( $p>0.05$ ,  $n=8$ ).

### **d) Integral force of contraction (AUC)**

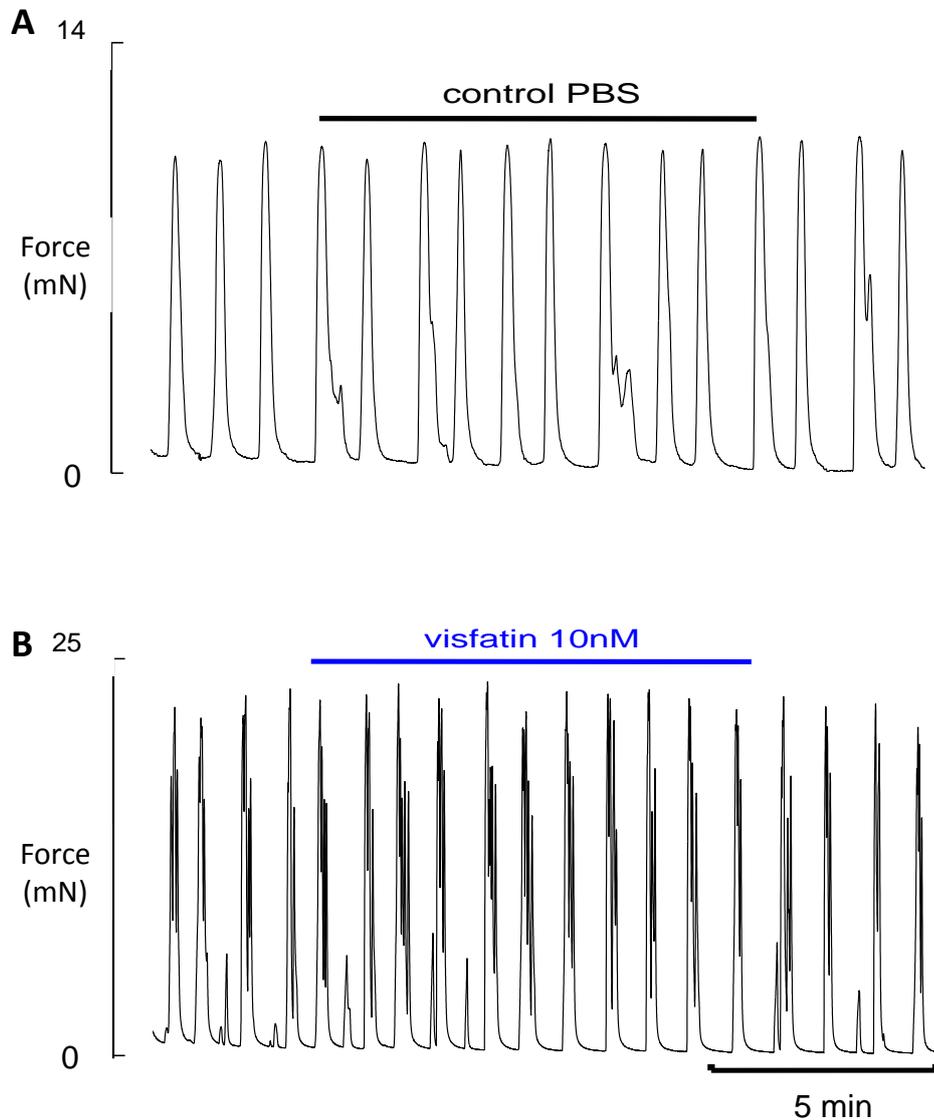
The mean AUC of both WT and ApoE<sup>-/-</sup> mouse myometrium after the application of 10nM visfatin was not significantly different from the 100 control, ( $p>0.05$ ,  $n=8$ ). The mean Integral of contractions of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium were  $10.9 \pm 2$  and  $8.3 \pm 1.4$ . Visfatin (10nM) had no effect on the AUC of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium ( $p>0.05$ ,  $n=8$ ). Spontaneous contractile activity was not significantly different between non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium ( $p>0.05$ ,  $n=8$ ).

**Conclusion:** visfatin (10nM) had no effect on the spontaneous contractions of both non-pregnant WT ( $n=8$ ) and ApoE<sup>-/-</sup> mouse myometrium ( $n=8$ ) ( $p>0.05$ ).

**Table 3.1** Difference in the spontaneous responses to visfatin (10nM) between non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium. P value <0.05 indicates that the trend was significant by Student's *t test*.

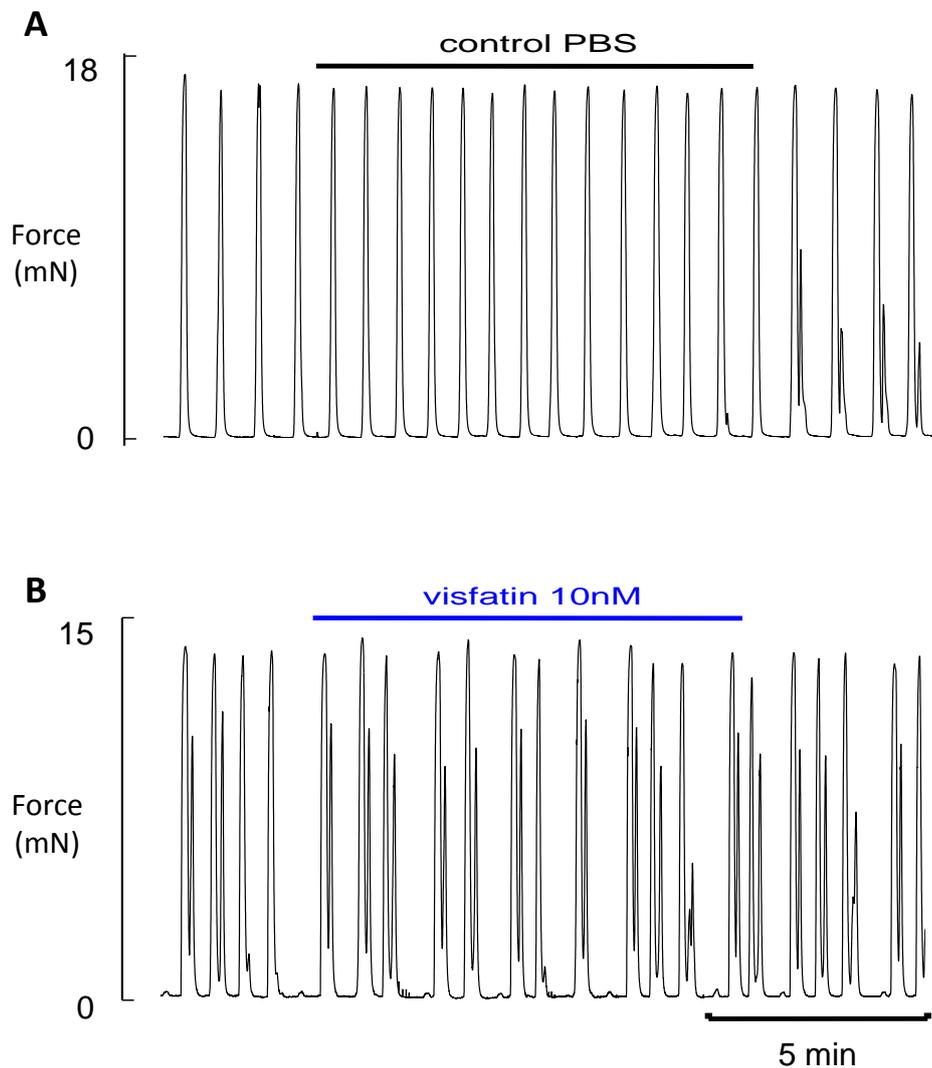
	<b>WT mouse (n=8)</b>	<b>ApoE<sup>-/-</sup> mouse (n=8)</b>	<b>P value</b>
<b>Force amplitude (mN)</b>	13.2 ± 2	9.7 ± 1.4	P value>0.05
<b>Duration (minutes)</b>	0.7 ± 0.1	0.2 ± 0.04	P value>0.05
<b>Frequency (contractions/20 minutes)</b>	0.1 ± 0.005	1.3 ± 0.3	P value>0.05
<b>AUC (au)</b>	10.9 ± 2	8.3 ± 1.4	P value>0.05

## Non-pregnant Wild Type



**Figure 3.9 Non-pregnant WT mouse myometrium: Effect of 10-minute application of 10mM visfatin on spontaneously contracting WT mouse myometrium.** A) a paired tissue strip exposed to PBS for the same length of time B) a representative trace for the effect of visfatin (10nM) on the spontaneous contractions of non-pregnant WT mouse myometrium. Visfatin (10nM) had no effect on non-pregnant WT mouse myometrium.

## Non-pregnant ApoE<sup>-/-</sup> mouse



**Figure 3.10 Non-pregnant ApoE<sup>-/-</sup> mouse myometrium: Effect of 10-minute application of 10mM visfatin on spontaneously contracting ApoE<sup>-/-</sup> mouse myometrium.** A) a paired tissue strip exposed to PBS for the same length of time B) a representative trace for the effect of visfatin (10nM) on the spontaneous contractions of non-pregnant ApoE<sup>-/-</sup> mouse myometrium. Visfatin (10nM) had no effect on non-pregnant ApoE<sup>-/-</sup> mouse myometrium.

### 3.3.3.2 The effect of visfatin on oxytocin-induced non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium.

Oxytocin is known to increase the contractile force in both mouse and human myometrium. To investigate the effect of visfatin under agonist stimulation to create a physiological environment closer to labour, the effects of visfatin (10nM) on oxytocin-induced contractions were studied. 1nM of oxytocin was added to PSS and applied to myometrial strips. Representative control traces for oxytocin-induced WT and ApoE<sup>-/-</sup> mouse myometrium with the paired application of PBS in oxytocin are illustrated in **Figures 3.11A** and **3.12A**. As shown in the original traces, visfatin (10nM) had a significant inhibitory effect on oxytocin-induced non-pregnant WT and no effect on oxytocin-induced ApoE<sup>-/-</sup> mouse myometrium (**Figures 3.11B** and **3.12B**). Once visfatin was removed and the myometrium returned to oxytocin, contractions resumed and returned to control values. **Table 3.2** summarises the contractility differences in the effects of visfatin (10nM) on oxytocin-induced non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium.

#### a) Force amplitude

The mean amplitude of both WT and ApoE<sup>-/-</sup> mouse myometrium was not shown to be significantly different after the application of 10nM visfatin compared to the control ( $p > 0.05$ ,  $n=8$ ). The mean force amplitude of oxytocin-induced contractions of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium recorded after the application of visfatin 10nM were  $7 \pm 1.1$  mN and  $6.2 \pm 1.8$  mN, respectively. There was no significant difference in the amplitude between WT and ApoE<sup>-/-</sup> mouse myometrium ( $p > 0.05$ ,  $n=8$ ).

#### b) Duration

The mean duration of both WT and ApoE<sup>-/-</sup> mouse myometrium was not shown to be significantly different after the application of 10nM visfatin compared to the control ( $p > 0.05$ ,  $n=8$ ). The mean duration of oxytocin-induced contractions of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium recorded after the application of visfatin 10nM were  $0.2 \pm 0.01$  minutes and  $0.2 \pm 0.04$  minutes. However, it was shown not to be significantly different from the control ( $p > 0.05$ ,  $n=8$ ).

#### c) Frequency

The mean frequency of both WT and ApoE<sup>-/-</sup> mouse myometrium was not shown to be significantly different after the application of 10nM visfatin compared to the control ( $p>0.05$ ,  $n=8$ ). The mean frequency of oxytocin-induced contractions of non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium recorded after the application of visfatin 10nM were  $5 \pm 0.7$  and  $1.5 \pm 0.3$ . Student *t test* showed this not to be significantly different ( $p>0.05$ ,  $n=8$ ).

**d) Integral force of contraction (AUC)**

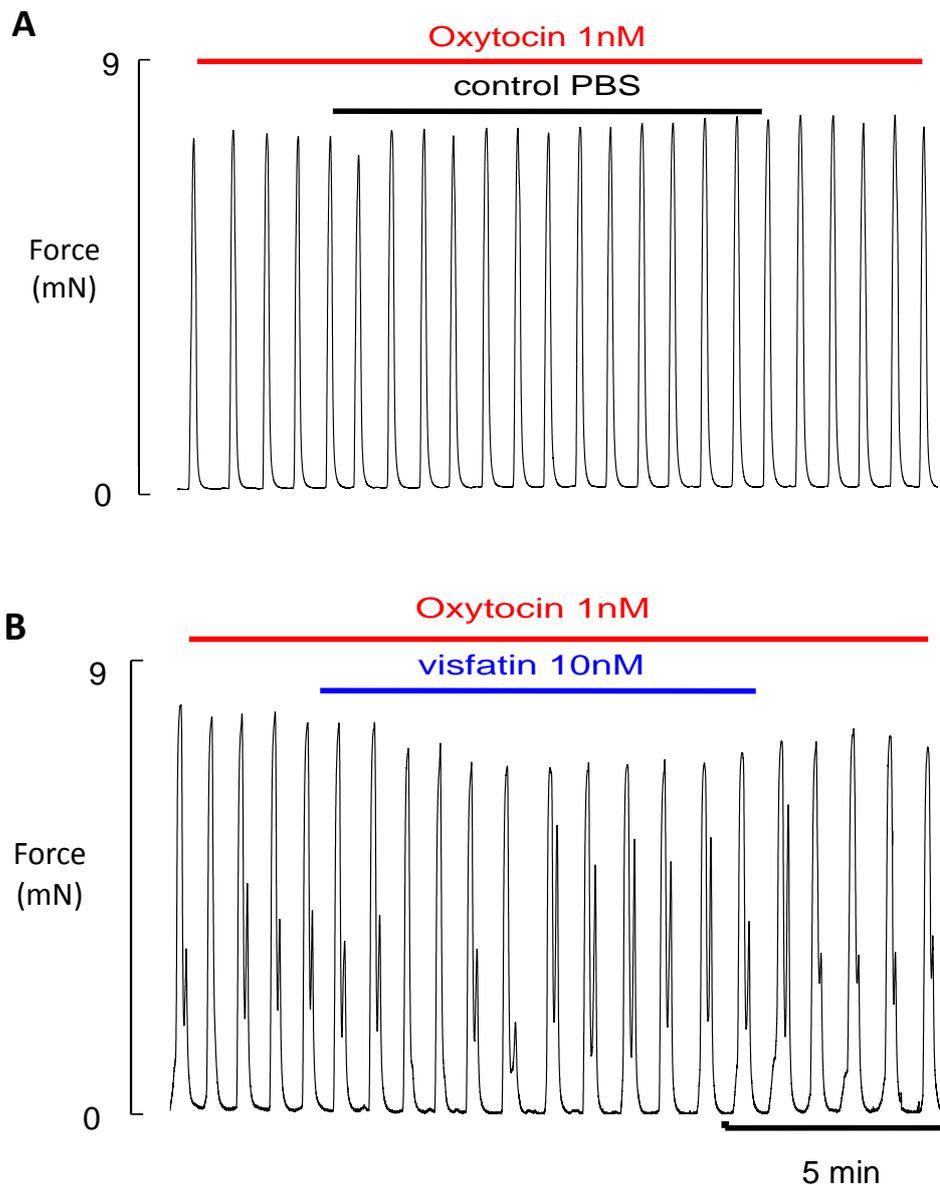
The mean AUC of WT mouse myometrium was shown to be significantly lower after the application of 10nM visfatin compared to the control ( $p<0.05$ ,  $n=8$ ). The mean integral of contractions after the application of visfatin 10nM on non-pregnant WT and ApoE<sup>-/-</sup> mouse myometrium were  $8 \pm 2.1$  and  $7.2 \pm 0.8$ . The AUC of WT mouse myometrium was not significantly different from ApoE<sup>-/-</sup> mouse myometrium ( $p>0.05$ ,  $n=8$ ).

**Conclusion:** visfatin (10nM) had an inhibitory effect on the oxytocin-induced contractions of non- pregnant WT mouse myometrium.

**Table 3.2 Difference in the response to visfatin (10nM) between non-pregnant WT and ApoE<sup>-/-</sup> oxytocin-induced mouse myometrium. P value <0.05 indicates that the trend was significant by Student's *t* test.**

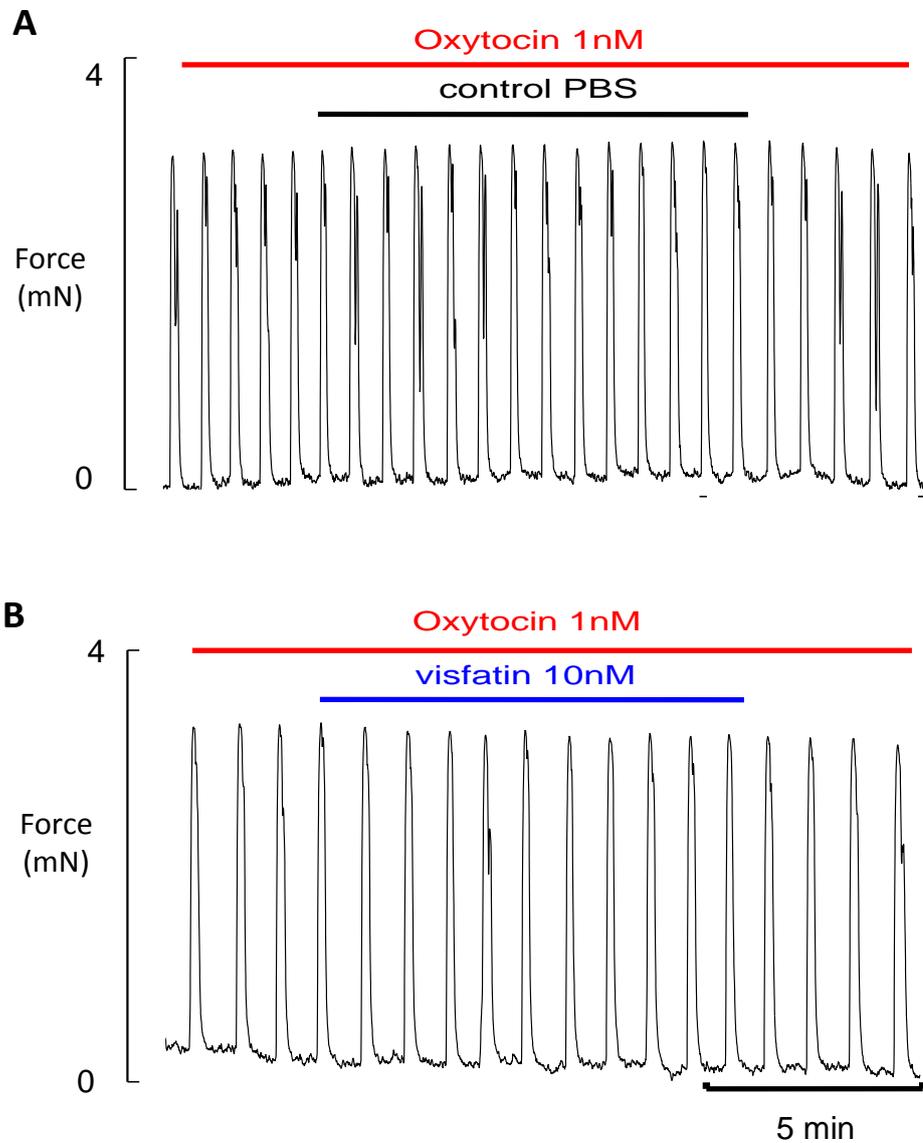
	<b>WT mouse (n=8)</b>	<b>ApoE<sup>-/-</sup> mouse (n=8)</b>	<b>P value</b>
<b>Force amplitude (mN)</b>	7 ± 1.1	6.2 ± 1.8	P value<0.05
<b>Duration (minutes)</b>	0.2 ± 0.01	0.2 ± .04	P value>0.05
<b>Frequency (contractions/20 minutes)</b>	5 ± 0.7	1.5 ± 0.3	P value>0.05
<b>AUC (au)</b>	8 ± 2.1*	7.2 ± 0.8	P value>0.05

## Non-pregnant WT mouse



**Figure 3.11 Non-pregnant mouse myometrium: Effect of 10 minute application of 10mM visfatin on oxytocin-induced WT mouse myometrium.** A) a paired tissue strip exposed to PBS for the same length of time B) a representative trace for the effect of visfatin (10nM) on the oxytocin-induced contractions of non-pregnant WT mouse myometrium which illustrates that Visfatin (10nM) caused a reduction of contractile activity of oxytocin-induced non-pregnant WT mouse myometrium.

## Non-pregnant ApoE<sup>-/-</sup> mouse



**Figure 3.12 Non-pregnant mouse myometrium: Effect of 10-minute application of 10mM visfatin on oxytocin-induced ApoE<sup>-/-</sup> mouse myometrium.** A) a paired tissue strip exposed to PBS for the same length of time B) a representative trace for the effect of visfatin (10nM) on the oxytocin-induced contractions of non-pregnant ApoE<sup>-/-</sup> mouse myometrium. Visfatin (10nM) had no effect on oxytocin-induced non-pregnant ApoE<sup>-/-</sup> mouse myometrium.

### 3.3.4 Examining the effect of visfatin on term pregnant WT and ApoE<sup>-/-</sup> mouse myometrial contractility

After studying the effects of visfatin on non-pregnant mouse myometrium, I moved on to study its effect on both WT and ApoE<sup>-/-</sup> term pregnant mouse myometrium. Visfatin was also tested on spontaneously contracted term pregnant myometrium and in the presence of oxytocin. Myometrial activity was compared directly to the original control contractions preceding the application of visfatin by Student's *t*-test.

#### 3.3.4.1 The effect of visfatin on the spontaneous contractile activity of term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium.

Serial concentrations of visfatin, from 10nM to 150mM, were applied extracellularly to spontaneously contracting term pregnant WT mouse myometrial strips for a 10-minute period and the effects on contractility were determined (10nM, n=9, 50nM, n=2, 100nM, n=1, 150nM, n=1). These were simply pilot studies to ensure that using a higher concentration wouldn't produce a response. Recordings for each concentration are shown in **Figure 3.13**. As seen in the original traces, visfatin had no effect on term pregnant WT mouse myometrial contractility, even with increasing concentrations of visfatin from 10nM up to 150nM. Therefore, for term pregnant ApoE<sup>-/-</sup> mouse myometrium, only 10nM concentration of visfatin was used (**Figure 3.14**). Even with longer applications of visfatin on term pregnant WT mouse myometrial (20 minutes), doses higher than 10nM had no effect (traces are not shown). As shown in the original recordings, visfatin (10nM) had no effect on term pregnant ApoE<sup>-/-</sup> mouse (n=4). **Table 3.3** summarises the contractile parameters of the responses to visfatin (10nM) between spontaneously contracted term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium.

##### a) Force amplitude

The mean amplitude of both WT and ApoE<sup>-/-</sup> mouse myometrium was not shown to be significantly different after the application of 10nM visfatin compared to the control ( $p > 0.05$ , n=9). The mean force amplitude of spontaneous contractions of term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium recorded

were  $12.6 \pm 2.3$  mN and  $9.3 \pm 1.3$  mN, respectively. Contraction amplitude between WT and ApoE<sup>-/-</sup> mouse was not shown to be significantly different ( $p > 0.05$ ,  $n=9$ ).

#### **b) Duration**

The mean duration of both WT and ApoE<sup>-/-</sup> mouse myometrium was not shown to be significantly different after the application of 10nM visfatin compared to the control ( $p > 0.05$ ,  $n=9$ ). The mean duration of spontaneous contractions of term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium were  $0.3 \pm 0.03$  minutes and  $0.2 \pm 0.02$  minutes. However, it was shown not to be significantly different ( $p > 0.05$ ,  $n=9$ ).

#### **c) Frequency**

The mean frequency of both WT and ApoE<sup>-/-</sup> mouse myometrium was not shown to be significantly different after the application of 10nM visfatin compared to the control ( $p > 0.05$ ,  $n=9$ ). The mean frequency of spontaneous contractions of term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium recorded were  $6.5 \pm 0.4$  and  $4.8 \pm 0.7$ . Student *t test* showed this not to be significantly different ( $p > 0.05$ ,  $n=9$ ).

#### **d) Integral force of contraction (AUC)**

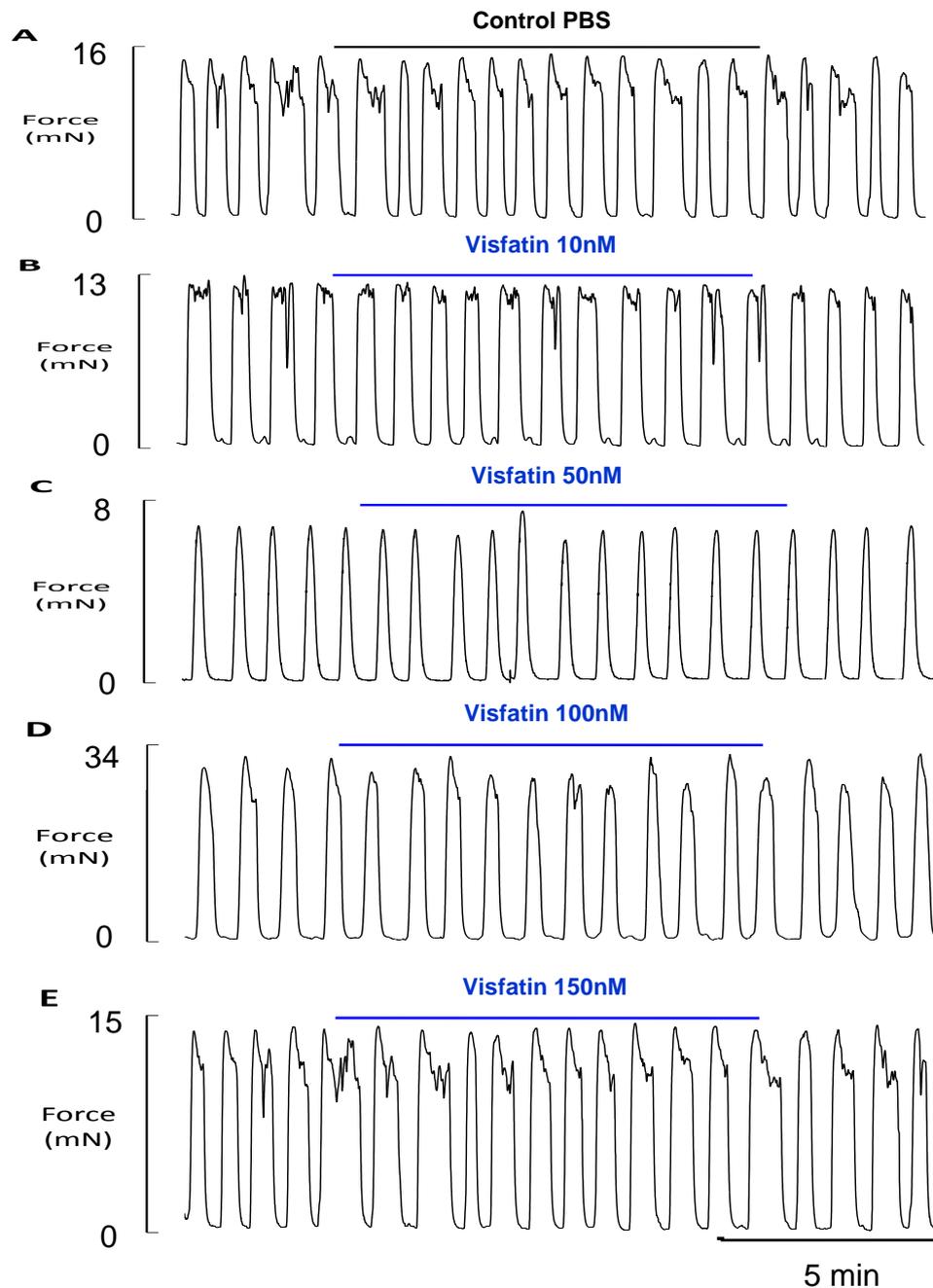
The mean AUC of both WT and ApoE<sup>-/-</sup> mouse myometrium was not shown to be significantly different after the application of 10nM visfatin compared to the control ( $p > 0.05$ ,  $n=9$ ). The mean integral of contractions after the application of visfatin 10nM on term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium were  $28.6 \pm 5.1$  and  $17.8 \pm 2.8$ . Spontaneous contractile activity of term pregnant WT mouse myometrium was not shown to be significantly different between WT and ApoE<sup>-/-</sup> mouse ( $p > 0.05$ ,  $n=9$ ).

**Conclusion:** visfatin (10nM) has no effect on the spontaneous contractions of both term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium ( $p > 0.05$ ,  $n=9$ ).

**Table 3.3** Difference in the effect of visfatin (10nM) between spontaneously contracted term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium. P value <0.05 indicates that the trend was significant by Student's *t* test.

	<b>WT mouse (n=9)</b>	<b>ApoE<sup>-/-</sup> mouse (n=9)</b>	<b>P value</b>
<b>Force amplitude (mN)</b>	12.6 ± 2.3	9.3 ± 1.3	P value > 0.05
<b>Duration (minutes)</b>	0.3 ± 0.03	0.2 ± 0.02	P value > 0.05
<b>Frequency (contractions/20 minutes)</b>	6.5 ± 0.4	4.8 ± 0.7	P value > 0.05
<b>AUC (au)</b>	28.6 ± 5.1	17.8 ± 2.8	P value > 0.05

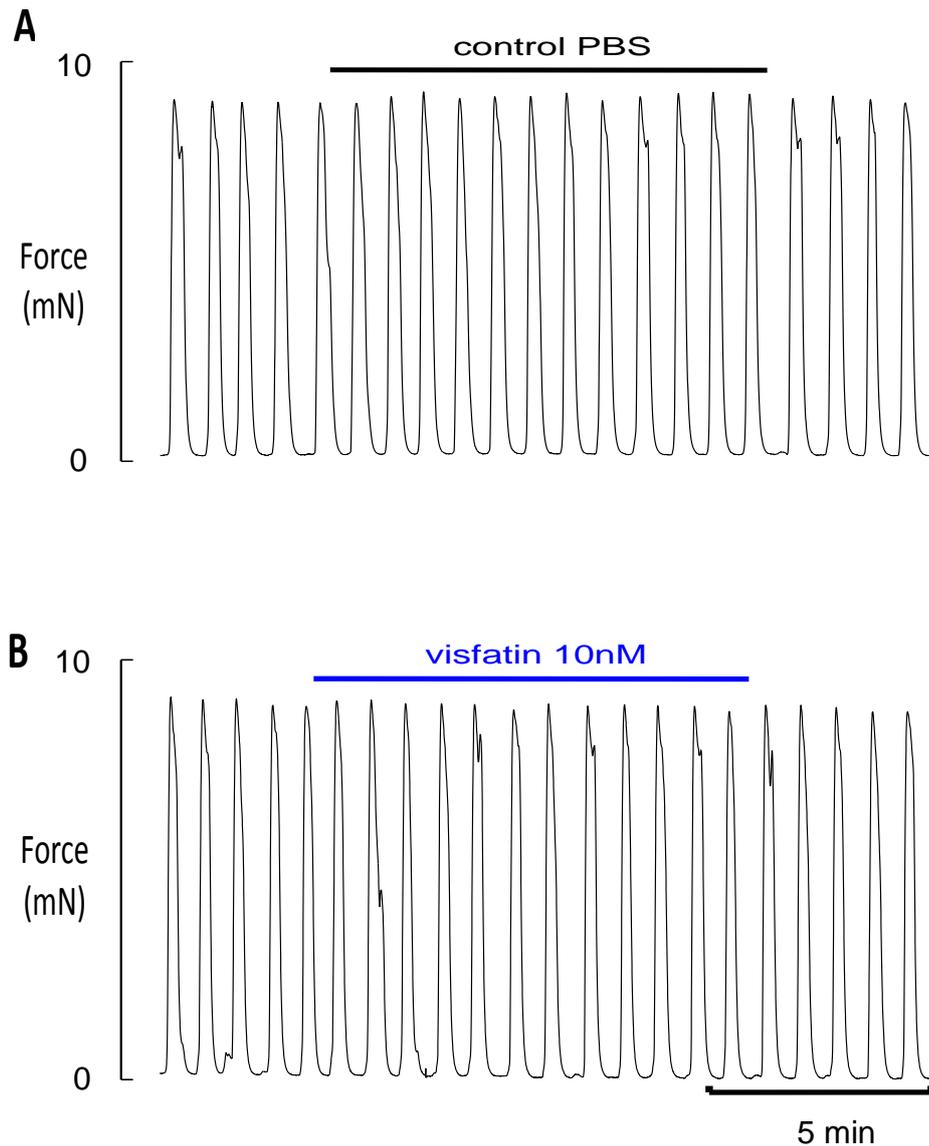
## Pregnant Wild Type mouse



**Figure 3.13 Term pregnant mouse myometrium: Effect of 10-minute application of visfatin on spontaneously contracting WT mouse myometrium.**

A) A paired tissue strip exposed to PBS for the same length of time. Representative traces for the effect of visfatin B) 10nM, C) 50nM, D) 100nM and E) 150nM on the spontaneous contractions of term pregnant WT mouse myometrium. Visfatin (10nM-150nM) had no effect on term pregnant WT mouse myometrium.

## Pregnant ApoE<sup>-/-</sup> mouse



**Figure 3.14** Term pregnant ApoE<sup>-/-</sup> mouse myometrium: Effect of 10-minute application of 10mM visfatin on spontaneously contracting ApoE<sup>-/-</sup> mouse myometrium. A) a paired tissue strip exposed to PBS for the same length of time B) a representative trace for the effect of visfatin (10nM) on the spontaneous contractions of term pregnant ApoE<sup>-/-</sup> mouse myometrium. Visfatin (10nM) had no effect on spontaneously contracting term pregnant ApoE<sup>-/-</sup> mouse myometrium.

### 3.3.4.2 The effect of visfatin on oxytocin-induced term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium.

These experiments were commenced by establishing a dose response assessment of visfatin concentration on term pregnant WT mouse myometrium. Serial concentrations of visfatin, from 10nM to 100mM, were applied extracellularly to oxytocin driven term pregnant WT mouse myometrial contractions for a 10-minute period and the effects on contractility were determined (10nM, n=20, 50nM, n=2, 100nM, n=1). Oxytocin (0.5nM) was added to PSS and applied to term pregnant myometrial strips. Typical recordings for each concentration are shown in **Figure 3.15**. As seen in the original traces, the response to visfatin was almost the same on increasing concentrations of visfatin up to 150nM. Therefore, for upcoming experiments on term pregnant mouse myometrium, a 10nM concentration of visfatin was chosen. Visfatin (10nM) caused a reduction of the contractile activity on term pregnant WT mouse myometrium (**Figure 3.16**). Representative control traces for oxytocin-induced WT and ApoE<sup>-/-</sup> mouse myometrium with paired application of PBS in oxytocin are illustrated in **Figures 3.15A** and **3.16A**. As shown in the original recordings, visfatin (10nM) had a significant inhibitory effect on oxytocin-induced term pregnant WT mouse myometrium (n=20) and no effect on ApoE<sup>-/-</sup> (n=4) mouse myometrium (**Figures 3.15B** and **3.16B**). The end number of oxytocin-induced term pregnant WT mouse myometrial tissue was high hence the visfatin effect measurements done alone and during studying the visfatin mechanism of action on myometrium were added. Even when the visfatin effect on oxytocin-induced term pregnant WT mouse measurements done alone was compared with ApoE<sup>-/-</sup> mouse myometrial contractility, the same statistically significant finding was found. Even with longer applications of visfatin on oxytocin induced term pregnant WT mouse myometrium (for 30 minutes), doses higher than 10nM had the almost similar effects of 10nM concentration (traces are not shown). Once visfatin was withdrawn and the myometrium returned to oxytocin, contractions did not return to control values. **Table 3.4** summarises the contractility differences in the effects of visfatin (10nM) on oxytocin-induced term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium.

### **a) Force amplitude**

The mean amplitude of both WT (n=20) and ApoE<sup>-/-</sup> (n=4) mouse myometrium was not found to be significantly different after the application of 10nM visfatin compared to the control (p>0.05). The mean force amplitude of oxytocin-induced contractions of term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium were 16.4 ± 2 mN and 23.4 ± 5.2mN, respectively.

### **b) Duration**

The mean duration of both WT (n=20) and ApoE<sup>-/-</sup> (n=4) mouse myometrium was not shown to be significantly different after the application of 10nM visfatin compared to the control (p>0.05). The mean duration of oxytocin-induced contractions of term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium were 7.4 ± 0.7 minutes and 6.7± 0.2 minutes. However, it was shown not to be significantly different (p>0.05).

### **c) Frequency**

The mean frequency of both WT (n=20) and ApoE<sup>-/-</sup> (n=4) mouse myometrium was not shown to be significantly different after the application of 10nM visfatin compared to the control (p>0.05). The mean frequency of oxytocin-induced contractions of term pregnant WT and ApoE<sup>-/-</sup> mouse myometrium were 0.2 ± 0.01 and 0.2 ± 0.03. However, it is not shown to be significantly different (p>0.05).

### **d) Integral force of contraction (AUC)**

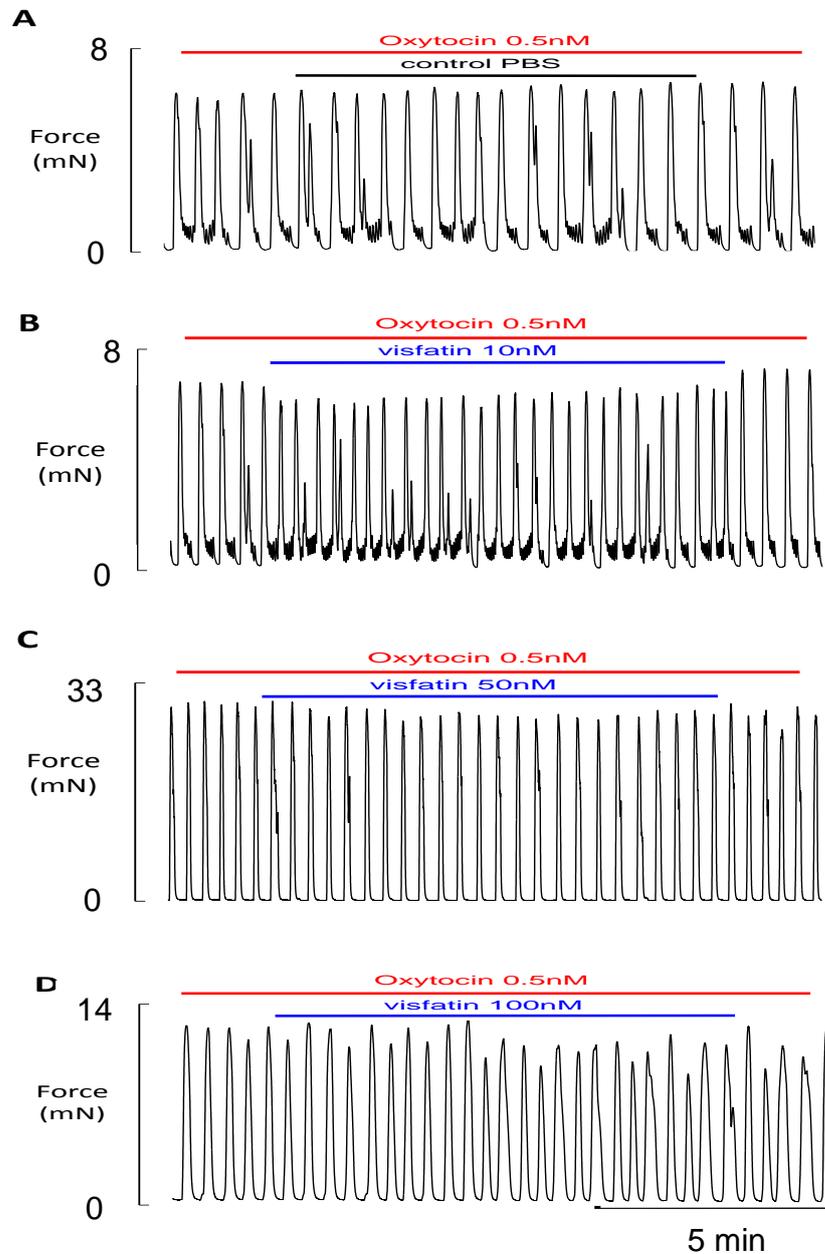
The mean AUC of WT (n=20) mouse myometrium was shown to be significantly lower after the application of 10nM visfatin compared to the control (p<0.05). The mean integral of contractions after the application of visfatin 10nM on non-pregnant WT (n=20) and ApoE<sup>-/-</sup> (n=4) mouse myometrium were 9.7 ± 1.4 and 15.7 ± 3. However, it was not found to be significantly different (p>0.05).

**Conclusion:** visfatin had a significant inhibitory effect on the AUC of oxytocin-induced term pregnant WT mouse myometrium.

**Table 3.4 Difference in the response to visfatin (10nM) between term pregnant WT and ApoE<sup>-/-</sup> oxytocin-induced mouse myometrium. P value <0.05 indicates that the trend was significant by Student's *t test*. \* Denotes significant difference in contractility compared to preceding control period p<0.05 \*\*p<0.005 \*\*\*p<0.0005**

	<b>WT mouse (n=20)</b>	<b>ApoE<sup>-/-</sup> mouse (n=4)</b>	<b>P value</b>
<b>Force amplitude (mN)</b>	16.4 ± 2	23.4.3 ± 5.2	P value>0.05
<b>Duration (minutes)</b>	7.4 ± 0.7	6.7 ± 0.2	P value>0.05
<b>Frequency (contractions/20 minutes)</b>	0.2 ± 0.01	0.2 ± 0.03	P value>0.05
<b>AUC (au)</b>	9.7 ± 1.4*	15.7 ± 3	P value>0.05

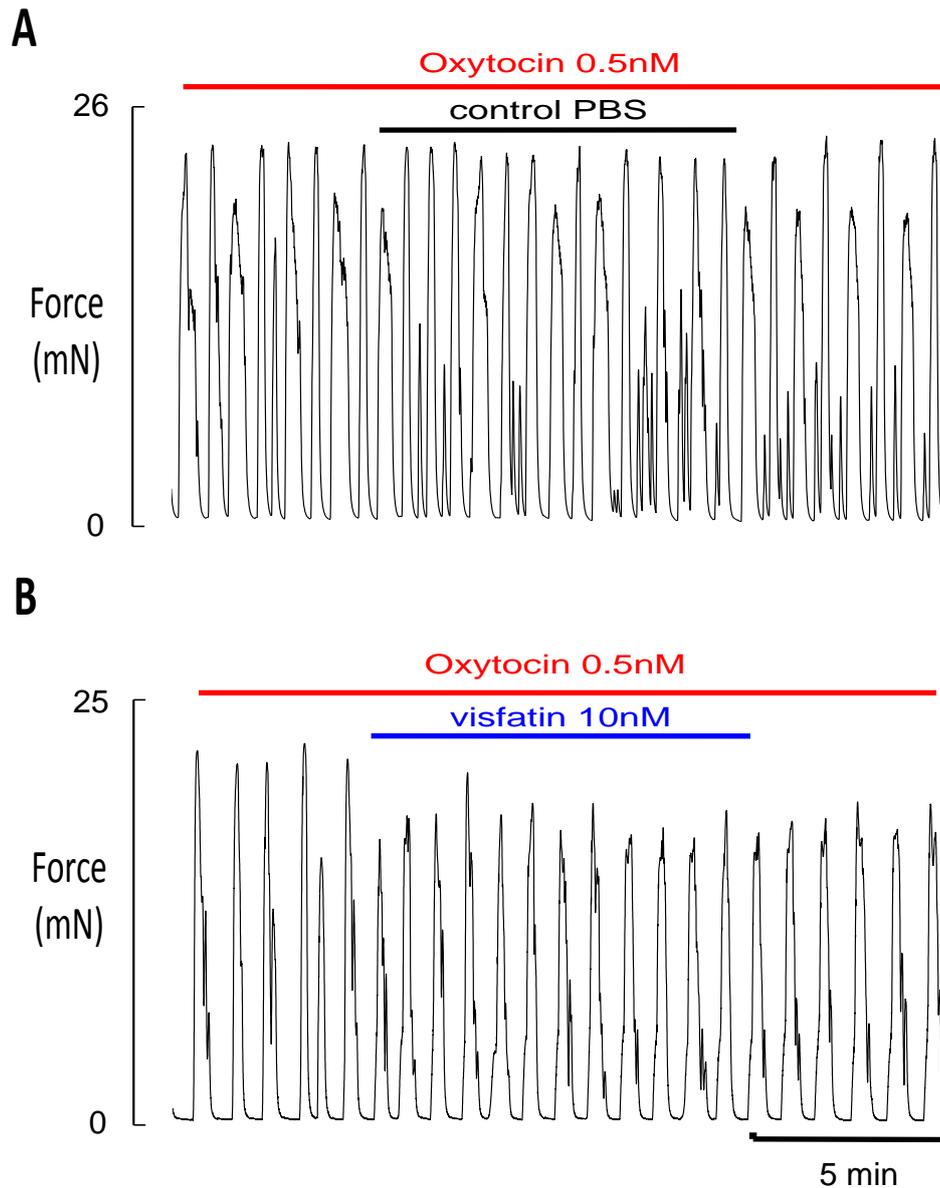
## Pregnant WT mouse



**Figure 3.15 Term pregnant mouse myometrium: Effect of 10-minute application of visfatin on oxytocin-induced WT mouse myometrium.**

A) A paired tissue strip exposed to PBS for the same length of time. Representative traces for the effect of visfatin B) 10nM, C) 50nM, and D) 100nM on the oxytocin-induced contractions of term pregnant WT mouse myometrium. Visfatin (10nM-100nM) caused a reduction of the contractile activity.

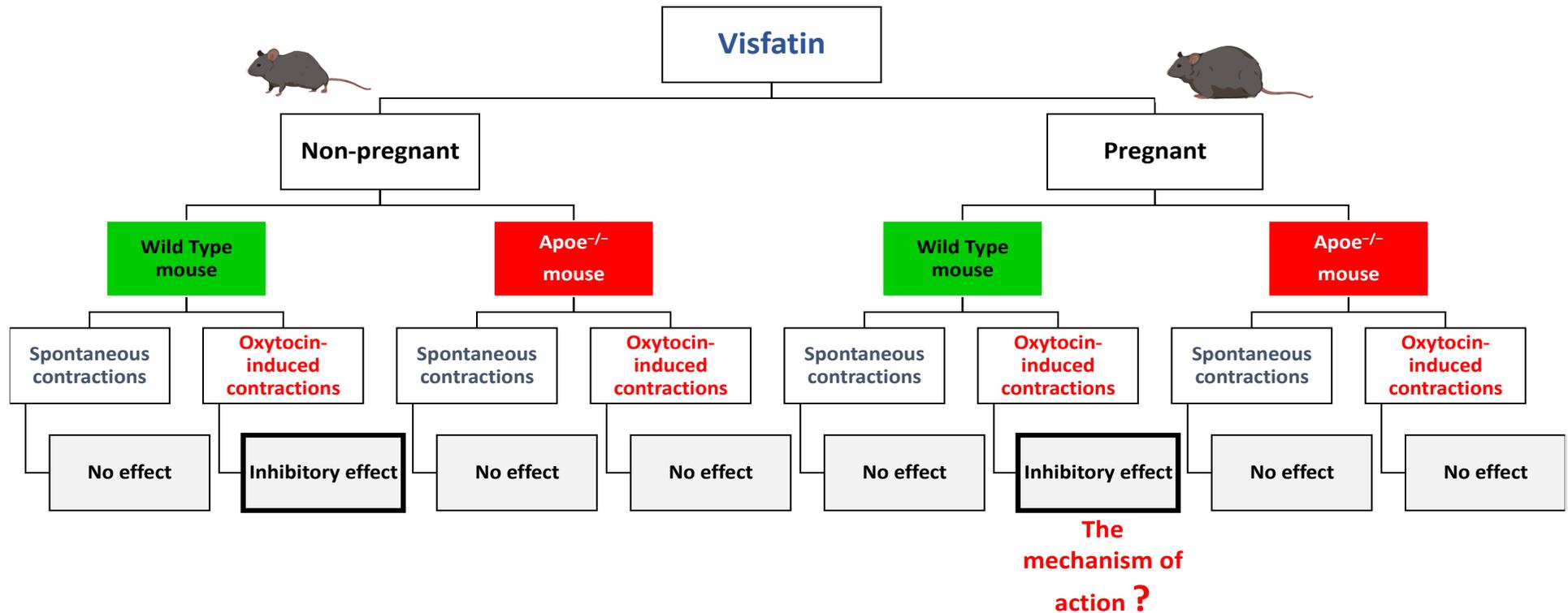
## Pregnant ApoE<sup>-/-</sup> mouse



**Figure 3.16 Term pregnant mouse myometrium: Effect of 10-minute application of 10mM visfatin on oxytocin-induced ApoE<sup>-/-</sup> mouse myometrium.** A) a paired tissue strip exposed to PBS for the same length of time B) a representative trace for the effect of visfatin (10nM) on the oxytocin-induced contractions of non-pregnant ApoE<sup>-/-</sup> mouse myometrium. Visfatin (10nM) had an inhibitory effect on oxytocin-induced term pregnant ApoE<sup>-/-</sup> mouse myometrium; however, this effect is not statistically significant.

Figure 3.17 Summary of the effects of visfatin on non-pregnant and pregnant WT and ApoE<sup>-/-</sup> mouse myometrium.

125



Visfatin had an inhibitory effect on both non-pregnant and pregnant WT oxytocin-induced mouse myometrium based on analysis of the integrated tension records. With no observed effect on ApoE<sup>-/-</sup> mouse myometrial contractility. To explore the mechanism of action of visfatin on myometrium, term pregnant WT mouse myometrium under the exposure of oxytocin drive was used.

### 3.3.5 Exploring the mechanism of action of visfatin on pregnant WT mouse myometrial contractility - NAD<sup>+</sup> pathway

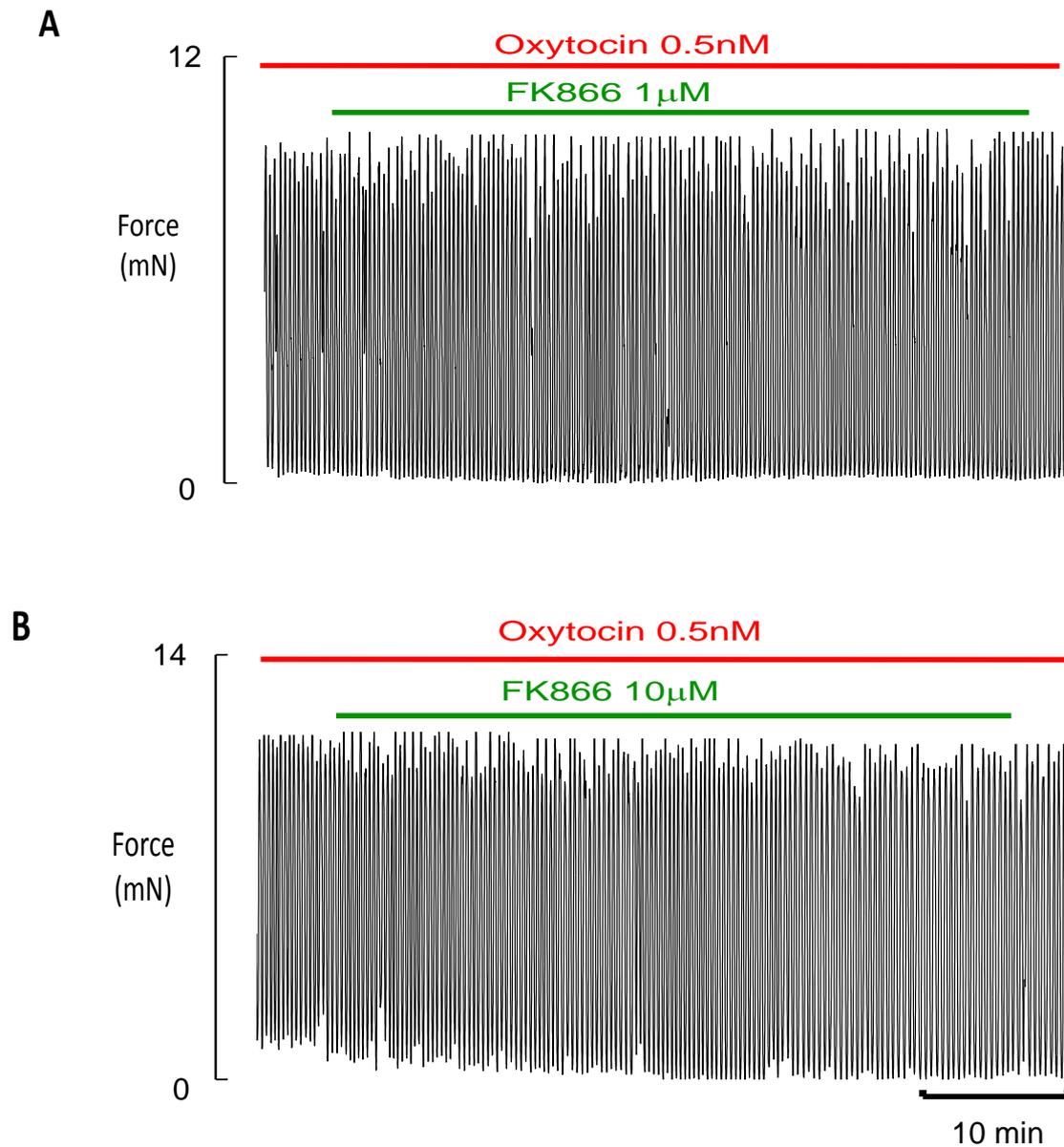
**Figure 3.17** summarises the effects of visfatin on non-pregnant and pregnant WT and ApoE<sup>-/-</sup> mouse myometrium. It had an inhibitory effect on both non-pregnant and pregnant WT oxytocin-induced mouse myometrium. To further study the mechanism of action of visfatin on myometrium, the NAD<sup>+</sup> pathway was examined on term pregnant WT mice. To examine whether visfatin inhibits oxytocin-induced term pregnant WT mouse myometrial contractility through NAD<sup>+</sup> pathway, FK866, NAD<sup>+</sup> and Nicotinic Acid were added to PSS with the direct application of visfatin and the effect on mouse myometrial contractility was recorded (**Figure 3.2**).

## 1- FK866

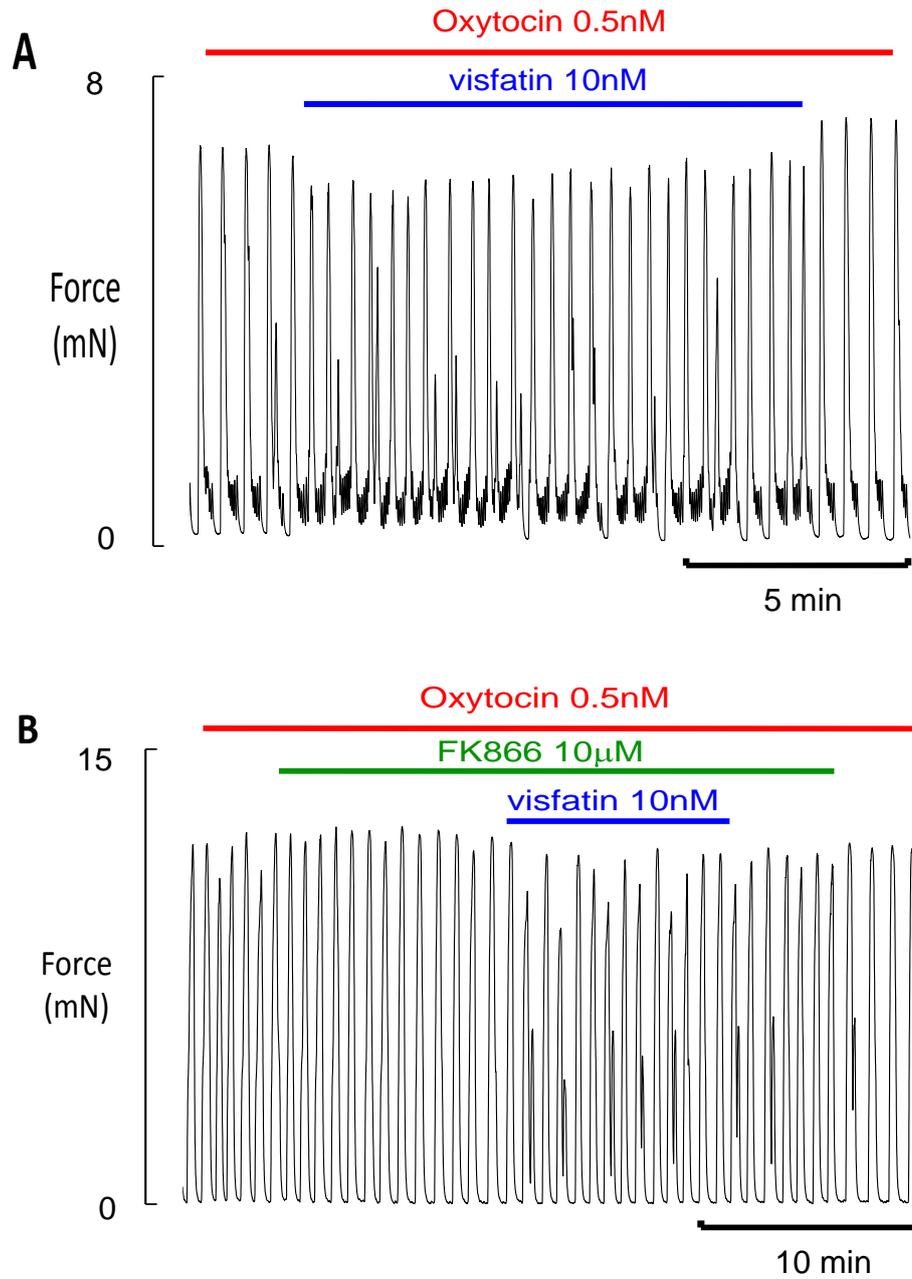
Initial control experiments were carried out to examine the effect of FK866 alone on myometrial contractility, and to determine how long stable contractions in its presence could be observed. Two concentrations of FK866 1 $\mu$ M and 10 $\mu$ M were examined (**Figures 3.18 A and B**). Neither concentration had an effect on myometrial contractility during 45min applications. Therefore FK866 at 10 $\mu$ M concentration was used to maximise its inhibitory activity.

10 $\mu$ M FK866 was added to contracting myometrium alone for 10 minutes to allow sufficient inhibition of the NAD<sup>+</sup> pathway and visfatin was then directly applied to the myometrial tissue for 10 minutes. The negative effect of visfatin on myometrial contractility was inhibited in the presence of FK866 (**Figure 3.19B**). There was no significant decrease in amplitude, duration, frequency or AUC in the presence of FK866 (**Figure 3.19B**). On comparison of mouse myometrium in response to 10nM visfatin with and without FK866, my data showed that inhibiting NAD<sup>+</sup> pathway significantly cancelling the inhibitory effect of visfatin on the AUC of contractions (**Figure 3.20d**). Once visfatin was removed and the myometrium returned to oxytocin, contractions did not return to control values.

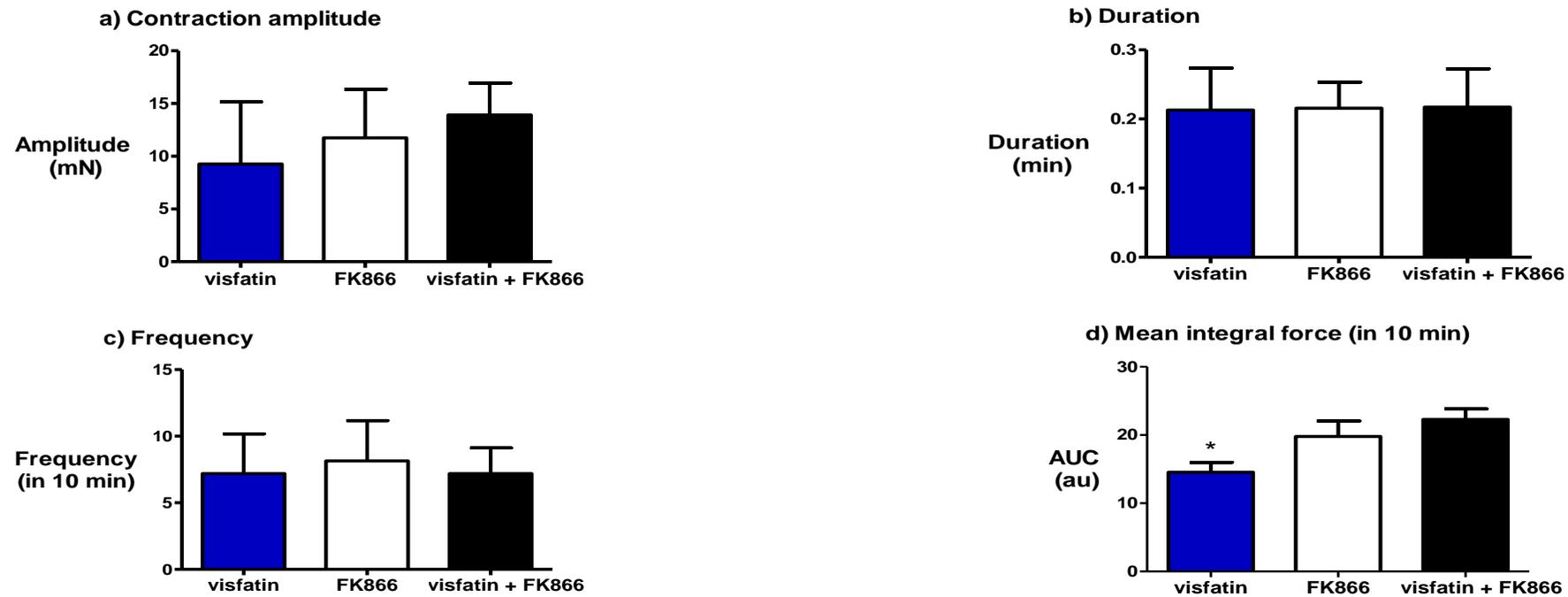
Visfatin 10nM was found to decrease the AUC of term pregnant WT mouse myometrial contractility, therefore a one-way ANOVA test was done by comparing the AUC's. The mean integral of contractions after the application of visfatin 10nM on non-pregnant WT mouse myometrium (n=20) was  $14.54 \pm 1.4$  which is significantly different from the control ( $p < 0.05$ ). The end number of oxytocin-induced term pregnant WT mouse myometrial tissue (the control) was high hence the visfatin effect measurements done alone (see section 3.3.4.2) and during studying the visfatin mechanism of action on myometrium in this section were added. There was no statistical difference between the AUC if FK866 was applied alone ( $19.8 \pm 2.3$ , n=7) or in combination with visfatin ( $22.3 \pm 1.5$ , n=8) ( $p > 0.05$ ) (**Figure 3.20d**). This indicates that FK866 has inhibited (cancelled) the effect of visfatin on myometrial contractility.



**Figure 3.18 The effect of FK866 on oxytocin-induced term pregnant WT mouse myometrium.** Representative traces showing the effect of FK866 A) 1 $\mu$ m and B) 10 $\mu$ m on oxytocin induced mouse myometrial contractility (45 min). FK866 on both concentrations produced no effect on oxytocin-induced pregnant WT mouse myometrium.



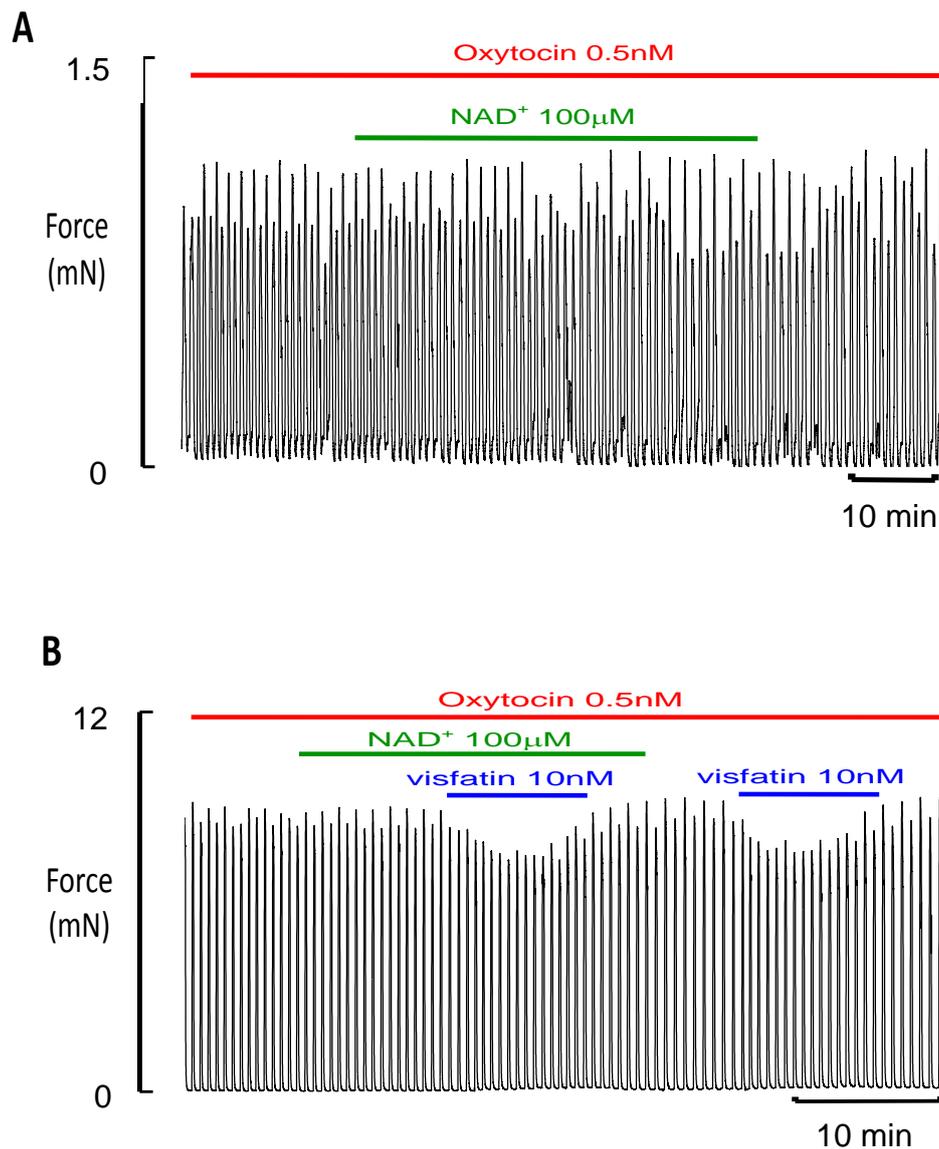
**Figure 3.19** The effect of FK866 and visfatin on oxytocin-induced term pregnant WT mouse myometrium. Representative traces illustrating the effect of 10nM visfatin in B) the absence and C) the presence of 10μm FK866. FK866 reduced the inhibitory effect visfatin on oxytocin-induced term pregnant WT mouse myometrium.



**Figure 3.20** The contractile profiles for the effect of FK866 (10 $\mu$ M) and visfatin (10nM) on oxytocin-induced term pregnant WT mouse myometrium. Visfatin 10nM had a significant inhibitory effect on the AUC (n=20, p<0.05). There was no statistical difference between the AUC if FK866 applied alone (n=7) or in combination with visfatin (n=8) or if both compared to control (p>0.05). FK866 cancelled the effect of visfatin on myometrial contractility. \* Denotes significant difference in contractility compared to preceding control period p<0.05 \*\*p<0.005 \*\*\*p<0.0005.

## 2- Nicotinamide Adenine Dinucleotide (NAD<sup>+</sup>)

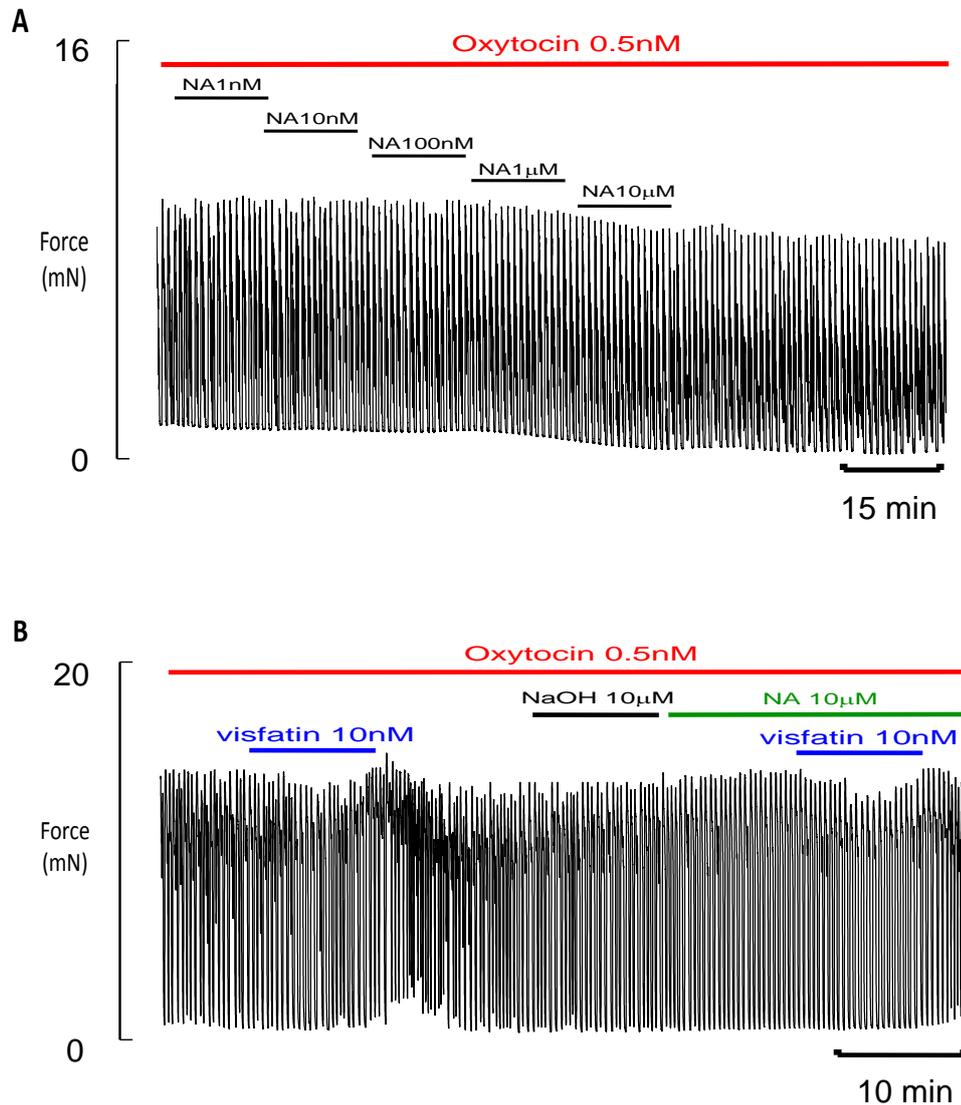
Application of NAD<sup>+</sup> metabolite was used to accelerate the NAD<sup>+</sup> pathway (**Figure 3.2**). NAD<sup>+</sup> is a metabolic product of glycolysis (Wang et al., 2006). Initial control experiments were carried out to examine the effect of NAD<sup>+</sup> alone on myometrial contractility, and to determine how long stable contractions in its presence could be observed (**Figure 3.21A**). A 100 $\mu$ M concentration of NAD<sup>+</sup> was used and applied for 90 minutes. NAD<sup>+</sup> (100 $\mu$ M) caused no effect on myometrial contractility, therefore it could be used to examine the mechanism of action of visfatin on myometrial contractility. 100 $\mu$ M NAD<sup>+</sup> was added to the contracting myometrium alone for 20 minutes to allow potentiation of the NAD<sup>+</sup> pathway and visfatin was then directly applied to the myometrial tissue for 10 minutes (n=3). The negative effect of visfatin on myometrial contractility was enhanced in the presence of NAD<sup>+</sup> (**Figure 3.21B**).



**Figure 3.21 The effect of NAD<sup>+</sup> on oxytocin-induced pregnant WT mouse myometrium.** A) A representative trace showing the effect of 100μm NAD<sup>+</sup> on mouse myometrial contractility. NAD<sup>+</sup> (100μm) caused no effect on oxytocin-induced myometrial contractility. B) Representative trace for the effect of 10nM visfatin in the absence and the presence of 100μm NAD<sup>+</sup>. NAD<sup>+</sup> enhanced the effect visfatin on oxytocin-induced pregnant WT mouse myometrium

## 2- Nicotinic Acid

The effect of another cellular metabolite was also examined. Application of nicotinic acid (NA) was used to indirectly increase NAD<sup>+</sup> production. Initial control experiments were carried out to examine the effect of NA alone on myometrial contractility (**Figure 3.22A**). Serial concentrations of NA were used and applied for 15 minutes (1nM, 10nM, 100nM, 1μM and 10μM). NA caused no effect on myometrial contractility, therefore it can be used to examine the mechanism of action of visfatin on myometrial contractility. NA (10μM) was added to the contracting myometrium alone for 15 minutes to allow sufficient stimulation of the NAD<sup>+</sup> pathway and visfatin was then directly applied to the myometrial tissue for 10 minutes (n=2). The negative effect of visfatin on myometrial contractility was enhanced in the presence of NA (**Figure 3.22B**).



**Figure 3.22 The effect of nicotinic acid on oxytocin-induced pregnant WT mouse myometrium.** A) A representative trace showing the effect of NA (1nM, 10nM, 100nM, 1μM and 10μM) on mouse myometrial contractility after application for 15 minutes. NA even in high concentration (10μM) has no effect on myometrial contractility (the baseline moved down). B) Representative trace for the effect of 10nM visfatin in the absence and the presence of 10μm NA. NA enhanced the effect visfatin on oxytocin-induced pregnant WT mouse myometrium.

### 3.4 Discussion

A better understanding of the pathophysiological impact of obesity on pregnancy and labour could provide effective targets for the prevention and management of obesity complications in pregnant women. The results of this study have shown no differences in contractility between non-pregnant WT and the dyslipidaemic animal model, the ApoE<sup>-/-</sup> mouse in spontaneous contractions and in responses to oxytocin drive and MβCD application. Interestingly, the stimulatory effect of oxytocin was higher in WT compared to ApoE<sup>-/-</sup> mouse myometrium; however, this difference was not statistically significant. This indicates a lack of evidence that having a hyperlipidaemic environment might disrupt myometrial contractility. The effects of dyslipidaemia may be underestimated in my study as other factors, such as hormonal, paracrine, autocrine effects produced by adipose tissue accumulation are missing or reduced in *in vitro* experiments.

It was disappointing that the lowering of cholesterol with MβCD, and its disruption of membrane caveolae, showed no difference between WT and ApoE<sup>-/-</sup> mice. Cholesterol has been shown to increase the activity of K<sup>+</sup> channels producing large outward currents in uterine myocytes (Shmygol et al., 2007b), and that will decrease contractility. The oxytocin receptor activity was reported to be reduced in lipid rafts when disrupted by MβCD (Klein et al., 1995). Although it was noticed that our in-house female ApoE<sup>-/-</sup> mice were having difficulties becoming pregnant and breeding them in our lab was a challenging task; however, their reproductive physiology was reported by others to be normal (Kashyap et al., 1995).

It was shown that cholesterol has a profound inhibitory effect on force production of both spontaneous and oxytocin induced contractions impairing the ability of the uterus to contract effectively during labour (Zhang et al., 2010a). Some insights were gleaned from the simultaneous recording of intracellular calcium and spontaneous contraction in the presence of cholesterol which found that this inhibition occurs due to reduced Ca<sup>+2</sup> influx (Smith et al., 2005). Gam *et al* recently found that myometrial mitochondrial quantity and capacity in the isolated state was not affected in obese pregnant women, nevertheless, they observed an increase in myometrial lipid content and reduction in myocyte

density which might explain poor uterine contractility in obese women (Gam et al., 2017).

In this chapter, It was shown that visfatin has no effect on non-pregnant and pregnant WT and ApoE<sup>-/-</sup> mouse spontaneous myometrial contractility or on oxytocin-induced non-pregnant and term pregnant ApoE<sup>-/-</sup> mouse myometrial contractility. This might be due to the adaptation to high lipids and visfatin levels in the chronic hyperlipidaemic environment suggested to develop in ApoE<sup>-/-</sup> mouse to maintain cholesterol homeostasis. However, it was shown that visfatin, has a significant inhibitory effect on the AUC, which is an index of the total contractile activity, of oxytocin-induced non-pregnant and term pregnant WT mouse. These results suggest that visfatin has a relaxant effect only if the tissue was under physiological conditions (i.e. oxytocin). This inhibitory effect was detected at a relatively low concentration for *in vitro* experiments. A lack of a concentration dependence of the inhibitory effect of visfatin on mouse myometrium was observed. This is a general characteristic of a receptor mediated effect to be likely a concentration dependent effect. It might be due to the little amount of inhibition produced by visfatin on myometrial contractility. A possible supramaximal response at 10nM might also occurred. However, it was found to be a concentration dependent effect on human myometrium and the IC<sub>50</sub> of the AUC was 1.08nM (Mumtaz et al., 2015).

Strips were compared directly from the same myometrial tissue maintaining reproducible results on myometrial contractility and controlling for physical conditions, stretch, vehicle and time. There were no significant effects of vehicles. Time was chosen carefully to give the drug sufficient time to work and long enough to avoid tissue fatigue. The effects of visfatin on myometrial contractility were reliable, obvious, reproducible and significant, and in agreement to its effects on both rat and human.

The inhibitory effect of visfatin was observed at relatively low concentrations (10nM) and is within the physiological maternal plasma range for visfatin during pregnancy (Mazaki-Tovi et al., 2009b). The data obtained from this present study is in line with our previous study on pregnant rat and human myometrium which found that visfatin produced a significant reduction in the amplitude and AUC of spontaneous contractions and a significant reduction in

the AUC of oxytocin-induced myometrial contractions *in vitro* (Mumtaz et al., 2015). However no effect on spontaneous contractions was found in this study.

Other adipokines investigated in connection with uterine contractility have been found to have an inhibitory effect on myometrial contractility *in vitro*. Leptin has been documented to have a relaxant effect on spontaneous and oxytocin-induced rat and human myometrial contractions (Mumtaz et al., 2015) and this effect was cumulative on human myometrium (Moynihan et al., 2006). Apelin was found to exert a potent dose-dependent inhibitory effect on spontaneous and oxytocin-induced pregnant human myometrial contractility (Hehir and Morrison, 2012). Despite the contradictory published data about the effect of ghrelin on myometrial contractility, three studies have reported that it has a relaxant effect on myometrial contractility. Mostafa and Samir reported that ghrelin has an inhibitory effect on virgin rat spontaneous and oxytocin-induced myometrial (Mostafa and Samir, 2013) and Hehir *et al* indicated that this effect is concentration-dependent on pregnant human myometrium (Hehir et al., 2008). O'Brien *et al* found that ghrelin plays a significant role in the maintenance of uterine relaxation during pregnancy (O'Brien et al., 2010). Thus, all four of these adipokines - visfatin, leptin, apelin and ghrelin - have been reported to relax human myometrium *in vitro*, which leads to the suggestion that there may be a metabolic modulation of myometrium by adipokines which are produced in high concentrations in the dyslipidaemic environment frequently developed in obese pregnant women. Therefore, my results, along with the previous data from the effect of other adipokines on myometrial contractility, introduce a narrative aspect that adipokines released from the excess adipose tissue developed with obesity modulate myometrial contractility and may contribute to dysfunctional labour, and failed post term induction (Arrowsmith et al., 2011).

There are some studies of the effect of visfatin on other smooth muscle contractility, particularly on vascular smooth muscle. It has a vasodilating effect on isolated noradrenaline stimulated aortic smooth muscle and this effect was found to be mediated through endothelium-derived nitric oxide (Yamawaki et al., 2009). Visfatin significantly tempers endothelium-dependent vasodilation in small bradykinin-stimulated coronary arteries, an effect thought to be as a result of endothelial cell NADPH oxidase activation by membrane lipid raft clustering (Xia

et al., 2011), which are potent modulators of myometrial force (Noble et al., 2006, Shmygol et al., 2007b). Perivascular adipokines have been reported to lose their anti-contractile activity with obesity indicating that responses to adipokines can be affected by underlying obesity (Boydens et al., 2012, Meyer et al., 2013).

In this study, it was demonstrated that visfatin reduces contractility in term pregnant WT mouse myometrium when it is augmented with oxytocin. But what is the cellular mechanism of action behind this reduced contractile activity? Many studies have indicated that visfatin may play a role in dysfunctional labour; however, no previous functional studies have investigated the effect of visfatin on myometrial contractility. A potential mechanism which might underlie the effect of visfatin on term pregnant WT mouse, the NAD<sup>+</sup> pathway, was investigated in this study. This pathway was also reported to be important in visfatin's vasodilator effects on mesenteric microvessels in rat and human (Vallejo et al., 2011). NAMPT enzyme catalyses the rate-limiting step in the biosynthesis of NAD<sup>+</sup> cofactor from nicotinamide which is essential for energy production and cellular metabolism (Garten et al., 2009). Its metabolic role in priming the myometrial contractions could be involved under conditions of stress or hypoxia (Mumtaz et al., 2015). Human visfatin gene expression has been shown to be stimulated by hypoxia (Bae et al., 2006, Segawa et al., 2006). Although normal uterine contractions are found to be associated with transient hypoxia (Alotaibi et al., 2015), hypoxia is known to decrease contractile strength per se (Wray, 2007). Stretch also induces the profound production of visfatin (Kendal-Wright et al., 2010, Kendal-Wright et al., 2008), a mechanism which can explain the increased supply of energy and metabolites required for the single cell to effectively modify its cytoskeleton structure and gene expression necessary for the extra mechanical work needed during successful labour.

To the best of my knowledge, no previous study has examined the mechanism of action of visfatin on myometrium. To assess whether or not visfatin works through the NAD<sup>+</sup> pathway, pharmacological modulation of the myometrium by FK866, NAD<sup>+</sup> and NA in the presence of visfatin were examined. By using FK866 as an inhibitor of the NAD<sup>+</sup> pathway, it was found that visfatin might work via this pathway. Findings from the application of NAD<sup>+</sup> and NA further consolidate this mechanism of action. FK866 cancelled the effect of visfatin whilst NAD<sup>+</sup> and NA enhanced its effect on WT oxytocin-induced myometrium. A direct

inhibitory effect of excessive NAD<sup>+</sup> application was expected; however it was shown that it has an enhancing effect of visfatin which made the mechanism more complicated. Therefore, visfatin inhibitory effect is likely to be independent of the direct enzyme action of visfatin. In addition, the enhancing effect of NA suggests that inhibitory effect is upstream of visfatin. Nonetheless, it is not yet clearly indicated whether visfatin acting extracellularly or intracellularly on myometrium. Visfatin, therefore, probably might work through increasing the NAD<sup>+</sup> pathway. These are preliminary observations hence visfatin theoretically might work on the myometrium through different mechanisms. It would be interesting for future studies to investigate if PGE2 pathway is important for visfatin's actions in human myometrium. In addition, it is not known whether visfatin is acting extracellularly or intracellularly. This is a subject of a future work.

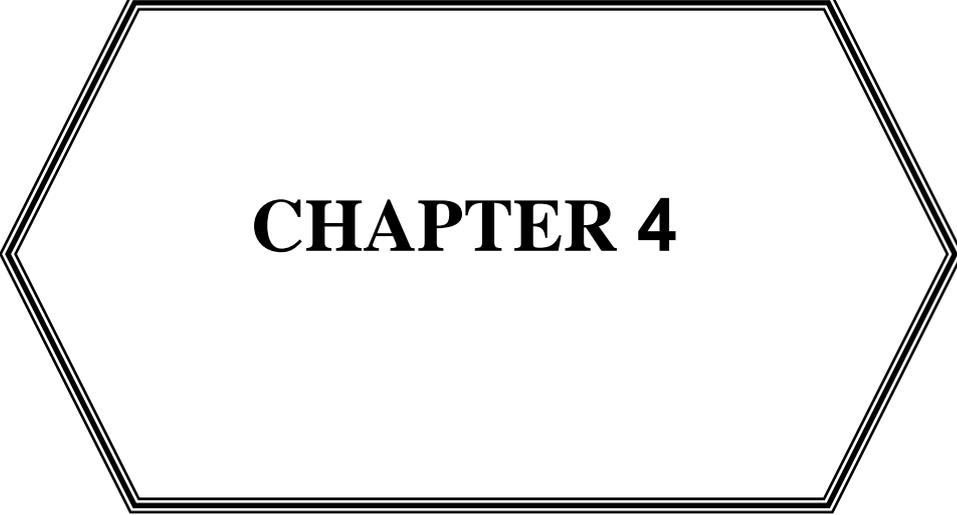
My results, along with previous data on other adipokines consolidate the suggestion that adipokines play an essential role in pregnancy and parturition (Denison et al., 2008) but its role in pregnant women is not fully known. Apart from its production in adipose tissue, visfatin is not only found to be expressed in human myometrium, but also in foetal membranes and placenta during normal gestation and parturition (Esplin et al., 2005, Marvin et al., 2002, Ognjanovic and Bryant-Greenwood, 2002). The acute relaxant effect that we observed for visfatin on myometrium may counteract a contractile drive coming from its release from the placenta during pregnancy. Hence visfatin has been documented to cause upregulation of pro-contractile and pro-inflammatory cytokines expression such as IL-6 and IL-8 which have been suggested to have a physiologic role during normal gestation (Ognjanovic and Bryant-Greenwood, 2002, Osmers et al., 1995, Prins et al., 2012). Moreover, the visfatin gene itself (in amniotic epithelium) is also upregulated in response to inflammatory stimuli, suggesting a role in pre-term labour (Ognjanovic et al., 2005). Normal pregnancy and infection-associated preterm labour are both associated with increased levels of visfatin, and visfatin changes have been reported in pregnancies complicated by gestational diabetes mellitus, preeclampsia, preterm labour and foetal growth restriction (Pavlová et al., 2015). However, the effects of circulating visfatin on labour onset and delivery needs further studies and *in vivo* approaches.

### 3.5 Conclusion

This study demonstrates through the use of *in vitro* contractility studies that there are no differences in the contractility of WT and ApoE<sup>-/-</sup> non-pregnant mouse myometrium. It was also found that visfatin has a relaxant effect on pregnant WT mouse myometrium *in vitro* and NAD<sup>+</sup> pathway might to be involved in the mechanism of action of visfatin on myometrium. My data clearly shows that visfatin only works when contractility is augmented by oxytocin. This data adds to our earlier data in human and rat myometrium showing that visfatin can reduce myometrial contractility, especially under physiological conditions. Together the data suggests that increased output of visfatin, possibly due to dyslipidaemia in obese pregnant women, may impair uterine contractility resulting in obesity-related complications. This study may provide a basis on which new approaches for the prevention and treatment of pregnancy-related complications in obese women can be developed.

### 3.6 Limitations of the study

One of the limitations of this study is that it only reflects the effect of acute changes in contractility in response to visfatin irrespective of body weight. It is suggested that examining the chronic effects and complications associated with high visfatin levels in a cross-sectional study involving obese pregnant women would build on these findings. It was very difficult to know where visfatin is acting on the myometrium. In addition, high organ bath PO<sub>2</sub> may increase NAD<sup>+</sup> saturating system and affect the results. It would be of interest to examine the direct measurements of NAD<sup>+</sup> and [Ca<sup>2+</sup>]<sub>i</sub> and visfatin's effects under hypoxic conditions. The mechanism of the effects of visfatin on human myometrial contractility should be a topic for future study. Although organ bath experiments are technically robust and shows good validity and reproducibility, they do have some limitations. Dissection can be a tricky procedure and unless that the strip size and temperature are fixed, and the tension applied was standardised, the results observed will be affected.



# **CHAPTER 4**

## **Chapter 4: Examining the effect of maternal BMI on pregnant human myometrial contractility *in vitro*.**

### **4.1 Introduction**

The prevalence of obesity has risen accounting for a worldwide epidemic (WHO, 2000). Obesity rate increases along with increasing numbers of women of childbearing age who are overweight and obese, not to mention the increasing rate of morbid obesity. Pregnancy, by itself, also contributes towards maternal obesity through large gestational weight gain and postnatal weight retention (Heslehurst et al., 2011a).

High BMI has an impact on many aspects of female reproductive function including the core reproductive signalling pathways leading to multiple maternal and foetal complications (OECD, 2018). This is a subject of increasing research interest. Gestational diabetes mellitus, preeclampsia, postpartum haemorrhage, obstructive labour, postdate pregnancy and infertility are all recognized to be related to maternal obesity (Baeten et al., 2001, Cedergren, 2004, Vesco et al., 2009, Magann et al., 2013, Gilead et al., 2012, Bautista-Castaño et al., 2013, Wang et al., 2002). Moreover, obesity was proven to be an independent risk factor for elective and emergency C-section delivery (Gilead et al., 2012, Poobalan et al., 2009). It was established that obese women associated with a significantly higher rate of induction of labour (IOL) and that IOL for these women was correlated with increasing rates of C-section delivery compared to normal weight women (Arrowsmith et al., 2011). As progress in labour is critically dependent on myometrial contractions, this data further suggests that the uterine environment and the myometrial signalling pathways are adversely influenced by obesity.

As discussed in Chapter 3, multiple studies have established that maternal obesity is strongly associated with a wide spectrum of adverse pregnancy and delivery outcomes, particularly dysfunctional labour and intrapartum C-section deliveries. These observational studies, collectively with the functional studies of cholesterol and adipokines on myometrium, suggest that maternal obesity has an inhibitory effect on myometrial function. Although modulation of myometrial membrane composition by direct application of modulatory substances has been

studied; the variations in maternal BMI were barely considered. Based on *in vitro* myometrial functional contractility studies, Zhang *et al.* observed poor uterine contractility in obese women, in the form of reduced amplitude, frequency of uterine contraction and  $\text{Ca}^{2+}$  influx, associated with increased rates of prolonged labour, C-section deliveries and postpartum haemorrhage, which are commonly encountered in obese mothers (Zhang *et al.*, 2007a). Similar findings were found by Cedergren; he reported that the risk of emergency C-section deliveries due to ineffective myometrial contractility was correlated with increasing maternal BMI (Cedergren, 2009). Other studies, however, have found no differences in *in vitro* myometrial contractility between obese and normal weight pregnant women (Higgins *et al.*, 2010, Sweeney *et al.*, 2013). It has been found that the time to commencement of spontaneous contractions in human pregnant myometrium increases with increasing maternal BMI *in vitro* (Crankshaw *et al.*, 2017).

Some additional factors underlying poor myometrial contractility in obese women have been addressed. Fat accumulation in the birth canal may lead to the development of birth resistance and reduced muscle contraction in pregnant women, and obstruction of labour and cephalopelvic disproportion (Zhou *et al.*, 2019). The human ether-a-go-go-related gene (hERG) activity, which codes for a specific potassium channel, has been found to increase in obese women and this change could result in weaker uterine contractions with increasing BMI (Parkington *et al.*, 2014). It has also been observed that myometrial p160 ROCK-1 protein expression is significantly reduced in obese women towards the end of pregnancy, which may contribute to poor uterine contractility at labour, due to its contribution to  $\text{Ca}^{+2}$  sensitisation through phosphorylation of MLCP and possibly other signalling pathways (O'Brien *et al.*, 2013). However, it was found that myometrial mitochondrial function and morphology were not altered at term with obesity apart from the observed increase in fat content and myocyte density (Gam *et al.*, 2015, Gam *et al.*, 2017).

Based on the relaxant effect of visfatin which is an adipokine believed to be raised in obesity, on myometrial contractility (see Chapter 3), It was hypothesised that spontaneous contractility in myometrium is reduced in obese women and would be further reduced with the augmentation of oxytocin. The study in this chapter was designed to provide further elaboration in the *in vitro* variation of myometrial contractile activity among pregnant women with different

BMI categories. This study, specifically, aimed to investigate the relationship between maternal BMI and the ability of the myometrium to contract spontaneously and in response to an uterotonic hormone, oxytocin. A secondary objective was to investigate the effect of maternal obesity in the time taken to start spontaneous myometrial contractions.

## **4.2 Methods**

### **4.2.1 Tissue collection and preparation**

Myometrial strips were dissected from non-labouring term uterine biopsies obtained with informed consent to participate from women undergoing an elective C-section with a singleton pregnancy at the Liverpool women's hospital, Liverpool, United Kingdom (**Appendix 4**). The women were recruited by the hospital's midwives. The ethics standards for human experimentation established by the Declaration of Helsinki were followed (WHO, 1997). Ethics approval for sample collection was obtained from the University of Liverpool Ethics Committee (Reference. 10/H1002/49, see **appendix 2**). I was blinded to the demographic details of the sample until experiments had been performed and data statistically analysed. Once performed, the women's information obtained from the Liverpool Women's Hospital database; including BMI at time of the first trimester, maternal age, gestational age at the time of delivery, parity, pre-existing medical diseases, pregnancy-related complications, indication for planned C-section delivery and birth weight were obtained for each woman. Women were excluded if they had not given consent, if they had uterine abnormalities, placenta praevia, extensive adhesions, significant intraoperative haemorrhage, malignancy or pregnancy-related complications including pre-existing and gestational diabetes and hypertensive diseases. Eligible participants were then classified into three BMI categories based on their first trimester BMI: (1) normal (BMI 18.50 - 24.99 kg/m<sup>2</sup>), (2) overweight (BMI: 25.00 - 29.99kg/m<sup>2</sup>), (3) obese (class I combined with class II) (BMI30.00 – 39.99 kg/m<sup>2</sup>) and morbidly obese (class III) (BMI ≥40.00 kg/m<sup>2</sup>) according to WHO definitions (Diet, 2003). All experimental tissues were placed in physiological saline solution (PSS) and tissues were stored at 4°C and used within 18 hours of collection.

### **4.2.2 Contractility measurements**

Contractility studies were carried out as described in Chapter 2. In summary, the human myometrium was dissected along the plane of the muscle, cleaned and longitudinal myometrial strips (~1 mm × 4 mm) were dissected from the human biopsies and attached to a force transducer under 2 mN of tension in an organ bath filled with 5 ml of physiological saline superfused with PSS at a rate of at 2 ml/min and gassed with 100% O<sub>2</sub>. Experiments were performed at 37 °C and myometrial strips were allowed to equilibrate for at least 2 hours to obtain reproducible spontaneous phasic contractions. Matched vehicle controls were performed in PSS. Contractility data were recorded via a tension transducer (World Precision Instruments, Aston, United Kingdom) and the signal amplified and stored in a commercial data acquisition system (Labscribe 2; World Precision Instruments, Aston, United Kingdom). To examine differences in the myometrial contractility between different BMI categories, average measurements of force of contraction, duration, frequency and mean integral force in 20 minutes were determined and comparisons made between the categories. The response to oxytocin was calculated after 15 minutes of exposure. The comparison was carried out for spontaneous and oxytocin-induced contractions. Oxytocin was added to PSS at a final concentration of 0.5nM.

### **4.2.3 Data and statistical analysis**

Statistical analysis was carried out in prism GraphPad Prism version 5.01 (GraphPad Software, San Diego, USA). A D'Agostino & Pearson test was used to determine the normal distribution of the data. The comparisons of the time for the initiation of spontaneous contractions were done by Spearman rank correlation test because the data was not normally distributed. To statistically compare more than two groups, one-way ANOVA was used for normally distributed data; so comparison of force records was performed using mean ± se and Kruskal-Wallis test with Dunn's Multiple Comparison test was used for data which is not normally distributed; so comparison of force records was performed using median ± IQR. Bar chart was used to present the normally distributed data and Box and Wisker plot was used to present the data which is not normally distributed. P value was taken as showing a significant difference when P value

<0.05 and (n) is the number of myometrial tissue strips examined from different women.

## 4.3 Results

### 4.3.1 Demographic data

A summary of the demographic characteristics of the women in this study are shown in **Table 4.1**. Myometrial biopsies were obtained from 42 term singleton pregnant women with an average maternal age of  $32.2 \pm 0.8$  years and an average gestational age of  $274.4 \pm 1$  days. Maternal BMI ranged from 19.5 to  $50.7 \text{ kg/m}^2$  and according to WHO definitions of BMI, 38.1% were normal weight, 26.2% were overweight, 26.2% were obese and 9.5% were morbidly obese. Among the study population, 19.1% were nulliparous, 45.2% were primiparous and 35.7% were multiparous. The average neonatal birthweight was  $3698 \pm 81.9$  g. Indications for planned C-section delivery were previous C-section delivery (n=29), breech presentation (n=2), previous traumatic vaginal delivery (n=5), maternal request (n=5), and foetal reasons (n=1).

The demographics for the women included in this study are presented for each BMI category in **Table 4.2**. Statistical analysis using one-way ANOVA test showed that there was no significant difference in maternal ages, gestational ages, parity and neonatal birthweights between different BMI categories (p value >0.05). The myometrial contractility of all 42 women was assessed for time to establishment of activity, spontaneous contraction and in response to an agonist, oxytocin. For analysis of contractile parameters, including time to establishment of activity, data are first analysed then divided into the following sub-categories (1) normal weight (n=16), (2) the overweight (n=11), (3) obese and morbidly obese (n=15) women. Since morbidly obese women are small in number (n=4), It was decided to be combined with obese women (n=11) in one category.

**Table 4.1 Summary of demographics characteristics for my study sample.**

This table shows the study sample characteristics of maternal age (year, mean  $\pm$  se), gestational age (days, mean  $\pm$  se), maternal weight (Kg, median  $\pm$  IQR), maternal height (m, median  $\pm$  IQR) and the percentage (%) of BMI categories, parity and neonatal birthweight (g, mean  $\pm$  se).

<b>Demographic Characteristics (n = 42)</b>	
<b>Maternal age</b> (years) (se)	32.2 (0.8)
<b>Gestational age</b> (days) (se)	274.4 (1)
<b>Maternal Weight</b> (Kgs) (IQR)	72.7 (25.7)
<b>Maternal Height</b> (m) (IQR)	1.64 (0.1)
<b>Maternal BMI</b> (kg/m <sup>2</sup> )	<b>n (%)</b>
Normal weight	16 (38.1)
Overweight	11 (26.2)
Obese	11 (26.2)
Morbidly obese	4 (9.5)
<b>Parity</b>	<b>n (%)</b>
Nulliparous	8 (19.1)
Primiparous	19 (45.2)
Multiparous	15 (35.7)
<b>Birthweight</b> (g) (se)	3703 (83.8)

**Table 4.2 Demographics for each BMI category.**

This table showing maternal ages, gestational ages, parity, reason for C-section and neonatal birthweight for each women and average results for each BMI category. No significant difference was found between the BMI categories in regards to other demographic characteristics (P value >0.05).

**Normal weight women n=16**

BMI (kg/m <sup>2</sup> )	maternal Age (years)	Gestational Age (days)	Parity	Indication for C-section delivery	Neonatal Birthweight (g)
19.5	25	272	0	Maternal request	2565
19.6	32	276	0	Maternal request	3740
19.8	31	276	1	Previous C-section	4150
20.6	32	267	1	Previous C-section	3870
20.9	36	284	0	Breech	3615
22.6	32	268	1	Previous C-section	4590
22.8	28	274	1	Previous traumatic delivery	3855
23	38	274	2	Previous traumatic delivery	4240
23.4	31	269	3	Previous C-section	3270
23.5	36	276	1	Previous C-section	3690
23.7	unknown	269	1	Previous C-section	3340
23.8	42	273	2	Previous C-section	3520
24	31	263	2	Previous C-section	2615
24.4	37	275	3	Previous C-section	3880
24.6	35	276	0	maternal request	3370
24.6	27	287	1	Previous C-section	4200
<b>Average</b>					
22.6	32.9	273.7	1		3656.9

**Overweight women n=11**

BMI (kg/m <sup>2</sup> )	maternal Age (years)	Gestational Age (days)	Parity	Indication for C-section delivery	Neonatal Birthweight (g)
25.1	32	274	3	Previous C-section	3165
25.4	31	259	1	Previous C-section	2800
26	40	273	2	Previous C-section	3035
26.8	43	274	1	Previous C-section	3795
27.2	28	290	1	Previous C-section	4220
27.5	37	274	2	Previous C-section	3740
28.1	40	275	0	maternal request	3000
28.2	33	280	1	Previous traumatic delivery	3910
29	30	283	0	foetal reasons	4180
29.2	26	280	0	Breech	4920

29.7	38	269	2	Previous C-section	3170
<b>Average</b>					
27.5	34.4	275.5	1		3630.5

### **Obese women n=11**

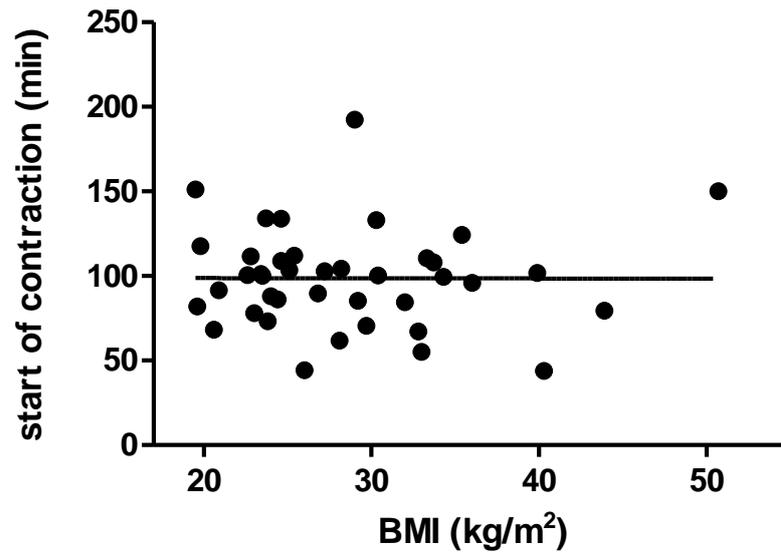
<b>BMI (kg/m<sup>2</sup>)</b>	<b>maternal Age (years)</b>	<b>Gestational Age (days)</b>	<b>Parity</b>	<b>Indication for C-section delivery</b>	<b>Neonatal Birthweight (g)</b>
30.3	27	273	2	Previous C-section	3150
30.4	33	271	3	Previous traumatic vaginal delivery	3170
32	36	276	2	Previous C-section	4605
32.8	26	275	3	Previous C-section	3465
33	29	273	4	Previous traumatic vaginal delivery	3550
33.3	32	266	3	Previous C-section	3670
33.7	34	272	1	Previous C-section	3710
34.3	28	272	1	Previous C-section	3465
35.4	23	270	1	Previous C-section	4224
36	28	272	1	Previous C-section	3505
39.9	34	273	1	Previous C-section	3590
<b>Average</b>					
33.7	30.0	272.1	2		3645.8

### **Morbidly Obese women n=4**

<b>BMI (kg/m<sup>2</sup>)</b>	<b>maternal Age (years)</b>	<b>Gestational Age (days)</b>	<b>Parity</b>	<b>Indication for C-section delivery</b>	<b>Neonatal Birthweight (g)</b>
40.3	32	281	0	maternal request	4515
43.9	34	273	1	Previous C-section	4280
44.9	30	277	1	Previous C-section	3970
50.7	23	290	1	Previous C-section	4200
<b>Average</b>					
45.0	29.8	280.3	1		4241.3

### **4.3.2 Comparing the time to commencement of spontaneous activity**

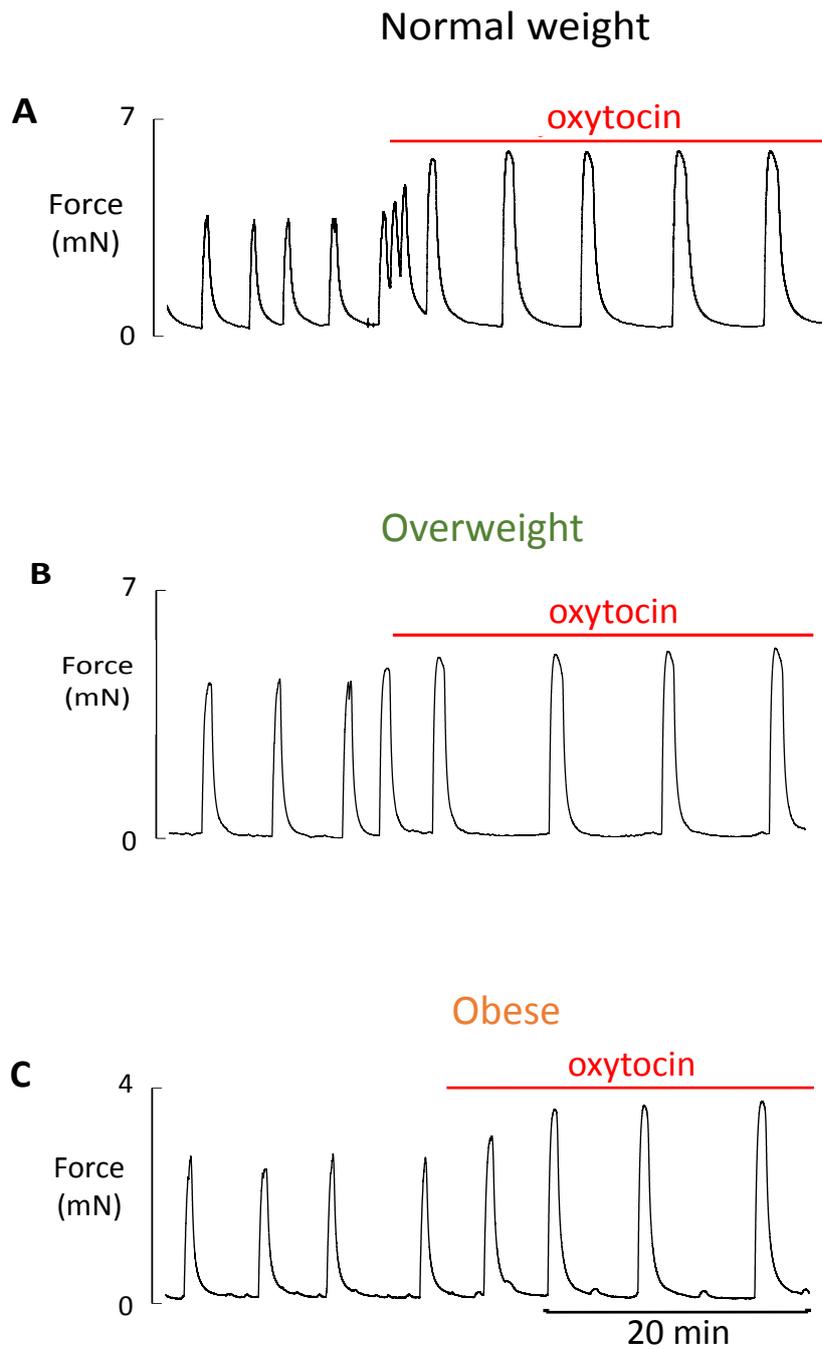
No association was found between the time to commencement of spontaneous contractions occurred *in vitro* and maternal BMI. This was tested using Spearman correlation test ( $R^2 = 0.000006$ , P value  $>0.05$ ,  $n=40$ ) (**Figure 4.1**). Two samples were exposed to an extra tension which was applied after a period of time of the original tension due to failure to successfully start spontaneous contractions. Therefore, they were not included in the analysis.



**Figure 4.1 Correlation between maternal BMI (kg/m<sup>2</sup>) and commencement of spontaneous activity (min).** Scatter plots showing no relationship between maternal BMI (kg/m<sup>2</sup>) and the time to start myometrial contractility ( $R^2 = -0.07$ ,  $P$  value  $>0.05$ ,  $n=40$ ).  $P$  value  $>0.05$  indicates that the trend was not significant by Spearman correlation test.

### 4.3.3 Comparing spontaneous contractile activity between different BMI categories

To examine whether increased maternal BMI could be related to poor myometrial contractility in obese women, variation in spontaneous activity between women with different BMI categories was examined. Spontaneous contractions from women with different BMI categories were established and the strip was left to contract for approximately 2 hours to obtain a control period from which to analyse the correlation between maternal BMI and the myometrial contraction indices including contraction amplitude, duration, frequency and AUC in a 10-minute period was tested. This was done for 16 normal weight, 11 overweight and 15 obese women. Representative traces showing spontaneous myometrial contractions in each BMI category are shown in **Figure 4.2**. To test the difference in the spontaneous myometrial contractions between maternal BMI categories, one-way ANOVA test was used for the amplitude and duration (mean  $\pm$  se) and Kruskal-Wallis test was used for frequency and AUC (median  $\pm$  IQR) (**Table 4.3**). No significant difference was found between the spontaneous myometrial contractions of different maternal BMI categories.

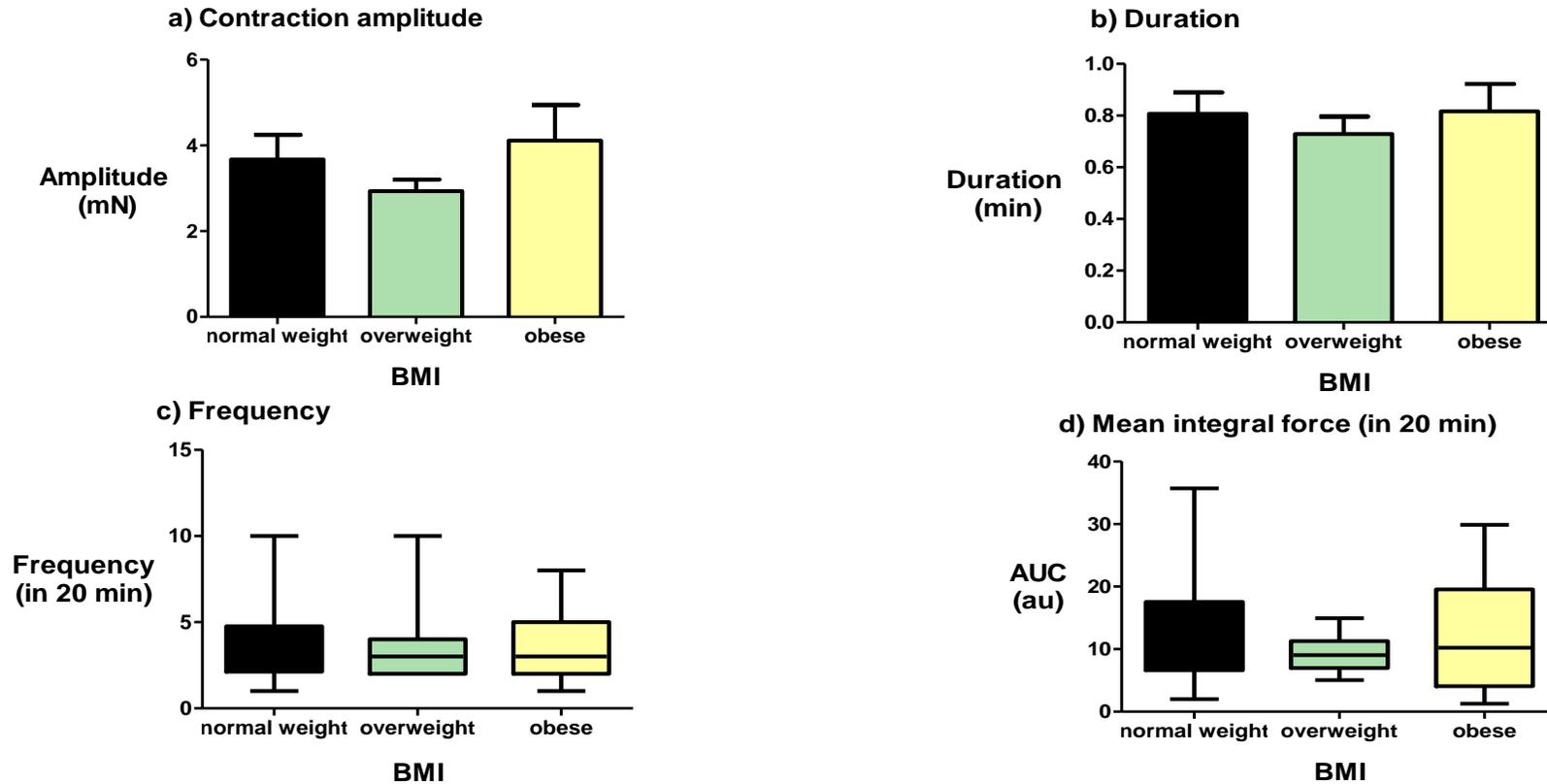


**Figure 4.2 Spontaneous and oxytocin-induced myometrial contractility in all BMI categories.** Representative traces of the myometrium of A) normal weight, B) overweight, C) obese women. No difference was noticed between the different BMI categories.

**Table 4.3 Difference in the contractile indices of spontaneous myometrial contractions in different BMI categories.** The data tested using one-way ANOVA test for the amplitude and duration (mean  $\pm$  se) and Kruskal-Wallis test for frequency and AUC (median (IQR)). After testing all the contractile parameters, no significant difference was found between the spontaneous myometrial contractions of different maternal BMI categories. P value  $<0.05$  indicates that the trend was significant by Student's *t* test.

	<b>Normal weight (n=16)</b>	<b>Overweight (n=11)</b>	<b>Obese (n=15)</b>	<b>P value</b>
<b>Force amplitude (mN)</b>	3.7 $\pm$ 0.6	2.9 $\pm$ 0.3	4.1 $\pm$ 0.8	>0.05
<b>Duration (minutes)</b>	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1	>0.05
<b>Frequency (contractions/ 20 minutes)</b>	3.5 (2.1-4.7)	3 (2-4)	3 (2-5)	>0.05
<b>AUC (au)</b>	11 (6.6-17.5)	9 (7-11.3)	10.2 (4.1-19.5)	>0.05

**Figure 4.3 Comparing spontaneous myometrial contractile activity in different BMI categories.**



155

Bar charts showing the mean values for a) contraction amplitude, b) duration and Box and whisker plots showing the median values for c) frequency and d) AUC for a period of 20 minutes. By comparing all the contractile parameters, there was no significant difference in the spontaneous myometrial contractility between the different BMI categories ( $p>0.05$ ).

#### 4.3.4 Comparing oxytocin-induced contractile activity between different BMI categories

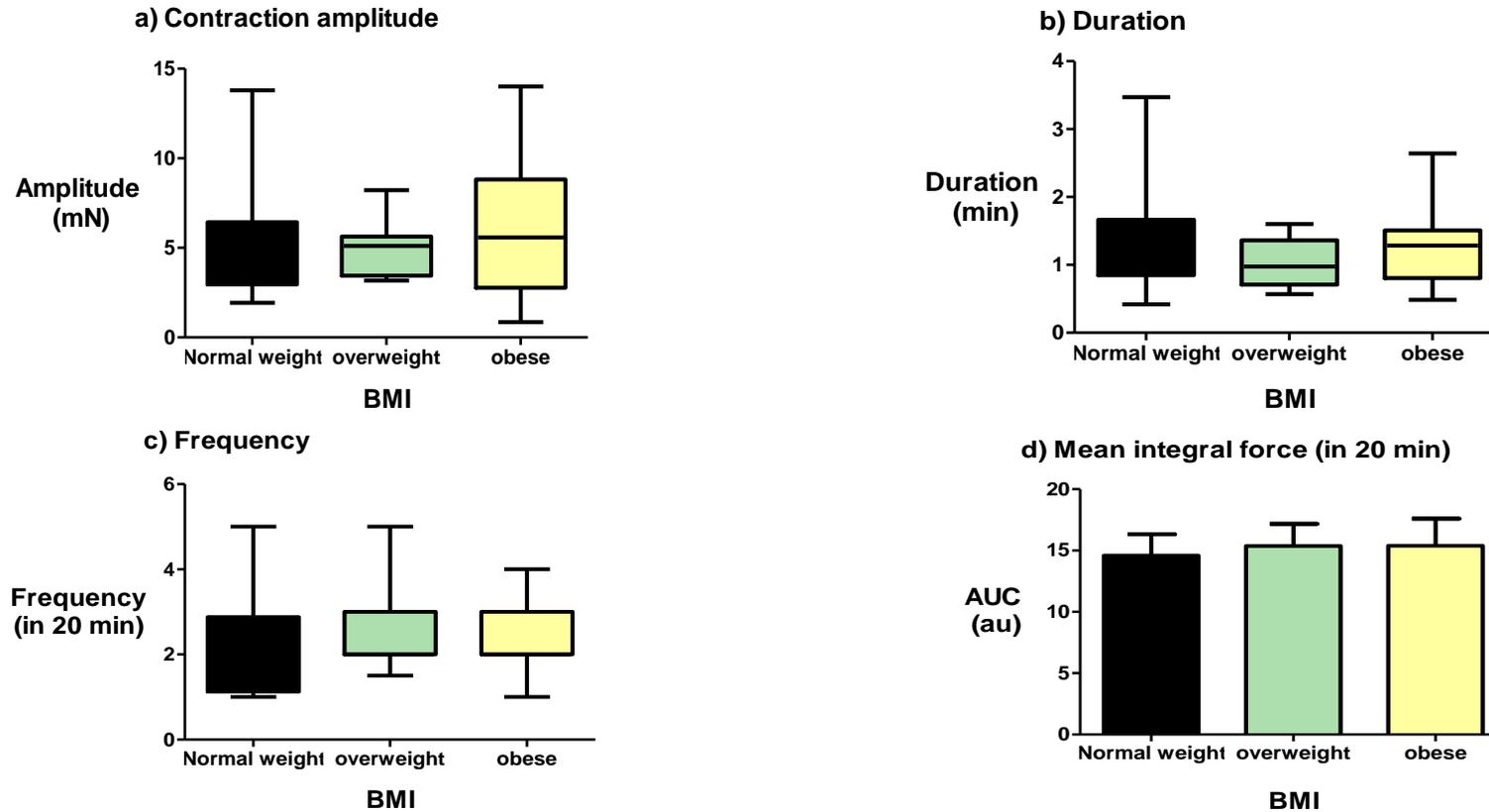
Variation in the myometrial tissue response to oxytocin between women with different BMI categories was also examined. A single dose of the drug was applied after the establishment of regular contractile activity resulting in a bath concentration of 0.5nM with the contractile response observed for 20 minutes. The correlation between maternal BMI and the myometrial contraction indices including contraction amplitude, duration, frequency and AUC was examined for 16 normal weight, 11 overweight and 15 obese women. Representative traces showing spontaneous myometrial contractions in each BMI category are shown in **Figure 4.2**.

Oxytocin has a significant stimulatory effect on all the maternal BMI categories's myometrial contractions. **Table 4.4** summaries the contractile indices of oxytocin-induced myometrial contractions for each maternal BMI category. All the maternal BMI categories showed a significant increase in the contraction amplitude compared to the control (P value<0.05). All the maternal BMI categories had a significantly longer duration compared to the control (P value<0.05). The contraction frequency was significantly lower in both normal weight and obese women compared to the control (P value<0.05). Both overweight and obese women showed a significant increase in the AUC compared to the control (P value<0.05). To test the difference in the oxytocin-induced myometrial contractions between maternal BMI categories, the Kruskal-Wallis test was used except for the AUC parameter where a one-way ANOVA test was used. No significant difference was found between the oxytocin-induced myometrial contractions in different maternal BMI categories (**Table 4.4**).

**Table 4.4 Difference in the contractile indices of oxytocin-induced myometrial contractions in different maternal BMI category.** The data tested using Kruskal-Wallis test (median (IQR)) except for AUC which was tested using one-way ANOVA test. After testing all the contractile parameters, no significant difference was found between the oxytocin-induced myometrial contractions of different maternal BMI categories. Oxytocin has a stimulatory effect on the myometrium in all different BMI categories. P value <0.05 indicates that the trend was significant by Student's *t test*. \* Denotes significant difference in contractility compared to preceding control period p<0.05 \*\*p<0.005 \*\*\*p<0.0005.

	<b>Normal weight (n=16)</b>	<b>Overweight (n=11)</b>	<b>Obese (n=15)</b>	<b>P value</b>
<b>Force amplitude (mN)</b>	4.3 (3-6.4)**	5.1 (3.4-5.6)***	5.6 (2.8-8.8)**	>0.05
<b>Duration (minutes)</b>	1.1 (0.85-1.7)	1 (0.7-1.4)	1.3 (0.8-1.5)***	>0.05
<b>Frequency (contractions/ 20 minutes)</b>	2 (1.1-2.9)***	2 (2-3)	2 (2-3)*	>0.05
<b>AUC (au)</b>	14.6 ± 1.7	15.4 ± 1.8**	15.4 ± 2.2**	>0.05

**Figure 4.4 Comparing oxytocin-induced myometrial contractile activity in different BMI categories.**



Box and whisker plots showing the median values for a) contraction amplitude, b) duration and c) frequency and bar charts showing the mean values for and d) AUC for a period of 20 minutes. By comparing all the contractile parameters, there was no significant difference in the oxytocin-induced myometrial contractility between the different BMI categories ( $p>0.05$ ).

## 4.4 Discussion

This chapter adds to the knowledge of the effects of obese environment on myometrial contractility during gestation. In contrast to my original hypothesis, the results of this chapter show no relationship between increasing maternal BMI and impairment of the ability of the human myometrium to contract spontaneously or in response to an agonist, oxytocin. No significant variations were observed in the time taken for the commencement of spontaneous myometrial contractility between pregnant women with different BMI categories. Even with a relatively small sample size, our findings from human myometrial samples are valid to the obstetric population because the maternal BMI distribution in our sample set is comparable to that in the obstetric female population (Kanagalingam et al., 2005).

Oxytocin was found to have a significant stimulatory effect on all the maternal BMI categories myometrial contractility. This is expected as oxytocin is a strong uterine agonist produced physiologically during labour (Arrowsmith and Wray, 2014, Blanks and Thornton, 2003). It is interesting to note that obese women response to oxytocin is unexpectedly higher than normal weight women. Oxytocin receptors are highly expressed in the pregnant myometrium (Gimpl and Fahrenholz, 2001) and increase at term and parturition in both the human and rat (Kimura et al., 1996, Larcher et al., 1995). Oxytocin binds to oxytocin receptors to increase internal  $Ca^{2+}$  through G-protein activation of phospholipase C (PLC), which liberates inositol-1,4,5, triphosphate (IP3) and releases internally stored  $Ca^{2+}$  ions from the SR. It also stimulates the opening of L-type calcium channels and inhibits  $Ca^{2+}$  extrusion by suppression of the  $Ca^{2+}$ -ATPase pump. Interestingly, uterine expression of oxytocin receptors was recently found to be significantly higher in the virgin female Wistar rats maintained with high-fat, high-cholesterol diet compared to those who were maintained with standard laboratory chow, which is consistent with my finding (Muir et al., 2016).

The hypothesis that an intrinsic biological impairment in myometrial contractility may occur in women with high maternal BMI is controversial and inconclusive. Higgins *et al* published a relatively similar study with a larger sample size, also finds no correlation between maternal BMI and the myometrial contractility in pregnant women (Higgins et al., 2010). Sweeney *et al*. found that there were no changes in the smooth muscle content or extracellular matrix of

human myometrium with increased maternal BMI (Sweeney et al., 2013). In addition, expression of oxytocin receptor in human myometrium was reported to not be affected by maternal BMI (Grotegut et al., 2013) and this observation is in line with my results which show no effect of maternal obesity on oxytocin-induced myometrial contractility. As tissues were obtained from different women, there is naturally some variation in the basic spontaneous contractility between samples. Samples from a large number of women would minimise this variation (Crankshaw and Morrison, 2011). Further work to relate maternal plasma cholesterol and myometrial cholesterol content and their changes during pregnancy and parturition according to maternal BMI therefore suggested.

The results from my study are in contrast to previous studies examining spontaneous activity *in vitro*. It has been reported that the human myometrium contracts with decreased frequency and amplitude of simultaneously measured  $Ca^{+2}$  transients force in pregnant women with high BMI (Zhang et al., 2007a). Crankshaw et al found that the time to start spontaneous contractions of human pregnant myometrium, maximal amplitude and mean force of contraction *in vitro* increases with increasing maternal BMI (Crankshaw et al., 2017). These different results potentially reflect technical variations between the studies with respect to the degree of stretch applied, flow rate, the organ bath solutions, pH and temperature, or on another way it may reflect the sample number and variability in the inclusion criteria.

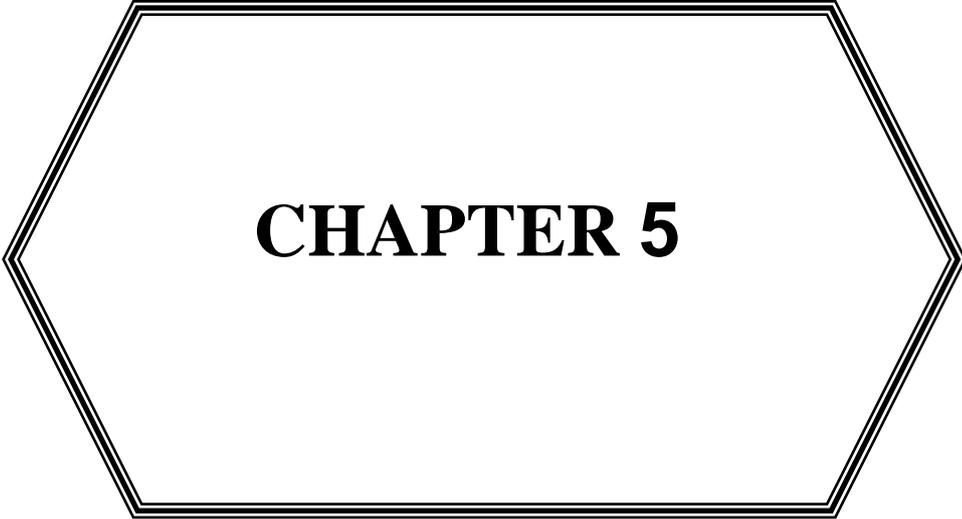
The metabolic status of obese women is often altered and thus other endocrine factors involved in pregnancy and labour may be altered in women with high BMI. Obese pregnant women have a long-term chronic exposure to high levels of hyperlipidaemia for the period of pregnancy where the myometrium might physiologically adapt to these changes and maintain a pro-contractile phenotype at delivery (Higgins et al., 2010). This suggests that the *in vivo* adverse influences of obesity on parturition cannot be explained by isolated *in vitro* myometrial examination isolated from hormonal and nervous input. It also indicates that there may be a larger picture of alternative mechanisms which explain the clinically observed increased risk of pregnancy-related complications in obese women. Chen and Scholl reported that effects of elevated free fatty acids on pregnancy outcomes were independent of the effects of maternal BMI (Chen and Scholl, 2008).

## **4.5 Conclusion**

My study indicated a lack of evidence for an effect of maternal obesity on spontaneous contractions, oxytocin-induced contractions, or the commencement of spontaneous contractions, in human myometrium *in vitro*. It concludes that the observed complications of maternal obesity on pregnancy and parturition *in vivo* cannot be explained by a direct, *in vitro*, effect on myometrial contractile mechanisms. This suggests that there may be alternative mechanisms underlying the pregnancy and delivery-related complications observed in obese mothers. This indicates further exploration to provide proper prevention and management protocols for these complications in obese mothers.

## **4.6 Limitation of the study**

As mentioned in Chapter 3, it must be acknowledged that an isolated *in vitro* approach was used. Although it is an efficient method, it does mean that the myometrium is separated from hormonal and neuronal modulatory effects. The myometrial biopsies used were from the lower uterine segments, which may not adequately reproduce the reactions of the fundus. Nonetheless, lower uterine segment biopsies were observed to have no difference in contractile properties compared to uterine biopsies obtained from the upper uterine segment (Luckas and Wray, 2000). Finally, although this study' sample size is representative to the obstetric population, further increase in the sample size is recommended to avoid any sampling bias.



# **CHAPTER 5**

# **Chapter 5: Investigating the association between maternal adipokines dysregulation and prolonged labour on obese pregnant women – a cross-sectional study**

## **5.1 Introduction:**

### **5.1.1 Maternal obesity and pregnancy adverse outcomes**

Obesity is a common disorder worldwide and its prevalence has increased particularly among women of childbearing age (Jungheim et al., 2012). The prevalence of obesity in Saudi women is high, 41% compared to 35% normal weight and 23% overweight women (Al-Kadi et al., 2018). Several studies have demonstrated that maternal obesity is associated with a wide spectrum of adverse pregnancy outcomes including gestational diabetes mellitus, preeclampsia, post-dated pregnancy, postpartum haemorrhage and foetal death (Yogev and Catalano, 2009, Heslehurst et al., 2008, Stubert et al., 2018). These complications have led to more elective and emergency C-sections deliveries in these women compared to those of normal weight (Berendzen and Howard, 2013, Abenhaim and Benjamin, 2011). Many factors, including mechanical and biochemical, have been examined to explain the close association between maternal obesity and pregnancy and delivery-related complications.

In addition to its fundamental role as a fat storage organ, adipose tissue functions as an endocrine organ and secretes a large spectrum of bioactive mediators, known as adipocytokines, including leptin, resistin, visfatin, tumour necrosis factor-alpha, interleukin-6 and adiponectin (Axelsson et al., 2005). Adipokines are indicated to be relevant to the pathogenesis of common metabolic complications including obesity, insulin resistance, type 2 diabetes mellitus, metabolic syndrome, cardiovascular diseases and atherosclerosis (Rabe et al., 2008, Antuna-Puente et al., 2008, Deng and Scherer, 2010, Freitas Lima et al., 2015, Kralisch et al., 2007). Adipokines are also found to have a role in the development of obesity-related complications in pregnant women (Hauner,

2005). A recent review of the literature indicated that adipokines dysregulation could be associated with pregnancy-related complications (AlSaif et al., 2015).

### **5.1.2 Leptin and reproduction**

Leptin, a critical hormone that regulates body nutritional status, is secreted by adipose tissue, is under the control of obesity genes and is increased in obesity (Haynes et al., 1998, Al Maskari and Alnaqdy, 2006, Alex, 2014). It is also secreted by the placenta (Domali and Messinis, 2002, Anderson et al., 2005), and increases in maternal blood during pregnancy, and then interestingly, decreases around parturition (Krizova et al., 2004, Nuamah et al., 2004). It has inflammatory, autonomic and endocrine effects (Haynes et al., 1998).

Leptin's role in the pathophysiology of several pregnancy related complications has been noted, including preeclampsia (Anderson et al., 2005, Song et al., 2016, Kalinderis et al., 2015, Taylor et al., 2015, Ozkan et al., 2005, Adali et al., 2009, Hendler et al., 2005a) and gestational diabetes mellitus (Pérez-Pérez et al., 2013, Ategbro et al., 2006, Chen et al., 2010, Kautzky-Willer et al., 2001, Vitoratos et al., 2001). Leptin was recently proposed as an early pregnancy biomarker of gestational diabetes mellitus (Powe, 2017) and elevated leptin levels have been suggested to have a prognostic significance for the development of preeclampsia even before the clinical onset of the disease (Miehle et al., 2012).

Leptin has been reported to have a cumulative inhibitory effect on spontaneous and oxytocin-induced myometrial contractility in biopsies from pregnant women having term elective C-sections (Wuntakal and Hollingworth, 2010, Moynihan et al., 2006). We previously showed that leptin at 1  $\mu$ M produced a small but significant inhibitory effect on spontaneous and oxytocin-induced human myometrial contractions, with the AUC, falling to around 80% of that found with control conditions (Mumtaz et al., 2015). Similar but less potent inhibitory effects of leptin have been found on contractions of isolated rat myometrium. If it acts in a way similar to that reported in vascular smooth muscle, decreasing intracellular  $Ca^{2+}$  release from the sarcoplasmic reticulum (Fortuno et al., 2002), then this could explain the fall in uterine force (Wray et al., 2003, Noble et al., 2009).

After testing *in vitro* application of leptin on human myometrium, it was logical to examine maternal leptin levels in plasma and relate it to prolonged labour in obese women. My idea was strengthened by the positive relation between the duration of labour and cord blood leptin levels reported by Logan *et al* (Logan *et al.*, 2016). There were; however, no existing studies of maternal leptin and labour outcome. Nonetheless, during my study in 2018, it was reported that plasma leptin levels are not associated with the duration of active phase (stage 1) of labour (Carlhäll *et al.*, 2018).

### **5.1.3 Visfatin and reproduction**

Visfatin is a novel adipocyte-derived cytokine reported to have many effects including pro-inflammatory, paracrine, autocrine and endocrine effects (Stastny *et al.*, 2012). The role of visfatin during pregnancy has not yet been clarified. It induces its effect in the same manner as the insulin/insulin receptor signal transduction pathway (Brown *et al.*, 2010). Visfatin is constitutively expressed in the myometrium, foetal (amnion and chorion) and maternal (decidual) portions of the foetal membranes and placenta during normal gestation (Ognjanovic *et al.*, 2003).

Plasma visfatin concentration increases during the development of obesity, with significantly higher expression in morbidly obese women (Beltowski, 2006). Levels have been found to increase with advanced gestational age during pregnancy (Morgan *et al.*, 2008) Although Mazaki-Tovi *et al* found that median maternal plasma concentrations of visfatin peak between 19 and 26 weeks of gestation in normal weight women then decrease towards the end of pregnancy (Mazaki-Tovi *et al.*, 2009b), these authors also found that this pattern of changes in circulating visfatin concentrations was absent in obese pregnant women.

As with leptin, visfatin has been found to be associated with many pregnancy and delivery-related complications such as preeclampsia, foetal growth restriction and impaired glucose metabolism (AlSaif *et al.*, 2015). Several studies have investigated the relationship between visfatin and preeclampsia with contradictory results. Plasma visfatin levels have been found to be increased in preeclampsia in some studies (Fasshauer *et al.*, 2008, Adali *et al.*, 2009), whereas other studies showed similar (Mazaki-Tovi *et al.*, 2010) or even decreased levels (Hu *et al.*, 2008). Other studies have investigated the

association between visfatin and gestational diabetes mellitus. Maternal visfatin levels were found to be lower (Haider et al., 2007, Chan et al., 2006, Mazaki-Tovi et al., 2009a) and higher (Lewandowski et al., 2007) in patients with gestational diabetes mellitus than in normal pregnant women. An association between circulating maternal visfatin and altered foetal growth, has also been identified (Briana and Malamitsi-Puchner, 2010). Plasma maternal visfatin in the third trimester was found to be higher in patients with foetal growth restriction (FGR) (Fasshauer et al., 2007, Malamitsi-Puchner et al., 2007). Another study; however, in gestational diabetes mellitus patients, found higher visfatin levels in large for gestational age (LGA) neonates (Mazaki-Tovi et al., 2009a). It has also been suggested that visfatin could play an important role in the regulation of infant adiposity by breast milk (Bienertova-Vasku et al., 2012). Therefore, visfatin is closely related to pregnancy- related complications

It was previously reported that visfatin, at a relatively low concentration (10nM), exerted a significant inhibitory effect on both spontaneous and oxytocin-induced contractions of pregnant rat and human myometrial tissue *in vitro* in comparison to controls (Mumtaz et al., 2015). It produced both a reduction in the amplitude and area under the curve (AUC) of spontaneous contractions and a significant reduction in the AUC of oxytocin-induced contractions in rat and human myometrium. Of the four adipokines studied to date, three (leptin, visfatin and apelin) reduce contractility, with visfatin being the most potent. It was demonstrated in Chapter 3 that the NAD<sup>+</sup> pathway might be involved in the mechanism of action of visfatin on myometrium.

The inflammatory process plays a critical role during normal labour (Romero et al., 2006), but the role of visfatin during labour is still unknown. Preterm labour with intra-amniotic infection or inflammation was found to be associated with high maternal plasma visfatin concentrations (Mazaki-Tovi et al., 2008, Mazaki-Tovi et al., 2009c). Visfatin plays a role in the inflammatory process of many inflammatory disorders and its role in the inflammatory cascade of labour, including the induction of several inflammatory cytokines and prostaglandins, is one research focus (Lappas, 2012). Its roles in the inflammation and smooth muscle contractility are overlapping and complicated. Therefore, studying visfatin, and being able to directly compared to leptin and

uterine effects in normal weight and obese pregnant women will provide increased understanding of obesity-related complication.

#### **5.1.4 Maternal obesity and prolonged/dysfunctional labour**

Obese pregnant women show a higher risk of post-dated pregnancies and a lower risk of preterm delivery (Wuntakal and Hollingworth, 2010, Arrowsmith et al., 2012), and high maternal BMI at booking increases the chance of induced labour with no association with prolonged labour (Arrowsmith et al., 2012). In addition, Robinson *et al* observed that increasing maternal BMI is not associated with prolonged labour (Robinson et al., 2011), and a more recent study has concluded that maternal BMI has no significant effect on the total duration of active labour (Ellekjaer et al., 2017). However, many studies have suggested that labour progresses more slowly as maternal BMI increases (Vahratian et al., 2004, Olesen et al., 2006, Kominiarek et al., 2011, Beyer et al., 2011, Hilliard et al., 2012, Norman et al., 2012, Samy et al., 2015, Hautakangas et al., 2018, Shahi et al., 2017, Carlhäll et al., 2018). These studies established that the risk of prolonged labour increases in obese women, particularly morbidly obese women, and that the duration of the active phase of labour increases significantly with increasing maternal BMI. Although the biological mechanisms linking obesity to dysfunctional labour are largely unknown.

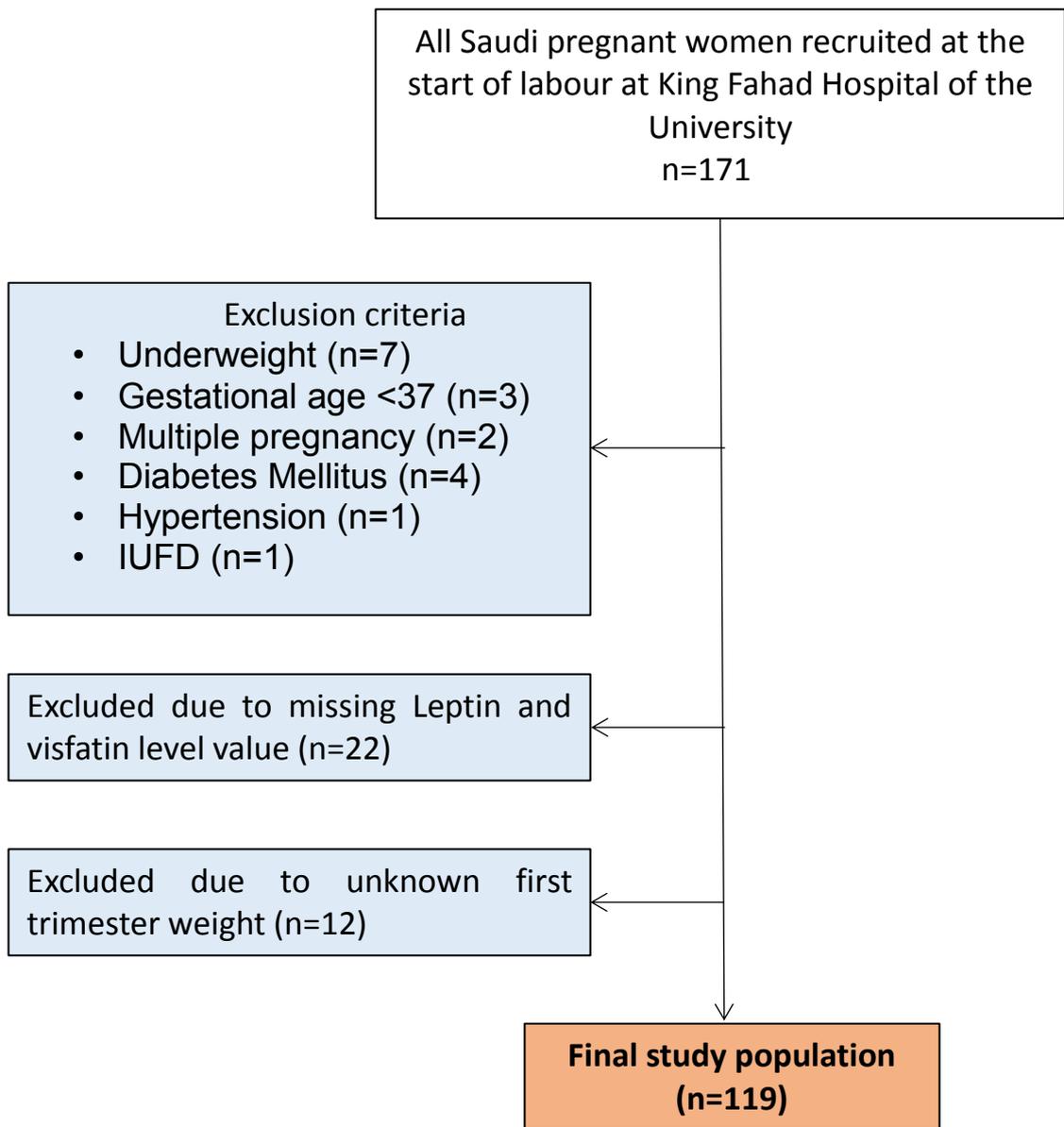
It was previously suggested that adipocytokines released from the excessive adipose mass that occurs in obesity may modulate uterine function. The work described in this chapter will test our main hypothesis that there is a close relationship between adipokines dysregulation and prolonged first stage of labour in obese pregnant women. It uses a cross-sectional study of pregnant women with different BMI categories followed by ELISA test for the selected adipokines in the participant's plasma. Though, the general recommendations for diagnosing dysfunctional labour are not defined according to the maternal BMI category however, high maternal BMI has been found to cause many delivery-related complications, including prolonged labour. The findings from this chapter might find a relationship between maternal BMI and prolonged labour and a possible clinical role of plasma leptin and visfatin in this process.

## 5.2 Methods

### 5.2.1 Study design and participants

This study was designed as a cross-sectional study of Saudi women with a singleton pregnancy delivered in King Fahad Hospital of the University, Imam Abdulrahman Bin Faisal University, Alkhobar, Saudi Arabia. This hospital provides maternity care for 7,000 women per annum and is the largest government hospital in Alkhobar. All women between 18 and 45 years old were asked to participate in this study by the collection of maternal blood samples at the start of labour. Patients were recruited primarily by me when attending antenatal clinics at King Fahad Hospital of the University, Imam Abdulrahman Bin Faisal University, Alkhobar, Saudi Arabia. Patients who attended at night were recruited by the midwives at the department of Obstetrics and Gynaecology (Rabab Emshamea, Shaikha Aldossary and Asrar Alrashed).

Patients were given the participants' information sheets to read. Those who consented were asked to fill out and sign the informed consent forms. The patient information sheets and informed consent forms were available in English and Arabic. The patient's information obtained included clinically important demographic data such as maternal and gestational age, parity, previous and current pregnancy complications, medical history and first trimester body weight. After obtaining informed written consent, the women were recruited to the study and were further investigated to determine whether they met the study criteria. Women who were underweight ( $BMI < 18.50 \text{ kg/m}^2$ ), those having preterm delivery (gestation week  $< 37+0$ ), premature rupture of the membrane, elective C-section delivery and malformed fetuses were excluded. Women from whom a blood sample was not taken and thus were missing leptin and visfatin values or had incomplete information on their first trimester BMI, were also excluded. After further examination, a total of 119 out of 171 pregnant women were found to satisfy the pre-determined inclusion criteria for the study (**Figure 5.1**).



**Figure 5.1. Flow chart of the included and excluded women in the study.** Of the 171 Saudi women recruited, 119 satisfied the inclusion criteria. IUFD, Intrauterine Foetal Death.

## 5.2.2 Sampling strategy and variables included in the study

Study variables that were evaluated included maternal and gestational age, maternal height and weight at the first trimester of pregnancy, parity, medical history, maternal and neonatal outcomes and maternal plasma leptin and visfatin levels. Maternal outcomes included mode of delivery (non-operative vaginal delivery, operative vaginal delivery and C-section delivery), oxytocin use for augmentation, induction of labour and epidural anaesthesia. Neonatal outcomes including baby gender, birth weight and Apgar scores at 5 minutes post-delivery. Pregnancy-related complications were also assessed including: postdates, preeclampsia, eclampsia, GDM, polyhydramnios, oligohydramnios and IUGR. The diagnostic criteria of each pregnancy-related complication is shown in **table 5.1**. Maternal BMI was calculated based upon maternal weight (Kg) and height (m) measurements provided during booking at the first trimester of pregnancy. Eligible participants were then classified into three categories; (1) normal (BMI 18.50 - 24.99 kg/m<sup>2</sup>), (2) high BMI (BMI: 25.00 - 29.99kg/m<sup>2</sup>), (3) obese BMI  $\geq$ 30.00 kg/m<sup>2</sup>) and morbidly obese (BMI  $\geq$ 40.00 kg/m<sup>2</sup>) according to WHO definitions (Diet, 2003).

Maternal age defined as the age of the mother in years at the time of delivery. Gestational age was based on ultrasound scan performed during the first trimester. Term delivery was defined as delivery between 37 and 41<sup>+2</sup> (259-289 days) gestation. Postdate delivery was defined as delivery on or after 41<sup>+3</sup> (290 days) gestation. Parity was defined as the number of times that the participant had given birth to a foetus with a gestational age of 24 weeks or more, regardless of whether the child was born alive or was stillborn. The term “nulliparous” defines women who have never given birth to a baby. Primiparous is a term indicated for women who have given only one birth to a baby, and multiparous is a term described women who have given birth to more than one baby.

**Table 5.1. Summary table of the diagnostic criteria for pregnancy-related complications.**

The complication	The diagnostic criteria
Preeclampsia	woman with the new onset of hypertension (systolic blood pressure $\geq 140$ mmHg or diastolic blood pressure $\geq 90$ mmHg on at least two occasions at least four hours apart) and proteinuria after 20 weeks of gestation
Eclampsia	The development of seizures in a woman with preeclampsia
Postdates	Delivery on or after 41 <sup>+3</sup> (290 days) gestation
Gestational Diabetes	Diagnosis is made if one or more of the plasma glucose values meet or exceed the following thresholds: 92 mg/dL, fasting blood sugar, 180 mg/dL glucose tolerance test after 1 hr and 153 mg/dL glucose tolerance test after 2 hr
Polyhydramnios	Amniotic fluid index (AFI) $\geq 24$ cm
Oligohydramnios	Amniotic fluid index (AFI) $\leq 5$ cm
IUGR	If there is a 3-week difference between the referenced gestational age measurement and the actual measurement

Non-operative vaginal delivery defines a childbirth which takes place without any form of instrument intervention. Operative vaginal delivery was defined as a delivery in which the operator uses instruments such as forceps or a ventouse to extract the foetus via the vagina route, with the assistance of maternal pushing. C-section delivery is a surgical procedure used to deliver a baby through an incision made in the abdomen and the uterus of the mother. C-section delivery for failed induction was indicated if there was no cervical dilatation after 10 hours of intravenous oxytocin and 12 hours of vaginal prostaglandins in those who had unfavourable cervix at the initial assessment. C-section delivery was performed for a prolonged second stage of labour if the foetal head had not successfully descended below the ischial spines for two hours without active pushing and one hour with active pushing. The sample was also divided into two categories according to the duration of labour: normal length and prolonged. Prolonged labour is defined as labour lasts for  $\geq 20$  hours in nulliparous women, and  $\geq 14$  hours in multiparous women. In general terms, postpartum haemorrhage is defined as 500ml of blood loss. Vaginal delivery is usually expected to result in approximately 500ml or less of blood loss while C-section delivery is commonly associated with more blood loss. Therefore, the more clinically relevant definition of postpartum haemorrhage in these types of deliveries will be a blood loss of  $>500$  ml for vaginal deliveries and  $>1000$  ml for C-section delivery and thus was significant in this study.

Neonatal birth weight was measured immediately after delivery. Normal Saudi Neonatal birth weight is between 2,500-4,000 g (Wong, 1990, El-Gilany and Hammad, 2010). Neonatal birthweight  $< 2,500$  g is considered to be low birth weight (LBW). Macrosomia is defined as a birth weight of  $> 4,000$  g. Apgar score after 1 and 5 minutes was measured and recorded by the attending physician and it was considered normal if between 7 and 10 (2014). Neonatal complications observed including LBW ( $<2500$  g), macrosomia ( $\geq 4000$  g) and Apgar score  $< 7$  after 5 minutes.

### **5.2.3 Ethical considerations:**

Data was handled in compliance with the new GDPR law, 2018. It was confidentially held and stored manually and electronically. The handling of personal data complied with King Fahad Hospital of the University Personal Data

Protection Act. This study was approved externally by the Saudi hospital's ethical committee (Reference. PGS-2018-01-026, see **Appendix 6**) and was recognised by Liverpool Research Ethics committee (Reference. 2685, see **Appendix 7**). Patient approval and consent is part of the legal requirements for any research involving human subjects.

Each woman recruited for the study was initially approved and was handed a patient information sheet giving a detailed description of what the study involved and the relevant risks (**see Appendix 8**). The research subject and the details of involvement were further clarified orally for each interviewed woman. Questions were answered and those who agreed to join the study were recruited and signed a consent form signifying informed consent to take part (**see Appendix 8**).

Patients were consented when they were admitted to the hospital with labour pain by the primary investigator except if the admission was out of work hours, in which case, they were recruited after obtaining informed consent by the labour room and inpatients ward midwives who had been previously trained. Consent forms were stored in a locked office with a key kept with the primary investigator and electronic data was stored in a password-protected personal computer to maintain patient confidentiality. The files collecting the informed consent forms and the patient information sheets were transferred from Saudi Arabia to the UK in a locked bag which was kept in the aeroplane seat cabinet.

#### **5.2.4 Sample size determination**

Six to seven thousand pregnant women attending the antenatal clinics at King Fahad Hospital of the University each year and there are 1,300-1,500 deliveries per year according to the hospital records. A previous retrospective chart review results from the Saudi Arabian Eastern region showed that 39.3%, 23.6% and 28.7% of patients of measured BMI are normal weight, overweight and obese, respectively (El-Gilany and El-Wehady, 2009). Another study examined the maternal plasma concentration of visfatin with advanced gestation at the first, second and third trimester in normal weight and obese women used a sample size of 93 at the third trimester (Mazaki-Tovi et al., 2009b). By calculating the sample size, predicting that the confidence level is 95% and the confidence interval 5% and the population proportion 50%, the sample size required is at least 96. The following equation was used:

$$\text{Sample size} = \frac{\frac{z^2 \times p(1-p)}{e^2}}{1 + \left( \frac{z^2 \times p(1-p)}{e^2 N} \right)}$$

Where N = population size (1500); e = Margin of error (confidence interval); z = z-score. The z-score is the number of standard deviations a given proportion is away from the mean. It is equal to 1.96 if the desired confidence level = 95%.

### 5.2.5 Analysis of plasma leptin and visfatin levels

A maternal blood sample was collected shortly after women were admitted to the hospital at the first stage of labour which was determined by the women's self-reporting of pain. For leptin and visfatin analysis, 2ml of blood was collected in vacutainer tube and transferred to the hospital haematology lab. The blood then centrifuged, aliquoted and plasma was stored at  $-80^{\circ}\text{C}$  for ELISA analysis.

The plasma leptin and visfatin concentrations were measured by an automated ELISA machine according to the manufacturer's instructions (ETI-MAX 3000, Diasorin S.p.A, Saluggia, Italy). An indirect (sandwich) ELISA technique was used. Human leptin and visfatin were captured by pre-coated monoclonal antibodies on a 96-well microtiter plate (R&D systems, USA), followed by addition of conjugated enzyme-linked monoclonal secondary antibodies. The substrate solution was then added to the secondary antibodies, Spectrophotometric examination of the enzyme activity was done automatically by the machine at 450 nm after adding stop solution to stop the enzymatic reaction. The wells were washed by a wash buffer in between each step (3 times) to remove unbound substances. Increased absorbance was directly proportional to the amount of captured human leptin in the samples, and quantification was derived from a generated standard curve with reference calibrators of known concentrations. The specificity of the assay was 100% for both human leptin and visfatin with no cross-reactivity observed. The Intra-assay and inter-assay variations are 3.2% and 3.5%, respectively.

## 5.2.6 Data management and statistical analysis

The BMI of the women in relationship to maternal and neonatal outcomes and maternal leptin and visfatin levels were analysed. A D'Agostino & Pearson test was used to determine the normal distribution of the data. For continuous data that was normally distributed (maternal age, maternal height, neonatal birth weight), the mean and the standard error values were calculated. The significance was tested by paired Student's t-tests to analyse two groups, or a one-way ANOVA test with Bonferroni's multiple comparison post hoc test to analyse more than two groups. To address the relationship between maternal BMI and maternal plasma leptin levels, a one-way ANOVA test was used.

For continuous variables that are not normally distributed (gestational age, maternal weight, maternal BMI and maternal leptin and visfatin levels), significance was tested by Mann-Whitney test between two groups, or if more than two groups were tested, a Kruskal-Wallis ANOVA test with Dunns multiple comparison post hoc test was performed and the median and interquartile range (IQR) were calculated. To examine the relationship between the length of labour and maternal leptin and visfatin, a Mann-Whitney test was used. The Mann-Whitney test was also used to test the association between BMI and Apgar scores after 5 minutes. To examine maternal plasma leptin and visfatin in relationship to oxytocin usage, induction of labour and pregnancy-related complications, Kruskal-Wallis ANOVA test to test the association between maternal BMI and maternal plasma visfatin levels and the relationship between the mode of delivery and maternal leptin and visfatin levels was used. Bar chart was used to present the normally distributed data and Box and Wisker plot was used to present the data which is not normally distributed.

Percentages were calculated for categorical data used in this study including maternal BMI, parity, induction of labour, gestational age, duration of labour, pregnancy-related complications and baby gender. Bivariate analysis was used to study the relationship between maternal BMI and maternal characteristics including parity, oxytocin usage, induction of labour, epidural anaesthesia, mode of delivery, and pregnancy-related complications. Bivariate analysis was also used to study the association between maternal BMI and neonatal baby gender. This analysis was performed by Chi square ( $X^2$ ) test. Linear regression analysis

was used to study the correlation between maternal BMI and continuous variables including maternal and gestational age, neonatal birth weight, maternal plasma leptin and visfatin. It was also performed to test the association between maternal and gestational age, mode of delivery and neonatal birth weight with maternal leptin and visfatin levels.

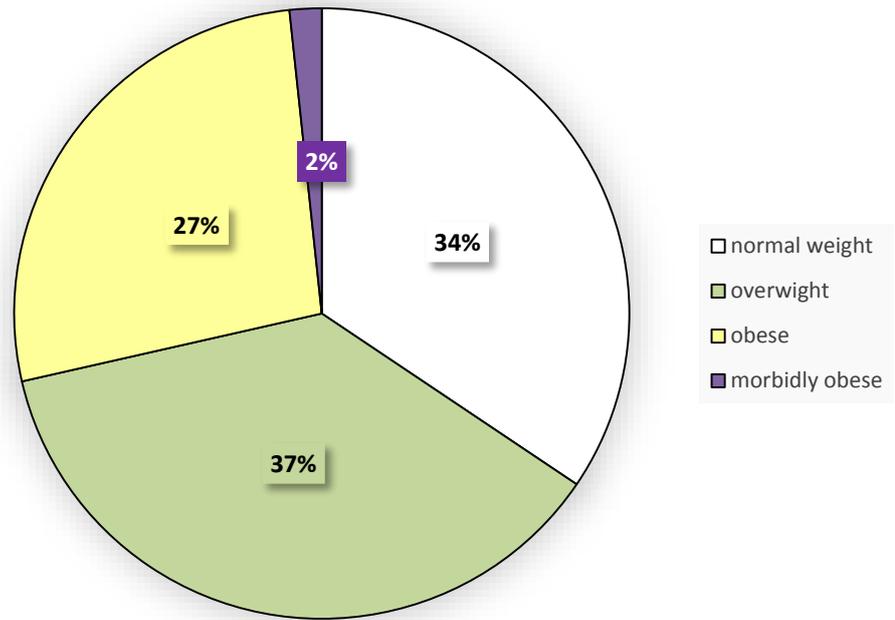
Statistical analysis was performed using GraphPad Prism version 5.01 (GraphPad Software, San Diego, USA). For all statistical tests,  $p$  value  $< .05$  was statistically significant. 'n' is the number of samples, each representing a different woman. An asterisk (\*) denotes significant difference in contractility compared to preceding control period  $p < 0.05$ , \*\*  $p < 0.005$  and \*\*\*  $p < 0.0005$ .

## 5.3 Results:

### 5.3.1 Study sample characteristics:

A summary of the key study sample characteristics is presented in **Table 5.2**. The total study population was 119 Saudi singleton pregnant women who were recruited at the start of labour. The study population is ethnically homogenous. Only 3 participants refused to be recruited in the study which represents a 2% refusal rate. The mean maternal age of the participants was  $30 \pm 0.5$  years with half of the sample being 30 years old or younger. The median gestational age was  $273 \pm 112$  days. The incidence of maternal obesity in this study population was 29 % ( $n=34$ ), with 27% ( $n=32$ ) of them were classified as having BMI at obese level and 2% ( $n=2$ ) of them were classified as having morbidly obese level of BMI. Maternal BMI level corresponding to overweight levels was observed in 37% ( $n=44$ ) of the population whilst 34% ( $n=41$ ) of women were classified as having a BMI within the normal range (**Figure 5.2**). Therefore, more than 65% of the participants were either overweight or obese. Almost half of the study population (51%) were multiparous, and the other half were distributed almost equally between nulliparous (24%) and primiparous (25%). Labour started spontaneously in 77% of the study sample whilst 33% of the women underwent labour induction. The most commonly occurring pregnancy-related complications encountered in this study was gestational diabetes (11%) followed by oligohydramnios (4%), intrauterine growth retardation (IUGR) (2%) and polyhydramnios (1%). No cases of preeclampsia or eclampsia were reported. The

majority of women (89%) had term delivery while 11% of them were postdates and 9% of them had prolonged labour. Of the total sample, only one woman had postpartum haemorrhage and none of them were smokers. The average neonatal birth weight was 3069 g. Among all the neonates delivered, 7% were LBW. Apgar scores after 1 minute were between 5 and 9 and Apgar scores after 5 minutes were between 7 and 10. Male and female gender were equally distributed in the offspring of the study population.



**Figure 5.2. Study population distribution according to maternal BMI.**

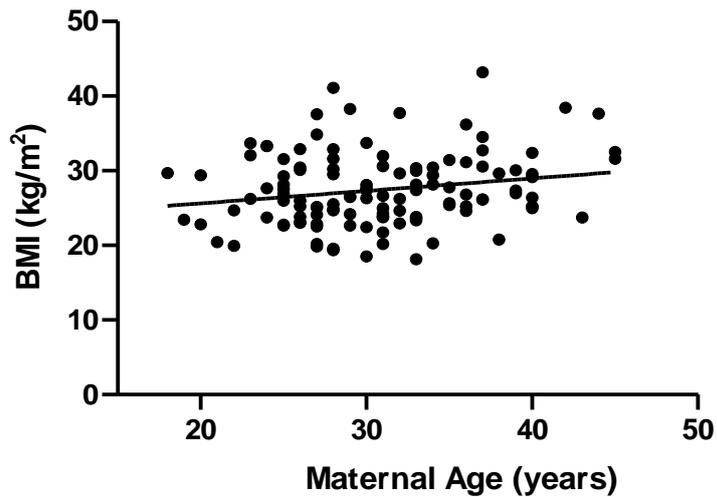
The percentage of normal weight women in the study population was 34% compared to 37% was for overweight and 29% was for obese women.

**Table 5.2. Summary table of study sample characteristics after application of exclusion criteria.** The table shows the study sample characteristics of maternal age (year, mean  $\pm$  se), gestational age (days, median (IQR)), maternal weight (Kg, median (IQR)), maternal height (m, mean  $\pm$  se) and the percentage (%) of BMI categories, parity and pregnancy/delivery related complications (n=119). IUGR; Intrauterine Growth Retardation.

<b>Study sample characteristics</b>	
Maternal age (years) (se)	30.3 (0.5)
Gestational age (days) (IQR)	273 (266-280)
Maternal Weight (Kgs) (IQR)	65 (59-75)
Maternal Height (m) (se)	1.56 (0.005)
Maternal BMI (kg/m <sup>2</sup> )	n (%)
Normal weight	41 (34)
Overweight	44 (37)
Obese	32 (27)
Morbidly obese	2 (2)
Parity	n (%)
Nulliparous	28 (23)
Primiparous	30 (26)
Multiparous	61 (51)
Prolonged labour n (%)	11 (9)
Postdates n (%)	13 (11)
Gestational Diabetes n (%)	13 (11)
Polyhydramnios n (%)	1 (1)
Oligohydramnios n (%)	5 (4)
IUGR n (%)	2 (2)

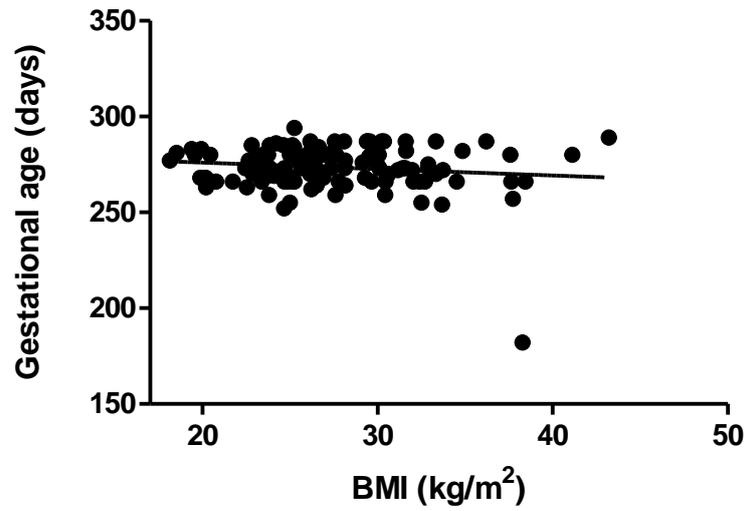
### 5.3.2 The relationship between maternal BMI and maternal demographic characteristics

This study aimed to examine the relationship between maternal BMI and maternal demographic characteristics. The maternal demographic characteristics tested including maternal age, gestational age and parity. Maternal BMI was found to increase with maternal age and this relationship was statistically significant ( $R^2=0.04$ ,  $P$  value $<0.05$ ); however, an  $R^2$  with 4% of variation is not enough to explain/predict the variation in the outcome (BMI) (**Figure 5.3**). There is no relation between maternal BMI and gestational age ( $R^2=0.01$ ,  $P$  value $>0.05$ ) (**Figure 5.4**). Maternal BMI was observed to be significantly higher with increasing parity ( $R^2=0.06$ ,  $P$  value $<0.05$ ,  $n=119$ ); however 6%  $R^2$  explains some of the variation but it is not enough to predict the changes in the outcome (parity) (**Figure 5.5**).



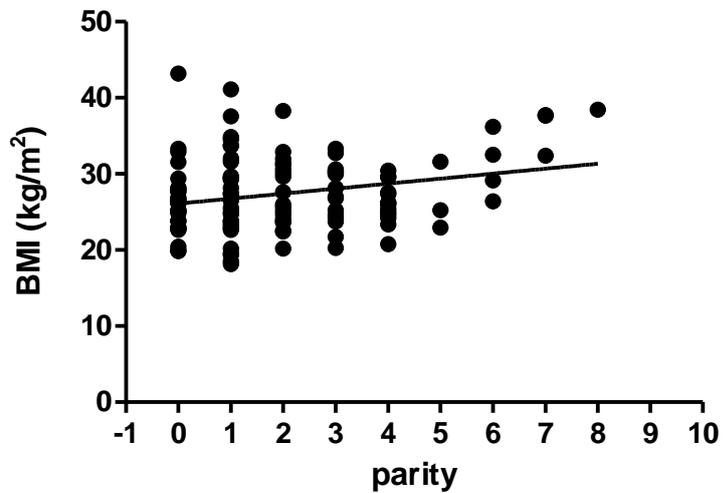
**Figure 5.3 The correlation between maternal BMI and maternal age.**

A scatter plot showing no relationship between maternal BMI (kg/m<sup>2</sup>) and maternal age (years) ( $R^2=0.04$ , P value $<0.05$ ,  $n=119$ ). P value  $<0.05$  indicates that the relationship is statistically significant by the Pearson correlation test. However,  $R^2$  (4%) is very low to predict the variation.



**Figure 5.4 The correlation between maternal BMI and gestational age.**

A scatter plot showing no relationship between maternal BMI (kg/m<sup>2</sup>) and gestational age (days) (R<sup>2</sup>=0.01, P value>0.05, n=119). P value >0.05 indicates that the trend was insignificant by the Pearson correlation test.



**Figure 5.5 The correlation between maternal BMI and parity.**

A scatter plot showing no relationship between maternal BMI (kg/m<sup>2</sup>) and parity ( $R^2=0.06$ , P value<0.05, n=119). P value <0.05 indicates that the relationship is statistically significant by the Pearson correlation test. However,  $R^2$  (6%) is very low to predict the variation.

### 5.3.3 The relationship between maternal BMI and pregnancy-related complications

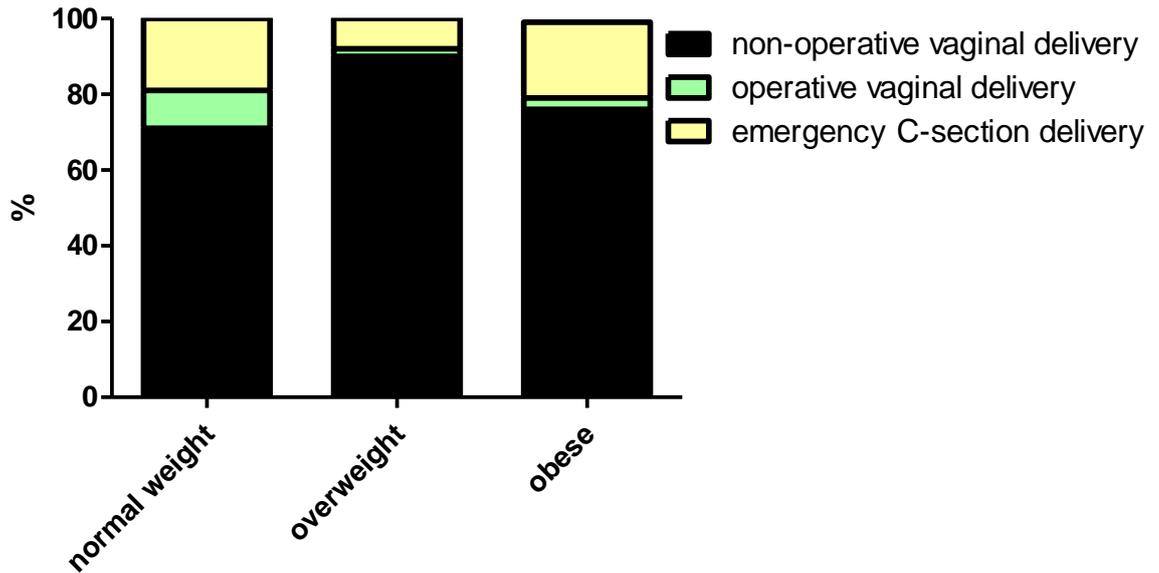
The risks of pregnancy-related complications according to maternal BMI categories were also examined. Of the 119 women examined, 7% of normal weight women developed GDM compared to 9% of overweight and 17% of obese women ( $X^2=2$ ,  $df=2$ ,  $P$  value $>0.05$ ). Furthermore, 4% of normal weight women developed oligohydramnios compared to 2% of overweight and 5% of obese women ( $X^2=2$ ,  $df=2$ ,  $P$  value $>0.05$ ). Only one obese women delivered a neonate with IUGR and none of them has polyhydramnios which made it impossible to test for an association between maternal BMI and these pregnancy-related complications.

### 5.3.4 The effect of maternal BMI on obstetric maternal interventions and maternal outcomes

The obstetric maternal interventions examined in this study were oxytocin use, induction of labour and epidural anaesthesia. Oxytocin was administered for augmentation of labour in 46% of normal weight, 63% of overweight and 50% of obese women ( $X^2=2$ ,  $df=2$ ,  $P$  value $>0.05$ ). Labour was induced in 14% of normal weight, 22% of overweight and 32% of obese women ( $X^2=3$ ,  $df=2$ ,  $P$  value $>0.05$ ). Epidural anaesthesia was used in 6% of normal weight, 4% of overweight and 5% of obese women ( $X^2=0.7$ ,  $df=2$ ,  $P$  value $>0.05$ ).

The relationship between maternal BMI and the mode of delivery was also investigated. Most of the study sample population (80%) had non-operative vaginal delivery compared to 5% who had an operative vaginal delivery and 15% who required an emergency C-section delivery (**Figure 5.6**). The incidence and the end numbers of each mode of delivery in relationship to different maternal BMI categories are shown in **Table 5.3**. The incidence of non-operative vaginal delivery in normal weight women was 70 % compared to 90% in overweight and 76% in obese women ( $X^2=5$ ,  $df=2$ ,  $P$  value $>0.05$ ). The incidence of operative vaginal delivery in normal weight women was 10 % in contrast to 2% in overweight and 3% in obese women ( $X^2=2$ ,  $df=2$ ,  $P$  value $>0.05$ ). The emergency C-section delivery rate in normal weight women was 20% compared to 8% in overweight and 19% in

obese women ( $X^2=2$ ,  $df=2$ ,  $P$  value $>0.05$ ). Indications for C-section encountered in this study included: foetal distress, failed induction, the prolonged second stage of labour, unsuccessful operative delivery and breach presentation.



**Figure 5.6 The mode of delivery in relation to maternal BMI.**

Most of the women delivered with non-operative vaginal delivery. No significant difference between non-operative vaginal, operative vaginal and C-section deliveries in normal weight (n=41), overweight (n=44) and obese (n=34) women (P value>0.05, n=119). P value >0.05 indicates that the trend was insignificant by chi-square test.

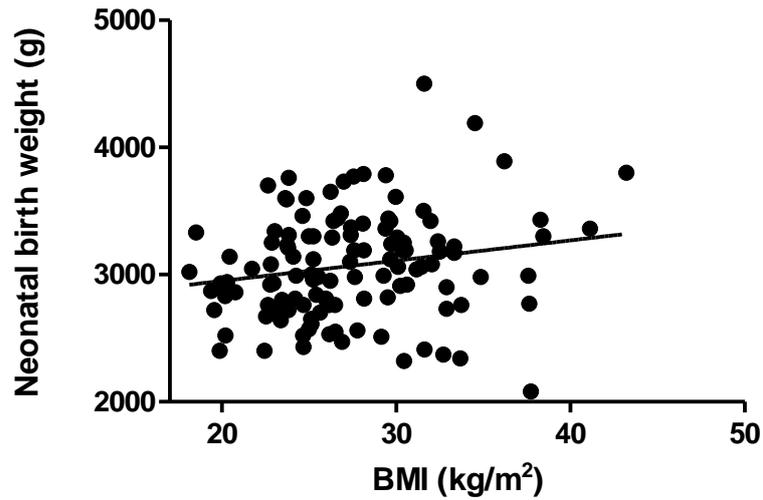
**Table 5.3. The relationship between the mode of delivery and maternal BMI.**

P value >0.05 indicates that the trend was insignificant by chi-square test (n=119).

	<b>non-operative vaginal delivery n (%)</b>	<b>operative vaginal delivery n (%)</b>	<b>C-section delivery n (%)</b>
<b>Normal weight BMI, (%) , (n=41)</b>	29 (70)	4 (10)	8 (20)
<b>Overweight BMI (%) , (n=44)</b>	40 (90)	1 (2)	4 (8)
<b>Obese BMI (%) , (n=34)</b>	26 (76)	1 (3)	7 (21)
<b>P value</b>			>0.05

### 5.3.5 The relationship between maternal BMI and neonatal characteristics

The relationship between maternal BMI and neonatal characteristics was also assessed. The normal weight women were less likely to deliver a female baby (incidence of baby girls was 34%), compared with overweight (61% female babies) and obese (56% female babies) mothers ( $X^2=6$ ,  $df=2$ ,  $P$  value $<0.05$ ). Neonatal birth weight was found to increase with increasing BMI and this association was found to be statistically significant ( $r^2=0.03$ ,  $P$  value $<0.05$ ) (**figure 5.7**); however, an  $R^2$  with 3% variation is not enough to predict the variation in the model. Of the 119 women, 7% of normal weight women delivered a LBW baby compared to 2% of overweight and 14% of obese women ( $X^2=4$ ,  $df=2$ ,  $P$  value $>0.05$ ). All neonatal Apgar scores after 5 minutes were within the normal range in all maternal BMI groups. There was no significant relationship between maternal BMIs of women who delivered neonates having Apgar scores between 7 and 8 after 5 minutes ( $27 \pm 10$ ,  $n=4$ ) compared to those who delivered neonates having Apgar scores between 9 and 10 ( $26 \pm 7$ ,  $n=115$ ) ( $P$  value $>0.05$ ).



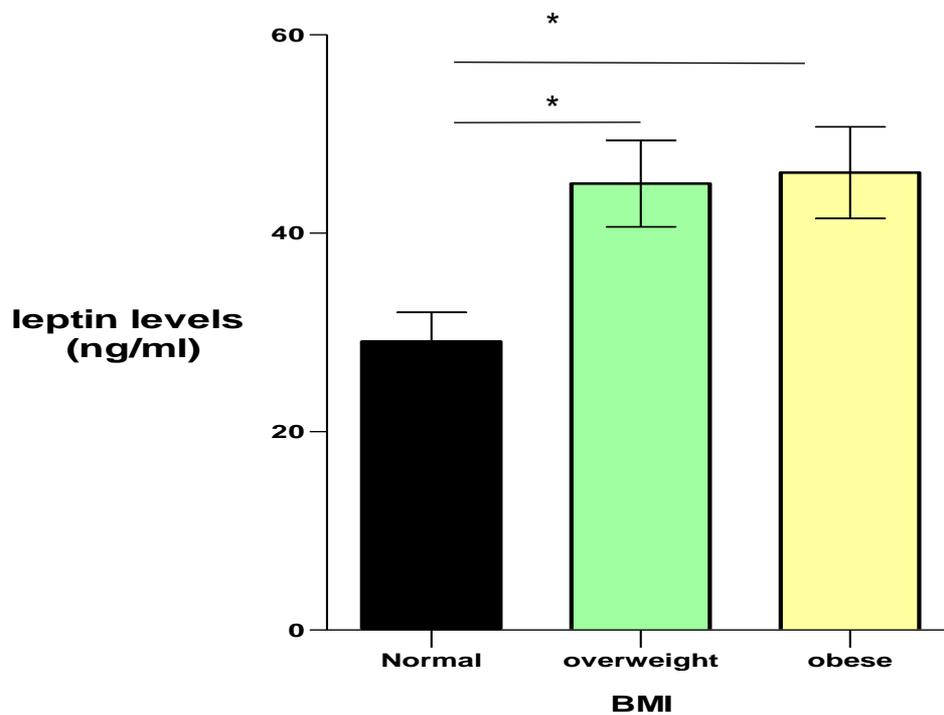
**Figure 5.7 The relationship between maternal BMI and neonatal birthweight.**

A scatter plot demonstrating a positive linear relationship between maternal BMI ( $\text{kg/m}^2$ ) and neonatal birthweight (g) ( $R^2=0.03$ , P value $<0.05$ ,  $n=119$ ). P value  $<0.05$  indicates that the trend was significant by the Pearson correlation test. However,  $R^2$  (3%) is very low to predict the association.

### **5.3.6 The relationship between maternal BMI and maternal plasma leptin levels at the start of labour**

Maternal plasma leptin concentrations at the start of labour were measured using the ELISA technique, as described earlier. Leptin was detected in the plasma of all samples and all the values lay within the standard curve detection levels (**Appendix 9**). The mean maternal plasma leptin levels were 33 ng/ml  $\pm$  32 (n=98).

The relationship between maternal plasma leptin and maternal BMI was examined. Comparisons of maternal plasma leptin levels between BMI categories showed that its levels were significantly higher in obese women (46.1 ng/ml  $\pm$  4.6, n=29) and overweight (45 ng/ml  $\pm$  4.4, n=36) in comparison to normal weight women (29.1 ng/ml  $\pm$  2.9, n=33) (P value<0.05) with no significant difference between overweight and obese women (P value>0.05) (**figure 5.8**). The data was normally distributed (mean  $\pm$  se).



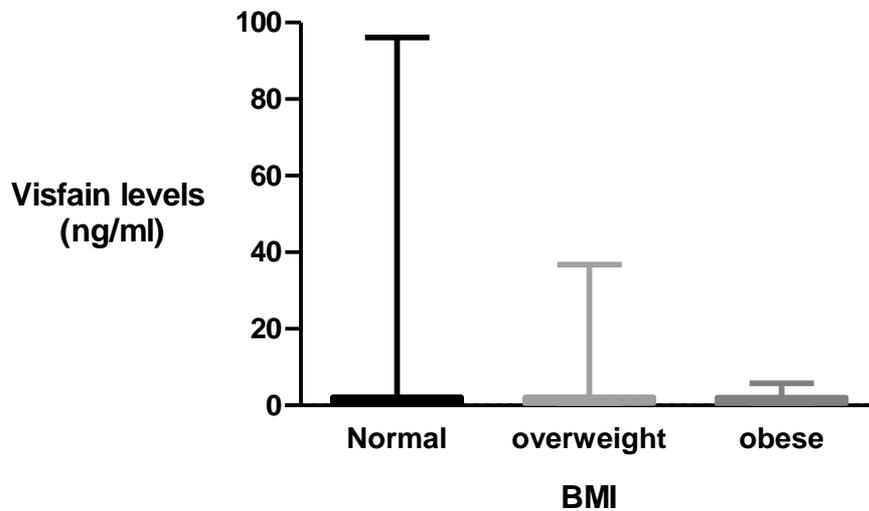
**Figure 5.8 Maternal Leptin levels at the start of labour at different maternal BMI (mean  $\pm$  se)**

Maternal plasma leptin levels are significantly higher in obese (46.1 ng/ml  $\pm$  4.6, n=29) and overweight women (45 ng/ml  $\pm$  4.4, n=36) in comparison to normal weight women (29.1 ng/ml  $\pm$  2.9, n=33) (P value<0.05). P value <0.05 indicates that the trend was significant by one way ANOVA test.

### **5.3.7 The relationship between maternal BMI and maternal plasma visfatin levels at the start of labour**

Maternal plasma visfatin concentrations at the start of labour were also measured using the ELISA technique. Visfatin was detected in the plasma of all subjects. All the values lay within the standard curve detection levels (**Appendix 9**). The median maternal plasma visfatin levels is 0.9 ng/ml (0.6-2), (n=116).

Comparisons of maternal plasma visfatin levels between BMI categories showed no significant difference in its levels between normal weight women (0.9 ng/ml (0.8-2.1), n=39) and overweight (0.7 ng/ml (0.6-2.1), n=43) in comparison to obese women (0.95 ng/ml (0.5-2), n=34) (P value>0.05) (**figure 5.9**). The data was not normally distributed (median  $\pm$  IQR).



**Figure 5.9 Maternal visfatin levels at the start of labour at different maternal BMIs (median (IQR)).**

Box and Wisker plot showing that maternal plasma visfatin levels are not significantly different between normal weight women (0.9 (0.8-2.1) ng/ml, n=39), overweight (0.7 (0.6-2.1) ng/ml, n=43) and obese women (0.95 (0.5-2) ng/ml, n=34) (P value>0.05). P value >0.05 indicates that the trend was not significant by one way ANOVA test.

### **5.3.8. The relationship between prolonged labour and maternal plasma leptin and visfatin levels at the start of labour in obese women**

The main hypothesis was that obese women are more prone to have prolonged labour if they have high leptin and visfatin levels. However, of the 119 women, only a small proportion (9%, n=11) experienced prolonged labour and only 3% (n=3) of obese women had prolonged labour. These numbers are insufficient to establish a relationship between prolonged labour and maternal leptin and visfatin levels in obese women due to insufficient events (prolonged labour) in the sample set. When the whole study sample population (regardless of BMI category) was considered, there was no relationship between the duration of labour and maternal plasma leptin and visfatin levels.

### **5.4.9. The relationship between maternal characteristics and maternal plasma leptin and visfatin levels during labour**

A summary of the key maternal characteristics in relation to maternal plasma leptin and visfatin levels at the start of labour is given in **table 5.4**. No relationship was found between maternal age and maternal plasma leptin levels ( $R^2=0.01$ ,  $P$  value $>0.05$ ,  $n=98$ ) and visfatin levels ( $R^2=0.05$ ,  $P$  value $<0.05$ ,  $n=116$ ). There was no association between gestational age (including postdates) and maternal plasma leptin ( $R^2=0.005$ ,  $P$  value $>0.05$ ,  $n=98$ ) and visfatin levels ( $R^2=0.0008$ ,  $P$  value $>0.05$ ,  $n=116$ ). In addition, parity was found to have no relationship with maternal plasma leptin ( $R^2=0.01$ ,  $P$  value $>0.05$ ,  $n=98$ ) and visfatin concentrations ( $R^2=0.01$ ,  $P$  value $>0.05$ ,  $n=116$ ).

**Table 5.4. Maternal characteristics in relation to maternal plasma leptin and visfatin levels during labour.**

		leptin (pg/ml)			visfatin (pg/ml)			
Characteristic	N	1st quartile	median	3rd quartile	n	1st quartile	median	3rd quartile
age <25	17	23.5	<b>40.5</b>	64.8	21	0.6	<b>1.5</b>	2.4
age >25	81	20.5	<b>33.5</b>	47.3	95	0.6	<b>0.8</b>	1.4
Normal BMI	33	17.5	<b>23.5</b>	38.4	39	0.8	<b>0.9</b>	2.1
overweight BMI	36	26.9	<b>37.6</b>	64.4	43	0.6	<b>0.7</b>	2.1
obese BMI	29	30.2	<b>37.9</b>	67.1	34	0.5	<b>0.9</b>	2
Nulliparous	23	19.6	<b>30.2</b>	74.5	27	0.6	<b>0.9</b>	1.9
Primiparous	23	28.7	<b>40.5</b>	63.9	29	0.7	<b>1.1</b>	2.2
Multiparous	52	19.5	<b>32.8</b>	44.5	60	0.5	<b>0.8</b>	1.5
Gestational Diabetes yes	12	22.6	<b>40.1</b>	64.1	13	0.5	<b>1.4</b>	4.5
Gestational Diabetes no	86	19.6	<b>33.4</b>	52	103	0.6	<b>0.9</b>	1.9
Polyhydramnios yes	1		<b>76.8</b>				<b>2.4</b>	
Polyhydramnios no	98	20.2	<b>33.6</b>	52	115	0.6	<b>0.9</b>	2
Oligohydramnios yes	4	15.1	<b>27.2</b>	45	5	0.7	<b>1.4</b>	4.7
Oligohydramnios no	94	20.5	<b>33.6</b>	55	111	0.6	<b>0.9</b>	2
IUGR yes	2	11.6	<b>29.4</b>	47.3	2	0.6	<b>1.8</b>	3
IUGR no	96	20.5	<b>33.6</b>	53.6	114	0.6	<b>0.9</b>	2
Normal length labour	90	20.2	<b>34.6</b>	52	105	0.6	<b>0.9</b>	1.8
Prolonged labour	7	14.7	<b>21.6</b>	85.6	10	.0.5	<b>1.5</b>	12.7
Term pregnancy	87	20.6	<b>34.3</b>	51.2	103	0.6	<b>0.9</b>	2.1
postdate pregnancy	11	17.9	<b>30.9</b>	64.8	13	0.5	<b>0.9</b>	2.65
Induced delivery	22	18.8	<b>35.1</b>	65.8	27	0.7	<b>1.2</b>	3
Spontaneous delivery	77	19.6	<b>33.3</b>	47.3	90	0.6	<b>0.8</b>	1.4
Epidural anaesthesia yes	16	19.8	<b>34</b>	67.1	17	0.5	<b>0.7</b>	3.8
epidural anaesthesia no	82	20.2	<b>33.5</b>	52	99	0.6	<b>0.9</b>	2
oxytocin use yes	47	19.5	<b>30.5</b>	49.4	62	0.6	<b>1</b>	2.1
Oxytocin use no	51	20.5	<b>37.5</b>	54.4	54	0.5	<b>0.8</b>	1.5
vaginal delivery	79	23.5	<b>37.1</b>	58.7	94	0.6	<b>0.9</b>	1.9
Instrumental delivery	4	16.1	<b>21.9</b>	28.4	6	0.7	<b>2.1</b>	28.9
C-section delivery	15	16.2	<b>27.6</b>	47.6	16	0.5	<b>0.8</b>	1.3

### **5.3.10 The relationship between pregnancy-related complications and maternal plasma leptin and visfatin levels at the start of labour**

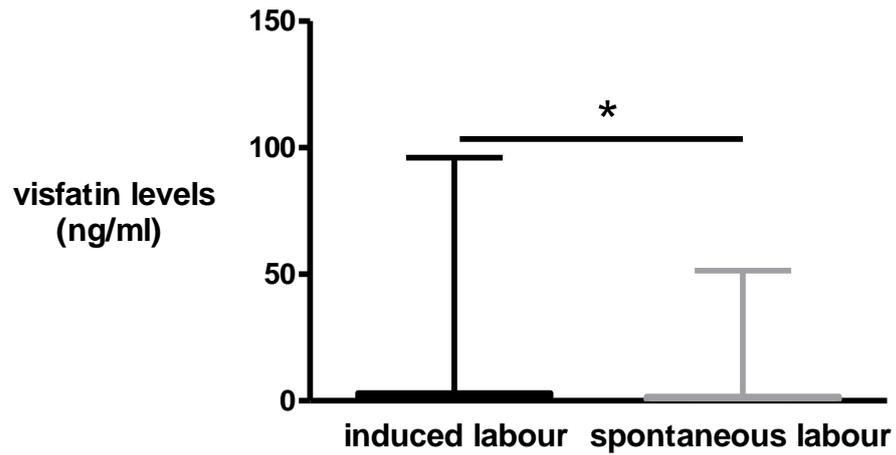
The relationship of pregnancy-related complications with maternal plasma leptin and visfatin levels was tested at the start of labour in normal and complicated pregnancy. The pregnancy-related complications tested were GDM and oligohydramnios and no association was found between these complications and maternal plasma leptin and visfatin levels ( $P$  value $>0.05$ ). Other pregnancy-related complications, including polyhydramnios ( $n=1$ ) and IUGR ( $n=2$ ), had insufficient numbers to be tested.

### **5.3.11 The association between maternal outcomes and maternal plasma leptin and visfatin levels at the start of labour**

The obstetric maternal interventions were initially tested and no association was found between oxytocin use and maternal plasma leptin and visfatin levels ( $P$  value $>0.05$ ). There was also no association between the induction of labour and maternal plasma leptin levels ( $P$  value $>0.05$ ). However, it was found that maternal plasma visfatin levels are significantly higher in women who experienced induction of labour (1.2 (0.7-3) ng/ml,  $n=27$ ) in contrast to women who delivered spontaneously (0.8 (0.5-1.7) ng/ml,  $n=89$ ) ( $P$  value $<0.05$ ) (**figure 5.10**). There was no relationship between the mode of delivery and maternal plasma leptin and visfatin levels ( $P$  value $>0.05$ ).

### **5.3.12 The relationship between neonatal characteristics and maternal plasma leptin and visfatin levels at the start of labour**

In regard to neonatal characteristics, there was no correlation between neonatal birth weight (including SGA) and maternal plasma leptin and visfatin levels. Apgar scores after 5 minutes were within the normal range in all the sample population and it was unnecessary to relate them to maternal plasma leptin and visfatin levels.



**Figure 5.10 The association between induction of labour and maternal plasma visfatin levels (median (IQR))**

Box and Wisker plot showing that maternal plasma visfatin levels are significantly higher in women having induction of labour (1.2 (0.7-3) ng/ml, n=27) compared to women who delivered spontaneously (0.8 (0.5-1.7) ng/ml, n=89) (P value<0.05). P value <0.05 indicates that the trend was significant by Mann Whitney test.

## 5.4 Discussion

In this study, an attempt was made to establish an association between maternal leptin and visfatin, and prolonged labour in Saudi obese pregnant women. It was hypothesised that obese women with high plasma leptin and visfatin, are more prone to prolonged/dysfunctional labour. This hypothesis was based on my findings from *in vitro* studies described in Chapter 3. In this study a population of 119 women, with a relatively representative prevalence of obesity, it was difficult to test for the association between maternal plasma leptin and visfatin and prolonged labour in obese women due to the low numbers of obese women who had prolonged labour.

No significant relationship was found between maternal BMI and both maternal and neonatal characteristics. It was observed that maternal plasma leptin levels, but not visfatin, increased with maternal BMI. No significant correlation was found between maternal plasma leptin and visfatin levels and maternal and neonatal characteristics. It was also found that maternal plasma visfatin levels are higher in women who were induced in comparison to those who delivered spontaneously.

Saudi Arabia is among the highest obesity and overweight rates in the world (Al-Kadi et al., 2018). The incidence of maternal obesity in my study was high (29%) with more than 65% of participants either overweight or obese. This rate is in line with the prevalence of obesity and overweight (65.9%) among females aged 15 or older in Saudi Arabia reported by WHO (Al-Quwaidhi et al., 2014). A more recent assessment conducted in several Saudi maternity hospitals in Riyadh reported that they had 68% of the participants were either overweight or obese (Wahabi et al., 2016), which is slightly higher than in my sample.

The maternal weight, which was used to calculate the maternal BMI in this study, was the weight taken at the first trimester of pregnancy and not weight at delivery hence most of the pregnancy and delivery-related risks in obese women are related to early pregnancy maternal BMI (Fitzsimons et al., 2009). Maternal BMI at delivery can be misleading as later in pregnancy, not only can the maternal body fat mass contribute to the weight, the amount of gestational weight gain, total body water, placenta weight, amniotic fluid volume and the foetal and uterus weight can have a considerable contribution to maternal BMI (Scholl et al., 1991).

It was determined that there was no relationship between maternal age and maternal BMI. My data, therefore, contradicts several previous studies which found that there was a strong positive association between maternal BMI and advancing maternal age (Pomerleau et al., 2000, Makgoba et al., 2012, Francaite-Daugeliene et al., 2016), that is partly explained by hormonal imbalance and restricted physical activity in older women. It was previously found that high maternal BMI is associated with increasing gestational age (Stotland et al., 2007, Denison et al., 2008) however, no relationship was found between maternal BMI and gestational age in my study.

Unlike other studies (Wolfe et al., 1997, Rosenberg et al., 2003, Ertem et al., 2008, El-Gilany and El-Wehady, 2009, Paulino et al., 2016, Iversen et al., 2018), maternal BMI in my study sample was found to have no association with increasing parity. Childbearing is associated with permanent weight gain (Abrams et al., 2013, Rosenberg et al., 2003). The mechanisms underlying the association between maternal obesity and parity are uncertain; however, new evidence suggests that there is an increase in the release of placental corticotropin-releasing hormone and cortisol concentrations during pregnancy (Magiakou et al., 1996). Both hormones have been found to contribute to the pathophysiology of abdominal obesity (Pasquali et al., 2006), which is known to be partly mediated by insulin resistance (Bjorntorp, 1993). Besides, non-biological factors during pregnancy including unhealthy lifestyles and socioeconomic and psychosocial stress may also lead to hypothalamic-pituitary-adrenal hyperactivity (Li et al., 2016). Peripheral insulin resistance is also triggered by pregnancy and ultimately increases calorie storage and triacylglycerol deposition in visceral adipose tissues and so might also play an independent role (Dahlgren, 2006).

Limited studies have examined the association between maternal obesity and pregnancy-related complications in Saudi pregnant women and concluded that GDM and preeclampsia are the most common complications in obese Saudi pregnant women (Wahabi et al., 2014, El-Gilany and Hammad, 2010). Although it is widely known that obesity is strongly associated with pregnancy-related complications, no association was found between maternal BMI and the pregnancy-related complications encountered in this study.

The intrapartum medical management associated with obese pregnant women was found to be different from normal weight women. It was found that

induction of labour, failed induction, oxytocin augmentation and epidural anaesthesia are significantly more used in obese nulliparous women in comparison to normal weight women (Carlson and Lowe, 2014). Oxytocin treatment for arrested dilatation required a larger dose (Frey et al., 2015) and is less effective in obese pregnant women (Soni et al., 2013). The indications for oxytocin usage and induction of labour in this hospital was a physician's decision. In general, It was observed that they were indicated for complicated labour; however, most women in my study sample who received oxytocin or underwent induced labour were having uncomplicated labour. Despite these discussed efforts to make the labour less complicated, obese pregnant women have a progressive reduction in non-operative vaginal delivery rate (Lynch et al., 2008) with an increased rate of operative vaginal delivery (Sydsjö et al., 2010). They are also more likely to end labour with emergency C-section delivery (Sheiner et al., 2004, Chu et al., 2007c). They also had a significantly higher rate of IOL ending in C-section delivery (Arrowsmith et al., 2011). Nevertheless, no correlation was found between maternal obesity and operative vaginal delivery or C-section delivery in my study sample. No association was found between maternal BMI and neonatal characteristics. Although several studies have found that there is a significant correlation between neonatal birth weight and maternal BMI (Hull et al., 2008, Upadhyay et al., 2011, Singh et al., 2016, Zoya et al., 2017, Papazian et al., 2017).

Primarily it was found that the mean maternal plasma leptin levels were higher with increasing maternal BMI (29ng/ml in normal weight compared to 46ng/ml in obese women). This finding is consistent with a number of other studies that have been published previously (Hamilton et al., 1995a, Ramsay et al., 2002, Al Maskari and Alnaqdy, 2006, Considine et al., 1996). It is biologically plausible that such an association would be predicted as adipose tissue mass increases with increasing BMI. The maternal plasma leptin levels in different BMI categories are very diverse in these studies which made it difficult for comparative purposes. Our results are close to the recent similar study of Carlhäll *et al* group (20ng/ml in normal weight compared to 50ng/ml in obese women) (Carlhäll et al., 2018), although the nature of the study population was different as they only included nulliparous women. They found that maternal plasma leptin in obese women was correlated with pre-pregnancy BMI rather than gestational weight

gain. Obesity has also been proven to be associated with leptin resistance (Myers et al., 2010). Leptin levels are higher during pregnancy in obese women in comparison to normal weight women (Yang, 2005, van der Wijden et al., 2013, Franco-Sena et al., 2015). The rate of this increase during pregnancy is lower in obese women (Misra and Trudeau, 2011, Castellano Filho et al., 2013).

Several studies have documented that leptin might produce a modulatory effect on human myometrial contractility (Moynihan et al., 2006, Wendremaire et al., 2011, Harrod et al., 2011, Wuntakal et al., 2013, Wendremaire et al., 2013, Mumtaz et al., 2015). Two of these studies, one of which we published, have observed a relaxant effect of leptin on human spontaneous and oxytocin induced myometrial contractility *in vitro* (Moynihan et al., 2006, Mumtaz et al., 2015). Moynihan *et al* demonstrated that leptin has a cumulative inhibitory effect on the amplitude and frequency of myometrial contractility. I have found that leptin, in a relatively high concentration (1 $\mu$ M), produced a reduction in the amplitude and AUC of myometrial contractions (Moynihan et al., 2006). The mechanism of action of leptin on myometrial contractility is unknown, but it has been suggested that , it stimulates BK channels which causes hyperpolarization and thus smooth muscle relaxation (O'Malley et al., 2005).

Plasma visfatin levels are known to have a significant relationship with BMI (Berndt et al., 2005, Zahorska-Markiewicz et al., 2007, Bełtowski, 2006). Nevertheless, this relationship was not found in my study sample. Berndt et al have used a sample size of 189, which is relatively close to my study sample number however, they included both men and women. Zahorska-Markiewicz *et al.* used a smaller study sample of 37 women, of whom 21 of them are obese. My data also showed that maternal plasma visfatin has no relationship with maternal age. This is unlike a study done previously which found a negative relationship between plasma visfatin concentrations and maternal age (Chan et al., 2006). This might partly explain the reason behind insulin resistance observed in old women. A possible explanation for this reduction in the levels of visfatin could be due to the reduction in muscle mass in older women which is an important source of visfatin. Additionally, maternal plasma visfatin levels found to be higher in women underwent induction of labour. This can be explained by the inflammatory role of visfatin during labour.

## **5.5 Conclusion:**

In this study, the main aim was to address an association between adipokines and prolonged labour in obese women and compare it to normal weight women. This aim was not met as the number of women in the sample who had a prolonged labour was small. Maternal obesity doesn't appear to be related to dysfunctional labour in Saudi women. This study confirmed that maternal plasma leptin levels are higher with increasing maternal BMI. It should be acknowledged that this study can be considered as a preliminary pilot study due to its underpowered sample size. It was demonstrated that maternal plasma visfatin levels have a positive correlation with induction of labour. With the increasing rate of maternal obesity in Saudi Arabia, it is of substantial interest to identify new targets for obstetricians to control both pregnancy and delivery-related complications in obese women. Modulating adipokines dysregulation in obese women might be a future option in the management of obstetric complications associated with maternal obesity.

## **5.6 Limitations of the study:**

Recruitment of the women during labour has its own methodological limitation; for example women were often be in pain and unable to be engaged with any kind of conversation. This was a hard challenge for me during the data collection period. Increasing the sample number is often of advantage to get more reliable results and to avoid random errors. No relationship was found between prolonged labour and maternal obesity because the number was small. Increasing the sample number might help to sub classify the sample to find more clinically significant associations such as classifying the sample according to parity. It chosen to classify the study sample into women who have a normal length of labour and those who have prolonged labour, though it will be more informative to calculate the labour time and relate it to maternal obesity. It was decided that because Carlhäll *et al* measured the time of active phase of labour and found it unrelated to maternal obesity (Carlhäll et al., 2018).

At the first trimester, obese women are classed as a high-risk group and given lifestyle modification counselling, including nutritional advice, thus I cannot account for any changes that might have taken place nor can I account for the changes in maternal weight during pregnancy. Although early pregnancy

maternal BMI measurements are representative of pregnant women's body weight, calculating the total amount of weight gain during pregnancy (GWG) and relating it to maternal BMI at the first trimester could enhance the study findings. Another limitation of this study involved merging obese and morbidly obese groups into a single group due to the relatively small number of morbidly obese women in my study sample (n=2)

Some methodological limitations of the study should be identified. I designed my study as a cross-sectional study which has some limitations. A cross-sectional study, also known as a prevalence study or transversal study, is a type of observational study that analyses data from a population, or a representative group, at a specific point in time or time interval. In a cross-sectional study, exposure and outcomes are ascertained simultaneously. It has level III evidence as it is placed lower down the ranking at the hierarchy of evidence; however, it can provide very useful data. Advantages of cross-sectional studies including the relatively low cost and less time-consuming design. It is often described as taking a snapshot of data with multiple variables happening at a specific time however, the timing of the snapshot is not guaranteed to be representative. So, data may not be premeditated to answer a specific question. The study timeline was designed to be 6 months which was enough to collect more than the calculated sample number, 96 women. However, prolonged labour is a delivery outcome and it is statistically impossible to control the outcomes encountered in your sample to meet your desired sample size.

A cross-sectional study has a low internal validity; which measures the degree to which the results are correct for the specific study sample included. Internal validity is affected by random errors (chance) and systematic errors (bias). Having a low sample size can raise the possibility of random errors as a result of chance. Significance testing is used to avoid random errors in statistics by calculating P value. Nevertheless, low P value, does not always indicate that the significant difference is clinically significant or of clinical advantage (Brahman, 1991).

Cross sectional observational studies usually susceptible to three types of systematic errors: selection, information (observational) and confounding bias. Reducing systematic errors strengthen the validity of the study. Selection bias is when the selected groups are different in variables other than the primarily

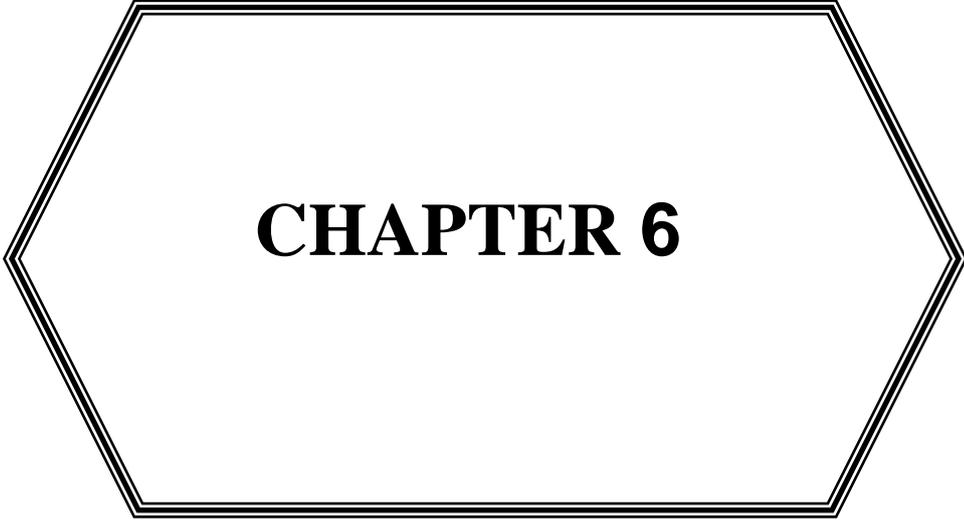
studied outcome and these variables affect the measured outcome. It usually occurs in the design phase of the study. To reduce the risk of selection bias, a clear definition of the study population should be identified in the study protocol which was done by determining clear and relative inclusion and exclusion criteria. The study sample was also restricted to women who agreed to participate in the study and might not represent the total population. Nevertheless, the non-response rate in my sample was very low which minimizes the selection bias.

Information (observational) bias happens when the investigator inaccurately measures, records or misclassifies the collected data. It usually occurs at the data collection stage of the study. Interviewer and questionnaire subjective bias was avoided by designing a clear and well-defined participant datasheet. The data taken from the medical record files were prospectively documented which restricts the recall bias risk. Information bias can also occur due to the study participant's misreporting. For patients who did not have their weight at the first trimester of pregnancy recorded in their files, self-reported weight was used. Although many studies have found that self-reported weight and BMI is reliable (Seijo et al., 2018, Natamba et al., 2016), it may be a possible source of bias.

Confounding bias can be encountered if the investigator fails to adjust for known factors associated with the exposure of outcome and is also associated with the development of the outcome of interest independently of exposure. The confounder, in this case, is not part of the causal pathway; i.e. an intermediate variable, therefore adjusting for intermediate variables which are not confounders might cause a bias instead of eliminating it. The confounding variable can affect the association between the exposure and the outcome of interest which ultimately, can misestimate the true effect. Only true well proven known confounders should be controlled for in statistical analyses. Further adjustments of confounders in my sample should be considered; however, that was difficult to achieve due to the small sample size. If I was successful finding a relationship between prolonged labour and leptin and visfatin levels in obese women, confounding factors including maternal age, parity, induction of labour and neonatal birth weight should be taken into consideration as they were found to affect the length of labour. Hence, nulliparous women and women who deliver

spontaneously have longer labour length than multiparous women and women who have induced labour (Zhang et al., 2010a, Ostborg et al., 2017).

A cross sectional study has a high external validity; which is defined as the degree to which the results can be applied to other individuals and settings outside the context of the study. The results can be generalised to the Saudi population or other similar populations and settings but not to different populations without eliminating the ethnic difference. The prevalence of maternal obesity is also different between populations, therefore, generalisation of this study results to other different populations is inappropriate.



# **CHAPTER 6**

# **Chapter 6: Investigating the expression of gap junction proteins, Cx46 and Cx26, in the obese human myometrium**

## **6.1 Introduction**

Connexions (Cx), or gap junction proteins are essential cellular structures involved in the regulation and coordination of multiple physiological processes (Mese et al., 2007). They are highly specialised regions of the cell plasma membrane that contain collections of transmembrane proteins functioning as channels to link the cytoplasmic compartments of neighbouring cells, ensuring direct cell to cell communication (Kumar and Gilula, 1996, Goodenough and Paul, 2009, Yeager and Nicholson, 1996). These proteins provide sites of low electrical resistance that allow the intercellular exchange of small metabolites, nutrients, second messengers and ions (Loewenstein, 1981). Structurally, gap junctions are composed of a hexameric assembly of four transmembrane domains and two extracellular loops, and details of which are provided in Chapter 1 (Bruzzone et al., 1996).

The role of gap junctions in the process of parturition is well known. They facilitate the regulation and coordination of myometrial contractile machinery during labour (Ou et al., 1997). There is a rapid increase in the number of functional gap junctions at the onset of labour, which allows the synchronised contractility of the myometrium (Garfield et al., 1977, Garfield et al., 1979, Garfield et al., 1988). The gap junction total proteins amount increases 5-fold prior to the initiation of labour (Winterhager et al., 1991). In addition, gap junction permeability is altered during pregnancy and parturition (Cole and Garfield, 1986). Increasing the function of gap junction seems to be a necessary, but inadequate, factor in initiating normal labour in human (Young and Hession, 1999). A notable characteristic of gap junctions is that they have a relatively short half-life, which reflects their physiologically dynamic nature; gap junction coupling alternately upregulates and downregulates during the delicate physiological process of parturition (Laird, 2006). There are multiple gap junction proteins

which were found to be expressed in the myometrium including, Cx26, Cx40, Cx43, and Cx45 (Winterhager and Kidder, 2015). Sex steroid hormones play a significant regulatory role in the formation of uterine gap junction proteins (Garfield et al., 1980). Steroid hormones may operate via modulation of prostaglandin synthesis or may directly affect gap junction proteins synthesis in uterus (Garfield et al., 1977).

Cx43, also known as Gap Junction  $\alpha$ 1 (GJ $\alpha$ 1), is the major myometrial gap junction protein that permits synchronised myometrial contractions by facilitating intracellular propagation of electrical signals (Chow and Lye, 1994, Orsino et al., 1996, Willecke et al., 2002). This protein is expressed in low levels in non-pregnant rat uterus, and its levels increase dramatically during labour in both human and rat (Chow and Lye, 1994, Orsino et al., 1996, Ou et al., 1997). Risek *et al.* detected the presence of Cx43 in both rat circular (more prominent) and longitudinal smooth muscle layer of myometrium and decidual endometrium (stromal cells). The authors also found that the abundance of its transcript in term rat is 5.5 times higher than non-pregnant myometrium (Risek et al., 1990). The relative abundance of Cx43 mRNA is shown to increase by ~ 30% at term; in contrast, it remains unchanged in postpartum rat myometrium (Lang et al., 1991). Cx43 is also detected in ovaries from pregnant and non-pregnant rats, where its levels were found to increase during pregnancy (Risek et al., 1990). Loss of this protein was found to disrupt uterine contractility leading to prolonged labour in mouse, which is one of the key complications of obesity in women (Doring et al., 2006). Moreover, it was found that the mRNA expression and protein immunoreactivity of Cx43 in women experiencing prolonged labour were lower than in women undergoing normal length labour (Cluff et al., 2006). This may suggest a role for this protein in the physiological co-ordination of myometrial contractility.

It was suggested that Cx43 biosynthesis or its post-translational phosphorylation is altered in prolonged labour (Burden et al., 1999). Obese high fat high cholesterol (HFHC) fed pregnant rats showed a reduction in the myometrial expression of Cx43 at term pregnancy and during labour (Muir et al., 2016, Elmes et al., 2011). It has also been found that myometrial stretch is required for the maximum expression of Cx43 during labour in rats (Ou et al., 1997). Both progesterone and human chorionic gonadotropin (hCG) hormones

were found to suppress Cx43 myometrial expression, a mechanism crucial for the maintenance of myometrial quiescence and the prevention of preterm labour (G and Winterhager, 2015, Ambrus and Rao, 1994). Conversely, Cx43 myometrial levels are upregulated by high levels of oestrogen hormone production (Lye et al., 1993). In addition, Cx43 myometrial expression during labour is influenced by pro-inflammatory prostaglandins, mainly PGF<sub>2</sub> $\alpha$  (Cook et al., 2000, Xu et al., 2013) and PGI<sub>2</sub>, (Fetalvero et al., 2008). Pregnant rats were found to have lower levels of myometrial Cx43 and require more labour contractions for successful delivery after space flight (Burden et al., 1999).

Cx26 is the most abundant gap junction protein in the endometrium, particularly in the endometrial epithelium, luminal and glandular epithelium. Its levels in the myometrium, however, are low (Risek et al., 1990, Risek et al., 1995). Cx26 plays an essential role in embryo implantation, placenta formation, early foetal development and menstrual cycle secretory phase (Jahn et al., 1995, Winterhager et al., 1988, Winterhager et al., 1993). Unlike Cx43, Cx26 myometrial expression is high during late pregnancy and then falls during labour in rat (Orsino et al., 1996). Cx26 uterine expression was also found to be regulated hormonally by oestrogen and progesterone (Grummer et al., 1994, Risek et al., 1990). Its expression, however, is not altered by stretch (Ou et al., 1997) or space flight (Burden et al., 1999). Thus, the contribution of Cx26 to the regulation of myometrial contractility is unclear. Also, there is no evidence suggesting that Cx26 myometrial expression changes with obesity.

Although current studies suggested that myometrial contractility of obese women is altered by cholesterol and adipokines disruption, no research to date has examined the effect of obesity on gap junction proteins expression in the human myometrium. Therefore, this chapter aimed to study the effect of obesity on the expression of key markers of myometrial contractility during pregnancy; Cx43 and Cx26. This might provide a potential mechanism underlying poor uterine contractility, which is commonly experienced by obese pregnant women.

## **6.2 Methods**

### **6.2.1 Tissue**

Human myometrial tissues were obtained from normal weight (BMI 18.50

- 24.99 kg/m<sup>2</sup>), overweight (BMI: 25.00 - 29.99kg/m<sup>2</sup>), obese (BMI 30.00-39.99 kg/m<sup>2</sup>) and morbidly obese women (BMI ≥40.00 kg/m<sup>2</sup>) during elective C-section deliveries. Biopsies of human myometrial tissue were obtained from a total of 39 healthy term pregnant women, between 38 and 40 weeks of gestation, with a maternal BMI between 16 and 48. The reasons for C-section deliveries were previous C-section, failed induction of labour, breach presentation and previous traumatic vaginal delivery. The mean maternal age at delivery was 31.8 ± 0.7 years. The average parity value of the women at the time of delivery was 1-2 (range 0-3). C-section delivery were carried out under regional anaesthesia. The maternal BMI measurements were taken from the patient's files in the first trimester. Immunohistochemical (IHC) analysis was performed in a blinded manner, whereby all participant's BMI values were only revealed after completion of experiments and data analysis, to reduce any bias. Details of how the tissue was collected, prepared, fixed, processed, embedded and sectioned are described in the general material and methods section in Chapter 2.

### **6.2.2 IHC procedure**

The expression of two gap junction proteins, Cx43 and Cx26, was compared in myometrial samples from obese and non-obese women. Sections (5µm thickness) were cut in the Liverpool Women's Hospital physiology lab and baked before IHC experiments. Before starting the IHC procedure, dewaxing and rehydration of the samples were required to prepare the myometrial tissues for the water-based solutions. The detailed IHC protocol is described in Chapter 2, Section 2.2.

Briefly, human myometrial sections were placed into boiling sodium citrate buffer for antigen retrieval and 3% hydrogen peroxide (Sigma Aldrich) was used to block endogenous peroxidase activity, before overnight incubation with rat Cx43 (1:250, Thermo Scientific) and mouse Cx26 antibodies (1:100, Thermo Scientific). The primary antibody was chosen according to the species reactivity to human tissue and the suitability for IHC test. Then, sections were washed in TBS and incubated for 30 minutes with the secondary antibodies: peroxidase-conjugated horse serum for anti-rabbit IgG (Vector Labs) and goat serum for anti-mouse IgG (Vector Labs). The primary antibody concentrations used in this study were determined following optimisation experiments (described in the following

Results section). DAB chromogen (Sigma Aldrich) was added to develop the required stain (brown stain). Stained samples were dehydrated, mounted onto microscope slides, then visualised and photographed under a light microscope.

One section from normal weight term pregnant myometrium was incubated overnight in the blocking solution without adding the primary antibody, to be used as a negative control. Tissues (animal and human) known to express the protein of interest was used as an external positive control. Three positive controls were used in total: the heart (rat and human) for Cx43 and the liver (human) for Cx26 antibodies. One sample was repeatedly tested in each experimental set with the same antibody to obtain an internal positive control. The positive control tissues were taken from the university's human tissue bank.

### **6.2.3 Scoring technique and statistical analysis**

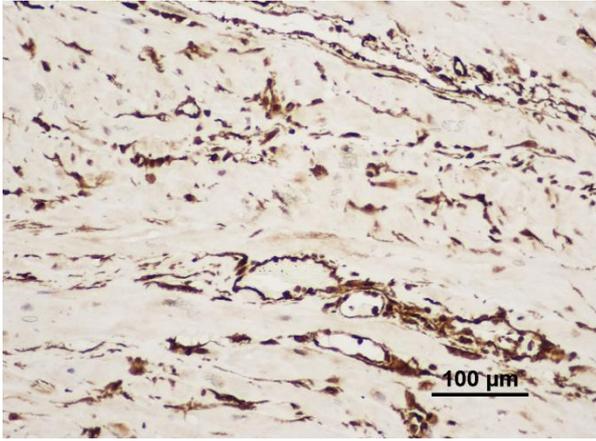
Samples were analysed by high content screening for rapid quantitation and comparison of data from multiple samples (scoring). The 20X objective was used to capture 10 different images per section, following which the average staining score was calculated. This was performed for all sections in each IHC experiment. Both stain intensity (weak, intermediate, strong) and percentage of positive cells (0-25%, 26-50%, 51-75% and >75%) were manually scored. The intensity was determined subjectively by comparing the slides to determine the lowest (mild) and the highest (strong) intensity. The sections were scored by multiplying the intensity (I) by the percentage of positive cells (P) using the formula  $Q = I \times P$ . The scoring was performed by viewing the sections with a Nikon Eclipse 50i microscope (Nikon Corporation, Tokyo 100-8331, Japan) under the 20X power objective. For statistical analysis, comparison of myometrial expression of Cx43 and Cx26 was performed using the average score  $\pm$  se, followed by one-way analysis of variance (ANOVA). P values < 0.05 indicated a statistically significant difference. Each n indicated a human myometrial sample.

## **6.3 Results**

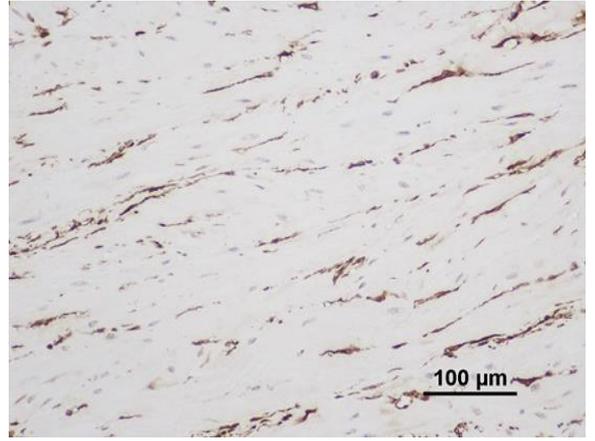
### **6.3.1 Cx43 antibody optimisation**

Primary antibody optimisation experiments were performed to define the minimum antibody concentration required to generate optimal IHC results. The

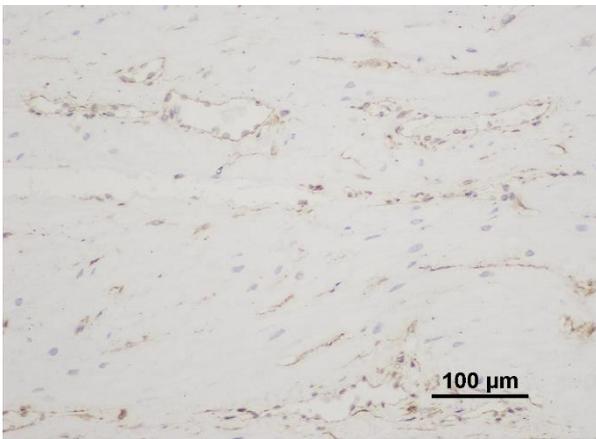
following antibody concentrations were used for rat Cx43 monoclonal antibody titration, 1:50, 1:100, 1:250 and 1:500 (**Figure 6.1**). These concentrations were chosen after identifying previous studies using these antibodies on myometrial tissue. Section 6.1A was stained with the highest antibody concentration used (1:50), section 6.1B was stained at a 1:100 concentration, section 6.1C at 1:250, section 6.1D at 1:500 and section 6.1E was not labelled with primary antibody (negative control). Section 6.1F is rat heart and was stained at a 1:250 concentration (positive control), which was optimal.



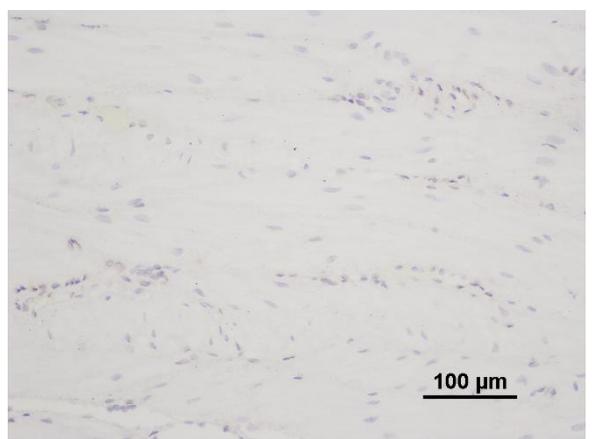
A. Human myometrium (1:50)



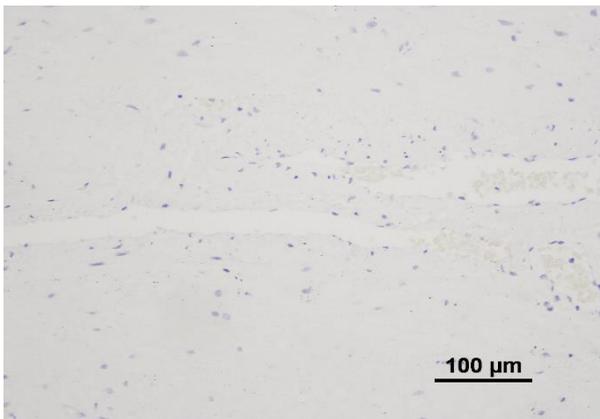
B. Human myometrium (1:100)



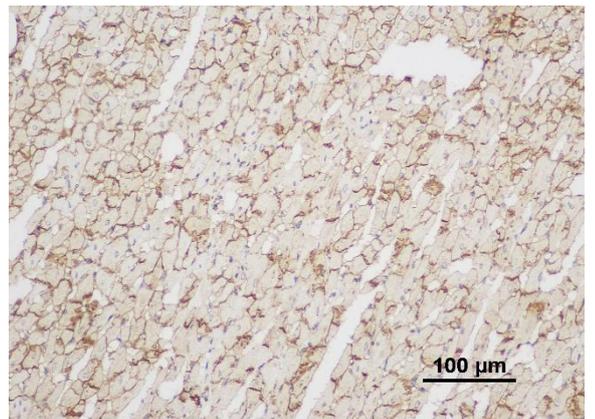
C. Human myometrium (1:250)



D. Human myometrium (1:500)



E. Human myometrium (No primary antibody)

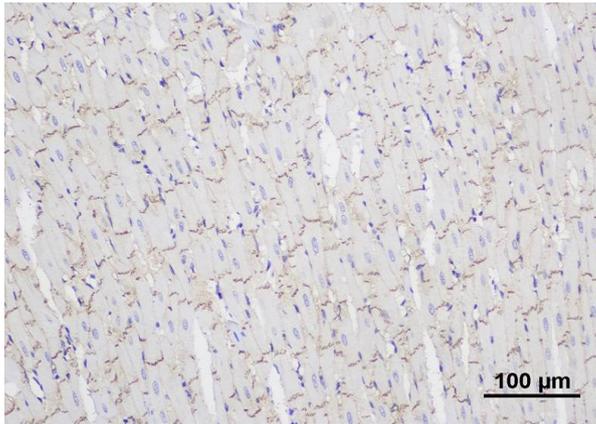


F. Rat heart (1:250)

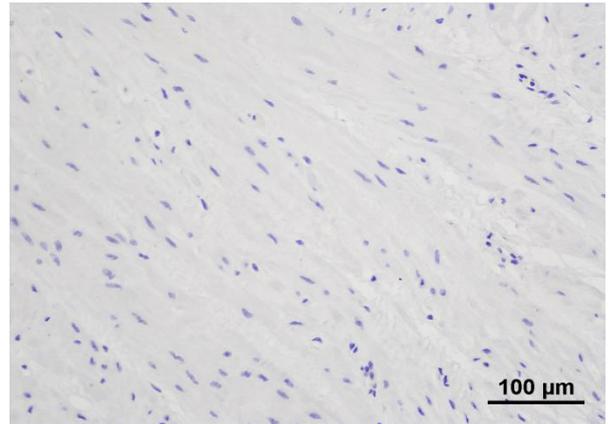
**Figure 6.1 Optimisation of the rat anti-Cx43 primary antibody concentration for IHC of human myometrial sections (5μM thickness, 20X).**

### **6.3.2 Comparing the myometrial expression of Cx43 in normal weight, overweight and obese pregnant women:**

Light microscopic examination of DAB-stained myometrial sections obtained from 38 women of different body weight was carried out. The expression of Cx43 was examined by DAB staining, which is equivalent to Cx43 expression. Positive and negative controls for Cx43 antibody staining are shown in **Figure 6.2**. The human heart, which is known to express the Cx43 epitope, was used as a positive control, and showed clear DAB staining between the intercalated discs (**Figure 6.2A**). The negative control is a myometrial section with no primary antibody used. This section did not show any staining, which indicates that DAB staining is specific to the Cx43 antibody and absence of non-specific staining (**Figure 6.2B**). Representative images of the myometrium from normal weight ( $5.6 \pm 0.61$ ,  $n=10$ ), overweight ( $6.6 \pm 0.6$ ,  $n=10$ ), obese ( $6.6 \pm 0.45$ ,  $n=10$ ) and morbidly obese ( $4.7 \pm 0.57$ ,  $n=9$ ) pregnant women are shown in **Figure 6.3 A-D**. Staining was found in the myometrium of all samples; however, Cx43 myometrial expression was not significantly different between normal weight, overweight and obese women ( $p>0.05$ ).

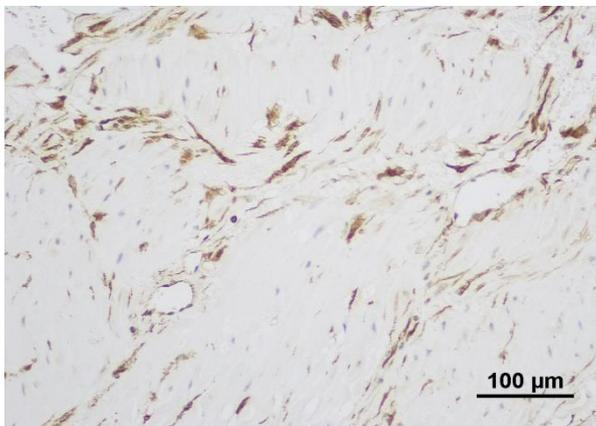


A. Human heart (1:250)

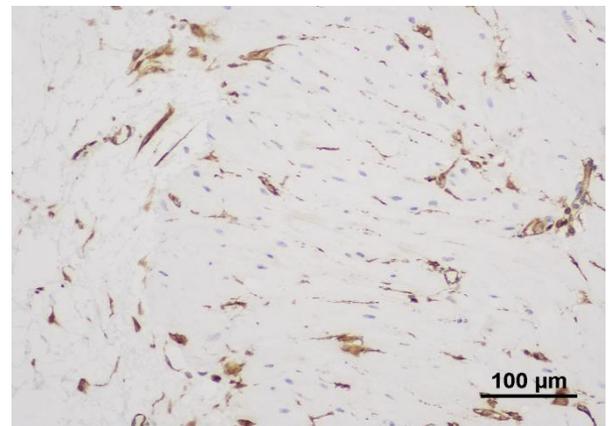


B. Human myometrium  
(no primary antibody)

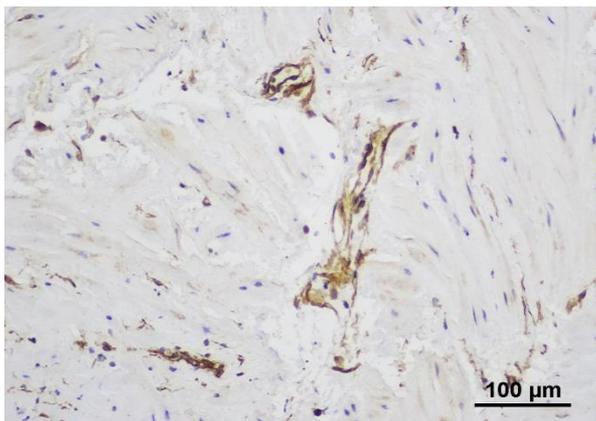
**Figure 6.2 CX-43 antibody positive and negative controls (5μM thickness, 20X).**



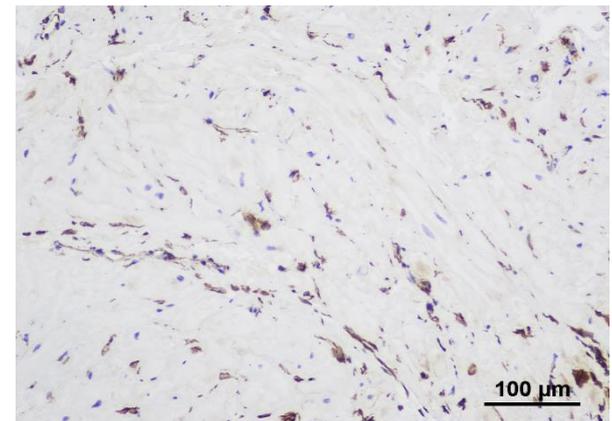
A. Normal weight



B. Overweight



C. Obese



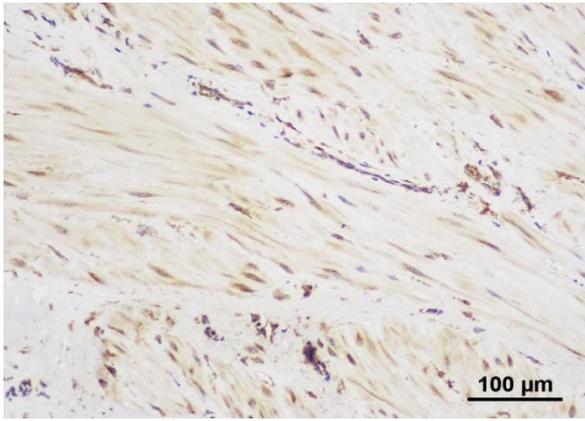
D. Morbidly obese

**Figure 6.3 Expression of Cx43 in the human myometrium (5μM thickness-20X).**

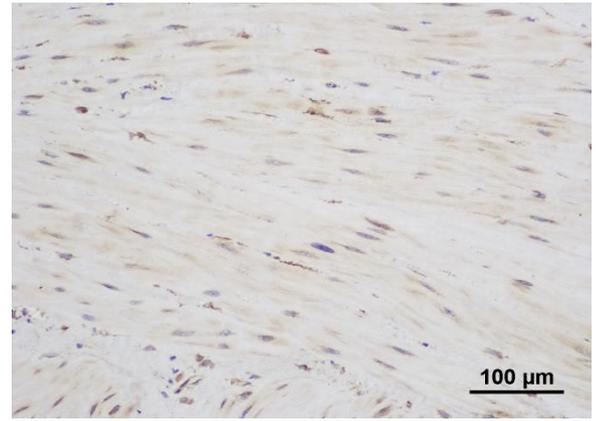
Expression of Cx43 is shown using DAB staining in myometrial tissue from A) normal weight, B) overweight, C) obese and D) morbidly obese women.

### 6.3.3 Cx26 Antibody optimisation

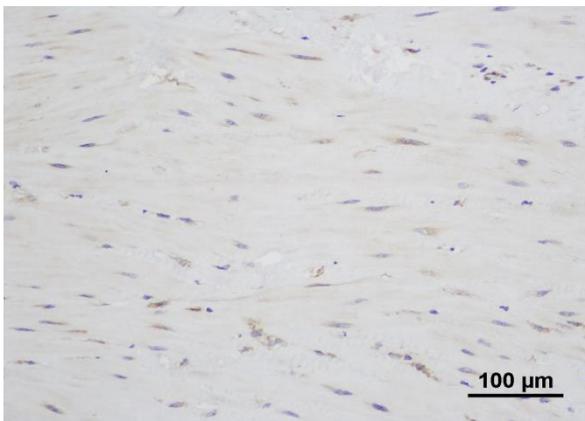
The following antibody concentrations were used for the mouse Cx26 monoclonal antibody titration experiment: 1:50, 1:100, 1:250, 1:500 and 1:1000 (**Figure 6.4** A-F). Section 6.4A was stained with the highest antibody concentration used (1:50), section 6.4B was stained with 1:100 of antibody concentration, section 6.4C was stained with 1:250 of antibody concentration, section 6.4D was stained with 1:500 of antibody concentration, section 6.4E was stained with no primary antibody (negative control), and section 6.4F is rat liver, which was stained with 1:100 of antibody concentration (positive control). The antibody concentration of 1:100 was found to be optimal for staining.



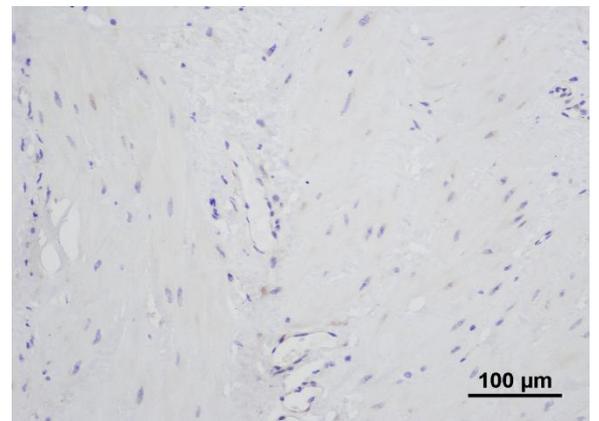
A. Human myometrium (1:50)



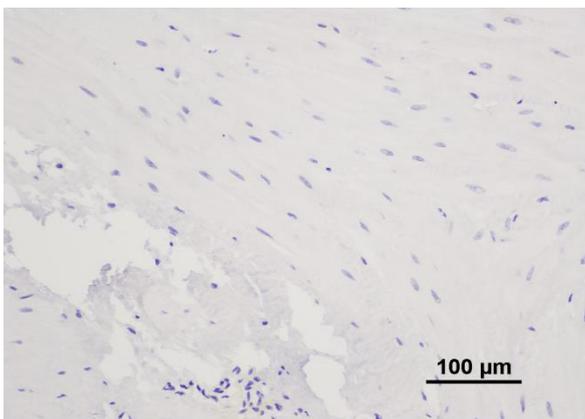
B. Human myometrium (1:100)



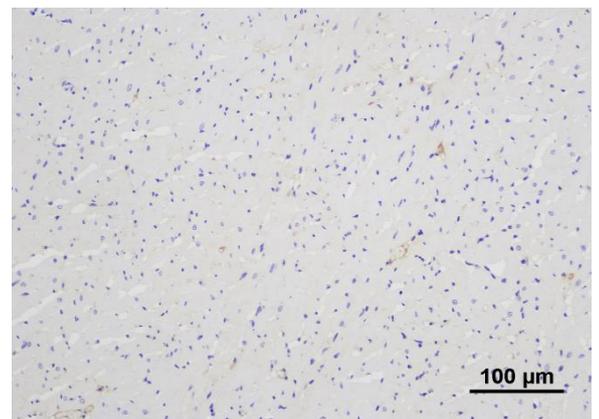
C. Human myometrium (1:250)



D. Human myometrium (1:500)



E. Human myometrium (No primary antibody)

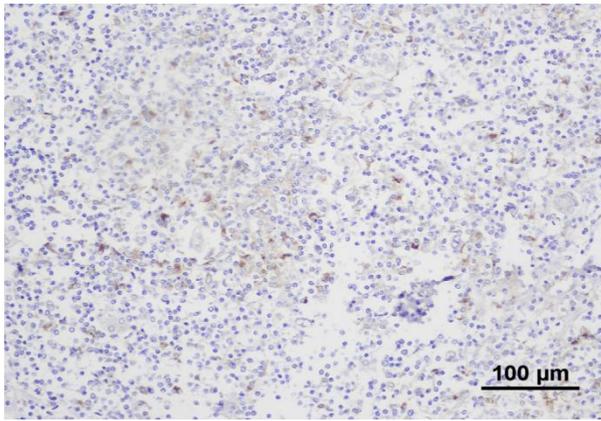


F. Rat Liver (1:100)

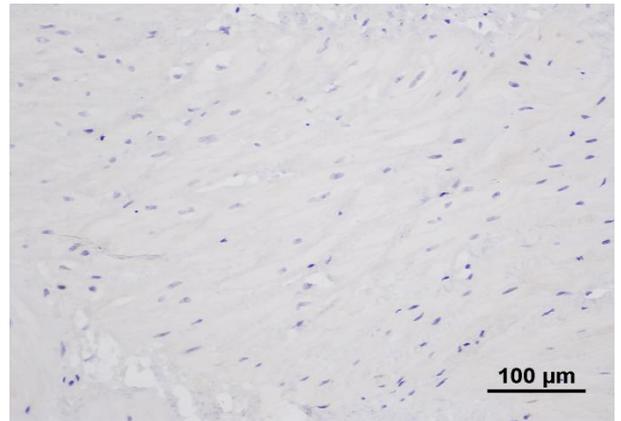
**Figure 6.4 Optimisation of the rat anti-Cx26 primary antibody concentration for IHC of human myometrial sections (5μM thickness, 20X).**

### 6.3.4 Comparing the myometrial expression of Cx26 in normal weight, overweight and obese pregnant women

As carried out above for Cx43 myometrial expression, detection of DAB staining by light microscope was used to determine the expression of Cx26 in the myometrial tissue of normal weight, overweight and obese women. Positive and negative controls for Cx26 antibody staining are shown in **Figure 6.5**. The human liver was used as a positive control, showing clear DAB staining of hepatocytes (**Figure 6.5A**). The negative control is a myometrial section that was not labelled with primary antibody, showing no staining (**Figure 6.5B**). Representative images of the myometrium from normal weight ( $3.1 \pm 1$ ,  $n=10$ ), overweight ( $3.8 \pm 0.9$ ,  $n=10$ ), obese ( $3.3 \pm 0.8$ ,  $n=10$ ) and morbidly obese ( $2.1 \pm 1.7$ ,  $n=7$ ) pregnant women are shown in **Figure 6.6** (A-C). It was found that Cx26 human myometrial expression is not significantly different between normal weight, overweight and obese pregnant women ( $p>0.05$ ).

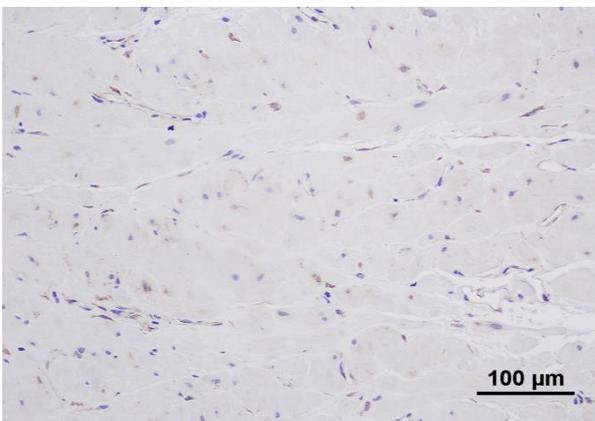


A. Human Liver (1:250)

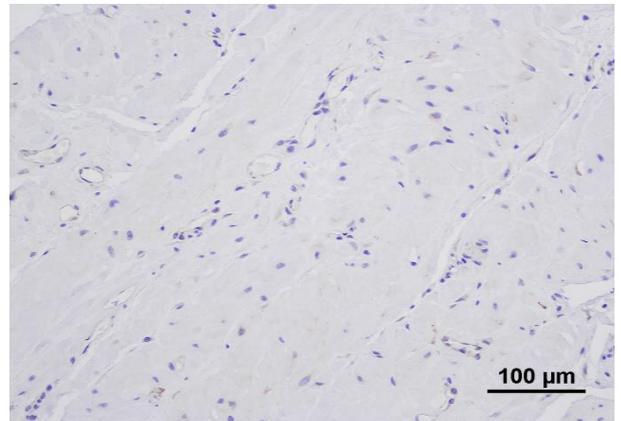


B. Human myometrium (no primary antibody)

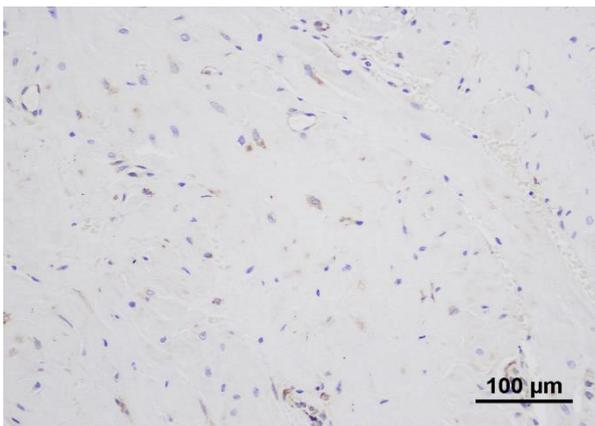
**Figure 6.5 CX-26 antibody positive and negative controls (5µM thickness-20X).**



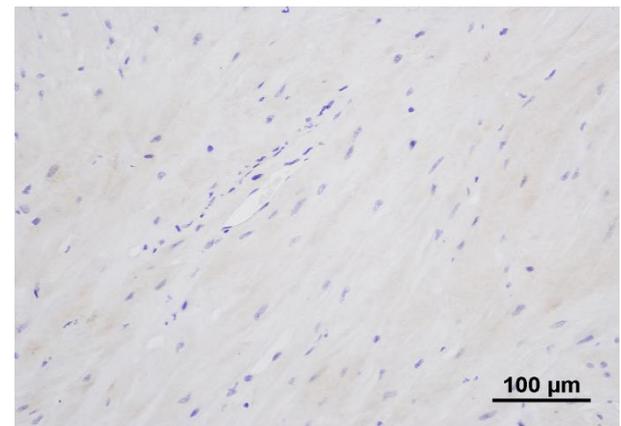
A. Normal weight



B. Overweight



C. Obese



C. Morbidly obese

**Figure 6.6 Expression of Cx26 in the human myometrium (5µM thickness-20X).**

Expression of Cx26 is shown using DAB staining in myometrial tissue from A) normal weight, B) overweight, C) and obese D) morbidly obese women.

## 6.4 Discussion

Despite considerable research into the pathophysiological factors underlying the impact of obesity on pregnancy and labour, no direct mechanisms have been identified. This is at least in part due to our limited understanding of the biochemical and hormonal effects of obesity on the myometrium. Understanding the complex effects of adipose tissues on myometrial contractility is of increasing scientific interest, as it may lead to the prevention of pregnancy and delivery-related complications in obese women. Poor myometrial contractility can lead to multiple delivery-related complications in obese women, which can ultimately cause significant increase in the risk of a C-section deliveries (Zhang et al., 2007a). The aim of this study was to investigate whether obesity would have an effect on the myometrial expression of gap junction proteins, Cx43 and Cx26, thereby suggesting a potential mechanism underpinning the poor myometrial contractility observed in obese pregnant women (Crane et al., 1997, Cedergren, 2004, Weiss et al., 2004, Zhang et al., 2007a). The expression of Cx43 and Cx26 in pregnant human myometrial sections, obtained during C-section delivery, was examined using light microscopy and quantified. The data in this chapter was obtained from the myometrial tissue of pregnant women of different BMIs.

Contrary to the hypothesis of this study, the results have shown that the myometrial expression of gap junction proteins, Cx43 and Cx26, is not altered by obesity in pregnant women. The hypothesis was based on the previously reported effects of a chronic HFHC diet on the expression of Cx43 and Cx26 in pregnant rat myometrium, in comparison to animals eating regular chow (Smith et al., 2005). However, the causes of obesity in the women whose tissues were used in the current study are unknown, and may be high fat-diet, lack of physical activity, genetic predisposition, metabolic parameters, behavioural differences, as well as medical or hormonal causes (Wright and Aronne, 2012). These diverse factors may exert interrelated effects on myometrial gap junction function and formation. Interestingly, another maternal factor which was also shown to decrease myometrial contractility and to be related to many pregnancy adverse outcomes (Luke and Brown, 2007), advanced maternal age, was found to have no effect on myometrial Cx43 expression (Elmes et al., 2015).

Elmes et al. found that a HFHC diet significantly decreases rat myometrial expression of the contractile proteins, CAV-1 and Cx43, during labour (Elmes et al., 2011). The same group also found that the HFHC diet decreased uterine expression of Cx43 and CAV-1, in the myometrium of both term non-labouring and term labouring rats (Muir et al., 2016). Moreover, the authors revealed, using organ bath experiments that the rats fed with the HFHC diet displayed poorly coordinated contractions in comparison to control rats. This effect may be explained by the high cholesterol content of the diet or larger fat mass. Increased myometrial content of cholesterol was found to decrease both human and rat myometrial contractility (Smith et al., 2005, Jie et al., 2007) and, mechanistically, this may be due to reduced expression of gap junction proteins in rats fed with the HFHC diet. The plasma cholesterol levels were not provided in the patient files for this study. However, a significant increase in plasma cholesterol levels is commonly observed in obese women (Klop et al., 2013). Previous studies investigating the effects of obesity on myometrial gap junction protein expression were performed in rat models, which are likely to express tissue specific characteristics that are different from human (Muir et al., 2016, Elmes et al., 2011). These *in vitro* studies suggested that obesity may alter the expression of contractile proteins in the myometrium of pregnant rats.

Further studies could next determine the expression of other myometrial gap junction proteins, Cx40 and Cx45, in obese pregnant women and compare these with normal weight pregnant women. These gap junction proteins are found to be typically co-expressed with Cx43 in the human myometrium (Kilarski et al., 1998). Furthermore, it could be beneficial to measure the myometrial prostaglandins expression in obese women, as well as to compare the expression of Cx43 and Cx26 in the endometrium of normal weight, overweight and obese pregnant women. This would give insight into the differential roles of the gap junction proteins on the function of the endometrium versus the myometrium.

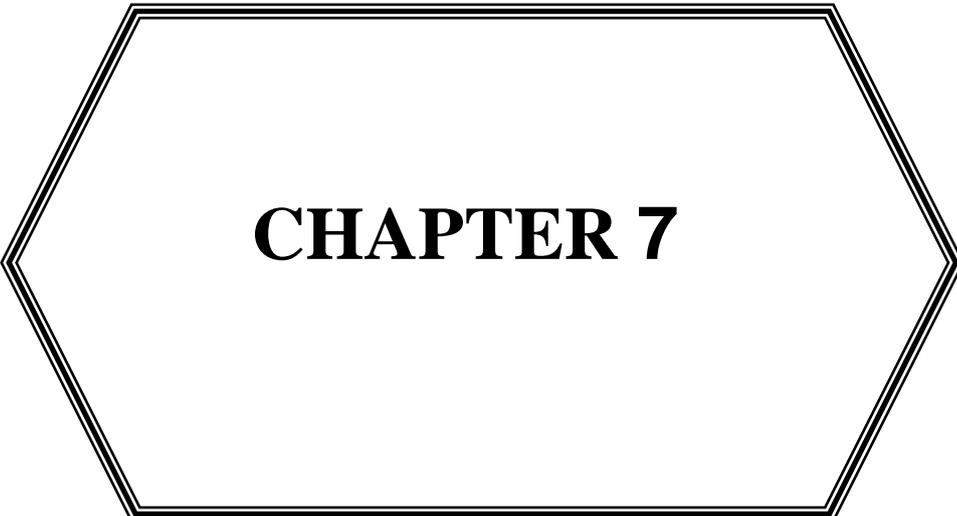
## **6.5 Conclusion**

The results of this chapter have excluded one of the mechanisms proposed to underlie poor myometrial contractility in obese pregnant women. In this chapter, the myometrial expression of Cx43 and Cx26 in normal weight, overweight, obese and morbidly obese women were compared. This involved

immunohistochemical quantification of myometrial gap junction proteins, Cx43 and Cx26, in obese pregnant women and comparison of IHC scores with those in overweight pregnant women. This is believed to be the first study to determine the expression of myometrial gap junction proteins Cx43 and Cx26 in obese women. The reasons behind the poor contractility in obese women may, therefore, might involve alterations of other myometrial gap junction proteins that have not been examined in this chapter. It is possible that changes in the expression of gap junction proteins may not be of pathological consequence to uterine contractility, which is shown to be poor in obese pregnant women.

## **6.6 Limitations of the study**

Immunohistochemical studies are limited in that they provide only a semi-quantitative approach to determining protein expression. Western blotting and real-time PCR are more accurate quantitative methods. In addition to its quantitative properties, IHC was used here to visualise the cellular localisation of connexions in the plasma membrane of the uterine myocytes and to exclude Cx43 and Cx26 expression in other cellular structures (e.g. blood vessels). Notably, oxytocin was given to some women, whose tissues were used for this study, prior to delivery. Thus, an interaction of oxytocin with Cx43 cannot be excluded. This study was performed on a relatively small number of samples; larger studies are needed to confirm or refute these findings. The samples used here were already available at the time of the current study; however, increasing the sample number should be a primary aim of future work.

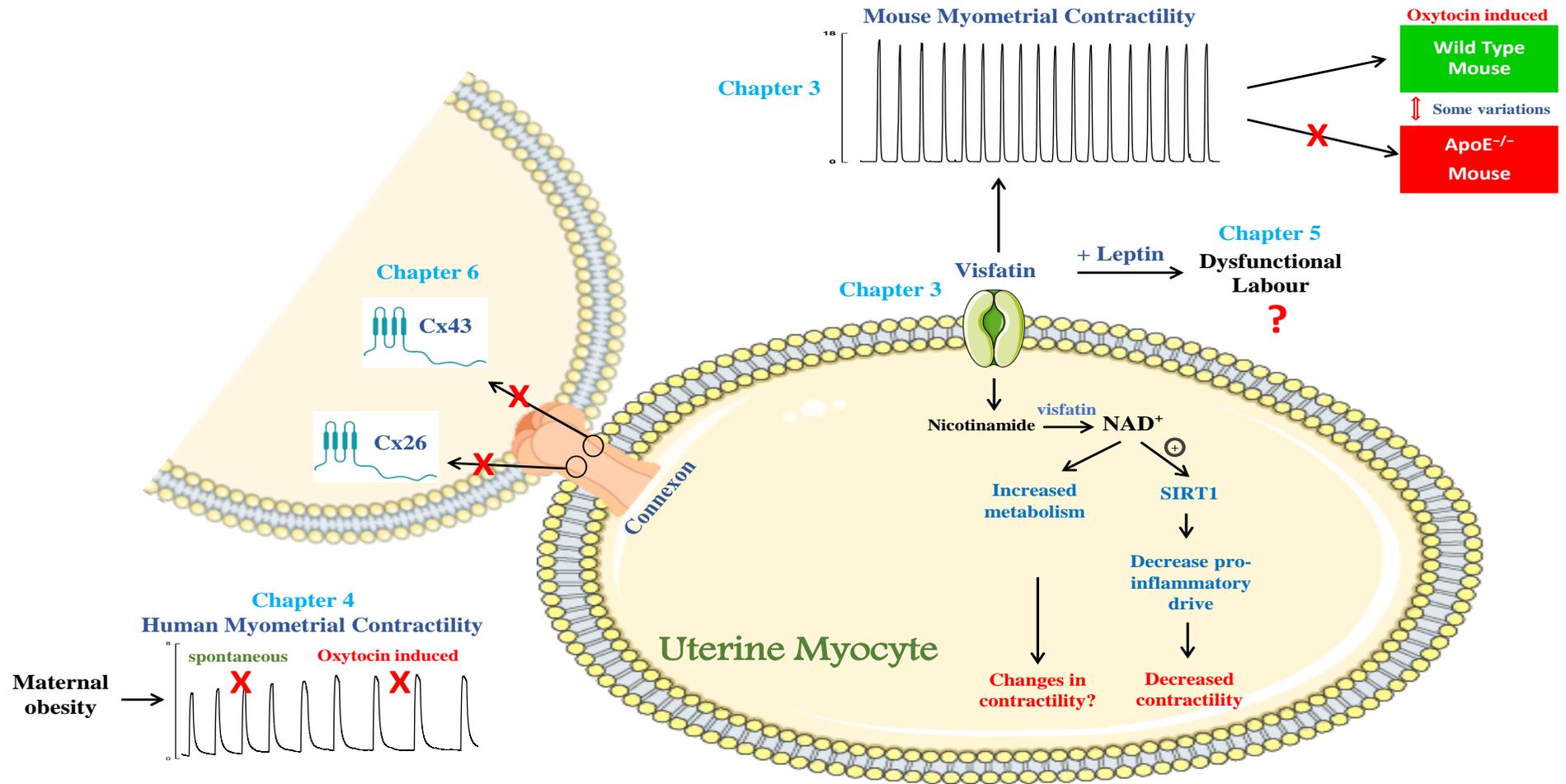


# **CHAPTER 7**

# Chapter 7: Discussion

## 7.1 Overview

This chapter provides a discussion of this thesis work as a whole to synthesise findings and suggest directions for future research. The aims of the work described within this thesis were to investigate some theories which might be contributed to poor myometrial contractility associated with maternal obesity (Figure 7.1). This was accomplished firstly by studying *in vitro* myometrial contractility in wild type mouse and comparing it to a genetically modified mouse model producing chronic hypercholesterolemia, ApoE<sup>-/-</sup> mouse. It was found that visfatin had an inhibitory effect on oxytocin-induced term pregnant WT mouse myometrium. Having shown effects, its mechanism of action on myometrium was investigated. This led me to undertake a clinical study, where an attempt was made to establish a link between visfatin and dysfunctional labour in obese women. In another approach to understanding how obesity decreases contractility, an attempt was made to find evidence supporting alterations in gap junction proteins with maternal obesity. This was done by studying the myometrial expression of two gap junction proteins, Cx43 and Cx26, in normal weight, overweight, obese and morbidly obese women. The ultimate aim was (i) to give a better understanding of the factors and mechanisms contributing to poor uterine contractility commonly observed in obese pregnant women, (ii) to aid clinicians in identifying women who are at greater risk of dysfunctional labour and (iii) to identify new targets to prevent obesity-related complications during labour. I consider that I have successfully addressed and achieved many of my specific aims and the work presented in this thesis has shed light on both the effect of dyslipidaemia and obesity on myometrial contractility, as well as identifying mechanisms which appear to be less important e.g. myometrial gap junction proteins expression. **Figure 7 .1** brings my findings together.



**Figure 7.1 Summary of the proposed mechanisms examined in this thesis which might be contributed to poor myometrial contractility associated with maternal obesity.** Nampt, nicotinamide phosphoribosyltransferase (visfatin); NAD<sup>+</sup>, nicotinamide adenine dinucleotide; SIRT1, silent information regulator 2-related protein. **X** refers to no effect was found.

## **7.2 Maternal obesity, dyslipidaemia and myometrial contractility**

My functional studies in mouse showed that no difference in the myometrial contractility between WT type and ApoE<sup>-/-</sup> mouse. Also, no association was shown between maternal obesity and the time to commencement of spontaneous contractions, spontaneous nor oxytocin-induced contractions in human myometrium. This might be due to the anatomical and the physiological variations in the myometrium between the two species which were discussed.

Most of our knowledge of myometrial contractility comes from in-depth studies of animal myometrium, especially rats (Wray et al., 2001). Although human tissues are a more physiologically relevant model for studying uterine contraction in human diseases, the study of animal myometrial tissue has recognisable advantages. These include both the more detailed background knowledge and the availability of the tissue at certain ages or gestation. Human tissue is usually obtained only at the time of elective C-section delivery. One noticeable difference in the *in vitro* recordings from mouse and human myometrium is the difference in frequency. Just as *in vivo*, the rate is much faster in mice than women. Despite this, the basis of contraction is the same and both are worthwhile models. For both species, there is always the question about what is the best number of samples to study, and experiments on a larger number of samples to minimize variation in some datasets, would have been helpful.

## **7.3 The effect of visfatin on mouse myometrial contractility**

Visfatin had an inhibitory effect on non-pregnant and pregnant WT mouse, and this is in line with its effects on pregnant rat and human myometrium (Mumtaz et al., 2015). My study also found that visfatin has no effect on ApoE<sup>-/-</sup> mouse myometrium. It is interesting to note the effect of visfatin was only exhibited if the myometrium was pregnant and augmented with oxytocin. However, visfatin had a relaxant effect on both spontaneous and oxytocin-induced contractility in rat and human myometrium. My data suggests that visfatin in favour to work during the physiological conditions occurring during pregnancy i.e. under oxytocin augmentation. This might suggest that visfatin requires the myometrium to be maximally stimulated in order to produce its effects. Notably, the ApoE<sup>-/-</sup> mouse

myometrium, non-pregnant and pregnant, was shown to not respond to visfatin application and this may be attributed to the physiological adaptation to high plasma lipids and visfatin levels, along with increased lipid content in mitochondria in the chronic hyperlipidaemic environment to maintain cellular lipid homeostasis (Gam et al., 2017). This explanation might also be applied to maintain a pro-contractile phenotype at delivery for obese women who were studied in Chapter 4 and showed no differences in myometrial contractility compared to normal weight women (Higgins et al., 2010). The data from pregnant human myometrium showed that visfatin had a cumulative effect on contractility - an interesting finding that was not shown in mouse myometrium. It appears that some of these adverse effects in myometrial contractility and their consequences can be partly contributed to hormonal imbalance encountered in obese women. This comprises not only adipokines, but also the interactions between obesity and the oxytocin system “oxytocin- oxytocin receptor interactions”.

While it seems perhaps premature to assume the type of visfatin receptors in myometrium, a front runner would appear to be insulin receptors, based on cultured cells experiments. Most of the studied adipokines (leptin, ghrelin and apelin) have shown to decrease myometrial contractility, which is in agreement with our data in visfatin. Furthermore, the plasma levels of these adipokines were found to increase with obesity (Leal and Mafra, 2013). This suggests that it might be a metabolic modulatory effects on myometrium by adipokines in obese pregnant women which may directly or indirectly contribute to dysfunctional labour.

In order to elucidate the cellular mechanism underlying the inhibitory effect of visfatin on myometrial contractility, the NAD<sup>+</sup> pathway was investigated. My data demonstrates a novel finding by which visfatin exerts its effects on myometrium. The NAD<sup>+</sup> salvage pathway was found to be the possible potential mechanism. Pharmacological modulation of the myometrium while it was exposed to visfatin, by both NAD<sup>+</sup> pathway inhibitor and enhancers, showed that NAD<sup>+</sup> pathway might be involved in visfatin effects on myometrial contractility though a full study with increased end numbers is required to confirm this. This pathway was also reported to be important in visfatin's vasodilator effects on another smooth muscle cells, mesenteric microvessels (Vallejo et al., 2011). It is difficult to interpret these data given the potential confusion between what's going

on outside and inside the cell as an effect of visfatin. Visfatin works as an enzyme and catalyses the rate-limiting step in the biosynthesis of NAD<sup>+</sup> cofactor from nicotinamide which is essential for cellular metabolism (Garten et al., 2009). This metabolic reaction was found to be enhanced by conditions characterised by increased energy utilization such as stress and hypoxia. This, in turn, indicates that visfatin also has some beneficial effects, particularly during pregnancy and labour, which were also suggested in some previous studies (Ognjanovic and Bryant-Greenwood, 2002, Esplin et al., 2005, Marvin et al., 2002, Ognjanovic et al., 2005). It might also be involved in uterine quiescence. Nonetheless, the physiologic role of visfatin in pregnancy and parturition remains inconclusive and requiring further investigations.

#### **7.4 Obesity, adipokines and dysfunctional labour**

My cross sectional study failed to find any statistically significant relationship between adipokines, visfatin and leptin, and dysfunctional labour in obese women. It does; however, provide a new dimension examining plasma leptin and visfatin levels and their relationship with maternal and neonatal outcomes. It is also novel, in that it examines a different ethnic group characterised by a very high obesity prevalence, Saudi women (Al-Kadi et al., 2018). The findings of my study were largely confounded by the unexpectedly low number of obese women who encountered dysfunctional / prolonged labour in my sample. I still consider; however, my hypothesis is still worth looking in the future with a larger sample size. Despite the difficulties with my sample size I have generated data showing that maternal plasma leptin levels are positively correlated with increasing maternal BMI. These data are consistent with a number of other studies (Hamilton et al., 1995a, Ramsay et al., 2002, Al Maskari and Alnaqdy, 2006, Considine et al., 1996). My data also showed that maternal plasma visfatin levels have a positive relationship with induction of labour. I consider that this may be explained by the pro-inflammatory role of visfatin during labour initiation.

It became clear towards the end of my PhD that the review I co-authored on the effects of dyslipidaemia on myometrial contractility, had stimulated PhD research work in a laboratory in Sweden – others have made one step further in the ladder to expand our knowledge in this area. A Swedish medical fellow,

Dr.Sara Carlhäll, has examined whether maternal plasma leptin levels were associated with disruptive myometrial contractility and prolonged active phase of labour regardless of maternal BMI (Carlhäll et al., 2018). Her study was a prospective cohort study with a large number of patients (914) and the duration of the active stage of labour was measured. In her population, she found no relationship between maternal plasma leptin levels and the duration of the active phase of labour. She attempted to examine the association between leptin and prolonged labour in obese women, however the number of obese women in her sample was very small – a difference between Scandinavian and Arabic populations.

## **7.5 Maternal obesity and gap junction proteins**

To further understand the aetiology of poor uterine contractility in obese women, the myometrial expression of gap junction proteins, Cx43 and Cx26, during pregnancy was examined and compared to normal weight women. To the best of my knowledge, this is the first study to examine the expression of myometrial gap junction proteins in obese women. However, my data revealed that no association between maternal obesity and the expression of Cx43 and Cx26 in the myometrium. This finding was unexpected and inconsistent with the findings from high fat high cholesterol diet fed rats that showed reduced myometrial expression of Cx43 and Cx26, which may express distinctive species differences from human (Muir et al., 2016, Elmes et al., 2011). It may be that immunohistochemistry is not sensitive enough to find differences with BMI. Although my finding on gap junction proteins, was against my hypothesis, it encouraged the exclusion of one of the mechanisms which I proposed as an explanation of the poor myometrial contractility in obese pregnant women (Figure 1.7).

In summary, my data indicates that there may be a larger picture of alternative mechanisms exist to explain the clinically observed increased risk of pregnancy-related complications encountered in obese women. Unluckily, several of my findings are statistically negative and move into a direction against my working hypothesis; however, it is necessary, when negative findings were obtained, to be expressed and published hence it enlighten our understanding of the underlying knowledge. This also could minimise further unnecessary extra

research testing the same hypothesis. I am confident enough that my methodological procedures are efficient for the purpose they have been designed for.

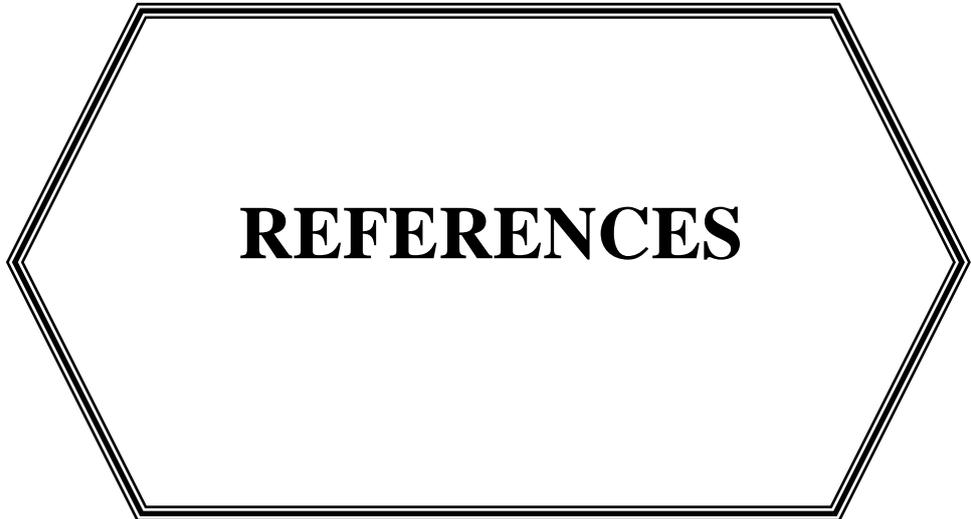
## 7.6 Future directions

There are several subsequent studies which could be pursued following my work. Higher myometrial cholesterol was found to be related to disruptive myometrial activity whilst reduction of cholesterol content enhanced contractions in *in vitro* examination. The missing gap in my data is whether maternal obesity is correlated with increased myometrial plasma membrane cholesterol content and whether increased plasma cholesterol levels are also correlated with poor myometrial contractility - which can be a useful predictor for the risk of adverse pregnancy and delivery outcomes, including dysfunctional labour. Measurements of electrical activity changes using patch clamp techniques in obese women would offer further understanding of the factors underlies poor myometrial contractility commonly associated with maternal obesity. To further understand the aetiology of poor uterine contractility in obese women, I would like to examine calcium signalling in obese human myometrium.

It would be informative to study visfatin receptors and visfatin's mechanism of action on human myometrium. Exploring the visfatin receptors in mouse myometrium will be also of advantage, as different gestational ages could be studied. It is suggestive to further examine the molecular mechanisms of visfatin on myometrium, particularly in relation to  $[Ca^{2+}]_i$ . To achieve that, confocal imaging for changes in calcium signalling simultaneously with force measurements. It would be very interesting to test the effects of visfatin in labouring myometrium and to further explore the role of oxytocin on labour progression in obese compared to normal weight women. Additional studies are needed to examine the effect and the specific mechanisms by which other adipokines modulate myometrial contractility. I would also like to find the relationship between maternal plasma visfatin and myometrial visfatin content and their changes during pregnancy and parturition according to body weight. *In vivo* evaluation to determine the possible physiological role of adipokines in the regulation of myometrial contractility will be the next step in understanding their contribution and putative therapeutic roles, only after more studies on both animal

and human myometrium, pregnant and non-pregnant myometrium are conducted. Using mouse obesity models with an obvious obesity phenotype would synergize with my Apo<sup>-/-</sup> mouse work such as ob/ob mouse. It would be interesting for future studies to investigate if PGE2 pathway is important for visfatin's actions in human myometrium

Further studies could next determine the expression of other myometrial gap junction proteins, Cx40 and Cx45, in obese pregnant women and compare these with normal weight pregnant women. These gap junction proteins are found to be typically co-expressed with Cx43 in the human myometrium. Furthermore, it could be beneficial to measure the myometrial prostaglandins expression in obese women.



**REFERENCES**

# References

## Uncategorized References

2014. Executive summary: Neonatal encephalopathy and neurologic outcome, second edition. Report of the American College of Obstetricians and Gynecologists' Task Force on Neonatal Encephalopathy. *Obstet Gynecol*, 123, 896-901.
- AARONSON, P. I., SARWAR, U., GIN, S., ROCKENBAUCH, U., CONNOLLY, M., TILLET, A., WATSON, S., LIU, B. & TRIBE, R. M. 2006. A role for voltage-gated, but not Ca<sup>2+</sup>-activated, K<sup>+</sup> channels in regulating spontaneous contractile activity in myometrium from virgin and pregnant rats. *Br J Pharmacol*, 147, 815-24.
- ABENHAIM, H. A. & BENJAMIN, A. 2011. Higher caesarean section rates in women with higher body mass index: are we managing labour differently? *J Obstet Gynaecol Can*, 33, 443-448.
- ABRAMS, B., HEGGESETH, B., REHKOPF, D. & DAVIS, E. 2013. Parity and body mass index in US women: a prospective 25-year study. *Obesity (Silver Spring, Md.)*, 21, 1514-1518.
- ADAIKAN, P. G. & ADEBIYI, A. 2005. Effect of functional modulation of Ca<sup>2+</sup>-activated Cl<sup>-</sup> currents on gravid rat myometrial activity. *Indian Journal of Pharmacology*, 37, 21-25.
- ADALI, E., YILDIZHAN, R., KOLUSARI, A., KURDOGLU, M., BUGDAYCI, G., SAHIN, H. G. & KAMACI, M. 2009. Increased visfatin and leptin in pregnancies complicated by pre-eclampsia. *The Journal of Maternal-Fetal & Neonatal Medicine*, 22, 873-879.
- AGUILAR, H. N. & MITCHELL, B. F. 2010. Physiological pathways and molecular mechanisms regulating uterine contractility. *Hum Reprod Update*, 16, 725-44.
- AKERMAN, F., LEI, Z. M. & RAO, C. V. 2002. Human umbilical cord and fetal membranes co-express leptin and its receptor genes. *Gynecol Endocrinol*, 16, 299-306.
- AL-KADI, A., MALIK, A. M. & MANSOUR, A. E. 2018. Rising incidence of obesity in Saudi residents. A threatening challenge for the surgeons. *International journal of health sciences*, 12, 45-49.
- AL-QUWAIDHI, A. J., PEARCE, M. S., CRITCHLEY, J. A., SOBNGWI, E. & O'FLAHERTY, M. 2014. Trends and future projections of the prevalence of adult obesity in Saudi Arabia, 1992-2022. *East Mediterr Health J*, 20, 589-95.
- AL MASKARI, M. Y. & ALNAQDY, A. A. 2006. Correlation between Serum Leptin Levels, Body Mass Index and Obesity in Omanis. *Sultan Qaboos University medical journal*, 6, 27-31.
- ALBERS, L. L. 1999. The duration of labor in healthy women. *J Perinatol*, 19, 114-9.
- ALBERTS, B., JOHNSON, A., LEWIS, J., RAFF, M., ROBERTS, K., AND WALTER 2008. Cell Junctions, Cell Adhesion, and the Extracellular Matrix. *Molecular Biology of the Cell*. 5th ed.: Garland Science.
- ALBRECHT, J. L., ATAL, N. S., TADROS, P. N., ORSINO, A., LYE, S. J., SADOVSKY, Y. & BEYER, E. C. 1996. Rat uterine myometrium contains the gap junction protein connexin45, which has a differing temporal expression pattern from connexin43. *Am J Obstet Gynecol*, 175, 853-8.
- ALEX, M. D. 2014. 20 YEARS OF LEPTIN: Leptin in common obesity and associated disorders of metabolism. *Journal of Endocrinology*, 223, T71-T81.
- ALHAZMI, A. A., AL HAJLAN, M. A. M., AL HAIDER, A. S., ALHAMAMI, Y. M. A., AL HARTHI, N. N. M., ALASMARI, M. H. & ALBUDAYDI, A. A. J. 2018. Rules of induction of labor, complication and benefits. *Egyptian Journal of Hospital Medicine*, 73, 6767-6772.
- ALLEN, V. M., BASKETT, T. F., O'CONNELL, C. M., MCKEEN, D. & ALLEN, A. C. 2009. Maternal and perinatal outcomes with increasing duration of the second stage of labor. *Obstet Gynecol*, 113, 1248-58.
- ALOTAIBI, M., ARROWSMITH, S. & WRAY, S. 2015. Hypoxia-induced force increase (HIFI) is a novel mechanism underlying the strengthening of labor contractions, produced by hypoxic stresses. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 9763-9768.

- ALSAIF, S., MUMTAZ, S. & WRAY, S. 2015. A short review of adipokines, smooth muscle and uterine contractility. *Life Sci*, 125, 2-8.
- AMBRUS, G. & RAO, C. V. 1994. Novel regulation of pregnant human myometrial smooth muscle cell gap junctions by human chorionic gonadotropin. *Endocrinology*, 135, 2772-9.
- AMERICAN COLLEGE OF OBSTETRICS AND GYNECOLOGY COMMITTEE ON PRACTICE BULLETINS-OBSTETRICS 2003. ACOG Practice Bulletin Number 49, December 2003: Dystocia and augmentation of labor. *Obstet Gynecol*, 102, 1445-54.
- AMOL, R. P., SUSANTH, V. S., ABDUL, S., MANICKAM, K., KANDASAMY, A., ANKITA, D. V., VIVEK, S., MANJIT, P., THAKUR UTTAM, S., AVINASH, G. T., SANTOSH, K. M. & SUBHASHREE, P. 2017. Hypercholesterolemia impairs oxytocin-induced uterine contractility in late pregnant mouse. *Reproduction*, 153, 565-576.
- ANDERSON, C. M., LOPEZ, F., ZHANG, H. Y., PAVLISH, K. & BENOIT, J. N. 2005. Characterization of changes in leptin and leptin receptors in a rat model of preeclampsia. *Am J Obstet Gynecol*, 193, 267-72.
- ANTUNA-PUENTE, B., FEVE, B., FELLAHI, S. & BASTARD, J. P. 2008. Adipokines: the missing link between insulin resistance and obesity. *Diabetes Metab*, 34, 2-11.
- ARNAUDEAU, S., LEPRETRE, N. & MIRONNEAU, J. 1994. Oxytocin mobilizes calcium from a unique heparin-sensitive and thapsigargin-sensitive store in single myometrial cells from pregnant rats. *Pflugers Arch*, 428, 51-9.
- ARROWSMITH, S., QUENBY, S., WEEKS, A., BURDYGA, T. & WRAY, S. 2012. Poor spontaneous and oxytocin-stimulated contractility in human myometrium from postdates pregnancies. *PLoS One*, 7, e36787.
- ARROWSMITH, S. & WRAY, S. 2014. Oxytocin: Its Mechanism of Action and Receptor Signalling in the Myometrium. *Journal of Neuroendocrinology*, 26, 356-369.
- ARROWSMITH, S., WRAY, S. & QUENBY, S. 2011. Maternal obesity and labour complications following induction of labour in prolonged pregnancy. *Bjog*, 118, 578-88.
- ATCHLEY, W. R. 1991. The Mouse: Its Reproduction and Development. Roberts Rugh. *The Quarterly Review of Biology*, 66, 490-490.
- ATEGBO, J. M., GRISSA, O., YESSOUFOU, A., HICHAMI, A., DRAMANE, K. L., MOUTAIROU, K., MILED, A., GRISSA, A., JERBI, M., TABKA, Z. & KHAN, N. A. 2006. Modulation of adipokines and cytokines in gestational diabetes and macrosomia. *J Clin Endocrinol Metab*, 91, 4137-43.
- ATHUKORALA, C., RUMBOLD, A. R., WILLSON, K. J. & CROWTHER, C. A. 2010. The risk of adverse pregnancy outcomes in women who are overweight or obese. *BMC Pregnancy Childbirth*, 10, 56.
- AXELSSON, J., HEIMBURGER, O., LINDHOLM, B. & STENVINKEL, P. 2005. Adipose tissue and its relation to inflammation: the role of adipokines. *J Ren Nutr*, 15, 131-6.
- AZAIS, H., LEROY, A., GHESQUIERE, L., DERUELLE, P. & HANSENS, S. 2017. Effects of adipokines and obesity on uterine contractility. *Cytokine Growth Factor Rev*, 34, 59-66.
- BABICH, L. G., SHLYKOV, S. G., KUSHNAROVA, A. M. & KOSTERIN, S. O. 2016. Ca(2+)-dependent regulation of the Ca(2+) concentration in the myometrium mitochondria. I. Trifluoperazine effects on mitochondria membranes polarization and [Ca(2+)](m). *Ukr Biochem J*, 88, 5-11.
- BABIYCHUK, E. B., SMITH, R. D., BURDYGA, T., BABIYCHUK, V. S., WRAY, S. & DRAEGER, A. 2004. Membrane cholesterol regulates smooth muscle phasic contraction. *Journal of Membrane Biology*, 198, 95-101.
- BAE, S.-K., KIM, S.-R., KIM, J. G., KIM, J. Y., KOO, T. H., JANG, H.-O., YUN, I., YOO, M.-A. & BAE, M.-K. 2006. Hypoxic induction of human visfatin gene is directly mediated by hypoxia-inducible factor-1. *FEBS Letters*, 580, 4105-4113.
- BAETEN, J. M., BUKUSI, E. A. & LAMBE, M. 2001. Pregnancy complications and outcomes among overweight and obese nulliparous women. *American Journal of Public Health*, 91, 436-440.

- BAKER, J. R., RANSON, R. M. & GOODRICH, E. S. 1932. Factors affecting the breeding of the field mouse (*Microtus agrestis*). Part II. Temperature and food. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, 112, 39-46.
- BARAU, G., ROBILLARD, P. Y., HULSEY, T. C., DEDECKER, F., LAFFITE, A., GERARDIN, P. & KAUFFMANN, E. 2006. Linear association between maternal pre-pregnancy body mass index and risk of caesarean section in term deliveries. *BJOG*, 113, 1173-7.
- BATRA, S. 1987. Increase by Estrogen of Calcium Entry and Calcium-Channel Density in Uterine Smooth-Muscle. *British Journal of Pharmacology*, 92, 389-392.
- BAUTISTA-CASTAÑO, I., HENRIQUEZ-SANCHEZ, P., ALEMÁN-PEREZ, N., GARCIA-SALVADOR, J., GONZALEZ-QUESADA, A., GARCÍA-HERNÁNDEZ, J. & SERRA-MAJEM, L. 2013. Maternal obesity in early pregnancy and risk of adverse outcomes. *PLoS one*, 8.
- BEŁTOWSKI, J. 2006. Apelin and visfatin: Unique "beneficial" adipokines upregulated in obesity? *Medical Science Monitor*, 12, RA112-RA119.
- BERENDZEN, J. A. & HOWARD, B. C. 2013. Association between cesarean delivery rate and body mass index. *Tenn Med*, 106, 35-7, 42.
- BERGGREN, E. K., GROH-WARGO, S., PRESLEY, L., HAUGUEL-DE MOUZON, S. & CATALANO, P. M. 2016. Maternal fat, but not lean, mass is increased among overweight/obese women with excess gestational weight gain. *Am J Obstet Gynecol*, 214, 745.e1-5.
- BERGLUND, S., PETTERSSON, H., CNATTINGIUS, S. & GRUNEWALD, C. 2010. How often is a low Apgar score the result of substandard care during labour? *BJOG*, 117, 968-978.
- BERNDT, J., KLOTING, N., KRALISCH, S., KOVACS, P., FASSHAUER, M., SCHON, M. R., STUMVOLL, M. & BLUHER, M. 2005. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes*, 54, 2911-6.
- BERTHOUD, V. M., MINOGUE, P. J., LAING, J. G. & BEYER, E. C. 2004. Pathways for degradation of connexins and gap junctions. *Cardiovasc Res*, 62, 256-67.
- BEYER, D. A., AMARI, F., LUDDERS, D. W., DIEDRICH, K. & WEICHERT, J. 2011. Obesity decreases the chance to deliver spontaneously. *Arch Gynecol Obstet*, 283, 981-8.
- BI, J., LI, H., YE, S. Q. & DING, S. 2012. Pre-B-cell colony-enhancing factor exerts a neuronal protection through its enzymatic activity and the reduction of mitochondrial dysfunction in in vitro ischemic models. *Journal of Neurochemistry*, 120, 334-346.
- BIEL, F. M., MARSHALL, N. E. & SNOWDEN, J. M. 2017. Maternal Body Mass Index and Regional Anaesthesia Use at Term: Prevalence and Complications. *Paediatr Perinat Epidemiol*, 31, 495-505.
- BIENERTOVA-VASKU, J., BIENERT, P., ZLAMAL, F., TOMANDL, J., TOMANDLOVA, M., DOSTALOVA, Z. & VASKU, A. 2012. Visfatin is secreted into the breast milk and is correlated with weight changes of the infant after the birth. *Diabetes Res Clin Pract*, 96, 355-61.
- BIGGERS, J. D., CURNOW, R. N., FINN, C. A. & MCLAREN, A. 1963. Regulation of the Gestation Period in Mice. *J Reprod Fertil*, 6, 125-38.
- BJORNTORP, P. 1993. Visceral obesity: a "civilization syndrome". *Obes Res*, 1, 206-22.
- BLACKBURN, S. T. 2013. *Maternal, fetal & neonatal physiology : a clinical perspective* Saunders, Elsevier.
- BLANKS, A. M. & THORNTON, S. 2003. The role of oxytocin in parturition. *BJOG*, 110 Suppl 20, 46-51.
- BLANKS, A. M., ZHAO, Z.-H., SHMYGOL, A., BRU-MERCIER, G., ASTLE, S. & THORNTON, S. 2007. Characterization of the molecular and electrophysiological properties of the T-type calcium channel in human myometrium. *The Journal of Physiology*, 581, 915-926.
- BLOMBERG, M. 2011a. Maternal and neonatal outcomes among obese women with weight gain below the new Institute of Medicine recommendations. *Obstet Gynecol*, 117, 1065-70.
- BLOMBERG, M. 2011b. Maternal obesity and risk of postpartum hemorrhage. *Obstet Gynecol*, 118, 561-8.

- BODNAR, L. M., CATOV, J. M., KLEBANOFF, M. A., NESS, R. B. & ROBERTS, J. M. 2007. Prepregnancy body mass index and the occurrence of severe hypertensive disorders of pregnancy. *Epidemiology*, 18, 234-9.
- BODNAR, L. M., SIEGA-RIZ, A. M., SIMHAN, H. N., HIMES, K. P. & ABRAMS, B. 2010. Severe obesity, gestational weight gain, and adverse birth outcomes. *Am J Clin Nutr*, 91, 1642-8.
- BOGAERTS, A., WITTERS, I., VAN DEN BERGH, B. R. H., JANS, G. & DEVLIEGER, R. 2013. Obesity in pregnancy: Altered onset and progression of labour. *Midwifery*, 29, 1303-1313.
- BOOTS, C. & STEPHENSON, M. D. 2011. Does obesity increase the risk of miscarriage in spontaneous conception: a systematic review. *Seminars in reproductive medicine*, 29, 507-513.
- BOOTS, C. E., BERNARDI, L. A. & STEPHENSON, M. D. 2014. Frequency of euploid miscarriage is increased in obese women with recurrent early pregnancy loss. *Fertility and Sterility*, 102, 455-459.
- BOYDENS, C., MAENHAUT, N., PAUWELS, B., DECALUWE, K. & VAN DE VOORDE, J. 2012. Adipose tissue as regulator of vascular tone. *Curr Hypertens Rep*, 14, 270-8.
- BRADLEY, K. N., FLYNN, E. R., MUIR, T. C. & MCCARRON, J. G. 2002. Ca(2+) regulation in guinea-pig colonic smooth muscle: the role of the Na(+)-Ca(2+) exchanger and the sarcoplasmic reticulum. *J Physiol*, 538, 465-82.
- BRAHMAN, L. E. 1991. Confidence Intervals Assess Both Clinical Significance and Statistical Significance. *Annals of Internal Medicine*, 114, 515-517.
- BRAINARD, A. M., KOROVKINA, V. P. & ENGLAND, S. K. 2007. Potassium channels and uterine function. *Semin Cell Dev Biol*, 18, 332-9.
- BRIANA, D. D. & MALAMITSI-PUCHNER, A. 2010. The role of adipocytokines in fetal growth. *Ann N Y Acad Sci*, 1205, 82-7.
- BRODERICK, R. & BRODERICK, K. A. 1990. Ultrastructure and Calcium Stores in the Myometrium. In: CARSTEN, M. E. & MILLER, J. D. (eds.) *Uterine Function: Molecular and Cellular Aspects*. Boston, MA: Springer US.
- BROWN, J. E., ONYANGO, D. J., RAMANJANEYA, M., CONNER, A. C., PATEL, S. T., DUNMORE, S. J. & RANDEVA, H. S. 2010. Visfatin regulates insulin secretion, insulin receptor signalling and mRNA expression of diabetes-related genes in mouse pancreatic beta-cells. *J Mol Endocrinol*, 44, 171-8.
- BRUZZONE, R., WHITE, T. W. & PAUL, D. L. 1996. Connections with connexins: the molecular basis of direct intercellular signaling. *Eur J Biochem*, 238, 1-27.
- BURDEN, H. W., ZARY, J. & ALBERTS, J. R. 1999. Effects of space flight on the immunohistochemical demonstration of connexin 26 and connexin 43 in the postpartum uterus of rats. *J Reprod Fertil*, 116, 229-34.
- BURDYGA, T., WRAY, S. & NOBLE, K. 2007. In situ calcium signaling: no calcium sparks detected in rat myometrium. *Ann N Y Acad Sci*, 1101, 85-96.
- BURDYGA, T. V. & WRAY, S. 1999. The effect of cyclopiazonic acid on excitation-contraction coupling in guinea-pig ureteric smooth muscle: role of the sarcoplasmic reticulum. *The Journal of physiology*, 517 ( Pt 3), 855-865.
- BUTWICK, A. J., ABREO, A., BATEMAN, B. T., LEE, H. C., EL-SAYED, Y. Y., STEPHANSSON, O. & FLOOD, P. 2018. Effect of Maternal Body Mass Index on Postpartum Hemorrhage. *Anesthesiology: The Journal of the American Society of Anesthesiologists*, 128, 774-783.
- CANVERENLER, E. 2015. *Adipocytokines in Particular Pregnancy Disorders*.
- CARE., N. H. 2019. Maternity Services Monthly Statistics November 2018, Experimental statistics.
- CARLHÄLL, S., KÄLLÉN, K. & BLOMBERG, M. 2013. Maternal body mass index and duration of labor. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 171, 49-53.

- CARLHÄLL, S., KÄLLÉN, K., THORSELL, A. & BLOMBERG, M. 2018. Maternal plasma leptin levels in relation to the duration of the active phase of labor. *Acta Obstetrica et Gynecologica Scandinavica*, 97, 1248-1256.
- CARLSON, N. S., HERNANDEZ, T. L. & HURT, K. J. 2015. Parturition dysfunction in obesity: time to target the pathobiology. *Reproductive biology and endocrinology : RB&E*, 13, 135-135.
- CARLSON, N. S. & LOWE, N. K. 2014. Intrapartum management associated with obesity in nulliparous women. *J Midwifery Womens Health*, 59, 43-53.
- CARMICHAEL, J. D., WINDER, S. J., WALSH, M. P. & KARGACIN, G. J. 1994. Calponin and smooth muscle regulation. *Can J Physiol Pharmacol*, 72, 1415-9.
- CARPENTER, J. R. Intrapartum Management of the Obese Gravida. 2016 2016 United States. J B LIPPINCOTT CO, 172.
- CASTELLANO FILHO, D. S., DO AMARAL CORREA, J. O., DOS SANTOS RAMOS, P., DE OLIVEIRA MONTESSI, M., AARESTRUP, B. J. & AARESTRUP, F. M. 2013. Body weight gain and serum leptin levels of non-overweight and overweight/obese pregnant women. *Med Sci Monit*, 19, 1043-9.
- CATTEAU, A., CAILLON, H., BARRIERE, P., DENIS, M. G., MASSON, D. & FREOUR, T. 2016. Leptin and its potential interest in assisted reproduction cycles. *Hum Reprod Update*, 22, 320-41.
- CATTERALL, W. A. 2011. Voltage-gated calcium channels. *Cold Spring Harbor perspectives in biology*, 3, a003947-a003947.
- CEDERGREN, M. 2006. Effects of gestational weight gain and body mass index on obstetric outcome in Sweden. *Int J Gynaecol Obstet*, 93, 269-74.
- CEDERGREN, M. I. 2004. Maternal morbid obesity and the risk of adverse pregnancy outcome. *Obstet Gynecol*, 103, 219-24.
- CEDERGREN, M. I. 2009. Non-elective caesarean delivery due to ineffective uterine contractility or due to obstructed labour in relation to maternal body mass index. *Eur J Obstet Gynecol Reprod Biol*, 145, 163-6.
- CERVERO, A., DOMINGUEZ, F., HORCAJADAS, J. A., QUINONERO, A., PELLICER, A. & SIMON, C. 2006. The role of the leptin in reproduction. *Curr Opin Obstet Gynecol*, 18, 297-303.
- CHAN, T. F., CHEN, Y. L., LEE, C. H., CHOU, F. H., WU, L. C., JONG, S. B. & TSAI, E. M. 2006. Decreased plasma visfatin concentrations in women with gestational diabetes mellitus. *J Soc Gynecol Investig*, 13, 364-7.
- CHEN, D., XIA, G., XU, P. & DONG, M. 2010. Peripartum serum leptin and soluble leptin receptor levels in women with gestational diabetes. *Acta Obstet Gynecol Scand*, 89, 1595-9.
- CHEN, X. & SCHOLL, T. O. 2008. Association of elevated free fatty acids during late pregnancy with preterm delivery. *Obstetrics and gynecology*, 112, 297-303.
- CHENG, Q., DONG, W., QIAN, L., WU, J. & PENG, Y. 2011. Visfatin inhibits apoptosis of pancreatic beta-cell line, MIN6, via the mitogen-activated protein kinase/phosphoinositide 3-kinase pathway. *J Mol Endocrinol*, 47, 13-21.
- CHENG, Y. W., SHAFFER, B. L., BRYANT, A. S. & CAUGHEY, A. B. 2010. Length of the first stage of labor and associated perinatal outcomes in nulliparous women. *Obstet Gynecol*, 116, 1127-35.
- CHIBA, T., NAKAZAWA, T., YUI, K., KANEKO, E. & SHIMOKADO, K. 2003. VLDL induces adipocyte differentiation in ApoE-dependent manner. *Arterioscler Thromb Vasc Biol*, 23, 1423-9.
- CHIEN, E. K., HARA, M., ROUARD, M., YANO, H., PHILLIPPE, M., POLONSKY, K. S. & BELL, G. I. 1997. Increase in Serum Leptin and Uterine Leptin Receptor Messenger RNA Levels during Pregnancy in Rats1. *Biochemical and Biophysical Research Communications*, 237, 476-480.
- CHIN, J. R., HENRY, E., HOLMGREN, C. M., VARNER, M. W. & BRANCH, D. W. 2012. Maternal obesity and contraction strength in the first stage of labor. *Am J Obstet Gynecol*, 207, 129.e1-6.

- CHOW, L. & LYE, S. J. 1994. Expression of the gap junction protein connexin-43 is increased in the human myometrium toward term and with the onset of labor. *Am J Obstet Gynecol*, 170, 788-95.
- CHU, D.-T., MALINOWSKA, E., JURA, M. & KOZAK, L. P. 2017. C57BL/6J mice as a polygenic developmental model of diet-induced obesity. *Physiological Reports*, 5, e13093.
- CHU, S. Y., CALLAGHAN, W. M., BISH, C. L. & D'ANGELO, D. 2009. Gestational weight gain by body mass index among US women delivering live births, 2004-2005: fueling future obesity. *Am J Obstet Gynecol*, 200, 271.e1-7.
- CHU, S. Y., CALLAGHAN, W. M., KIM, S. Y., SCHMID, C. H., LAU, J., ENGLAND, L. J. & DIETZ, P. M. 2007a. Maternal Obesity and Risk of Gestational Diabetes Mellitus. *Diabetes Care*, 30, 2070-2076.
- CHU, S. Y., KIM, S. Y., LAU, J., SCHMID, C. H., DIETZ, P. M., CALLAGHAN, W. M. & CURTIS, K. M. 2007b. Maternal obesity and risk of stillbirth: a metaanalysis. *Am J Obstet Gynecol*, 197, 223-8.
- CHU, S. Y., KIM, S. Y., SCHMID, C. H., DIETZ, P. M., CALLAGHAN, W. M., LAU, J. & CURTIS, K. M. 2007c. Maternal obesity and risk of cesarean delivery: a meta-analysis. *Obes Rev*, 8, 385-94.
- CIPOLLA, M. & OSOL, G. 1994. Hypertrophic and hyperplastic effects of pregnancy on the rat uterine arterial wall. *Am J Obstet Gynecol*, 171, 805-11.
- CLUFF, A. H., BYSTROM, B., KLIMAVICIUTE, A., DAHLQVIST, C., CEBERS, G., MALMSTROM, A. & EKMAN-ORDEBERG, G. 2006. Prolonged labour associated with lower expression of syndecan 3 and connexin 43 in human uterine tissue. *Reprod Biol Endocrinol*, 4, 24.
- CNATTINGIUS, S., BERGSTRÖM, R., LIPWORTH, L. & KRAMER, M. S. 1998. Prepregnancy Weight and the Risk of Adverse Pregnancy Outcomes. *New England Journal of Medicine*, 338, 147-152.
- CNATTINGIUS, S., VILLAMOR, E., JOHANSSON, S., BONAMY, A.-K., PERSSON, M., WIKSTRÖM, A. K. & GRANATH, F. 2013. *Maternal Obesity and Risk of Preterm Delivery*.
- COELHO, M., OLIVEIRA, T. & FERNANDES, R. 2013. Biochemistry of adipose tissue: an endocrine organ. *Archives of medical science : AMS*, 9, 191-200.
- COLE, W. C. & GARFIELD, R. E. 1986. Evidence for physiological regulation of myometrial gap junction permeability. *Am J Physiol*, 251, C411-20.
- COLEMAN, H. A., HART, J. D., TONTA, M. A. & PARKINGTON, H. C. 2000. Changes in the mechanisms involved in uterine contractions during pregnancy in guinea-pigs. *The Journal of physiology*, 523 Pt 3, 785-798.
- COLLINS, P. L., MOORE, J. J., LUNDGREN, D. W., CHOUBINEH, E., CHANG, S. M. & CHANG, A. S. 2000. Gestational Changes in Uterine L-Type Calcium Channel Function and Expression in Guinea Pig1. *Biology of Reproduction*, 63, 1262-1270.
- CONSIDINE, R. V., SINHA, M. K., HEIMAN, M. L., KRIAUCIUNAS, A., STEPHENS, T. W., NYCE, M. R., OHANNESIAN, J. P., MARCO, C. C., MCKEE, L. J., BAUER, T. L. & CARO, J. F. 1996. Serum Immunoreactive-Leptin Concentrations in Normal-Weight and Obese Humans. *New England Journal of Medicine*, 334, 292-295.
- COOK, J. L., ZARAGOZA, D. B., SUNG, D. H. & OLSON, D. M. 2000. Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: myometrial activation, stimulation, and preterm labor. *Endocrinology*, 141, 1718-28.
- CRANE, S. S., WOJTOWYCZ, M. A., DYE, T. D., AUBRY, R. H. & ARTAL, R. 1997. Association between pre-pregnancy obesity and the risk of cesarean delivery. *Obstet Gynecol*, 89, 213-6.
- CRANKSHAW, D. J. & MORRISON, J. J. 2011. Methodology and pharmacological analysis of effects of uterotonic compounds in human myometrium in vitro. *American Journal of Obstetrics and Gynecology*, 205, 155.e1-155.e6.
- CRANKSHAW, D. J., O'BRIEN, Y. M., CROSBY, D. A. & MORRISON, J. J. 2017. Maternal body mass index and spontaneous contractility of human myometrium in pregnancy. *J Perinatol*, 37, 492-497.

- CROY, A., YAMADA, A. T., DEMAYO, F. J. & ADAMSON, L. 2014. The Guide to Investigation of Mouse Pregnancy. *Academic Press*.
- CUMMINGS, K. F., HELMICH, M. S., OUNPRASEUTH, S. T., DAJANI, N. K. & MAGANN, E. F. 2018. The Third Stage of Labour in the Extremely Obese Parturient. *Journal of Obstetrics and Gynaecology Canada*, 40, 1148-1153.
- CURTIS, T. M., TUMELTY, J., STEWART, M. T., ARORA, A. R., LAI, F. A., MCGAHON, M. K., SCHOLFIELD, C. N. & MCGEOWN, J. G. 2008. Modification of smooth muscle Ca<sup>2+</sup>-sparks by tetracaine: evidence for sequential RyR activation. *Cell Calcium*, 43, 142-54.
- CYPESS, A. M., LEHMAN, S., WILLIAMS, G., TAL, I., RODMAN, D., GOLDFINE, A. B., KUO, F. C., PALMER, E. L., TSENG, Y. H., DORIA, A., KOLODNY, G. M. & KAHN, C. R. 2009. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med*, 360, 1509-17.
- DABERTRAND, F., FRITZ, N., MIRONNEAU, J., MACREZ, N. & MOREL, J. L. 2007. Role of RYR3 splice variants in calcium signaling in mouse nonpregnant and pregnant myometrium. *Am J Physiol Cell Physiol*, 293, C848-54.
- DAĞ, Z. Ö. & DILBAZ, B. 2015. Impact of obesity on infertility in women. *Journal of the Turkish German Gynecological Association*, 16, 111-117.
- DAHL, T. B., HOLM, S., AUKRUST, P. & HALVORSEN, B. 2012. Visfatin/NAMPT: A Multifaceted Molecule with Diverse Roles in Physiology and Pathophysiology. *Annual Review of Nutrition*, 32, 229-243.
- DAHLGREN, J. 2006. Pregnancy and insulin resistance. *Metab Syndr Relat Disord*, 4, 149-52.
- DALRYMPLE, A., SLATER, D. M., POSTON, L. & TRIBE, R. M. 2004. Physiological induction of transient receptor potential canonical proteins, calcium entry channels, in human myometrium: influence of pregnancy, labor, and interleukin-1 beta. *J Clin Endocrinol Metab*, 89, 1291-300.
- DAVIS-MOSS, R. & HOFFERTH, S. 2018. *Inadequate Gestational Weight Gain and Malnutrition-Related Causes of Infant Death*.
- DAWSON, M. J. & WRAY, S. 1985. The effects of pregnancy and parturition on phosphorus metabolites in rat uterus studied by <sup>31</sup>P nuclear magnetic resonance. *J Physiol*, 368, 19-31.
- DAYANGAÇ, A., KUTLU, S. & KONAR, V. 2010. Stimulatory effects of ghrelin on spontaneous contractions in the rat myometrium. *Ghrelin'in sıçan miyometriyumundaki spontan kasılmalara uyarıcı etkileri.*, 34, 35-38.
- DENG, Y. & SCHERER, P. E. 2010. Adipokines as novel biomarkers and regulators of the metabolic syndrome. *Annals of the New York Academy of Sciences*, 1212, E1-E19.
- DENISON, F. C., PRICE, J., GRAHAM, C., WILD, S. & LISTON, W. A. 2008. Maternal obesity, length of gestation, risk of postdates pregnancy and spontaneous onset of labour at term. *BJOG*, 115, 720-5.
- DESOYE, G., SCHWEDITSCH, M. O., PFEIFFER, K. P., ZECHNER, R. & KOSTNER, G. M. 1987. Correlation of hormones with lipid and lipoprotein levels during normal pregnancy and postpartum. *J Clin Endocrinol Metab*, 64, 704-12.
- DEVLIEGER, R., BENHALIMA, K., DAMM, P., VAN ASSCHE, A., MATHIEU, C., MAHMOOD, T., DUNNE, F. & BOGAERTS, A. 2016. Maternal obesity in Europe: where do we stand and how to move forward?: A scientific paper commissioned by the European Board and College of Obstetrics and Gynaecology (EBCOG). *European Journal of Obstetrics and Gynecology and Reproductive Biology*, 201, 203-208.
- DEWAR, A. D. 1968. Litter size and the duration of pregnancy in mice. *Q J Exp Physiol Cogn Med Sci*, 53, 155-61.
- DIET, N. A. T. P. O. C. D. 2003. *World Health Organ Tech Rep Ser*, 916, i-viii, 1-149, backcover.
- DIMITRIADIS, G. K., ADYA, R., TAN, B. K., JONES, T. A., MENON, V. S., RAMANJANEYA, M., KALTSAS, G., MIRAS, A. D. & RANDEVA, H. S. 2019. Effects of visfatin on brown adipose tissue energy regulation using T37i cells. *Cytokine*, 113, 248-255.

- DOLPHIN, A. C. 2006. A short history of voltage-gated calcium channels. *British journal of pharmacology*, 147 Suppl 1, S56-S62.
- DOMALI, E. & MESSINIS, I. E. 2002. Leptin in pregnancy. *J Matern Fetal Neonatal Med*, 12, 222-30.
- DOMINIC, C. J. 1966. Observations on the reproductive pheromones of mice. I. Source. *J Reprod Fertil*, 11, 407-14.
- DOPICO, A. M., BUKIYA, A. N. & SINGH, A. K. 2012. Large conductance, calcium- and voltage-gated potassium (BK) channels: regulation by cholesterol. *Pharmacol Ther*, 135, 133-50.
- DORING, B., SHYNLOVA, O., TSUI, P., ECKARDT, D., JANSSEN-BIENHOLD, U., HOFMANN, F., FEIL, S., FEIL, R., LYE, S. J. & WILLECKE, K. 2006. Ablation of connexin43 in uterine smooth muscle cells of the mouse causes delayed parturition. *J Cell Sci*, 119, 1715-22.
- EDISON, R. J., BERG, K., REMALEY, A., KELLEY, R., ROTIMI, C., STEVENSON, R. E. & MUENKE, M. 2007. Adverse Birth Outcome Among Mothers With Low Serum Cholesterol. *Pediatrics*, 120, 723-733.
- EDRY, I., SELA-ABRAMOVICH, S. & DEKEL, N. 2006. Meiotic arrest of oocytes depends on cell-to-cell communication in the ovarian follicle. *Mol Cell Endocrinol*, 252, 102-6.
- EHRENBERG, H. M., IAMS, J. D., GOLDENBERG, R. L., NEWMAN, R. B., WEINER, S. J., SIBAI, B. M., CARITIS, S. N., MIOODOVNIK, M. & DOMBROWSKI, M. P. 2009. Maternal obesity, uterine activity, and the risk of spontaneous preterm birth. *Obstet Gynecol*, 113, 48-52.
- EHRENBERG, H. M., MERCER, B. M. & CATALANO, P. M. 2004. The influence of obesity and diabetes on the prevalence of macrosomia. *Am J Obstet Gynecol*, 191, 964-8.
- EL-GILANY, A.-H. & HAMMAD, S. 2010. Body mass index and obstetric outcomes in pregnant in Saudi Arabia: a prospective cohort study. *Annals of Saudi medicine*, 30, 376-380.
- EL-GILANY, A. H. & EL-WEHADY, A. 2009. Prevalence of obesity in a Saudi obstetric population. *Obes Facts*, 2, 217-20.
- ELLEKJAER, K. L., BERGHOLT, T. & LØKKEGAARD, E. 2017. Maternal obesity and its effect on labour duration in nulliparous women: a retrospective observational cohort study. *BMC pregnancy and childbirth*, 17, 222-222.
- ELLIS, J. A., BROWN, C. M., BARGER, B. & CARLSON, N. S. 2019. Influence of Maternal Obesity on Labor Induction: A Systematic Review and Meta-Analysis. *J Midwifery Womens Health*, 64, 55-67.
- ELMES, M., SZYSZKA, A., PAULIAT, C., CLIFFORD, B., DANIEL, Z., CHENG, Z., WATHES, C. & MCMULLEN, S. 2015. Maternal age effects on myometrial expression of contractile proteins, uterine gene expression, and contractile activity during labor in the rat. *Physiological Reports*, 3, e12305.
- ELMES, M. J., TAN, D. S., CHENG, Z., WATHES, D. C. & MCMULLEN, S. 2011. The effects of a high-fat, high-cholesterol diet on markers of uterine contractility during parturition in the rat. *Reproduction*, 141, 283-90.
- ERTEM, M., BAHCECI, M., TUZCU, A., SAKA, G., OZTURK, U. & GOKALP, D. 2008. The association between high parity and obesity in women living in South-eastern Turkey. *Eat Weight Disord*, 13, e4-7.
- ESPLIN, M. S., FAUSETT, M. B., PELTIER, M. R., HAMBLIN, S., SILVER, R. M., BRANCH, D. W., ADASHI, E. Y. & WHITING, D. 2005. The use of cDNA microarray to identify differentially expressed labor-associated genes within the human myometrium during labor. *American Journal of Obstetrics and Gynecology*, 193, 404-413.
- FAIN, J. N., MADAN, A. K., HILER, M. L., CHEEMA, P. & BAHOUTH, S. W. 2004. Comparison of the Release of Adipokines by Adipose Tissue, Adipose Tissue Matrix, and Adipocytes from Visceral and Subcutaneous Abdominal Adipose Tissues of Obese Humans. *Endocrinology*, 145, 2273-2282.
- FAIRBROTHER, U., KIDD, E., MALAGAMUWA, T. & WALLEY, A. 2018. Genetics of Severe Obesity. *Current Diabetes Reports*, 18, 85.
- FARAH, N., MAHER, N., BARRY, S., KENNELLY, M., STUART, B. & TURNER, M. J. 2009. Maternal morbid obesity and obstetric outcomes. *Obes Facts*, 2, 352-4.

- FASSHAUER, M., BLUHER, M., STUMVOLL, M., TONESSEN, P., FABER, R. & STEPAN, H. 2007. Differential regulation of visfatin and adiponectin in pregnancies with normal and abnormal placental function. *Clin Endocrinol (Oxf)*, 66, 434-9.
- FASSHAUER, M., WALDEYER, T., SEEGER, J., SCHREY, S., EBERT, T., KRATZSCH, J., LOSSNER, U., BLUHER, M., STUMVOLL, M., FABER, R. & STEPAN, H. 2008. Serum levels of the adipokine visfatin are increased in pre-eclampsia. *Clin Endocrinol (Oxf)*, 69, 69-73.
- FEKETE, E. 1954. Gain in weight of pregnant mice in relation to litter size. *J. Hered*, 45, 88-89.
- FERRAZZI, E., BREMBILLA, G., CIPRIANI, S., LIVIO, S., PAGANELLI, A. & PARAZZINI, F. 2019. Maternal age and body mass index at term: Risk factors for requiring an induced labour for a late-term pregnancy. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 233, 151-157.
- Ferreira, D. C., Costa, T. F., Aguiar, S. L. F., Marques, A. R. S., Ramos, S. A., Gomes, K. B. & Alvarez-Leite, J. I. 2011. Association of Apolipoprotein E polymorphisms and metabolic syndrome in subjects with extreme obesity. *Clinica Chimica Acta*, 412, 1559-1562.
- FETALVERO, K. M., ZHANG, P., SHYU, M., YOUNG, B. T., HWA, J., YOUNG, R. C. & MARTIN, K. A. 2008. Prostacyclin primes pregnant human myometrium for an enhanced contractile response in parturition. *J Clin Invest*, 118, 3966-79.
- FITZSIMONS, K. J., MODDER, J. & GREER, I. A. 2009. Obesity in pregnancy: risks and management. *Obstet Med*, 2, 52-62.
- FORTUNO, A., RODRIGUEZ, A., GOMEZ-AMBROSI, J., MUNIZ, P., SALVADOR, J., DIEZ, J. & FRUHEBECK, G. 2002. Leptin inhibits angiotensin II-induced intracellular calcium increase and vasoconstriction in the rat aorta. *Endocrinology*, 143, 3555-60.
- FRANCAITE-DAUGELIENE, M., PETRENKO, V., BALIUTAVICIENE, D. & VELICKIENE, D. 2016. Retrospective analysis of age-adjusted body mass index among pre-pregnant women in the Lithuanian urban area during three decades. *BMJ Open*, 6, e010927.
- FRANCO-SENA, A. B., DE OLIVEIRA, L. C., DE JESUS PEREIRA PINTO, T., FARIAS, D. R., VAZ JDOS, S. & KAC, G. 2015. Factors associated with prospective leptin concentrations throughout pregnancy in pregestational normal weight, overweight and obese women. *Clin Endocrinol (Oxf)*, 82, 127-35.
- FRANKS, L. M. & PAYNE, J. 1970. The influence of age on reproductive capacity in C57BL mice. *J Reprod Fertil*, 21, 563-5.
- FREDERICH, R. C., HAMANN, A., ANDERSON, S., LOLLMANN, B., LOWELL, B. B. & FLIER, J. S. 1995. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med*, 1, 1311-4.
- FREITAS LIMA, L. C., BRAGA, V. D. A., DO SOCORRO DE FRANÇA SILVA, M., CRUZ, J. D. C., SOUSA SANTOS, S. H., DE OLIVEIRA MONTEIRO, M. M. & BALARINI, C. D. M. 2015. Adipokines, diabetes and atherosclerosis: an inflammatory association. *Frontiers in physiology*, 6, 304-304.
- FREY, H. A., TUULI, M. G., ENGLAND, S. K., ROEHL, K. A., ODIBO, A. O., MACONES, G. A. & CAHILL, A. G. 2015. Factors associated with higher oxytocin requirements in labor. *J Matern Fetal Neonatal Med*, 28, 1614-9.
- FRIEDMAN, E. A. 1955. Primigravid labor; a graphicostatistical analysis. *Obstet Gynecol*, 6, 567-89.
- FROLOVA, A., STOUT, M. J., TUULI, M. G., MACONES, G. A. & CAHILL, A. G. 2018a. 803: Impact of obesity on second stage of labor dystocia in nulliparous women. *American Journal of Obstetrics & Gynecology*, 218, S478.
- FROLOVA, A. I., WANG, J. J., CONNER, S. N., TUULI, M. G., MACONES, G. A., WOOLFOLK, C. L. & CAHILL, A. G. 2018b. Spontaneous Labor Onset and Outcomes in Obese Women at Term. *Am J Perinatol*, 35, 59-64.
- FUKUHARA, A., MATSUDA, M., NISHIZAWA, M., SEGAWA, K., TANAKA, M., KISHIMOTO, K., MATSUKI, Y., MURAKAMI, M., ICHISAKA, T., MURAKAMI, H., WATANABE, E., TAKAGI, T., AKIYOSHI, M., OHTSUBO, T., KIHARA, S., YAMASHITA, S., MAKISHIMA, M., FUNAHASHI,

- T., YAMANAKA, S., HIRAMATSU, R., MATSUZAWA, Y. & SHIMOMURA, I. 2005. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science*, 307, 426-30.
- FYFE, E. M., RIVERS, K. S., THOMPSON, J. M., THIYAGARAJAN, K. P., GROOM, K. M., DEKKER, G. A. & MCCOWAN, L. M. 2013. Elevated maternal lipids in early pregnancy are not associated with risk of intrapartum caesarean in overweight and obese nulliparous women. *BMC Pregnancy and Childbirth*, 13, 143.
- FYFE, E. M., THOMPSON, J. M. D., ANDERSON, N. H., GROOM, K. M. & MCCOWAN, L. M. 2012. Maternal obesity and postpartum haemorrhage after vaginal and caesarean delivery among nulliparous women at term: a retrospective cohort study. *BMC pregnancy and childbirth*, 12, 112-112.
- G, M. K. & WINTERHAGER, E. 2015. Physiological roles of connexins in labour and lactation. *Reproduction*, 150, R129-36.
- GAM, C., LARSEN, L. H., MORTENSEN, O. H., ENGELBRECHTSEN, L., POULSEN, S. S., QVORTRUP, K., MATHIESEN, E. R., DAMM, P. & QUISTORFF, B. 2017. Unchanged mitochondrial phenotype, but accumulation of lipids in the myometrium in obese pregnant women. *J Physiol*, 595, 7109-7122.
- GAM, C., MORTENSEN, O. H., LARSEN, L. H., POULSEN, S. S., QVORTRUP, K., MATHIESEN, E. R., DAMM, P. & QUISTORFF, B. 2018. Diabetes, myometrium, and mitochondria in pregnant women at term. *Acta Diabetol*, 55, 999-1010.
- GAM, C., MORTENSEN, O. H., QVORTRUP, K., DAMM, P. & QUISTORFF, B. 2015. Effect of high-fat diet on rat myometrium during pregnancy-isolated myometrial mitochondria are not affected. *Pflugers Arch*, 467, 1539-1549.
- GARFIELD, R. E., BLENNERHASSETT, M. G. & MILLER, S. M. 1988. Control of myometrial contractility: role and regulation of gap junctions. *Oxf Rev Reprod Biol*, 10, 436-90.
- GARFIELD, R. E. & HAYASHI, R. H. 1981. Appearance of gap junctions in the myometrium of women during labor. *American Journal of Obstetrics and Gynecology*, 140, 254-260.
- GARFIELD, R. E., KANNAN, M. S. & DANIEL, E. E. 1980. Gap junction formation in myometrium: control by estrogens, progesterone, and prostaglandins. *Am J Physiol*, 238, C81-9.
- GARFIELD, R. E. & MANER, W. L. 2007. Physiology and electrical activity of uterine contractions. *Seminars in cell & developmental biology*, 18, 289-295.
- GARFIELD, R. E., RABIDEAU, S., CHALLIS, J. R. & DANIEL, E. E. 1979. Ultrastructural basis for maintenance and termination of pregnancy. *Am J Obstet Gynecol*, 133, 308-15.
- GARFIELD, R. E., SIMS, S. & DANIEL, E. E. 1977. Gap junctions: their presence and necessity in myometrium during parturition. *Science*, 198, 958-60.
- GARTEN, A., PETZOLD, S., KÖRNER, A., IMAI, S.-I. & KIESS, W. 2009. Nampt: linking NAD biology, metabolism and cancer. *Trends in Endocrinology & Metabolism*, 20, 130-138.
- GARTNER, L. P. A. H., J. L & HIATT, J. L. 2009. Color Atlas of Histology. *Lippincott Williams & Wilkins. Maryland, USA*.
- GAUDET, L., FERRARO, Z. M., WEN, S. W. & WALKER, M. 2014. Maternal obesity and occurrence of fetal macrosomia: a systematic review and meta-analysis. *Biomed Res Int*, 2014, 640291.
- GERALD, M. K. & ELKE, W. 2015. Physiological roles of connexins in labour and lactation. *REPRODUCTION*, 150, R129-R136.
- GHERGHICEANU, M. & POPESCU, L. M. 2006. Caveolar nanospaces in smooth muscle cells. *J Cell Mol Med*, 10, 519-28.
- GILEAD, R., YANIV SALEM, S., SERGIENKO, R. & SHEINER, E. 2012. Maternal "isolated" obesity and obstetric complications. *The Journal of Maternal-Fetal & Neonatal Medicine*, 25, 2579-2582.
- GOK, D. E., YAZICI, M., UCKAYA, G., BOLU, S. E., BASARAN, Y., OZGURTAS, T., KILIC, S. & KUTLU, M. 2011. The role of visfatin in the pathogenesis of gestational diabetes mellitus. *J Endocrinol Invest*, 34, 3-7.
- GOODENOUGH, D. A. & PAUL, D. L. 2009. Gap junctions. *Cold Spring Harb Perspect Biol*, 1, a002576.

- GOSSET, M., BERENBAUM, F., SALVAT, C., SAUTET, A., PIGENET, A., TAHIRI, K. & JACQUES, C. 2008. Crucial role of visfatin/pre-B cell colony-enhancing factor in matrix degradation and prostaglandin E2 synthesis in chondrocytes: possible influence on osteoarthritis. *Arthritis Rheum*, 58, 1399-409.
- GOSSMAN, W., FAGAN, S. E., SOSA-STANLEY, J. N. & PETERSON, D. C. 2019. Anatomy, Abdomen and Pelvis, Uterus. *StatPearls*. Treasure Island (FL): StatPearls Publishing
- StatPearls Publishing LLC.
- GOSTYNSKI, M., GUTZWILLER, F., KUULASMAA, K., DORING, A., FERRARIO, M., GRAFNETTER, D., PAJAK, A. & PROJECT, W. M. 2004. Analysis of the relationship between total cholesterol, age, body mass index among males and females in the WHO MONICA Project. *Int J Obes Relat Metab Disord*, 28, 1082-90.
- GOULD, D. 2000. Normal labour: a concept analysis. *Journal of Advanced Nursing (Wiley-Blackwell)*, 31, 418-427.
- GRACEFFA, P., ADAM, L. P. & MORGAN, K. G. 1996. Strong interaction between caldesmon and calponin. *J Biol Chem*, 271, 30336-9.
- GRAVINA, F. S., JOBLING, P., KERR, K. P., DE OLIVEIRA, R. B., PARKINGTON, H. C. & VAN HELDEN, D. F. 2011. Oxytocin depolarizes mitochondria in isolated myometrial cells. *Exp Physiol*, 96, 949-56.
- GRAVINA, F. S., PARKINGTON, H. C., KERR, K. P., DE OLIVEIRA, R. B., JOBLING, P., COLEMAN, H. A., SANDOW, S. L., DAVIES, M. M., IMTIAZ, M. S. & VAN HELDEN, D. F. 2010. Role of mitochondria in contraction and pacemaking in the mouse uterus. *British journal of pharmacology*, 161, 1375-1390.
- GREENBERG, M. B., CHENG, Y. W., SULLIVAN, M., NORTON, M. E., HOPKINS, L. M. & CAUGHEY, A. B. 2007. Does length of labor vary by maternal age? *American Journal of Obstetrics & Gynecology*, 197, 428.e1-428.e7.
- GRIEVE, E., FENWICK, E., YANG, H. C. & LEAN, M. 2013. The disproportionate economic burden associated with severe and complicated obesity: a systematic review. *Obes Rev*, 14, 883-94.
- GROTEGUT, C. A., GUNATILAKE, R. P., FENG, L., HEINE, R. P. & MURTHA, A. P. 2013. The influence of maternal body mass index on myometrial oxytocin receptor expression in pregnancy. *Reprod Sci*, 20, 1471-7.
- GRUMMER, R., CHWALISZ, K., MULHOLLAND, J., TRAUB, O. & WINTERHAGER, E. 1994. Regulation of connexin26 and connexin43 expression in rat endometrium by ovarian steroid hormones. *Biol Reprod*, 51, 1109-16.
- GULLAM, J. E., BLANKS, A. M., THORNTON, S. & SHMYGOL, A. 2009. Phase-plot analysis of the oxytocin effect on human myometrial contractility. *Eur J Obstet Gynecol Reprod Biol*, 144 Suppl 1, S20-4.
- GUNDERSON, E. P. 2009. Childbearing and obesity in women: weight before, during, and after pregnancy. *Obstetrics and gynecology clinics of North America*, 36, 317-ix.
- GÜNGÖRDÜK, K., OLGAC, Y., GÜLSEREN, V. & KOCAER, M. 2018. Active management of the third stage of labor: A brief overview of key issues. *Turkish journal of obstetrics and gynecology*, 15, 188-192.
- HAIDER, D. G., HANDISURYA, A., STORKA, A., VOJTASSAKOVA, E., LUGER, A., PACINI, G., TURA, A., WOLZT, M. & KAUTZKY-WILLER, A. 2007. Visfatin response to glucose is reduced in women with gestational diabetes mellitus. *Diabetes Care*, 30, 1889-91.
- HAJAGOS-TÓTH, J., DUCZA, E., SAMAVATI, R., VARI, S. G. & GASPAR, R. 2017. Obesity in pregnancy: a novel concept on the roles of adipokines in uterine contractility. *Croatian medical journal*, 58, 96-104.
- HAKAMATA, H., SAKAGUCHI, H., ZHANG, C., SAKASHITA, N., SUZUKI, H., MIYAZAKI, A., TAKEYA, M., TAKAHASHI, K., KITAMURA, N. & HORIUCHI, S. 1998. The very low- and intermediate-density lipoprotein fraction isolated from apolipoprotein E-knockout mice transforms macrophages to foam cells through an apolipoprotein E-independent pathway. *Biochemistry*, 37, 13720-7.

- HAMILTON, B. S., PAGLIA, D., KWAN, A. Y. & DEITEL, M. 1995a. Increased obese mRNA expression in omental fat cells from massively obese humans. *Nat Med*, 1, 953-6.
- HAMILTON, B. S., PAGLIA, D., KWAN, A. Y. M. & DEITEL, M. 1995b. Increased obese mRNA expression in omental fat cells from massively obese humans. *Nature Medicine*, 1, 953-956.
- HANLEY, J.-A., WEEKS, A. & WRAY, S. 2015. Physiological increases in lactate inhibit intracellular calcium transients, acidify myocytes and decrease force in term pregnant rat myometrium. *The Journal of physiology*, 593, 4603-4614.
- HARA, N., YAMADA, K., SHIBATA, T., OSAGO, H., HASHIMOTO, T. & TSUCHIYA, M. 2007. Elevation of cellular NAD levels by nicotinic acid and involvement of nicotinic acid phosphoribosyltransferase in human cells. *J Biol Chem*, 282, 24574-82.
- HARPER, L. M., CAUGHEY, A. B., ROEHL, K. A., ODIBO, A. O. & CAHILL, A. G. 2014. Defining an abnormal first stage of labor based on maternal and neonatal outcomes. *Am J Obstet Gynecol*, 210, 536.e1-7.
- HARRIS, A. L. 2007. Connexin channel permeability to cytoplasmic molecules. *Progress in Biophysics and Molecular Biology*, 94, 120-143.
- HARROD, J. S., RADA, C. C., PIERCE, S. L., ENGLAND, S. K. & LAMPING, K. G. 2011. Altered contribution of RhoA/Rho kinase signaling in contractile activity of myometrium in leptin receptor-deficient mice. *American Journal Of Physiology. Endocrinology And Metabolism*, 301, E362-E369.
- HASMANN, M. & SCHEMAINDA, I. 2003. FK866, a highly specific noncompetitive inhibitor of nicotinamide phosphoribosyltransferase, represents a novel mechanism for induction of tumor cell apoptosis. *Cancer Res*, 63, 7436-42.
- HAUNER, H. 2005. Secretory factors from human adipose tissue and their functional role. *Proc Nutr Soc*, 64, 163-9.
- HAUTAKANGAS, T., PALOMAKI, O., EIDSTO, K., HUHTALA, H. & UOTILA, J. 2018. Impact of obesity and other risk factors on labor dystocia in term primiparous women: a case control study. *BMC Pregnancy Childbirth*, 18, 304.
- HAYNES, W. G., MORGAN, D. A., WALSH, S. A., SIVITZ, W. I. & MARK, A. L. 1998. Cardiovascular consequences of obesity: role of leptin. *Clin Exp Pharmacol Physiol*, 25, 65-9.
- HEAD, B. P., PATEL, H. H. & INSEL, P. A. 2014. Interaction of membrane/lipid rafts with the cytoskeleton: Impact on signaling and function: Membrane/lipid rafts, mediators of cytoskeletal arrangement and cell signaling. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1838, 532-545.
- HEDDERSON, M. M., WILLIAMS, M. A., HOLT, V. L., WEISS, N. S. & FERRARA, A. 2008. Body mass index and weight gain prior to pregnancy and risk of gestational diabetes mellitus. *Am J Obstet Gynecol*, 198, 409.e1-7.
- HEHIR, M. P., GLAVEY, S. V. & MORRISON, J. J. 2008. Uterorelaxant effect of ghrelin on human myometrial contractility. *Am J Obstet Gynecol*, 198, 323.e1-5.
- HEHIR, M. P. & MORRISON, J. J. 2012. The adipokine apelin and human uterine contractility. *Am J Obstet Gynecol*, 206, 359 e1-5.
- HELGUERA, G., OLCESE, R., SONG, M., TORO, L. & STEFANI, E. 2002. Tissue-specific regulation of Ca(2+) channel protein expression by sex hormones. *Biochim Biophys Acta*, 1569, 59-66.
- HENDLER, I., BLACKWELL, S. C., MEHTA, S. H., WHITTY, J. E., RUSSELL, E., SOROKIN, Y. & COTTON, D. B. 2005a. The levels of leptin, adiponectin, and resistin in normal weight, overweight, and obese pregnant women with and without preeclampsia. *Am J Obstet Gynecol*, 193, 979-83.
- HENDLER, I., GOLDENBERG, R. L., MERCER, B. M., IAMS, J. D., MEIS, P. J., MOAWAD, A. H., MACPHERSON, C. A., CARITIS, S. N., MIODOVNIK, M., MENARD, K. M., THURNAU, G. R. & SOROKIN, Y. 2005b. The Preterm Prediction Study: association between maternal body mass index and spontaneous and indicated preterm birth. *Am J Obstet Gynecol*, 192, 882-6.

- HENRÍQUEZ PINO, J. 2017. *Arrangement of Muscle Fibers in the Myometrium of the Human Uterus: A Mesoscopic Study*.
- HENSON, M. C. & CASTRACANE, V. D. 2006. Leptin in Pregnancy: An Update<sup>1</sup>. *Biology of Reproduction*, 74, 218-229.
- HESLEHURST, N., BELL, R. & RANKIN, J. 2011a. Tackling maternal obesity: the challenge for public health. *Perspect Public Health*, 131, 161-2.
- HESLEHURST, N., MOORE, H., RANKIN, J., ELLS, L. J., WILKINSON, J. R. & SUMMERBELL, C. D. 2011b. How can maternity services be developed to effectively address maternal obesity? A qualitative study. *Midwifery*, 27, e170-7.
- HESLEHURST, N., RANKIN, J., WILKINSON, J. R. & SUMMERBELL, C. D. 2010. A nationally representative study of maternal obesity in England, UK: trends in incidence and demographic inequalities in 619 323 births, 1989-2007. *Int J Obes (Lond)*, 34, 420-8.
- HESLEHURST, N., SIMPSON, H., ELLS, L. J., RANKIN, J., WILKINSON, J., LANG, R., BROWN, T. J. & SUMMERBELL, C. D. 2008. The impact of maternal BMI status on pregnancy outcomes with immediate short-term obstetric resource implications: a meta-analysis. *Obes Rev*, 9, 635-83.
- HESLEHURST, N., VIEIRA, R., AKHTER, Z., BAILEY, H., SLACK, E., NGONGALAH, L., PEMU, A. & RANKIN, J. 2019. The association between maternal body mass index and child obesity: A systematic review and meta-analysis. *PLoS Med*, 16, e1002817.
- HIDALGO-LOPEZOSA, P., HIDALGO-MAESTRE, M. & RODRÍGUEZ-BORREGO, M. A. 2016. Labor stimulation with oxytocin: effects on obstetrical and neonatal outcomes. *Revista latino-americana de enfermagem*, 24, e2744-e2744.
- HIGGINS, C. A., MARTIN, W., ANDERSON, L., BLANKS, A. M., NORMAN, J. E., MCCONNACHIE, A. & NELSON, S. M. 2010. Maternal obesity and its relationship with spontaneous and oxytocin-induced contractility of human myometrium in vitro. *Reprod Sci*, 17, 177-85.
- HILL, M., REED, K. L. & COHEN, W. R. 2015. Oxytocin utilization for labor induction in obese and lean women. *J Perinat Med*, 43, 703-6.
- HILLIARD, A. M., CHAUHAN, S. P., ZHAO, Y. & RANKINS, N. C. 2012. Effect of obesity on length of labor in nulliparous women. *Am J Perinatol*, 29, 127-32.
- HOFMANN, S. M., PEREZ-TILVE, D., GREER, T. M., COBURN, B. A., GRANT, E., BASFORD, J. E., TSCHOP, M. H. & HUI, D. Y. 2008. Defective lipid delivery modulates glucose tolerance and metabolic response to diet in apolipoprotein E-deficient mice. *Diabetes*, 57, 5-12.
- HOFMEYER, G. J. 2004. Obstructed labor: using better technologies to reduce mortality. *International Journal of Gynecology & Obstetrics*, 85, S62-S72.
- HOLDA, J. R., OBERTI, C., PEREZ-REYES, E. & BLATTER, L. A. 1996. Characterization of an oxytocin-induced rise in [Ca<sup>2+</sup>]<sub>i</sub> in single human myometrium smooth muscle cells. *Cell Calcium*, 20, 43-51.
- HOROWITZ, A., MENICE, C. B., LAPORTE, R. & MORGAN, K. G. 1996. Mechanisms of smooth muscle contraction. *Physiol Rev*, 76, 967-1003.
- HU, H., CHIAMVIMONVAT, N., YAMAGISHI, T. & MARBAN, E. 1997. Direct inhibition of expressed cardiac L-type Ca<sup>2+</sup> channels by S-nitrosothiol nitric oxide donors. *Circ Res*, 81, 742-52.
- HU, W., WANG, Z., WANG, H., HUANG, H. & DONG, M. 2008. Serum visfatin levels in late pregnancy and pre-eclampsia. *Acta Obstet Gynecol Scand*, 87, 413-8.
- HUANG, Z. H., REARDON, C. A. & MAZZONE, T. 2006. Endogenous ApoE Expression Modulates Adipocyte Triglyceride Content and Turnover. *Diabetes*, 55, 3394-3402.
- HUG, C. & LODISH, H. F. 2005. Visfatin: A New Adipokine. *Science*, 307, 366-367.
- HULL, H. R., DINGER, M. K., KNEHANS, A. W., THOMPSON, D. M. & FIELDS, D. A. 2008. Impact of maternal body mass index on neonate birthweight and body composition. *Am J Obstet Gynecol*, 198, 416.e1-6.
- HUSZAR, G. & NAFTOLIN, F. 1984. The myometrium and uterine cervix in normal and preterm labor. *N Engl J Med*, 311, 571-81.
- IKENOUE, S., MIYAKOSHI, K., KASUGA, Y., OCHIAI, D., MATSUMOTO, T. & TANAKA, M. 2018. Impaired fetal growth in mothers with inadequate gestational weight gain: a

- retrospective study in Japanese uncomplicated pregnancy. *The Journal of Maternal-Fetal & Neonatal Medicine*, 1-5.
- INOUE, Y. & SPERELAKIS, N. 1991. Gestational change in Na<sup>+</sup> and Ca<sup>2+</sup> channel current densities in rat myometrial smooth muscle cells. *American Journal of Physiology-Cell Physiology*, 260, C658-C663.
- INSTITUTE OF MEDICINE AND NATIONAL RESEARCH COUNCIL COMMITTEE TO REEXAMINE, I. O. M. P. W. G. 2009. The National Academies Collection: Reports funded by National Institutes of Health. In: RASMUSSEN, K. M. & YAKTINE, A. L. (eds.) *Weight Gain During Pregnancy: Reexamining the Guidelines*. Washington (DC): National Academies Press (US)
- National Academy of Sciences.
- IRANI, R. A. & FOSTER, S. 2015. Overview of the mechanisms of induction of labor. *Semin Perinatol*, 39, 426-9.
- IVERSEN, D. S., KESMODEL, U. S. & OVESEN, P. G. 2018. Associations between parity and maternal BMI in a population-based cohort study. *Acta Obstet Gynecol Scand*, 97, 694-700.
- JAHN, E., CLASSEN-LINKE, I., KUSCHE, M., BEIER, H. M., TRAUB, O., GRUMMER, R. & WINTERHAGER, E. 1995. Expression of gap junction connexins in the human endometrium throughout the menstrual cycle. *Hum Reprod*, 10, 2666-70.
- JANA, B., JAROSZEWSKI, J., KUCHARSKI, J., KOSZYKOWSKA, M., GÓRSKA, J. & MARKIEWICZ, W. 2010. Participation of Prostaglandin E2 in Contractile Activity of Inflamed Porcine Uterus. *Acta Veterinaria Brno*, 79, 249-259.
- JAQUET, D., DEGHMOUN, S., CHEVENNE, D., COLLIN, D., CZERNICHOW, P. & LEVY-MARCHAL, C. 2005. Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth. *Diabetologia*, 48, 849-55.
- JAVED, I., BHUTTA, S. & SHOAIB, T. 2007. Role of partogram in preventing prolonged labour. *J Pak Med Assoc*, 57, 408-11.
- JIANG, S., JIANG, J., XU, H., WANG, S., LIU, Z., LI, M., LIU, H., ZHENG, S., WANG, L., FEI, Y., LI, X., DING, Y., WANG, Z. & YU, Y. 2017. Maternal dyslipidemia during pregnancy may increase the risk of preterm birth: A meta-analysis. *Taiwanese Journal of Obstetrics and Gynecology*, 56, 9-15.
- JIE, Z., KENDRICK, A., QUENBY, S. & WRAY, S. 2007. Contractility and calcium signaling of human myometrium are profoundly affected by cholesterol manipulation: implications for labor? *Reprod Sci*, 14, 456-66.
- JIN, W.-Y., LIN, S.-L., HOU, R.-L., CHEN, X.-Y., HAN, T., JIN, Y., TANG, L., ZHU, Z.-W. & ZHAO, Z.-Y. 2016. Associations between maternal lipid profile and pregnancy complications and perinatal outcomes: a population-based study from China. *BMC pregnancy and childbirth*, 16, 60-60.
- JONES, K., SHMYGOL, A., KUPITTAYANANT, S. & WRAY, S. 2004. Electrophysiological characterization and functional importance of calcium-activated chloride channel in rat uterine myocytes. *Pflügers Archiv*, 448, 36-43.
- JONSSON, M., NORDEN-LINDEBERG, S., OSTLUND, I. & HANSON, U. 2008. Acidemia at birth, related to obstetric characteristics and to oxytocin use, during the last two hours of labor. *Acta Obstet Gynecol Scand*, 87, 745-50.
- JOSEPHS, T., WAUGH, H., KOKAY, I., GRATAN, D. & THOMPSON, M. 2007. Fasting-induced adipose factor identified as a key adipokine that is up-regulated in white adipose tissue during pregnancy and lactation in the rat. *J Endocrinol*, 194, 305-12.
- JUHASOVA, J., KREFT, M., ZIMMERMANN, R. & KIMMICH, N. 2018. Impact factors on cervical dilation rates in the first stage of labor. *Journal of Perinatal Medicine*.
- JUNG, U. J. & CHOI, M.-S. 2014. Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease. *International Journal of Molecular Sciences*, 15, 6184-6223.

- JUNGHEIM, E. S., TRAVIESO, J. L., CARSON, K. R. & MOLEY, K. H. 2012. Obesity and reproductive function. *Obstetrics and gynecology clinics of North America*, 39, 479-493.
- KALINDERIS, M., PAPANIKOLAOU, A., KALINDERI, K., VYZANTIADIS, T. A., IOAKIMIDOU, A. & TARLATZIS, B. C. 2015. Serum levels of leptin and IP-10 in preeclampsia compared to controls. *Arch Gynecol Obstet*, 292, 343-7.
- KAMISHIMA, T., BURDYGA, T., GALLAGHER, J. A. & QUAYLE, J. M. 2007. Caveolin-1 and caveolin-3 regulate Ca<sup>2+</sup> homeostasis of single smooth muscle cells from rat cerebral resistance arteries. *American Journal of Physiology-Heart and Circulatory Physiology*, 293, H204-H214.
- KANAGALINGAM, M. G., FOROUHI, N. G., GREER, I. A. & SATTAR, N. 2005. Changes in booking body mass index over a decade: retrospective analysis from a Glasgow Maternity Hospital. *BJOG: An International Journal of Obstetrics & Gynaecology*, 112, 1431-1433.
- KANIEWSKA, M., GOLOFIT, P., HEUBNER, M., MAAKE, C. & KUBIK-HUCH, R. A. 2018. Suspensory Ligaments of the Female Genital Organs: MRI Evaluation with Intraoperative Correlation. *Radiographics*, 38, 2195-2211.
- KAO, C. Y. 1959. Long-term observations of spontaneous electrical activity of the uterine smooth muscle. *Am J Physiol*, 196, 343-50.
- KAPADIA, M. Z., PARK, C. K., BEYENE, J., GIGLIA, L., MAXWELL, C. & MCDONALD, S. D. 2015. Can we safely recommend gestational weight gain below the 2009 guidelines in obese women? A systematic review and meta-analysis. *Obes Rev*, 16, 189-206.
- KASHYAP, V. S., SANTAMARINA-FOJO, S., BROWN, D. R., PARROTT, C. L., APPLEBAUM-BOWDEN, D., MEYN, S., TALLEY, G., PAIGEN, B., MAEDA, N. & BREWER, H. B., JR. 1995. Apolipoprotein E deficiency in mice: gene replacement and prevention of atherosclerosis using adenovirus vectors. *The Journal of clinical investigation*, 96, 1612-1620.
- KAUTZKY-WILLER, A., PACINI, G., TURA, A., BIEGLMAYER, C., SCHNEIDER, B., LUDVIK, B., PRAGER, R. & WALDHAUSL, W. 2001. Increased plasma leptin in gestational diabetes. *Diabetologia*, 44, 164-72.
- KENDAL-WRIGHT, C. E., HUBBARD, D. & BRYANT-GREENWOOD, G. D. 2008. Chronic stretching of amniotic epithelial cells increases pre-B cell colony-enhancing factor (PBEF/visfatin) expression and protects them from apoptosis. *Placenta*, 29, 255-65.
- KENDAL-WRIGHT, C. E., HUBBARD, D., GOWIN-BROWN, J. & BRYANT-GREENWOOD, G. D. 2010. Stretch and inflammation-induced Pre-B cell colony-enhancing factor (PBEF/Visfatin) and Interleukin-8 in amniotic epithelial cells. *Placenta*, 31, 665-674.
- KENDAL, C. E. & BRYANT-GREENWOOD, G. D. 2007. Pre-B-cell Colony-enhancing Factor (PBEF/Visfatin) Gene Expression is Modulated by NF- $\kappa$ B and AP-1 in Human Amniotic Epithelial Cells. *Placenta*, 28, 305-314.
- KEVEME, E. B. 1983. Pheromonal influences on the endocrine regulation of reproduction. *Trends in Neurosciences*, 6, 381-384.
- KHAN, R. N., MATHAROO-BALL, B., ARULKUMARAN, S. & ASHFORD, M. L. 2001. Potassium channels in the human myometrium. *Exp Physiol*, 86, 255-64.
- KHAN, R. N., SMITH, S. K., MORRISON, J. J. & ASHFORD, M. L. 1997. Ca<sup>2+</sup> dependence and pharmacology of large-conductance K<sup>+</sup> channels in nonlabor and labor human uterine myocytes. *Am J Physiol*, 273, C1721-31.
- KILARSKI, W. M., DUPONT, E., COPPEN, S., YEH, H. I., VOZZI, C., GOURDIE, R. G., REZAPOUR, M., ULMSTEN, U., ROOMANS, G. M. & SEVERS, N. J. 1998. Identification of two further gap-junctional proteins, connexin40 and connexin45, in human myometrial smooth muscle cells at term. *Eur J Cell Biol*, 75, 1-8.
- KILARSKI, W. M., ROTHERY, S., ROOMANS, G. M., ULMSTEN, U., REZAPOUR, M., STEVENSON, S., COPPEN, S. R., DUPONT, E. & SEVERS, N. J. 2001. Multiple connexins localized to individual gap-junctional plaques in human myometrial smooth muscle. *Microsc Res Tech*, 54, 114-22.

- KILLICK, S., TRUSSELL, J., CLELAND, K. & MOREAU, C. 2009. Factors associated with subfertility among women attending an antenatal clinic in Hull. *Human Fertility*, 12, 191-197.
- KIM, S. H. & PLUTZKY, J. 2016. Brown Fat and Browning for the Treatment of Obesity and Related Metabolic Disorders. *Diabetes & metabolism journal*, 40, 12-21.
- KIM, S. R., BAE, Y. H., BAE, S. K., CHOI, K. S., YOON, K. H., KOO, T. H., JANG, H. O., YUN, I., KIM, K. W., KWON, Y. G., YOO, M. A. & BAE, M. K. 2008. Visfatin enhances ICAM-1 and VCAM-1 expression through ROS-dependent NF-kappaB activation in endothelial cells. *Biochim Biophys Acta*, 1783, 886-95.
- KLEIN, U., GIMPL, G. & FAHRENHOLZ, F. 1995. Alteration of the Myometrial Plasma Membrane Cholesterol Content with .beta.-Cyclodextrin Modulates the Binding Affinity of the Oxytocin Receptor. *Biochemistry*, 34, 13784-13793.
- KLOP, B., ELTE, J. W. & CABEZAS, M. C. 2013. Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients*, 5, 1218-40.
- KNOCK, G. A., SMIRNOV, S. V. & AARONSON, P. I. 1999. Voltage-gated K<sup>+</sup> currents in freshly isolated myocytes of the pregnant human myometrium. *J Physiol*, 518 ( Pt 3), 769-81.
- KOMINIAREK, M. A., ZHANG, J., VANVELDHUISEN, P., TROENDLE, J., BEAVER, J. & HIBBARD, J. U. 2011. Contemporary labor patterns: the impact of maternal body mass index. *Am J Obstet Gynecol*, 205, 244.e1-8.
- KOTA, S. K., GAYATRI, K., JAMMULA, S., KOTA, S. K., KRISHNA, S. V., MEHER, L. K. & MODI, K. D. 2013. Endocrinology of parturition. *Indian J Endocrinol Metab*, 17, 50-9.
- KRALISCH, S., SOMMER, G., DECKERT, C. M., LINKE, A., BLUHER, M., STUMVOLL, M. & FASSHAUER, M. 2007. Adipokines in diabetes and cardiovascular diseases. *Minerva endocrinologica*, 32, 161-171.
- KRIZOVA, J., ERETOVA, V., HALUZIKOVA, D., ANDERLOVA, K., HOUSOVA, J., KOTRLIKOVA, E. & HALUZIK, M. 2004. Soluble leptin receptor and leptin levels in pregnant women before and after delivery. *Endocr Res*, 30, 379-85.
- KRZYZANOWSKA, K., KRUGLUGER, W., MITTERMAYER, F., RAHMAN, R., HAIDER, D., SHNAWA, N. & SCHERNTHANER, G. 2006. Increased visfatin concentrations in women with gestational diabetes mellitus. *Clin Sci (Lond)*, 110, 605-9.
- KU, C. Y., BABICH, L., WORD, R. A., ZHONG, M., ULLOA, A., MONGA, M. & SANBORN, B. M. 2006. Expression of Transient Receptor Channel Proteins in Human Fundal Myometrium in Pregnancy. *Journal of the Society for Gynecologic Investigation*, 13, 217-225.
- KUMAR, N. M. & GILULA, N. B. 1996. The gap junction communication channel. *Cell*, 84, 381-8.
- KUPITTAYANANT, S., BURDYGA, T. & WRAY, S. 2001. The effects of inhibiting Rho-associated kinase with Y-27632 on force and intracellular calcium in human myometrium. *Pflugers Arch*, 443, 112-4.
- KUPITTAYANANT, S., LUCKAS, M. J. M. & WRAY, S. 2002. Effect of inhibiting the sarcoplasmic reticulum on spontaneous and oxytocin-induced contractions of human myometrium. *Bjog-an International Journal of Obstetrics and Gynaecology*, 109, 289-296.
- KUTRYK, M. J. & PIERCE, G. N. 1988. Stimulation of sodium-calcium exchange by cholesterol incorporation into isolated cardiac sarcolemmal vesicles. *J Biol Chem*, 263, 13167-72.
- KYPREOS, K. E., KARAGIANNIDES, I., FOTIADOU, E. H., KARAVIA, E. A., BRINKMEIER, M. S., GIAKOUMI, S. M. & TSOMPANIDI, E. M. 2009. Mechanisms of obesity and related pathologies: role of apolipoprotein E in the development of obesity. *Febs j*, 276, 5720-8.
- LAIRD, D. W. 2006. Life cycle of connexins in health and disease. *Biochem J*, 394, 527-43.
- LAIRD, D. W. 2010. The gap junction proteome and its relationship to disease. *Trends in Cell Biology*, 20, 92-101.
- LANG, L. M., BEYER, E. C., SCHWARTZ, A. L. & GITLIN, J. D. 1991. Molecular cloning of a rat uterine gap junction protein and analysis of gene expression during gestation. *Am J Physiol*, 260, E787-93.
- LAPPAS, M. 2012. Visfatin regulates the terminal processes of human labour and delivery via activation of the nuclear factor-kappaB pathway. *Mol Cell Endocrinol*, 348, 128-34.

- LAUGHON, S. K., BERGHELLA, V., REDDY, U. M., SUNDARAM, R., LU, Z. & HOFFMAN, M. K. 2014. Neonatal and maternal outcomes with prolonged second stage of labor. *Obstetrics and gynecology*, 124, 57-67.
- LAVENDER, T., HART, A. & SMYTH, R. M. D. 2013. Effect of partogram use on outcomes for women in spontaneous labour at term. *Cochrane Database of Systematic Reviews*.
- LEAL, V. D. O. & MAFRA, D. 2013. Adipokines in obesity. *Clinica Chimica Acta*, 419, 87-94.
- LEBLANC, N., LEDOUX, J., SALEH, S., SANGUINETTI, A., ANGERMANN, J., O'DRISCOLL, K., BRITTON, F., PERRINO, B. A. & GREENWOOD, I. A. 2005. Regulation of calcium-activated chloride channels in smooth muscle cells: a complex picture is emerging. *Can J Physiol Pharmacol*, 83, 541-56.
- LECKE, S. B., MORSCH, D. M. & SPRITZER, P. M. 2011. Leptin and adiponectin in the female life course. *Braz J Med Biol Res*, 44, 381-7.
- LEDDY, M. A., POWER, M. L. & SCHULKIN, J. 2008. The impact of maternal obesity on maternal and fetal health. *Reviews in obstetrics & gynecology*, 1, 170-178.
- LEE, S.-E., AHN, D.-S. & LEE, Y.-H. 2009. Role of T-type Ca<sup>2+</sup> Channels in the Spontaneous Phasic Contraction of Pregnant Rat Uterine Smooth Muscle. *Korean J Physiol Pharmacol*, 13, 241-249.
- LETH, R. A., ULDBJERG, N., NORGAARD, M., MOLLER, J. K. & THOMSEN, R. W. 2011. Obesity, diabetes, and the risk of infections diagnosed in hospital and post-discharge infections after cesarean section: a prospective cohort study. *Acta Obstet Gynecol Scand*, 90, 501-9.
- LEVY, D., SEIGNEURET, M., BLUZAT, A. & RIGAUD, J. L. 1990. Evidence for proton countertransport by the sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase during calcium transport in reconstituted proteoliposomes with low ionic permeability. *J Biol Chem*, 265, 19524-34.
- LEWANDOWSKI, K. C., STOJANOVIC, N., PRESS, M., TUCK, S. M., SZOSLAND, K., BIENKIEWICZ, M., VATISH, M., LEWINSKI, A., PRELEVIC, G. M. & RANDEVA, H. S. 2007. Elevated serum levels of visfatin in gestational diabetes: a comparative study across various degrees of glucose tolerance. *Diabetologia*, 50, 1033-7.
- LI, W., WANG, Y., SHEN, L., SONG, L., LI, H., LIU, B., YUAN, J. & WANG, Y. 2016. Association between parity and obesity patterns in a middle-aged and older Chinese population: a cross-sectional analysis in the Tongji-Dongfeng cohort study. *Nutrition & metabolism*, 13, 72-72.
- LIPPI, G., ALBIERO, A., MONTAGNANA, M., SALVAGNO, G. L., SCEVAROLLI, S., FRANCHI, M. & GUIDI, G. C. 2007. Lipid and lipoprotein profile in physiological pregnancy. *Clin Lab*, 53, 173-7.
- LIPSCOMBE, D., HELTON, T. D. & XU, W. 2004. L-Type Calcium Channels: The Low Down. *Journal of Neurophysiology*, 92, 2633-2641.
- LISONKOVA, S., MURACA, G. M., POTTS, J., LIAUW, J., CHAN, W. S., SKOLL, A. & LIM, K. I. 2017. Association Between Prepregnancy Body Mass Index and Severe Maternal Morbidity. *Jama*, 318, 1777-1786.
- LOEWENSTEIN, W. R. 1981. Junctional intercellular communication: the cell-to-cell membrane channel. *Physiol Rev*, 61, 829-913.
- LOGAN, C. A., THIEL, L., BORNEMANN, R., KOENIG, W., REISTER, F., BRENNER, H., ROTHENBACHER, D. & GENEIT, J. 2016. Delivery Mode, Duration of Labor, and Cord Blood Adiponectin, Leptin, and C-Reactive Protein: Results of the Population-Based Ulm Birth Cohort Studies. *PLoS One*, 11, e0149918.
- LONGBOTTOM, E. R., LUCKAS, M. J. M., KUPITTAYANANT, S., BADRICK, E., SHMIGOL, T. & WRAY, S. 2000. The effects of inhibiting myosin light chain kinase on contraction and calcium signalling in human and rat myometrium. *Pflugers Archiv-European Journal of Physiology*, 440, 315-321.
- LOVREN, F., PAN, Y., SHUKLA, P. C., QUAN, A., TEOH, H., SZMITKO, P. E., PETERSON, M. D., GUPTA, M., AL-OMRAN, M. & VERMA, S. 2009. Visfatin activates eNOS via Akt and MAP

- kinases and improves endothelial cell function and angiogenesis in vitro and in vivo: translational implications for atherosclerosis. *Am J Physiol Endocrinol Metab*, 296, E1440-9.
- LUCKAS, M. J. M. & WRAY, S. 2000. A comparison of the contractile properties of human myometrium obtained from the upper and lower uterine segments. *BJOG: An International Journal of Obstetrics & Gynaecology*, 107, 1309-1311.
- LUKE, B. & BROWN, M. B. 2007. Elevated risks of pregnancy complications and adverse outcomes with increasing maternal age. *Hum Reprod*, 22, 1264-72.
- LUTZ, T. A. & WOODS, S. C. 2012. Overview of animal models of obesity. *Current protocols in pharmacology*, Chapter 5, Unit5.61-Unit5.61.
- LYE, S. J., NICHOLSON, B. J., MASCARENHAS, M., MACKENZIE, L. & PETROCELLI, T. 1993. Increased expression of connexin-43 in the rat myometrium during labor is associated with an increase in the plasma estrogen:progesterone ratio. *Endocrinology*, 132, 2380-6.
- LYE, S. J. & PORTER, D. G. 1978. Demonstration that progesterone 'blocks' uterine activity in the ewe in vivo by a direct action on the myometrium. *J Reprod Fertil*, 52, 87-94.
- LYNCH, C. M., SEXTON, D. J., HESSION, M. & MORRISON, J. J. 2008. Obesity and Mode of Delivery in Primigravid and Multigravid Women. *Amer J Perinatol*, 25, 163-167.
- LYNES, C., MCLAIN, A. C., YEUNG, E. H., ALBERT, P., LIU, J. & BOGHOSSIAN, N. S. 2017. Interpregnancy weight change and adverse maternal outcomes: a retrospective cohort study. *Ann Epidemiol*, 27, 632-637.e5.
- MA, R. C. W., SCHMIDT, M. I., TAM, W. H., MCINTYRE, H. D. & CATALANO, P. M. 2016. Clinical management of pregnancy in the obese mother: before conception, during pregnancy, and post partum. *Lancet Diabetes Endocrinol*, 4, 1037-1049.
- MACKENZIE, I. Z. 2006. Induction of labour at the start of the new millennium. 131, 989.
- MACKENZIE, L. W. & GARFIELD, R. E. 1985. Hormonal control of gap junctions in the myometrium. *Am J Physiol*, 248, C296-308.
- MAGANN, E. F., DOHERTY, D. A., SANDLIN, A. T., CHAUHAN, S. P. & MORRISON, J. C. 2013. The effects of an increasing gradient of maternal obesity on pregnancy outcomes. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, 53, 250-257.
- MAGED, A. M., BELAL, D. S., MARIE, H. M., RASHWAN, H., ABDELAZIZ, S., GABR, A. A. & ELZAYAT, A. R. 2017. Prospective study of the effect of maternal body mass index on labor progress in nulliparous women in Egypt. *Int J Gynaecol Obstet*, 139, 329-335.
- MAGIAKOU, M. A., MASTORAKOS, G., RABIN, D., MARGIORIS, A. N., DUBBERT, B., CALOGERO, A. E., TSIGOS, C., MUNSON, P. J. & CHROUSOS, G. P. 1996. The maternal hypothalamic-pituitary-adrenal axis in the third trimester of human pregnancy. *Clin Endocrinol (Oxf)*, 44, 419-28.
- MAHLEY, R. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science*, 240, 622-630.
- MAKGOBA, M., SAVVIDOU, M. D. & STEER, P. J. 2012. An analysis of the interrelationship between maternal age, body mass index and racial origin in the development of gestational diabetes mellitus. *BJOG*, 119, 276-82.
- MALAMITSI-PUCHNER, A., BRIANA, D. D., BOUTSIKOU, M., KOUSKOUNI, E., HASSIAKOS, D. & GOURGIOTIS, D. 2007. Perinatal circulating visfatin levels in intrauterine growth restriction. *Pediatrics*, 119, e1314-8.
- MARCH, M. I., WARSOFF, S. L. & CHAUHAN, S. P. 2012. Fetal biometry: relevance in obstetrical practice. *Clin Obstet Gynecol*, 55, 281-7.
- MARGETIC, S., GAZZOLA, C., PEGG, G. G. & HILL, R. A. 2002. Leptin: a review of its peripheral actions and interactions. *International Journal of Obesity*, 26, 1407-1433.
- MARIN, J., ENCABO, A., BRIONES, A., GARCIA-COHEN, E. C. & ALONSO, M. J. 1999. Mechanisms involved in the cellular calcium homeostasis in vascular smooth muscle: calcium pumps. *Life Sci*, 64, 279-303.

- MARSEGLIA, L., MANTI, S., D'ANGELO, G., CUPPARI, C., SALPIETRO, V., FILIPPELLI, M., CHIRICO, V., GITTO, E., SALPIETRO, C. & ARRIGO, T. 2015. The Role of Visfatin in Pregnancy, Complications and Procreation. *J Pediatr Biochem*, 05, 002-007.
- MARTIN, C., CHAPMAN, K. E., THORNTON, S. & ASHLEY, R. H. 1999. Changes in the expression of myometrial ryanodine receptor mRNAs during human pregnancy. *Biochim Biophys Acta*, 1451, 343-52.
- MARVIN, K. W., KEELAN, J. A., EYKHOLT, R. L., SATO, T. A. & MITCHELL, M. D. 2002. Use of cDNA arrays to generate differential expression profiles for inflammatory genes in human gestational membranes delivered at term and preterm. *MHR: Basic science of reproductive medicine*, 8, 399-408.
- MASTORAKOS, G., VALSAMAKIS, G., PAPTAEODOROU, D. C., BARLAS, I., MARGELI, A., BOUSIADIS, A., KOUSKOUNI, E., VITORATOS, N., PAPADIMITRIOU, A., PAPASSOTIRIOU, I. & CREATSAS, G. 2007. The role of adipocytokines in insulin resistance in normal pregnancy: visfatin concentrations in early pregnancy predict insulin sensitivity. *Clin Chem*, 53, 1477-83.
- MATHEW, H., CASTRACANE, V. D. & MANTZOROS, C. 2018. Adipose tissue and reproductive health. *Metabolism - Clinical and Experimental*, 86, 18-32.
- MATSUKI, K., TAKEMOTO, M., SUZUKI, Y., YAMAMURA, H., OHYA, S., TAKESHIMA, H. & IMAIZUMI, Y. 2017. Ryanodine receptor type 3 does not contribute to contractions in the mouse myometrium regardless of pregnancy. *Pflugers Arch*, 469, 313-326.
- MATTAGAJASINGH, I., KIM, C.-S., NAQVI, A., YAMAMORI, T., HOFFMAN, T. A., JUNG, S.-B., DERICCO, J., KASUNO, K. & IRANI, K. 2007. SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 14855-14860.
- MATTHEW, A., SHMYGOL, A. & WRAY, S. 2004. Ca<sup>2+</sup> entry, efflux and release in smooth muscle. *Biol Res*, 37, 617-24.
- MAZAKI-TOVI, S., ROMERO, R., KUSANOVIC, J. P., EREZ, O., GOTSCH, F., MITTAL, P., THAN, N. G., NHAN-CHANG, C. L., HAMILL, N., VAISBUCH, E., CHAIWORAPONGSA, T., EDWIN, S. S., NIEN, J. K., GOMEZ, R., ESPINOZA, J., KENDAL-WRIGHT, C., HASSAN, S. S. & BRYANT-GREENWOOD, G. 2008. Visfatin/Pre-B cell colony-enhancing factor in amniotic fluid in normal pregnancy, spontaneous labor at term, preterm labor and prelabor rupture of membranes: an association with subclinical intrauterine infection in preterm parturition. *J Perinat Med*, 36, 485-96.
- MAZAKI-TOVI, S., ROMERO, R., KUSANOVIC, J. P., VAISBUCH, E., EREZ, O., THAN, N. G., CHAIWORAPONGSA, T., NHAN-CHANG, C.-L., PACORA, P., GOTSCH, F., YEO, L., KIM, S. K., EDWIN, S. S., HASSAN, S. S. & MITTAL, P. 2009a. Visfatin in human pregnancy: maternal gestational diabetes vis-à-vis neonatal birthweight. *Journal of perinatal medicine*, 37, 218-231.
- MAZAKI-TOVI, S., ROMERO, R., KUSANOVIC, J. P., VAISBUCH, E., EREZ, O., THAN, N. G., CHAIWORAPONGSA, T., NHAN-CHANG, C. L., PACORA, P., GOTSCH, F., YEO, L., KIM, S. K., EDWIN, S. S., HASSAN, S. S. & MITTAL, P. 2009b. Maternal visfatin concentration in normal pregnancy. *J Perinat Med*, 37, 206-17.
- MAZAKI-TOVI, S., ROMERO, R., VAISBUCH, E., EREZ, O., CHAIWORAPONGSA, T., MITTAL, P., KIM, S. K., PACORA, P., GOTSCH, F., DONG, Z., HASSAN, S. S. & KUSANOVIC, J. P. 2009c. Maternal plasma visfatin in preterm labor. *J Matern Fetal Neonatal Med*, 22, 693-704.
- MAZAKI-TOVI, S., VAISBUCH, E., ROMERO, R., KUSANOVIC, J. P., CHAIWORAPONGSA, T., KIM, S. K., NHAN-CHANG, C. L., GOMEZ, R., ALPAY SAVASAN, Z., MADAN, I., YOON, B. H., YEO, L., MITTAL, P., OGGE, G., GONZALEZ, J. M. & HASSAN, S. S. 2010. Maternal and neonatal circulating visfatin concentrations in patients with pre-eclampsia and a small-for-gestational age neonate. *J Matern Fetal Neonatal Med*, 23, 1119-28.
- MAZAKI-TOVI, S., VAISBUCH, E. D. I. & ROMERO, R. 2013. ADIPOKINES AND PATHOPHYSIOLOGY OF PREGNANCY COMPLICATIONS – THE ROLE OF LEPTIN AND ADIPONECTIN. *Fetal and Maternal Medicine Review*, 24, 232-259.

- MAZURKIEWICZ, J. C., WATTS, G. F., WARBURTON, F. G., SLAVIN, B. M., LOWY, C. & KOUKKOU, E. 1994. Serum lipids, lipoproteins and apolipoproteins in pregnant non-diabetic patients. *J Clin Pathol*, 47, 728-31.
- MCELVY, S. S., MIODOVNIK, M., MYATT, L., KHOURY, J. & SIDDIQI, T. A. 2000. Is human myometrial sampling at the time of cesarean delivery safe? *Am J Obstet Gynecol*, 183, 1583-6.
- MCGEOWN, J. G. 2004. Interactions between inositol 1,4,5-trisphosphate receptors and ryanodine receptors in smooth muscle: one store or two? *Cell Calcium*, 35, 613-619.
- MCKILLEN, K., THORNTON, S. & TAYLOR, C. W. 1999. Oxytocin increases the  $[Ca^{2+}]_i$  sensitivity of human myometrium during the falling phase of phasic contractions. *Am J Physiol*, 276, E345-51.
- MEI-DAN, E., PITTINI, A., BARRETT, J. & MELAMED, N. 2017. 945: Indication for induction of labor and risk for cesarean section. *American Journal of Obstetrics and Gynecology*, 216, S534-S535.
- MERSHON, J. L., MIKALA, G. & SCHWARTZ, A. 1994. Changes in the Expression of the L-Type Voltage-Dependent Calcium-Channel during Pregnancy and Parturition in the Rat. *Biology of Reproduction*, 51, 993-999.
- MESE, G., RICHARD, G. & WHITE, T. W. 2007. Gap junctions: basic structure and function. *J Invest Dermatol*, 127, 2516-24.
- METWALLY, M., ONG, K. J., LEDGER, W. L. & LI, T. C. 2008. Does high body mass index increase the risk of miscarriage after spontaneous and assisted conception? A meta-analysis of the evidence. *Fertil Steril*, 90, 714-26.
- MEYER, M. R., FREDETTE, N. C., BARTON, M. & PROSSNITZ, E. R. 2013. Regulation of vascular smooth muscle tone by adipose-derived contracting factor. *PLoS One*, 8, e79245.
- MIEHLE, K., STEPAN, H. & FASSHAUER, M. 2012. Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia. *Clinical Endocrinology*, 76, 2-11.
- MIKOSHIBA, K. 2007. IP3 receptor/ $Ca^{2+}$  channel: from discovery to new signaling concepts. *J Neurochem*, 102, 1426-1446.
- MIRONNEAU, J., MACREZ, N., MOREL, J. L., SORRENTINO, V. & MIRONNEAU, C. 2002. Identification and function of ryanodine receptor subtype 3 in non-pregnant mouse myometrial cells. *J Physiol*, 538, 707-16.
- MISRA, V. K. & TRUDEAU, S. 2011. The influence of overweight and obesity on longitudinal trends in maternal serum leptin levels during pregnancy. *Obesity (Silver Spring)*, 19, 416-21.
- MIYOSHI, H., BOYLE, M. B., MACKAY, L. B. & GARFIELD, R. E. 1996. Voltage-clamp studies of gap junctions between uterine muscle cells during term and preterm labor. *Biophys J*, 71, 1324-34.
- MOAYERI, M., HEIDA, K. Y., FRANX, A., SPIERING, W., DE LAAT, M. W. & OUDIJK, M. A. 2017. Maternal lipid profile and the relation with spontaneous preterm delivery: a systematic review. *Arch Gynecol Obstet*, 295, 313-323.
- MODDER J, F. K. 2010. CMACE/RCOG Joint Guideline. Management of Women with Obesity in Pregnancy. *The Centre for Maternal and Child Enquiries and the Royal College of Obstetricians and Gynaecologists*.
- MOHAMMED FAHAD ALOTAIBI. 2012. *An Investigation into the Effect of Hypoxia in the Uterus; Does Hypoxic Preconditioning Occur?*, University of Liverpool.
- MOLLER, J. V., JUUL, B. & LE MAIRE, M. 1996. Structural organization, ion transport, and energy transduction of P-type ATPases. *Biochim Biophys Acta*, 1286, 1-51.
- MONCADA, S. & HIGGS, E. A. 2006. Nitric oxide and the vascular endothelium. *Handb Exp Pharmacol*, 213-54.
- MONGA, M. & SANBORN, B. M. 2004. *Biology and physiology of the reproductive tract and control of myometrial contraction*.

- MONTELONGO, A., LASUNCION, M. A., PALLARDO, L. F. & HERRERA, E. 1992. Longitudinal study of plasma lipoproteins and hormones during pregnancy in normal and diabetic women. *Diabetes*, 41, 1651-9.
- MORGAN, J. M., DE SMEDT, H. & GILLESPIE, J. I. 1996. Identification of three isoforms of the InsP3 receptor in human myometrial smooth muscle. *Pflugers Arch*, 431, 697-705.
- MORGAN, K. L., RAHMAN, M. A., MACEY, S., ATKINSON, M. D., HILL, R. A., KHANOM, A., PARANJOTHY, S., HUSAIN, M. J. & BROPHY, S. T. 2014. Obesity in pregnancy: a retrospective prevalence-based study on health service utilisation and costs on the NHS. *BMJ Open*, 4, e003983.
- MORGAN, S. A., BRINGOLF, J. B. & SEIDEL, E. R. 2008. Visfatin expression is elevated in normal human pregnancy. *Peptides*, 29, 1382-9.
- MORIZAKI, N., MORIZAKI, J., HAYASHI, R. H. & GARFIELD, R. E. 1989. A functional and structural study of the innervation of the human uterus. *American Journal of Obstetrics and Gynecology*, 160, 218-228.
- MOSCHOS, S., CHAN, J. L. & MANTZOROS, C. S. 2002. Leptin and reproduction: a review. *Fertil Steril*, 77, 433-44.
- MOSTAFA, A. F. & SAMIR, S. M. 2013. What is the effect of ghrelin on rat uterine contractility in vitro? *J Basic Clin Physiol Pharmacol*, 24, 137-42.
- MOYNIHAN, A. T., HEHIR, M. P., GLAVEY, S. V., SMITH, T. J. & MORRISON, J. J. 2006. Inhibitory effect of leptin on human uterine contractility in vitro. *American Journal Of Obstetrics And Gynecology*, 195, 504-509.
- MUDD, L. M., HOLZMAN, C. B., CATOV, J. M., SENAGORE, P. K. & EVANS, R. W. 2012. Maternal lipids at mid-pregnancy and the risk of preterm delivery. *Acta Obstetrica et Gynecologica Scandinavica*, 91, 726-735.
- MUIR, R., BALLAN, J., CLIFFORD, B., MCMULLEN, S., KHAN, R., SHMYGOL, A., QUENBY, S. & ELMES, M. 2016. Modelling maternal obesity: the effects of a chronic high-fat, high-cholesterol diet on uterine expression of contractile-associated proteins and ex vivo contractile activity during labour in the rat. *Clin Sci (Lond)*, 130, 183-92.
- MULLER, P. S. & NIRMALA, M. 2018. Effects of Pre pregnancy Maternal Body Mass Index on Gestational Diabetes Mellitus. *International Journal of Engineering and Technology, International Journal of Engineering and Technology UAE*, 7, 279-282.
- MUMTAZ, S., ALSAIF, S., WRAY, S. & NOBLE, K. 2015. Inhibitory effect of visfatin and leptin on human and rat myometrial contractility. *Life Sci*, 125, 57-62.
- MÜNZBERG, H. & MORRISON, C. D. 2015. Structure, production and signaling of leptin. *Metabolism: clinical and experimental*, 64, 13-23.
- MURATA, M., PERANEN, J., SCHREINER, R., WIELAND, F., KURZCHALIA, T. V. & SIMONS, K. 1995. VIP21/caveolin is a cholesterol-binding protein. *Proc Natl Acad Sci U S A*, 92, 10339-43.
- MYERS, M. G., JR., LEIBEL, R. L., SEELEY, R. J. & SCHWARTZ, M. W. 2010. Obesity and leptin resistance: distinguishing cause from effect. *Trends Endocrinol Metab*, 21, 643-51.
- NAJAFI, F., HASANI, J., IZADI, N., HASHEMI-NAZARI, S.-S., NAMVAR, Z., MOHAMMADI, S. & SADEGHI, M. 2019. The effect of prepregnancy body mass index on the risk of gestational diabetes mellitus: A systematic review and dose-response meta-analysis. *Obesity Reviews*, 20, 472-486.
- NAKAJIMA, A. 1971. Action potential of human myometrial fibers. *American Journal of Obstetrics & Gynecology*, 111, 266-269.
- NASCIMENTO, I. B. D., SALES, W. B., FLEIG, R., SILVA, G. D. D. & SILVA, J. C. 2016. Excess weight and dyslipidemia and their complications during pregnancy: a systematic review. *Revista Brasileira de Saúde Materno Infantil*, 16, 93-101.
- NASIOUDIS, D., DOULAVERIS, G. & KANNINEN, T. T. 2019. Dyslipidemia in pregnancy and maternal-fetal outcome. *Minerva Ginecol*, 71, 155-162.
- NATAMBA, B. K., SANCHEZ, S. E., GELAYE, B. & WILLIAMS, M. A. 2016. Concordance between self-reported pre-pregnancy body mass index (BMI) and BMI measured at the first prenatal study contact. *BMC Pregnancy Childbirth*, 16, 187.

- NATIONAL HEALTH SERVICE 2016. Maternity Services Monthly Statistics, England - September and October 2015, Experimental statistics.
- NATIONAL HEALTH SERVICE 2019. Statistics on Obesity, Physical Activity and Diet, England.
- NEAL, J. L., LOWE, N. K., PATRICK, T. E., CABBAGE, L. A. & CORWIN, E. J. 2010. What is the slowest-yet-normal cervical dilation rate among nulliparous women with spontaneous labor onset? *Journal of obstetric, gynecologic, and neonatal nursing : JOGNN*, 39, 361-369.
- NEDERLOF, M., DE WALLE, H. E., VAN POPPEL, M. N., VRIJKOTTE, T. G. & GADEMAN, M. G. 2015. Deviant early pregnancy maternal triglyceride levels and increased risk of congenital anomalies: a prospective community-based cohort study. *Bjog*, 122, 1176-83.
- NEILSON, J., LAVENDER, T., QUENBY, S. & WRAY, S. 2003. Obstructed labour: Reducing maternal death and disability during pregnancy. *British Medical Bulletin*, 67, 191-204.
- NELSON, J. F., KARELUS, K., FELICIO, L. S. & JOHNSON, T. E. 1990. Genetic influences on the timing of puberty in mice. *Biol Reprod*, 42, 649-55.
- NELSON, M. T., CONWAY, M. A., KNOT, H. J. & BRAYDEN, J. E. 1997. Chloride channel blockers inhibit myogenic tone in rat cerebral arteries. *J Physiol*, 502 ( Pt 2), 259-64.
- NELSON, S. M., MATTHEWS, P. & POSTON, L. 2010. Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. *Human reproduction update*, 16, 255-275.
- NELSON, T. J., SUN, M. K., HONGPAISAN, J. & ALKON, D. L. 2008. Insulin, PKC signaling pathways and synaptic remodeling during memory storage and neuronal repair. *Eur J Pharmacol*, 585, 76-87.
- NESHEIM, B.-I. 1988. Duration of Labor: An analysis of influencing factors. *Acta Obstetrica et Gynecologica Scandinavica*, 67, 121-124.
- NEUMANN, K., INDORF, I., HÄRTEL, C., CIRKEL, C., RODY, A. & BEYER, D. A. 2017. C-Section Prevalence Among Obese Mothers and Neonatal Hypoglycemia: a Cohort Analysis of the Department of Gynecology and Obstetrics of the University of Lübeck. *Geburtshilfe und Frauenheilkunde*, 77, 487-494.
- NIELSEN, M. S., AXELSEN, L. N., SORGEN, P. L., VERMA, V., DELMAR, M. & HOLSTEIN-RATHLOU, N.-H. 2012. Gap junctions. *Comprehensive Physiology*, 2, 1981-2035.
- NKOKA, O., NTENDA, P. A. M., SENGHORE, T. & BASS, P. 2019. Maternal overweight and obesity and the risk of caesarean birth in Malawi. *Reproductive health*, 16, 40-40.
- NOBLE, K., FLOYD, R., SHMYGOL, A., SHMYGOL, A., MOBASHERI, A. & WRAY, S. 2010. Distribution, expression and functional effects of small conductance Ca-activated potassium (SK) channels in rat myometrium. *Cell Calcium*, 47, 47-54.
- NOBLE, K., MATTHEW, A., BURDYGA, T. & WRAY, S. 2009. A review of recent insights into the role of the sarcoplasmic reticulum and Ca entry in uterine smooth muscle. *European Journal of Obstetrics and Gynecology*, 144, S11-S19.
- NOBLE, K., ZHANG, J. & WRAY, S. 2006. Lipid rafts, the sarcoplasmic reticulum and uterine calcium signalling: an integrated approach. *J Physiol*, 570, 29-35.
- NOHR, E. A., VAETH, M., BAKER, J. L., SORENSEN, T., OLSEN, J. & RASMUSSEN, K. M. 2008. Combined associations of prepregnancy body mass index and gestational weight gain with the outcome of pregnancy. *Am J Clin Nutr*, 87, 1750-9.
- NORMAN, J. 1996. Nitric oxide and the myometrium. *Pharmacology & Therapeutics*, 70, 91-100.
- NORMAN, J. E. & REYNOLDS, R. M. 2011. The consequences of obesity and excess weight gain in pregnancy. *Proc Nutr Soc*, 70, 450-6.
- NORMAN, S. M., TUULI, M. G., ODIBO, A. O., CAUGHEY, A. B., ROEHL, K. A. & CAHILL, A. G. 2012. The effects of obesity on the first stage of labor. *Obstet Gynecol*, 120, 130-5.
- NORWITZ, E. R., ROBINSON, J. N. & CHALLIS, J. R. 1999. The control of labor. *N Engl J Med*, 341, 660-6.
- NUAMAH, M. A., YURA, S., SAGAWA, N., ITOH, H., MISE, H., KORITA, D., KAKUI, K., TAKEMURA, M., OGAWA, Y., NAKAO, K. & FUJII, S. 2004. Significant increase in maternal plasma leptin concentration in induced delivery: a possible contribution of pro-inflammatory cytokines to placental leptin secretion. *Endocr J*, 51, 177-87.

- NYSTEDT, A. & HILDINGSSON, I. 2014. Diverse definitions of prolonged labour and its consequences with sometimes subsequent inappropriate treatment. *BMC pregnancy and childbirth*, 14, 233-233.
- O'BRIEN, M., CARBIN, S., MORRISON, J. J. & SMITH, T. J. 2013. Decreased myometrial p160 ROCK-1 expression in obese women at term pregnancy. *Reprod Biol Endocrinol*, 11, 79.
- O'BRIEN, M., EARLEY, P., MORRISON, J. J. & SMITH, T. J. 2010. Ghrelin in the human myometrium. *Reprod Biol Endocrinol*, 8, 55.
- O'DWYER, V., O'KELLY, S., MONAGHAN, B., ROWAN, A., FARAH, N. & TURNER, M. J. 2013. Maternal obesity and induction of labor. *Acta Obstetrica et Gynecologica Scandinavica*, 92, 1414-1418.
- O'MALLEY, D., IRVING, A. J. & HARVEY, J. 2005. Leptin-induced dynamic alterations in the actin cytoskeleton mediate the activation and synaptic clustering of BK channels. *FASEB J*, 19, 1917-9.
- OECD 2018. Obesity Update.
- OGNJANOVIC, S. & BRYANT-GREENWOOD, G. D. 2002. Pre-B-cell colony-enhancing factor, a novel cytokine of human fetal membranes. *American Journal of Obstetrics and Gynecology*, 187, 1051-1058.
- OGNJANOVIC, S., KU, T. L. & BRYANT-GREENWOOD, G. D. 2005. Pre-B-cell colony-enhancing factor is a secreted cytokine-like protein from the human amniotic epithelium. *American Journal of Obstetrics and Gynecology*, 193, 273-282.
- OGNJANOVIC, S., TASHIMA, L. S. & BRYANT-GREENWOOD, G. D. 2003. The effects of pre-B-cell colony-enhancing factor on the human fetal membranes by microarray analysis. *Am J Obstet Gynecol*, 189, 1187-95.
- OKABE, K., INOUE, Y. & SOEDA, H. 1999. Estradiol inhibits Ca<sup>2+</sup> and K<sup>+</sup> channels in smooth muscle cells from pregnant rat myometrium. *European Journal of Pharmacology*, 376, 101-108.
- OLESEN, A. W., WESTERGAARD, J. G. & JQRN, O. 2006. Prenatal risk indicators of a prolonged pregnancy. The Danish Birth Cohort 1998–2001. *Acta Obstetrica et Gynecologica Scandinavica*, 85, 1338-1341.
- OLUWOLE, A. A., ADEGBESAN-OMILABU, M. A. & OKUNADE, K. S. 2014. Preterm delivery and low maternal serum cholesterol level: Any correlation? *Niger Med J*, 55, 406-10.
- ORGANIZATION, W. H. 2011. WHO Recommendations for Induction of Labour.
- ORSINO, A., TAYLOR, C. V. & LYE, S. J. 1996. Connexin-26 and connexin-43 are differentially expressed and regulated in the rat myometrium throughout late pregnancy and with the onset of labor. *Endocrinology*, 137, 1545-53.
- ORTEGA, A. & MASOLIVA, J. 1984. Cholesterol Effect on Enzyme-Activity of the Sarcolemmal (Ca<sup>2+</sup>Mg<sup>2+</sup>)-Atpase from Cardiac-Muscle. *Biochimica Et Biophysica Acta*, 773, 231-236.
- OSMERS, R. G. W., BLASER, J., KUHN, W. & TSCHESCHE, H. 1995. Interleukin-8 synthesis and the onset of labor. *Obstetrics & Gynecology*, 86, 223-229.
- OSTBORG, T. B., ROMUNDSTAD, P. R. & EGGEBO, T. M. 2017. Duration of the active phase of labor in spontaneous and induced labors. *Acta Obstet Gynecol Scand*, 96, 120-127.
- OU, C. W., ORSINO, A. & LYE, S. J. 1997. Expression of connexin-43 and connexin-26 in the rat myometrium during pregnancy and labor is differentially regulated by mechanical and hormonal signals. *Endocrinology*, 138, 5398-407.
- OUCHI, N., PARKER, J. L., LUGUS, J. J. & WALSH, K. 2011. Adipokines in inflammation and metabolic disease. *Nature reviews. Immunology*, 11, 85-97.
- OZKAN, S., EREL, C. T., MADAZLI, R. & AYDINLI, K. 2005. Serum leptin levels in hypertensive disorder of pregnancy. *Eur J Obstet Gynecol Reprod Biol*, 120, 158-63.
- PALESTINI, P., BOTTO, L., RIVOLTA, I. & MISEROCCHI, G. 2011. Remodelling of Membrane Rafts Expression in Lung Cells as an Early Sign of Mechanotransduction-Signalling in Pulmonary Edema.

- PAPAZIAN, T., ABI TAYEH, G., SIBAI, D., HOUT, H., MELKI, I. & RABBAA KHABBAZ, L. 2017. Impact of maternal body mass index and gestational weight gain on neonatal outcomes among healthy Middle-Eastern females. *PLoS One*, 12, e0181255.
- PARK, H. K. & AHIMA, R. S. 2015. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. *Metabolism*, 64, 24-34.
- PARKINGTON, H. C. & COLEMAN, H. A. 1988. Ionic mechanisms underlying action potentials in myometrium. *Clin Exp Pharmacol Physiol*, 15, 657-65.
- PARKINGTON, H. C. & COLEMAN, H. A. 1990. The Role of Membrane Potential in the Control of Uterine Motility. In: CARSTEN, M. E. & MILLER, J. D. (eds.) *Uterine Function: Molecular and Cellular Aspects*. Boston, MA: Springer US.
- PARKINGTON, H. C. & COLEMAN, H. A. 2001. Excitability in uterine smooth muscle. *Front Horm Res*, 27, 179-200.
- PARKINGTON, H. C., STEVENSON, J., TONTA, M. A., PAUL, J., BUTLER, T., MAITI, K., CHAN, E. C., SHEEHAN, P. M., BRENNECKE, S. P., COLEMAN, H. A. & SMITH, R. 2014. Diminished hERG K<sup>+</sup> channel activity facilitates strong human labour contractions but is dysregulated in obese women. *Nat Commun*, 5, 4108.
- PARKINGTON, H. C., TONTA, M. A., BRENNECKE, S. P. & COLEMAN, H. A. 1999. Contractile activity, membrane potential, and cytoplasmic calcium in human uterine smooth muscle in the third trimester of pregnancy and during labor. *Am J Obstet Gynecol*, 181, 1445-51.
- PASQUALI, R., VICENNATI, V., CACCIARI, M. & PAGOTTO, U. 2006. The hypothalamic-pituitary-adrenal axis activity in obesity and the metabolic syndrome. *Ann N Y Acad Sci*, 1083, 111-28.
- PAULINO, D. S., SURITA, F. G., PERES, G. B., DO NASCIMENTO, S. L. & MORAIS, S. S. 2016. Association between parity, pre-pregnancy body mass index and gestational weight gain. *J Matern Fetal Neonatal Med*, 29, 880-4.
- PAVLOVÁ, T., NOVÁK, J. & BIENERTOVÁ-VAŠKŮ, J. 2015. The role of visfatin (PBEF/Nampt) in pregnancy complications. *Journal of Reproductive Immunology*, 112, 102-110.
- PEHLIVANOĞLU, B., BAYRAK, S. & DOĞAN, M. 2013. A close look at the contraction and relaxation of the myometrium; the role of calcium. *Journal of the Turkish German Gynecological Association*, 14, 230-234.
- PENDSE, A. A., ARBONES-MAINAR, J. M., JOHNSON, L. A., ALTENBURG, M. K. & MAEDA, N. 2009. Apolipoprotein E knock-out and knock-in mice: atherosclerosis, metabolic syndrome, and beyond. *J Lipid Res*, 50 Suppl, S178-82.
- PÉREZ-PÉREZ, A., MAYMÓ, J. L., GAMBINO, Y. P., GUADIX, P., DUEÑAS, J. L., VARONE, C. L. & SÁNCHEZ-MARGALET, V. 2013. Activated Translation Signaling in Placenta from Pregnant Women with Gestational Diabetes Mellitus: Possible Role of Leptin. *Horm Metab Res*, 45, 436-442.
- PEREZ-REYES, E. 2003. Molecular physiology of low-voltage-activated t-type calcium channels. *Physiol Rev*, 83, 117-61.
- PEREZ-REYES, E. 2004. Paradoxical role of T-type calcium channels in coronary smooth muscle. *Mol Interv*, 4, 16-8.
- PEREZ, G. J., TORO, L., ERULKAR, S. D. & STEFANI, E. 1993. Characterization of large-conductance, calcium-activated potassium channels from human myometrium. *Am J Obstet Gynecol*, 168, 652-60.
- PERLOW, J. H. & MORGAN, M. A. 1994. Massive maternal obesity and perioperative cesarean morbidity. *Am J Obstet Gynecol*, 170, 560-5.
- PETERSEN, C. C., BERRIDGE, M. J., BORGESE, M. F. & BENNETT, D. L. 1995. Putative capacitative calcium entry channels: expression of Drosophila trp and evidence for the existence of vertebrate homologues. *Biochem J*, 311 ( Pt 1), 41-4.
- PEVZNER, L., POWERS, B. L., RAYBURN, W. F., RUMNEY, P. & WING, D. A. 2009. Effects of maternal obesity on duration and outcomes of prostaglandin cervical ripening and labor induction. *Obstet Gynecol*, 114, 1315-21.

- PHILLIPS, M. 2014. *Apolipoprotein E Isoforms and Lipoprotein Metabolism*.
- PIERCE, S. J., KUPITTAYANANT, S., SHMYGOL, T. & WRAY, S. 2003. The effects of pH change on Ca<sup>++</sup> signaling and force in pregnant human myometrium. *American Journal of Obstetrics & Gynecology*, 188, 1031-1038.
- PIKE AND LINDA 2003. Lipid rafts: bringing order to chaos. *J Lipid Res* 44, 655-67.
- PIKE, L. J., HAN, X. L., CHUNG, K. N. & GROSS, R. W. 2002. Lipid rafts are enriched in arachidonic acid and plasmenylethanolamine and their composition is independent of caveolin-1 expression: A quantitative electrospray ionization/mass spectrometric analysis. *Biochemistry*, 41, 2075-2088.
- PIPER, J. M., BOLLING, D. R. & NEWTON, E. R. 1991. The second stage of labor: factors influencing duration. *Am J Obstet Gynecol*, 165, 976-9.
- PLOWDEN, T., ZAREK, S., SCHISTERMAN, E., SJAARDA, L., SILVER, R. M., GALAI, N., DECHERNEY, A. & MUMFORD, S. L. 2015. Association between leptin and adverse pregnancy outcomes. *Fertility and Sterility*, 104, e108.
- POMERLEAU, J., PUDULE, I., GRINBERGA, D., KADZIAUSKIENE, K., ABARAVICIUS, A., BARTKEVICIUTE, R., VAASK, S., ROBERTSON, A. & MCKEE, M. 2000. Patterns of body weight in the Baltic Republics. *Public Health Nutr*, 3, 3-10.
- POOBALAN, A. S., AUCOTT, L. S., GURUNG, T., SMITH, W. C. S. & BHATTACHARYA, S. 2009. Obesity as an independent risk factor for elective and emergency caesarean delivery in nulliparous women – systematic review and meta-analysis of cohort studies. *Obesity Reviews*, 10, 28-35.
- POWE, C. E. 2017. Early Pregnancy Biochemical Predictors of Gestational Diabetes Mellitus. *Curr Diab Rep*, 17, 12.
- PRALLE, A., KELLER, P., FLORIN, E. L., SIMONS, K. & HORBER, J. K. 2000. Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. *J Cell Biol*, 148, 997-1008.
- PRENDERGAST, C., QUAYLE, J., BURDYGA, T. & WRAY, S. 2014. Atherosclerosis affects calcium signalling in endothelial cells from apolipoprotein E knockout mice before plaque formation. *Cell Calcium*, 55, 146-54.
- PRINS, J., GOMEZ-LOPEZ, N. & ROBERTSON, S. 2012. *Interleukin-6 in pregnancy and gestational disorder*.
- PULKKINEN, M. O., NYMAN, S., HAMALAINEN, M. M. & MATTINEN, J. 1998. Proton NMR spectroscopy of the phospholipids in human uterine smooth muscle and placenta. *Gynecol Obstet Invest*, 46, 220-4.
- PUTNEY, J. W., JR. & RIBEIRO, C. M. 2000. Signaling pathways between the plasma membrane and endoplasmic reticulum calcium stores. *Cell Mol Life Sci*, 57, 1272-86.
- QUENBY, S., PIERCE, S. J., BRIGHAM, S. & WRAY, S. 2004. Dysfunctional labor and myometrial lactic acidosis. *Obstet Gynecol*, 103, 718-23.
- QUEST, A. F. G., LEYTON, L. & PÁRRAGA, M. 2004. Caveolins, caveolae, and lipid rafts in cellular transport, signaling, and disease. *Biochemistry and Cell Biology*, 82, 129-144.
- R PARRATT, J., TAGGART, M. & WRAY, S. 1995. *Functional effects of intracellular pH alteration in the human uterus: Simultaneous measurements of pH and force*.
- RABE, K., LEHRKE, M., PARHOFER, K. G. & BROEDL, U. C. 2008. Adipokines and insulin resistance. *Molecular medicine (Cambridge, Mass.)*, 14, 741-751.
- RADULESCU, L., MUNTEANU, O., POPA, F. & CIRSTOIU, M. 2013. The implications and consequences of maternal obesity on fetal intrauterine growth restriction. *Journal of medicine and life*, 6, 292-298.
- RAMSAY, J. E., FERRELL, W. R., CRAWFORD, L., WALLACE, A. M., GREER, I. A. & SATTAR, N. 2002. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. *J Clin Endocrinol Metab*, 87, 4231-7.
- RAMSAY, J. E., GREER, I. & SATTAR, N. 2006. ABC of obesity. Obesity and reproduction. *BMJ*, 333, 1159-62.

- RANKIN, J., TENNANT, P. W. G., STOTHARD, K. J., BYTHELL, M., SUMMERBELL, C. D. & BELL, R. 2010. Maternal body mass index and congenital anomaly risk: a cohort study. *International Journal Of Obesity*, 34, 1371.
- RASMUSSEN, K. M., CATALANO, P. M. & YAKTINE, A. L. 2009. New guidelines for weight gain during pregnancy: what obstetrician/gynecologists should know. *Curr Opin Obstet Gynecol*, 21, 521-6.
- RASMUSSEN, S. A., CHU, S. Y., KIM, S. Y., SCHMID, C. H. & LAU, J. 2008. Maternal obesity and risk of neural tube defects: a metaanalysis. *Am J Obstet Gynecol*, 198, 611-9.
- REINL, E. L., CABEZA, R., GREGORY, I. A., CAHILL, A. G. & ENGLAND, S. K. 2015. Sodium leak channel, non-selective contributes to the leak current in human myometrial smooth muscle cells from pregnant women. *Mol Hum Reprod*, 21, 816-24.
- RILEY, H. 2011. Weight management before, during and after pregnancy – what are the ‘rules’? *Nutrition Bulletin*, 36, 212-215.
- RISEK, B., GUTHRIE, S., KUMAR, N. & GILULA, N. B. 1990. Modulation of gap junction transcript and protein expression during pregnancy in the rat. *J Cell Biol*, 110, 269-82.
- RISEK, B., KLIER, F. G., PHILLIPS, A., HAHN, D. W. & GILULA, N. B. 1995. Gap junction regulation in the uterus and ovaries of immature rats by estrogen and progesterone. *J Cell Sci*, 108 ( Pt 3), 1017-32.
- ROBERTS, J. M., BODNAR, L. M., PATRICK, T. E. & POWERS, R. W. 2011. The Role of Obesity in Preeclampsia. *Pregnancy hypertension*, 1, 6-16.
- ROBINSON, B. K., MAPP, D. C., BLOOM, S. L., ROUSE, D. J., SPONG, C. Y., VARNER, M. W., RAMIN, S. M., SOROKIN, Y., SCISCIONE, A., MERCER, B. M., THORP, J. M., JR., MALONE, F. D., HARPER, M., EHRENBERG, H., EUNICE KENNEDY SHRIVER NATIONAL INSTITUTE OF CHILD, H. & HUMAN DEVELOPMENT OF THE MATERNAL-FETAL MEDICINE UNITS, N. 2011. Increasing maternal body mass index and characteristics of the second stage of labor. *Obstet Gynecol*, 118, 1309-13.
- RODRÍGUEZ-MESA, N., ROBLES-BENAYAS, P., RODRÍGUEZ-LÓPEZ, Y., PÉREZ-FERNÁNDEZ, M. E. & COBO-CUENCA, I. A. 2019. Influence of Body Mass Index on Gestation and Delivery in Nulliparous Women: A Cohort Study. *International Journal of Environmental Research and Public Health*, 16.
- RÓG, T. & VATTULAINEN, I. 2014. Cholesterol, sphingolipids, and glycolipids: What do we know about their role in raft-like membranes? *Chemistry and Physics of Lipids*, 184, 82-104.
- ROMERO, R., ESPINOZA, J., GONCALVES, L. F., KUSANOVIC, J. P., FRIEL, L. A. & NIEN, J. K. 2006. Inflammation in preterm and term labour and delivery. *Semin Fetal Neonatal Med*, 11, 317-26.
- RONGVAUX, A., SHEA, R. J., MULKS, M. H., GIGOT, D., URBAIN, J., LEO, O. & ANDRIS, F. 2002. Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. *Eur J Immunol*, 32, 3225-34.
- ROSENBERG, L., PALMER, J. R., WISE, L. A., HORTON, N. J., KUMANYIKA, S. K. & ADAMS-CAMPBELL, L. L. 2003. A prospective study of the effect of childbearing on weight gain in African-American women. *Obes Res*, 11, 1526-35.
- ROSENBLATT, K., GURNANI, P., PASTOR, J., WRIGHT, C., EVANS, M., GALEN, R., CARUSO, D., LEON, J. & GREENWOOD, P. B. 2007. Improved sensitivity (SEN) & positive predictive value (PPV) for the detection of pre-term labor (PTL): A new multivariate quantitative protein microarray serum panel.
- ROTHBERG, K. G., HEUSER, J. E., DONZELL, W. C., YING, Y. S., GLENNEY, J. R. & ANDERSON, R. G. 1992. Caveolin, a protein component of caveolae membrane coats. *Cell*, 68, 673-82.
- ROWE, R., KNIGHT, M. & KURINCZUK, J. J. 2018. Outcomes for women with BMI>35kg/m2 admitted for labour care to alongside midwifery units in the UK: A national prospective cohort study using the UK Midwifery Study System (UKMidSS). *PLoS One*, 13, e0208041.

- RUDMANN, D. G. & FOLEY, G. L. 2013. Chapter 60 - Female Reproductive System. In: HASCHEK, W. M., ROUSSEAU, C. G. & WALLIG, M. A. (eds.) *Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition)*. Boston: Academic Press.
- SADDI-ROSA, P., OLIVEIRA, C. S., GIUFFRIDA, F. M. & REIS, A. F. 2010. Visfatin, glucose metabolism and vascular disease: a review of evidence. *Diabetol Metab Syndr*, 2, 21.
- SAKAI, N., TABB, T. & GARFIELD, R. E. 1992. Studies of connexin 43 and cell-to-cell coupling in cultured human uterine smooth muscle. *Am J Obstet Gynecol*, 167, 1267-77.
- SÁMANO, R., MARTÍNEZ-ROJANO, H., CHICO-BARBA, G., GODÍNEZ-MARTÍNEZ, E., SÁNCHEZ-JIMÉNEZ, B., MONTIEL-OJEDA, D. & TOLENTINO, M. 2017. Serum Concentration of Leptin in Pregnant Adolescents Correlated with Gestational Weight Gain, Postpartum Weight Retention and Newborn Weight/Length. *Nutrients*, 9.
- SAMY, M., SANAD, Z., EMARA, M. & MOHAMED ABDOU, S. 2015. Effect of obesity on the length of the first and second stages of labor. *Menoufia Medical Journal*, 28, 858-863.
- SANBORN, B. M. 1995. Ion channels and the control of myometrial electrical activity. *Semin Perinatol*, 19, 31-40.
- SANBORN, B. M. 2000. Relationship of ion channel activity to control of myometrial calcium. *J Soc Gynecol Investig*, 7, 4-11.
- SANDSTRÖM, A., ALTMAN, M., CNATTINGIUS, S., JOHANSSON, S., AHLBERG, M. & STEPHANSSON, O. 2017. Durations of second stage of labor and pushing, and adverse neonatal outcomes: a population-based cohort study. *Journal of perinatology : official journal of the California Perinatal Association*, 37, 236-242.
- SARAVANAKUMAR, K., RAO, S. G. & COOPER, G. M. 2006. Obesity and obstetric anaesthesia. *Anaesthesia*, 61, 36-48.
- SATPATHY, H. K., FLEMING, A., FREY, D., BARSOOM, M., SATPATHY, C. & KHANDALAVALA, J. 2008. Maternal obesity and pregnancy. *Postgrad Med*, 120, E01-9.
- SCHAEFER, E. J., GREGG, R. E., GHISELLI, G., FORTE, T. M., ORDOVAS, J. M., ZECH, L. A. & BREWER, H. B., JR. 1986. Familial apolipoprotein E deficiency. *The Journal of Clinical Investigation*, 78, 1206-1219.
- SCHNEID-KOFMAN, N., SHEINER, E., LEVY, A. & HOLCBERG, G. 2005. Risk factors for wound infection following cesarean deliveries. *Int J Gynaecol Obstet*, 90, 10-5.
- SCHNEIDER, L., VASCONCELLOS SCHMITT, J. S., DIAS, T. B., DA ROCHA, A. C. G., BAPTISTELLA DO NASCIMENTO, I. & SILVA, J. C. 2019. Evaluation of neonatal and obstetric outcomes according to increased or decreased body mass index of the pregnant woman. *Obesity Medicine*, 14, 100100.
- SCHOLL, T. O., HEDIGER, M. L., KHOO, C. S., HEALEY, M. F. & RAWSON, N. L. 1991. Maternal weight gain, diet and infant birth weight: correlations during adolescent pregnancy. *J Clin Epidemiol*, 44, 423-8.
- SCHREYER, S. A., VICK, C., LYSTIG, T. C., MYSTKOWSKI, P. & LEBOEUF, R. C. 2002. LDL receptor but not apolipoprotein E deficiency increases diet-induced obesity and diabetes in mice. *Am J Physiol Endocrinol Metab*, 282, E207-14.
- SCIFRES, C. M., FEGHALI, M. N., ALTHOUSE, A. D., CARITIS, S. N. & CATOV, J. M. 2014. Effect of excess gestational weight gain on pregnancy outcomes in women with type 1 diabetes. *Obstet Gynecol*, 123, 1295-302.
- SCOTT-PILLAI, R., SPENCE, D., CARDWELL, C. R., HUNTER, A. & HOLMES, V. A. 2013. The impact of body mass index on maternal and neonatal outcomes: a retrospective study in a UK obstetric population, 2004-2011. *Bjog*, 120, 932-9.
- SCROYEN, I., HEMMERYCKX, B. & LIJNEN, H. R. 2013. From mice to men--mouse models in obesity research: what can we learn? *Thromb Haemost*, 110, 634-40.
- SEDA, M., PINTO, F. M., WRAY, S., CINTADO, C. G., NOHEDA, P., BUSCHMANN, H. & CANDENAS, L. 2007. Functional and Molecular Characterization of Voltage-Gated Sodium Channels in Uteri from Nonpregnant Rats<sup>1</sup>. *Biology of Reproduction*, 77, 855-863.
- SEGAWA, K., FUKUHARA, A., HOSOGAI, N., MORITA, K., OKUNO, Y., TANAKA, M., NAKAGAWA, Y., KIHARA, S., FUNAHASHI, T., KOMURO, R., MATSUDA, M. & SHIMOMURA, I. 2006.

- Visfatin in adipocytes is upregulated by hypoxia through HIF1 $\alpha$ -dependent mechanism. *Biochemical and Biophysical Research Communications*, 349, 875-882.
- SEIJO, M., MINCKAS, N., CORMICK, G., COMANDE, D., CIAPPONI, A. & BELIZAN, J. M. 2018. Comparison of self-reported and directly measured weight and height among women of reproductive age: a systematic review and meta-analysis. *Acta Obstet Gynecol Scand*, 97, 429-439.
- SENECAL, J., XIONG, X. & FRASER, W. D. 2005. Effect of fetal position on second-stage duration and labor outcome. *Obstet Gynecol*, 105, 763-72.
- SETHI, J. K. & VIDAL-PUIG, A. 2005. Visfatin: the missing link between intra-abdominal obesity and diabetes? *Trends in Molecular Medicine*, 11, 344-347.
- SHABAN, M. M., BASSIOUNY, Y. A., ELZAHABY, I. M. & HASSAN, A. A. 2014. Body mass index and labour outcome in Egyptian women. *J Obstet Gynaecol*, 34, 248-50.
- SHAHEEN, A., NAZLI, R., FATIMA, S., ALI, R., KHAN, I. & KHATTAK, S. 2016. Adipokine Serum visfatin level in pregnancy induced hypertension and uncomplicated pregnancy. *Pakistan journal of medical sciences*, 32, 1419-1424.
- SHAHI, A., DABIRI, F., KAMJOO, A., YABANDEH, A. P., KHADEMI, Z. & DAVARIDOLATABADI, N. 2017. Association between body mass index (BMI) and duration of pregnancy in women referred to Shariati Hospital in Bandar Abbas. *Electron Physician*, 9, 3611-3615.
- SHARAMI, S. H., TANGESTANI, A., FARAJI, R., ZAHIRI, Z. & AMIRI, A. 2012. Role of dyslipidemia in preeclamptic overweight pregnant women. *Iranian journal of reproductive medicine*, 10, 105-112.
- SHAW, G. M., WISE, P. H., MAYO, J., CARMICHAEL, S. L., LEY, C., LYELL, D. J., SHACHAR, B. Z., MELSOP, K., PHIBBS, C. S., STEVENSON, D. K., PARSONNET, J. & GOULD, J. B. 2014. Maternal Prepregnancy Body Mass Index and Risk of Spontaneous Preterm Birth. *Paediatric & Perinatal Epidemiology*, 28, 302-311.
- SHEINER, E., LEVY, A., MENES, T. S., SILVERBERG, D., KATZ, M. & MAZOR, M. 2004. Maternal obesity as an independent risk factor for caesarean delivery. *Paediatr Perinat Epidemiol*, 18, 196-201.
- SHELDON, R. E., MASHAYAMOMBE, C., SHI, S. Q., GARFIELD, R. E., SHMYGOL, A., BLANKS, A. M. & VAN DEN BERG, H. A. 2014. Alterations in gap junction connexin43/connexin45 ratio mediate a transition from quiescence to excitation in a mathematical model of the myometrium. *J R Soc Interface*, 11, 20140726.
- SHI, X. L., WANG, G. L., ZHANG, Z., LIU, Y. J., CHEN, J. H., ZHOU, J. G., QIU, Q. Y. & GUAN, Y. Y. 2007. Alteration of volume-regulated chloride movement in rat cerebrovascular smooth muscle cells during hypertension. *Hypertension*, 49, 1371-7.
- SHMIGOL, A., EISNER, D. A. & WRAY, S. 1998. Carboxyeosin decreases the rate of decay of the [Ca<sup>2+</sup>]<sub>i</sub> transient in uterine smooth muscle cells isolated from pregnant rats. *Pflugers Archiv-European Journal of Physiology*, 437, 158-160.
- SHMIGOL, A. V., EISNER, D. A. & WRAY, S. 1999. The role of the sarcoplasmic reticulum as a Ca<sup>2+</sup> sink in rat uterine smooth muscle cells. *J Physiol*, 520 Pt 1, 153-63.
- SHMYGOL, A., BLANKS, A. M., BRU-MERCIER, G., GULLAM, J. E. & THORNTON, S. 2007a. Control of uterine Ca<sup>2+</sup> by membrane voltage: toward understanding the excitation-contraction coupling in human myometrium. *Ann N Y Acad Sci*, 1101, 97-109.
- SHMYGOL, A., NOBLE, K. & WRAY, S. 2007b. Depletion of membrane cholesterol eliminates the Ca<sup>2+</sup>-activated component of outward potassium current and decreases membrane capacitance in rat uterine myocytes. *The Journal of Physiology*, 581, 445-456.
- SHMYGOL, A. & WRAY, S. 2004. Functional architecture of the SR calcium store in uterine smooth muscle. *Cell Calcium*, 35, 501-8.
- SHORE, V., SHORE, B., SHORE, V. & SHORE, B. 1973. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry*, 12, 502-507.
- SIEGA-RIZ, A. M., VISWANATHAN, M., MOOS, M. K., DEIERLEIN, A., MUMFORD, S., KNAACK, J., THIEDA, P., LUX, L. J. & LOHR, K. N. 2009. A systematic review of outcomes of maternal

- weight gain according to the Institute of Medicine recommendations: birthweight, fetal growth, and postpartum weight retention. *Am J Obstet Gynecol*, 201, 339.e1-14.
- SILVESTRI, E., DE PERGOLA, G., ROSANIA, R. & LOVERRO, G. 2018. Obesity as disruptor of the female fertility. *Reproductive biology and endocrinology : RB&E*, 16, 22-22.
- SIMONS, K. & IKONEN, E. 2000. How cells handle cholesterol. *Science*, 290, 1721-6.
- SINGH, S., SHEHU, C. & NNADI, D. 2016. The relationship between maternal body mass index and the birth weight of neonates in North-West Nigeria. *Sahel Medical Journal*, 19, 185-189.
- SIVAN, E., WHITTAKER, P. G., SINHA, D., HOMKO, C. J., LIN, M., REECE, E. A. & BODEN, G. 1998. Leptin in human pregnancy: the relationship with gestational hormones. *Am J Obstet Gynecol*, 179, 1128-32.
- SLATER, D. M., ASTLE, S., WOODCOCK, N., CHIVERS, J. E., DE WIT, N. C. J., THORNTON, S., VATISH, M. & NEWTON, R. 2006. Anti-inflammatory and relaxatory effects of prostaglandin E2 in myometrial smooth muscle. *Molecular Human Reproduction*, 12, 89-97.
- SMITH, C. J., BAER, R. J., OLTMAN, S. P., BREHENY, P. J., BAO, W., ROBINSON, J. G., DAGLE, J. M., LIANG, L., FEUER, S. K., CHAMBERS, C. D., JELLIFFE-PAWLOWSKI, L. L. & RYCKMAN, K. K. 2018. Maternal dyslipidemia and risk for preterm birth. *PLoS one*, 13, e0209579-e0209579.
- SMITH, R. C., MCCLURE, M. C., SMITH, M. A., ABEL, P. W. & BRADLEY, M. E. 2007. The role of voltage-gated potassium channels in the regulation of mouse uterine contractility. *Reproductive Biology and Endocrinology*, 5, 41.
- SMITH, R. D., BABYCHUK, E. B., NOBLE, K., DRAEGER, A. & WRAY, S. 2005. Increased cholesterol decreases uterine activity: functional effects of cholesterol alteration in pregnant rat myometrium. *Am J Physiol Cell Physiol*, 288, C982-8.
- SOMLYO, A. P. 1985. Excitation-contraction coupling and the ultrastructure of smooth muscle. *Circulation Research*, 57, 497-507.
- SOMLYO, A. P. & SOMLYO, A. V. 1998. From pharmacomechanical coupling to G-proteins and myosin phosphatase. *Acta Physiol Scand*, 164, 437-48.
- SOMLYO, A. P. & SOMLYO, A. V. 2003. Ca<sup>2+</sup> sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev*, 83, 1325-58.
- SOMMER, C., SLETNER, L., MORKRID, K., JENUM, A. K. & BIRKELAND, K. I. 2015. Effects of early pregnancy BMI, mid-gestational weight gain, glucose and lipid levels in pregnancy on offspring's birth weight and subcutaneous fat: a population-based cohort study. *BMC Pregnancy Childbirth*, 15, 84.
- SONG, K. S., SCHERER, P. E., TANG, Z., OKAMOTO, T., LI, S., CHAFEL, M., CHU, C., KOHTZ, D. S. & LISANTI, M. P. 1996. Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins. *J Biol Chem*, 271, 15160-5.
- SONG, Y., GAO, J., QU, Y., WANG, S., WANG, X. & LIU, J. 2016. Serum levels of leptin, adiponectin and resistin in relation to clinical characteristics in normal pregnancy and preeclampsia. *Clinica Chimica Acta*, 458, 133-137.
- SONI, S., CHIVAN, N. & COHEN, W. R. 2013. Effect of maternal body mass index on oxytocin treatment for arrest of dilatation. *J Perinat Med*, 41, 517-21.
- SOWA, G., PYPAERT, M. & SESSA, W. C. 2001. Distinction between signaling mechanisms in lipid rafts vs. caveolae. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 14072-14077.
- SPECCHIA, M. L., VENEZIANO, M. A., CADEDDU, C., FERRIERO, A. M., MANCUSO, A., IANUALE, C., PARENTE, P., CAPRI, S. & RICCIARDI, W. 2014. Economic impact of adult obesity on health systems: a systematic review. *European Journal of Public Health*, 25, 255-262.
- STAMILIO, D. M. & SCIFRES, C. M. 2014. Extreme obesity and postcesarean maternal complications. *Obstet Gynecol*, 124, 227-32.
- STANG, J. & HUFFMAN, L. G. 2016. Position of the Academy of Nutrition and Dietetics: Obesity, Reproduction, and Pregnancy Outcomes. *J Acad Nutr Diet*, 116, 677-91.

- STARLING, A. P., BRINTON, J. T., GLUECK, D. H., SHAPIRO, A. L., HARROD, C. S., LYNCH, A. M., SIEGA-RIZ, A. M. & DABELEA, D. 2015. Associations of maternal BMI and gestational weight gain with neonatal adiposity in the Healthy Start study. *Am J Clin Nutr*, 101, 302-9.
- STASTNY, J., BIENERTOVA-VASKU, J. & VASKU, A. 2012. Visfatin and its role in obesity development. *Diabetes Metab Syndr*, 6, 120-4.
- STEIN, T. P., SCHOLL, T. O., SCHLUTER, M. D. & SCHROEDER, C. M. 1998. Plasma leptin influences gestational weight gain and postpartum weight retention. *Am J Clin Nutr*, 68, 1236-40.
- STERPU, I., ANFELTER, P., WRAY, S., KAIHOLA, H., AKERUD, H. & WIBERG-ITZEL, E. 2018. The association of second trimester biomarkers in amniotic fluid and fetal outcome. *J Matern Fetal Neonatal Med*, 1-6.
- STOTLAND, N. E., WASHINGTON, A. E. & CAUGHEY, A. B. 2007. Prepregnancy body mass index and the length of gestation at term. *Am J Obstet Gynecol*, 197, 378 e1-5.
- STUBERT, J., REISTER, F., HARTMANN, S. & JANNI, W. 2018. The Risks Associated With Obesity in Pregnancy. *Deutsches Arzteblatt international*, 115, 276-283.
- SWEENEY, E. M., CRANKSHAW, D. J., O'BRIEN, Y., DOCKERY, P. & MORRISON, J. J. 2013. Stereology of human myometrium in pregnancy: influence of maternal body mass index and age. *American Journal of Obstetrics & Gynecology*, 208, 324.e1-324.e6.
- SWEENEY, G. 2002. Leptin signalling. *Cellular Signalling*, 14, 655-663.
- SWIETACH, P., ROSSINI, A., SPITZER, K. W. & VAUGHAN-JONES, R. D. 2007. H<sup>+</sup> ion activation and inactivation of the ventricular gap junction: a basis for spatial regulation of intracellular pH. *Circ Res*, 100, 1045-54.
- SYDSJÖ, G., SYDSJÖ, A., BRYNHILDSEN, J. & JOSEFSSON, A. 2010. Trends in caesarean section and instrumental deliveries in relation to Body Mass Index: a clinical survey during 1978 - 2001. *Reproductive health*, 7, 18-18.
- TAGGART, M. & WRAY, S. 1993. Simultaneous measurement of intracellular pH and contraction in uterine smooth muscle. *Pflügers Archiv*, 423, 527-529.
- TAGGART, M. J. 2001. Smooth muscle excitation-contraction coupling: a role for caveolae and caveolins? *News Physiol Sci*, 16, 61-5.
- TAGGART, M. J., LEAVIS, P., FERON, O. & MORGAN, K. G. 2000. Inhibition of PKC $\alpha$  and rhoA translocation in differentiated smooth muscle by a caveolin scaffolding domain peptide. *Exp Cell Res*, 258, 72-81.
- TAGGART, M. J., MENICE, C. B., MORGAN, K. G. & WRAY, S. 1997. Effect of metabolic inhibition on intracellular Ca<sup>2+</sup>, phosphorylation of myosin regulatory light chain and force in rat smooth muscle. *The Journal of physiology*, 499 ( Pt 2), 485-496.
- TAGGART, M. J. & WRAY, S. 1995. The effect of metabolic inhibition on rat uterine intracellular pH and its role in contractile failure. *Pflügers Archiv*, 430, 125-131.
- TAGGART, M. J. & WRAY, S. 1998. Contribution of sarcoplasmic reticular calcium to smooth muscle contractile activation: gestational dependence in isolated rat uterus. *J Physiol*, 511 ( Pt 1), 133-44.
- TAIPALE, P. & HIILESMAA, V. 2001. Predicting delivery date by ultrasound and last menstrual period in early gestation. *Obstet Gynecol*, 97, 189-94.
- TAYLOR, B. D., NESS, R. B., OLSEN, J., HOUGAARD, D. M., SKOGSTRAND, K., ROBERTS, J. M. & HAGGERTY, C. L. 2015. Serum Leptin Measured in Early Pregnancy Is Higher in Women With Preeclampsia Compared With Normotensive Pregnant Women. *Hypertension*, 65, 594-599.
- TERRA, X., AUGUET, T., QUESADA, I., AGUILAR, C., LUNA, A. M., HERNANDEZ, M., SABENCH, F., PORRAS, J. A., MARTINEZ, S., LUCAS, A., PELLITERO, S., LLUTART, J., DEL CASTILLO, D. & RICHART, C. 2012. Increased levels and adipose tissue expression of visfatin in morbidly obese women: the relationship with pro-inflammatory cytokines. *Clin Endocrinol (Oxf)*, 77, 691-8.

- TEZUKA, N., ALI, M., CHWALISZ, K. & GARFIELD, R. E. 1995. Changes in transcripts encoding calcium channel subunits of rat myometrium during pregnancy. *Am J Physiol*, 269, C1008-17.
- THOMSEN, P., ROEPSTORFF, K., STAHLHUT, M. & VAN DEURS, B. 2002. Caveolae are highly immobile plasma membrane microdomains, which are not involved in constitutive endocytic trafficking. *Mol Biol Cell*, 13, 238-50.
- THOMSON, M. & HANLEY, J. 1988. Factors predisposing to difficult labor in primiparas. *American Journal of Obstetrics and Gynecology*, 158, 1074-1078.
- TOGASHI, K. 2007. Uterine contractility evaluated on cine magnetic resonance imaging. *Ann N Y Acad Sci*, 1101, 62-71.
- TORLONI, M. R., BETRAN, A. P., HORTA, B. L., NAKAMURA, M. U., ATALLAH, A. N., MORON, A. F. & VALENTE, O. 2009. Prepregnancy BMI and the risk of gestational diabetes: a systematic review of the literature with meta-analysis. *Obes Rev*, 10, 194-203.
- TRAYHURN, P. 2018. Brown Adipose Tissue-A Therapeutic Target in Obesity? *Frontiers in physiology*, 9, 1672-1672.
- TRAYHURN, P. & WOOD, I. S. 2004. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *British Journal of Nutrition*, 92, 347-355.
- TRIANTAFYLLOU, G. A., PASCHOU, S. A. & MANTZOROS, C. S. 2016. Leptin and Hormones: Energy Homeostasis. *Endocrinol Metab Clin North Am*, 45, 633-45.
- TRIBE, R. M., MORIARTY, P. & POSTON, L. 2000. Calcium homeostatic pathways change with gestation in human myometrium. *Biol Reprod*, 63, 748-55.
- TRIGGLE, D. J. 1996. Depolarization as a regulatory signal at voltage-gated calcium channels. *Zhongguo Yao Li Xue Bao*, 17, 193-6.
- TSAI, P.-J. S., DAVIS, J., THOMPSON, K. & BRYANT-GREENWOOD, G. 2015. Visfatin/Nampt and SIRT1: Roles in Postterm Delivery in Pregnancies Associated With Obesity. *Reproductive sciences (Thousand Oaks, Calif.)*, 22, 1028-1036.
- TULENKO, T. N., BIALECKI, R., GLEASON, M. & D'ANGELO, G. 1990. Ion channels, membrane lipids and cholesterol: a role for membrane lipid domains in arterial function. *Prog Clin Biol Res*, 334, 187-203.
- TURI, A., KISS, A. L. & MÜLLNER, N. 2001. ESTROGEN DOWNREGULATES THE NUMBER OF CAVEOLAE AND THE LEVEL OF CAVEOLIN IN UTERINE SMOOTH MUSCLE. *Cell Biology International*, 25, 785-794.
- UMAR, U., ISYAKU, K., ADAMU, Y., ABUBAKAR, S., KABO, N., NURA, I. & NAIMATU, A. 2017. Sonographic measurement of uterine dimensions in healthy nulliparous adults in Northwestern Nigeria. *Sahel Medical Journal*, 20, 1-7.
- UPADHYAY, BICCHA, R. P., SHERPA, M. T., SHRESTHA, R. & PANTA, P. P. 2011. Association between maternal body mass index and the birth weight of neonates. *Nepal Med Coll J*, 13, 42-5.
- VAHRATIAN, A., ZHANG, J., TROENDLE, J. F., SAVITZ, D. A. & SIEGA-RIZ, A. M. 2004. Maternal prepregnancy overweight and obesity and the pattern of labor progression in term nulliparous women. *Obstet Gynecol*, 104, 943-51.
- VALLEJO, S., ROMACHO, T., ANGULO, J., VILLALOBOS, L. A., CERCAS, E., LEIVAS, A., BERMEJO, E., CARRARO, R., SANCHEZ-FERRER, C. F. & PEIRO, C. 2011. Visfatin impairs endothelium-dependent relaxation in rat and human mesenteric microvessels through nicotinamide phosphoribosyltransferase activity. *PLoS One*, 6, e27299.
- VAN DER WIJDEN, C. L., DELEMARRE-VAN DER WAAL, H. A., VAN MECHELEN, W. & VAN POPPEL, M. N. 2013. The concurrent validity between leptin, BMI and skin folds during pregnancy and the year after. *Nutr Diabetes*, 3, e86.
- VAN EERDEN, P. 2011. Obesity in pregnancy. *South Dakota medicine : the journal of the South Dakota State Medical Association*, Spec No, 46-50.
- VAN GESTEL, I., MM, I. J., HOOGLAND, H. J. & EVERS, J. L. 2003. Endometrial wave-like activity in the non-pregnant uterus. *Hum Reprod Update*, 9, 131-8.

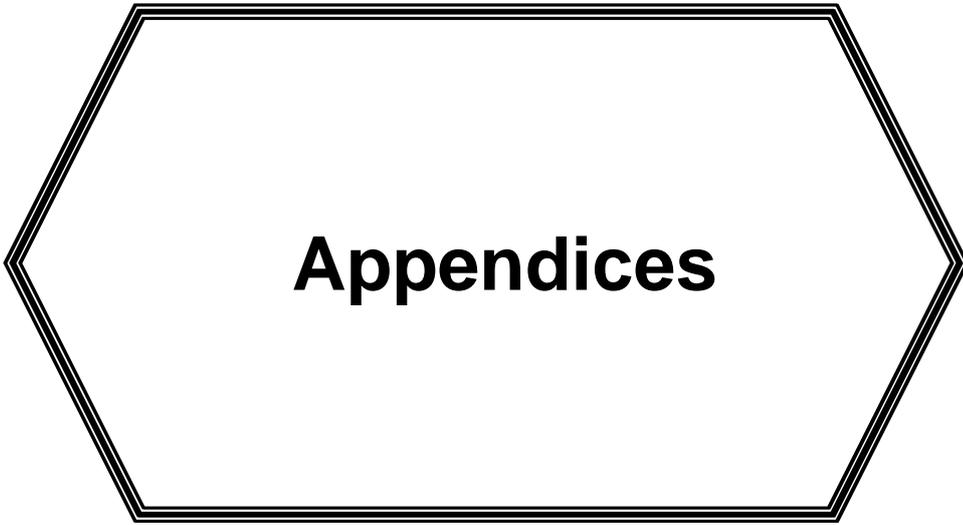
- VAN MEER, G., VOELKER, D. R. & FEIGENSON, G. W. 2008. Membrane lipids: where they are and how they behave. *Nature reviews. Molecular cell biology*, 9, 112-124.
- VAN REE, J. H., VAN DEN BROEK, W. J., DAHLMANS, V. E., GROOT, P. H., VIDGEON-HART, M., FRANTS, R. R., WIERINGA, B., HAVEKES, L. M. & HOFKER, M. H. 1994. Diet-induced hypercholesterolemia and atherosclerosis in heterozygous apolipoprotein E-deficient mice. *Atherosclerosis*, 111, 25-37.
- VERDIALES, M., PACHECO, C. & COHEN, W. 2009. Effect of maternal obesity on the course of labor. *Journal of perinatal medicine*, 37, 651-5.
- VERNINI, J. M., MORELI, J. B., COSTA, R. A. A., NEGRATO, C. A., RUDGE, M. V. C. & CALDERON, I. M. P. 2016. Maternal adipokines and insulin as biomarkers of pregnancies complicated by overweight and obesity. *Diabetology & Metabolic Syndrome*, 8, 68.
- VESCO, K. K., DIETZ, P. M., RIZZO, J., STEVENS, V. J., PERRIN, N. A., BACHMAN, D. J., CALLAGHAN, W. M., BRUCE, F. C. & HORN BROOK, M. C. 2009. Excessive gestational weight gain and postpartum weight retention among obese women. *Obstet Gynecol*, 114, 1069-75.
- VITORATOS, N., SALAMALEKIS, E., KASSANOS, D., LOGHIS, C., PANAYOTOPOULOS, N., KOUSKOUNI, E. & CREATSAS, G. 2001. Maternal plasma leptin levels and their relationship to insulin and glucose in gestational-onset diabetes. *Gynecol Obstet Invest*, 51, 17-21.
- VOGEL, U., SANDVIG, K. & VAN DEURS, B. 1998. Expression of caveolin-1 and polarized formation of invaginated caveolae in Caco-2 and MDCK II cells. *J Cell Sci*, 111 ( Pt 6), 825-32.
- VRACHNIS, N., BELITSOS, P., SIFAKIS, S., DAFOPOULOS, K., SIRISTATIDIS, C., PAPPA, K. I. & ILIODROMITI, Z. 2012. Role of adipokines and other inflammatory mediators in gestational diabetes mellitus and previous gestational diabetes mellitus. *Int J Endocrinol*, 2012, 549748.
- WAHABI, H., FAYED, A., ESMAEIL, S., ALZEIDAN, R., ELAWAD, M., TABASSUM, R., HANSOTI, S., MAGZOU, M. E., AL-KADRI, H., ELSHERIF, E., AL-MANDIL, H., AL-SHAikh, G. & ZAKARIA, N. 2016. Riyadh Mother and Baby Multicenter Cohort Study: The Cohort Profile. *PLoS one*, 11, e0150297-e0150297.
- WAHABI, H. A., FAYED, A. A., ALZEIDAN, R. A. & MANDIL, A. A. 2014. The independent effects of maternal obesity and gestational diabetes on the pregnancy outcomes. *BMC endocrine disorders*, 14, 47-47.
- WANG, J. X., DAVIES, M. J. & NORMAN, R. J. 2002. Obesity Increases the Risk of Spontaneous Abortion during Infertility Treatment. *Obesity Research*, 10, 551-554.
- WANG, P., XU, T. Y., GUAN, Y. F., SU, D. F., FAN, G. R. & MIAO, C. Y. 2009. Perivascular adipose tissue-derived visfatin is a vascular smooth muscle cell growth factor: role of nicotinamide mononucleotide. *Cardiovasc Res*, 81, 370-80.
- WANG, T., ZHANG, X., BHEDA, P., REVOLLO, J. R., IMAI, S. & WOLBERGER, C. 2006. Structure of Nampt/PBEF/visfatin, a mammalian NAD<sup>+</sup> biosynthetic enzyme. *Nat Struct Mol Biol*, 13, 661-2.
- WATHES, D. C. & PORTER, D. G. 1982. Effect of uterine distension and oestrogen treatment on gap junction formation in the myometrium of the rat. *J Reprod Fertil*, 65, 497-505.
- WATKINS, M. L., RASMUSSEN, S. A., HONEIN, M. A., BOTTO, L. D. & MOORE, C. A. 2003. Maternal obesity and risk for birth defects. *Pediatrics*, 111, 1152-8.
- WEISS, J. L., MALONE, F. D., EMIG, D., BALL, R. H., NYBERG, D. A., COMSTOCK, C. H., SAADE, G., EDDLEMAN, K., CARTER, S. M., CRAIGO, S. D., CARR, S. R., D'ALTON, M. E. & CONSORTIUM, F. R. 2004. Obesity, obstetric complications and cesarean delivery rate--a population-based screening study. *Am J Obstet Gynecol*, 190, 1091-7.
- WEISSGERBER, T. L. & WOLFE, L. A. 2006. Physiological adaptation in early human pregnancy: adaptation to balance maternal-fetal demands. *Appl Physiol Nutr Metab*, 31, 1-11.
- WELGE, J. A., WARSHAK, C. R. & WOOLLETT, L. A. 2018. Maternal plasma cholesterol concentration and preterm birth: a meta-analysis and systematic review of literature. *The Journal of Maternal-Fetal & Neonatal Medicine*, 1-9.

- WEN, T. & LV, Y. 2015. Inadequate gestational weight gain and adverse pregnancy outcomes among normal weight women in China. *International journal of clinical and experimental medicine*, 8, 2881-2886.
- WENDREMAIRE, M., BARDOU, M., PEYRONEL, C., HADI, T., SAGOT, P., MORRISON, J. J. & LIRUSSI, F. 2011. Effects of leptin on lipopolysaccharide-induced myometrial apoptosis in an in vitro human model of chorioamnionitis. *American Journal Of Obstetrics And Gynecology*, 205, 363.e1-9.
- WENDREMAIRE, M., LIRUSSI, F., SEDIKI, M., GOIRAND, F., PEYRONEL, C., LIONNAIS-COUVREUR, S., DUMAS, M., SAGOT, P. & BARDOU, M. 2010. The Leptin Receptor (Ob-R) Is Expressed and Functional in the Human Near-Term Myometrium. United States: SAGE PUBLICATIONS.
- WENDREMAIRE, M., MOURTIALON, P., GOIRAND, F., LIRUSSI, F., BARRICHON, M., HADI, T., GARRIDO, C., LE RAY, I., DUMAS, M., SAGOT, P. & BARDOU, M. 2013. Effects of leptin on lipopolysaccharide-induced remodeling in an in vitro model of human myometrial inflammation. *Biol Reprod*, 88, 45.
- WHITWORTH, M., BRICKER, L. & MULLAN, C. 2015. Ultrasound for fetal assessment in early pregnancy. *Cochrane Database Syst Rev*, Cd007058.
- WHO 1997. World Medical Association declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. *Jama*, 277, 925-6.
- WHO 2000. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser*, 894, i-xii, 1-253.
- WHO 2011a. Care in normal birth: a practical guide.
- WHO 2011b. recommendations for Induction of labour.
- WHO 2018. Overweight and obesity. *Geneva: World Health Organization*.
- WIBERG-ITZEL, E., PEMBE, A. B., WRAY, S., WIHLBACK, A. C., DARJ, E., HOESLI, I. & AKERUD, H. 2014. Level of lactate in amniotic fluid and its relation to the use of oxytocin and adverse neonatal outcome. *Acta Obstet Gynecol Scand*, 93, 80-5.
- WIBERG-ITZEL, E., WRAY, S. & AKERUD, H. 2018. A randomized controlled trial of a new treatment for labor dystocia. *J Matern Fetal Neonatal Med*, 31, 2237-2244.
- WILKINSON, J. E., BURMEISTER, L., BROOKS, S. V., CHAN, C.-C., FRIEDLINE, S., HARRISON, D. E., HEJTMANCIK, J. F., NADON, N., STRONG, R., WOOD, L. K., WOODWARD, M. A. & MILLER, R. A. 2012. Rapamycin slows aging in mice. *Aging Cell*, 11, 675-682.
- WILLECKE, K., EIBERGER, J., DEGEN, J., ECKARDT, D., ROMUALDI, A., GULDENAGEL, M., DEUTSCH, U. & SOHL, G. 2002. Structural and functional diversity of connexin genes in the mouse and human genome. *Biol Chem*, 383, 725-37.
- WILLIAMS, T. M. & LISANTI, M. P. 2004. The caveolin proteins. *Genome Biology*, 5, 214.
- WINTERHAGER, E., BRUMMER, F., DERMIETZEL, R., HULSER, D. F. & DENKER, H. W. 1988. Gap junction formation in rabbit uterine epithelium in response to embryo recognition. *Dev Biol*, 126, 203-11.
- WINTERHAGER, E., GRUMMER, R., JAHN, E., WILLECKE, K. & TRAUB, O. 1993. Spatial and temporal expression of connexin26 and connexin43 in rat endometrium during trophoblast invasion. *Dev Biol*, 157, 399-409.
- WINTERHAGER, E. & KIDDER, G. M. 2015. Gap junction connexins in female reproductive organs: implications for women's reproductive health. *Hum Reprod Update*, 21, 340-52.
- WINTERHAGER, E., STUTENKEMPER, R., TRAUB, O., BEYER, E. & WILLECKE, K. 1991. Expression of different connexin genes in rat uterus during decidualization and at term. *Eur J Cell Biol*, 55, 133-42.
- WMA DECLARATION OF HELSINKI 2013. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *Jama*, 310, 2191-4.
- WOLF, M., KETTYLE, E., SANDLER, L., ECKER, J. L., ROBERTS, J. & THADHANI, R. 2001. Obesity and preeclampsia: the potential role of inflammation. *Obstetrics & Gynecology*, 98, 757-762.

- WOLFE, W. S., SOBAL, J., OLSON, C. M., FRONGILLO, E. A., JR. & WILLIAMSON, D. F. 1997. Parity-associated weight gain and its modification by sociodemographic and behavioral factors: a prospective analysis in US women. *Int J Obes Relat Metab Disord*, 21, 802-10.
- WONG, S. S. 1990. Birth order and birth weight of Saudi newborns. *J R Soc Health*, 110, 96-7, 100.
- WRAY, S. 1993. Uterine contraction and physiological mechanisms of modulation. *Am J Physiol*, 264, C1-18.
- WRAY, S. 2007. Insights into the uterus. *Experimental Physiology*, 92, 621-631.
- WRAY, S. & BURDYGA, T. 2010. Sarcoplasmic reticulum function in smooth muscle. *Physiol Rev*, 90, 113-78.
- WRAY, S., JONES, K., KUPITTAYANANT, S., LI, Y., MATTHEW, A., MONIR-BISHTY, E., NOBLE, K., PIERCE, S. J., QUENBY, S. & SHMYGOL, A. V. 2003. Calcium signaling and uterine contractility. *J Soc Gynecol Investig*, 10, 252-64.
- WRAY, S., KUPITTAYANANT, S., SHMYGOL, A., SMITH, R. D. & BURDYGA, T. 2001. The physiological basis of uterine contractility: a short review. *Exp Physiol*, 86, 239-46.
- WRAY, S. & PRENDERGAST, C. 2019. The Myometrium: From Excitation to Contractions and Labour. *Adv Exp Med Biol*, 1124, 233-263.
- WRIGHT, S. M. & ARONNE, L. J. 2012. Causes of obesity. *Abdom Imaging*, 37, 730-2.
- WUNTAKAL, R. & HOLLINGWORTH, T. 2010. Leptin--a tocolytic agent for the future? *Med Hypotheses*, 74, 81-2.
- WUNTAKAL, R., KALER, M. & HOLLINGWORTH, T. 2013. Women with high BMI: Should they be managed differently due to antagonising action of leptin in labour? *Medical Hypotheses*, 80, 767-768.
- XIA, M., ZHANG, C., BOINI, K. M., THACKER, A. M. & LI, P. L. 2011. Membrane raft-lysosome redox signalling platforms in coronary endothelial dysfunction induced by adipokine visfatin. *Cardiovasc Res*, 89, 401-9.
- XIE, H., TANG, S.-Y., LUO, X.-H., HUANG, J., CUI, R.-R., YUAN, L.-Q., ZHOU, H.-D., WU, X.-P. & LIAO, E.-Y. 2007. Insulin-Like Effects of Visfatin on Human Osteoblasts. *Calcified Tissue International*, 80, 201-210.
- XINWANG, C., XIAO, J., JIE, Q., YOUFEI, G. & JIHONG, K. 2013. Adipokines in reproductive function: a link between obesity and polycystic ovary syndrome. *Journal of Molecular Endocrinology*, 50, R21-R37.
- XU, C., LONG, A., FANG, X., WOOD, S. L., SLATER, D. M., NI, X. & OLSON, D. M. 2013. Effects of PGF2alpha on the expression of uterine activation proteins in pregnant human myometrial cells from upper and lower segment. *J Clin Endocrinol Metab*, 98, 2975-83.
- XU, J., MENON, S. N., SINGH, R., GARNIER, N. B., SINHA, S. & PUMIR, A. 2015. The role of cellular coupling in the spontaneous generation of electrical activity in uterine tissue. *PLoS One*, 10, e0118443.
- YAMAKAGE, M. & NAMIKI, A. 2002. Calcium channels — basic aspects of their structure, function and gene encoding; anesthetic action on the channels — a review. *Canadian Journal of Anesthesia*, 49, 151-164.
- YAMAWAKI, H. 2011. Vascular effects of novel adipocytokines: focus on vascular contractility and inflammatory responses. *Biol Pharm Bull*, 34, 307-10.
- YAMAWAKI, H., HARA, N., OKADA, M. & HARA, Y. 2009. Visfatin causes endothelium-dependent relaxation in isolated blood vessels. *Biochemical and biophysical research communications*, 383, 503-508.
- YANG, M. J. 2005. Interrelationships of maternal serum leptin, body mass index and gestational age. *J Chin Med Assoc*, 68, 452-7.
- YANG, Y. & SAUVE, A. A. 2016. NAD(+) metabolism: Bioenergetics, signaling and manipulation for therapy. *Biochimica et biophysica acta*, 1864, 1787-1800.
- YAO, R., ANANTH, C. V., PARK, B. Y., PEREIRA, L. & PLANTE, L. A. 2014. Obesity and the risk of stillbirth: a population-based cohort study. *Am J Obstet Gynecol*, 210, 457.e1-9.

- YARAR, Y., CETIN, A. & KAYA, T. 2001. Chloride channel blockers 5-nitro-2-(3-phenylpropylamino) benzoic acid and anthracene-9-carboxylic acid inhibit contractions of pregnant rat myometrium in vitro. *J Soc Gynecol Investig*, 8, 206-9.
- YEAGER, M. & HARRIS, A. L. 2007. Gap junction channel structure in the early 21st century: facts and fantasies. *Current Opinion in Cell Biology*, 19, 521-528.
- YEAGER, M. & NICHOLSON, B. J. 1996. Structure of gap junction intercellular channels. *Curr Opin Struct Biol*, 6, 183-92.
- YILMAZ, M., GANGOPADHYAY, S. S., LEAVIS, P., GRABAREK, Z. & MORGAN, K. G. 2013. Phosphorylation at Ser(2)(6) in the ATP-binding site of Ca(2+)(+)/calmodulin-dependent kinase II as a mechanism for switching off the kinase activity. *Biosci Rep*, 33.
- YOGEV, Y. & CATALANO, P. M. 2009. Pregnancy and obesity. *Obstet Gynecol Clin North Am*, 36, 285-300, viii.
- YOUNG, R. C. 2007. Myocytes, myometrium, and uterine contractions. *Ann N Y Acad Sci*, 1101, 72-84.
- YOUNG, R. C. & HESSION, R. O. 1999. Three-dimensional structure of the smooth muscle in the term-pregnant human uterus. *Obstet Gynecol*, 93, 94-9.
- YOUNG, R. C., SMITH, L. H. & MCLAREN, M. D. 1993. T-type and L-type calcium currents in freshly dispersed human uterine smooth muscle cells. *American Journal of Obstetrics and Gynecology*, 169, 785-792.
- YOUNG, R. C. & ZHANG, P. 2005. Inhibition of in vitro contractions of human myometrium by mibefradil, a T-type calcium channel blocker: support for a model using excitation-contraction coupling, and autocrine and paracrine signaling mechanisms. *Journal of the Society for Gynecologic Investigation*, 12, e7-12.
- YOUNG, T. K. & WOODMANSEE, B. 2002. Factors that are associated with cesarean delivery in a large private practice: the importance of prepregnancy body mass index and weight gain. *Am J Obstet Gynecol*, 187, 312-8; discussion 318-20.
- ZAHORSKA-MARKIEWICZ, B., OLSZANECKA-GLINIANOWICZ, M., JANOWSKA, J., KOCELAK, P., SEMIK-GRABARCZYK, E., HOLECKI, M., DABROWSKI, P. & SKORUPA, A. 2007. Serum concentration of visfatin in obese women. *Metabolism*, 56, 1131-4.
- ZENG, Z., LIU, F. & LI, S. 2017. Metabolic Adaptations in Pregnancy: A Review. *Annals of Nutrition and Metabolism*, 70, 59-65.
- ZHANG, F., CHEN, Y., HEIMAN, M. & DIMARCHI, R. 2005. Leptin: structure, function and biology. *Vitam Horm*, 71, 345-72.
- ZHANG, J., BRICKER, L., WRAY, S. & QUENBY, S. 2007a. Poor uterine contractility in obese women. *BJOG*, 114, 343-8.
- ZHANG, J. & DUAN, T. 2018. The physiologic pattern of normal labour progression. *Bjog*, 125, 955.
- ZHANG, J., KENDRICK, A., QUENBY, S. & WRAY, S. 2007b. Contractility and Calcium Signaling of Human Myometrium Are Profoundly Affected by Cholesterol Manipulation: Implications for Labor? *Reproductive Sciences*, 14, 456-466.
- ZHANG, J., LANDY, H. J., BRANCH, D. W., BURKMAN, R., HABERMAN, S., GREGORY, K. D., HATJIS, C. G., RAMIREZ, M. M., BAILIT, J. L., GONZALEZ-QUINTERO, V. H., HIBBARD, J. U., HOFFMAN, M. K., KOMINIAREK, M., LEARMAN, L. A., VAN VELDHUISEN, P., TROENDLE, J., REDDY, U. M. & CONSORTIUM ON SAFE, L. 2010a. Contemporary patterns of spontaneous labor with normal neonatal outcomes. *Obstet Gynecol*, 116, 1281-7.
- ZHANG, J., TROENDLE, J., MIKOLAJCZYK, R., SUNDARAM, R., BEAVER, J. & FRASER, W. 2010b. The natural history of the normal first stage of labor. *Obstet Gynecol*, 115, 705-10.
- ZHANG, L. Q., HERUTH, D. P. & YE, S. Q. 2011. Nicotinamide Phosphoribosyltransferase in Human Diseases. *Journal of bioanalysis & biomedicine*, 3, 13-25.
- ZHOU, F., PAN, Y., HUANG, Z., JIA, Y., ZHAO, X., CHEN, Y., DIAO, J., WAN, Q. & CUI, X. 2013. Visfatin induces cholesterol accumulation in macrophages through up-regulation of scavenger receptor-A and CD36. *Cell Stress Chaperones*, 18, 643-52.

- ZHOU, L., YANG, H.-X., ZHAO, R.-F. & ZHANG, W.-Y. 2019. Association of pre-pregnancy body mass index and gestational weight gain with labor stage. *Chinese Medical Journal*, 132, 483-487.
- ZHU, Y., HEDDERSON, M. M., QUESENBERRY, C. P., FENG, J. & FERRARA, A. 2019. Central Obesity Increases the Risk of Gestational Diabetes Partially Through Increasing Insulin Resistance. *Obesity*, 27, 152-160.
- ZORBA, E., VAVILIS, D., VENETIS, C. A., ZOURNATZI, V., KELLARTZIS, D. & TARLATZIS, B. C. 2012. Visfatin serum levels are increased in women with preeclampsia: a case-control study. *J Matern Fetal Neonatal Med*, 25, 1668-73.
- ZOYA, T., J. F., Z. A., K. Z., S., GHOLAMREZA5 & MITRA6, A. M. 2017. Relationships of the First Trimester Maternal BMI with New-born Anthropometric Characteristics and Visfatin Levels throughout Pregnancy. *International Journal of Medical Research & Health Sciences*, 6, 17-23.



**Appendices**

# **Appendix 1**

## **Publications**

# **Appendix 2**

Ethics approval for the investigation of pathological and physiological effect of different agents, novel substances and biomarkers on human myometrial contractility

# **Appendix 3**

Patient information sheet and  
consent form for the collection of  
human uterine biopsy

# **Appendix 4**

Ethics approval for human  
immunohistochemistry testing of  
human tissue samples

# **Appendix 5**

ApoE<sup>-/-</sup> mouse data sheet  
(plasma cholesterol levels)

# **Appendix 6**

Ethics approval for the cross-sectional study from King Fahad Hospital of the University Research Ethics committee  
(Chapter 5)

# **Appendix 7**

Ethics approval for the cross-sectional study from Liverpool Research Ethics committee

# **Appendix 8**

Patient information sheet and  
consent form for the cross-  
sectional study

# **Appendix 9**

## ELISA Standard curves