

TABLE OF CONTENTS

ACKNOWLEDGMENTS	1
TABLE OF CONTENTS	3
INDEX OF FIGURES	8
INDEX OF TABLES	10
INDEX OF SUPPLEMENTARY FILES	11
ORIGINAL PUBLICATIONS	13
SUMMARY	15
RESUMEN	18
RESUM	21
ABBREVIATIONS	25
LITERATURE BACKGROUND	29
GENERAL INTRODUCCION.....	31
IMPORTANCE OF MICROBIOTA FOR HUMAN HEALTH	33
<i>Microbiota and disease</i>	33
<i>Early programming and human health</i>	35
MICROBIOTA ACQUISITION PROCESS AND EVOLUTION.....	39
<i>In utero environment</i>	39
Women microbiota during gestation and lactation.....	39
In utero microbial contact	41
Maternal diet and clinical factors	44
<i>From birth to first year of life: towards a complex microbiota in postnatal period</i> 45	
<i>Factors influencing neonatal microbiota development</i>	46
Gestational age: preterm neonates.....	46
Delivery mode	47
Antibiotic exposure and bacterial infections	49
Infant diet: Breastfeeding practice and introduction of solid foods.....	51
Genetic background.....	53
Geographical location and environment.....	53
GUT MICROBIOTA AND IMMUNE SYSTEM.....	55
<i>Physiology of the immune system in the gut</i>	55
<i>Microbiota and immune system development</i>	58
METHODOLOGIES TO STUDY MICROBIOTA	60
Culture-based techniques	61
Culturomics	62

Molecular approaches	62
Sequencing-based methods	63
Other methodologies	66
Future perspectives.....	67
RATIONALE OF THE THESIS	71
STUDY DESIGN AND PARTICIPANTS.....	72
Participants	72
Data and samples collection	72
OBJECTIVES OF THE THESIS	74
CHAPTER I – MATERNAL MICROBIOTA IS RELATED TO AMNIOTIC FLUID CYTOKINE PROFILE AND PLACENTA GENE EXPRESSION.....	77
ABSTRACT	79
INTRODUCTION.....	80
MATERIAL AND METHODS.....	81
Description of the study	81
Sample collection	81
Faecal DNA extraction and sequencing	82
Amniotic fluid analysis	82
<i>Immunological profile</i>	82
<i>Microbial SCFA profile</i>	83
Placental tissue specific gene expression	83
Statistical analysis	84
RESULTS.....	85
Description of participants	85
Maternal microbiota was related with some biochemical markers in blood.....	86
Maternal microbiota composition was related to amniotic fluid cytokines at delivery	88
Maternal biochemical markers and microbiota related to gene expression on placenta	89
SCFA determination in amniotic fluid	92
Neonatal microbiota was related to amniotic fluid cytokines concentration	93
DISCUSSION	94
CONCLUSIONS	100
SUPPLEMENTARY DATA.....	101
CHAPTER II - MATERNAL MICROBIOTA, CORTISOL CONCENTRATION AND POST-PARTUM WEIGHT RECOVERY ARE DEPENDENT ON MODE OF DELIVERY	105
ABSTRACT	107
INTRODUCTION.....	109
MATERIAL AND METHODS	110
Participants and sampling information.....	110

Biological samples	110
Cortisol concentration quantification	111
DNA extraction	111
Amplicon sequencing and bioinformatics	111
Statistical analysis	112
RESULTS.....	113
Study participants characteristics	113
Maternal microbiota composition at delivery time	115
Factors affecting maternal microbiota at delivery	117
Delivery mode was related to saliva cortisol concentration	120
Delivery mode was associated with post-partum maternal weight retention	121
DISCUSSION	122
CONCLUSIONS	127
SUPPLEMENTARY DATA.....	129
CHAPTER III - MATERNAL DIET DURING PREGNANCY AND	
INTESTINAL MARKERS ARE ASSOCIATED WITH EARLY GUT	
MICROBIOTA	133
ABSTRACT	135
INTRODUCTION.....	136
MATERIAL AND METHODS	137
Study design and volunteers	137
Clinical, anthropometric and obstetric characteristics.....	137
Assessment of maternal dietary intake	137
Maternal-neonatal biological samples	138
Maternal intestinal markers	138
DNA extraction and 16S rRNA amplicon sequencing	138
Statistical analysis	139
Ethical aspects	140
RESULTS.....	140
Characteristics of study participants.....	141
Relation between maternal diet and maternal gut microbiota composition.....	141
Maternal diet and intestinal markers	145
Relation between maternal diet, intestinal markers, and neonatal gut microbiota	
composition in a birth-dependent manner	136
DISCUSSION	151
CONCLUSIONS	155
SUPPLEMENTARY DATA.....	156
CHAPTER IV - DISTINCT MATERNAL MICROBIOTA CLUSTERS ARE	
ASSOCIATED WITH DIET DURING PREGNANCY: IMPACT ON	

NEONATAL MICROBIOTA AND INFANT GROWTH DURING THE FIRST 18 MONTHS OF LIFE

ABSTRACT	167
INTRODUCTION.....	168
MATERIAL AND METHODS	168
Study design and volunteers	168
Nutritional assessment.....	169
Child growth development	169
Faecal samples and DNA extraction	170
16S rRNA amplicon sequencing	171
Statistical analysis	171
RESULTS.....	172
Maternal microbiota clusters at birth and clinical data.....	172
Maternal microbial cluster composition and diversity	175
Maternal microbial clusters are linked to specific dietary compounds.....	176
Maternal microbial clusters are linked to neonatal microbiota at birth	178
Maternal microbial clusters are linked to infant growth development and risk of overweight.....	179
DISCUSSION	181
CONCLUSIONS	186

CHAPTER V - PERINATAL ENVIRONMENT SHAPES MICROBIOTA COLONIZATION AND INFANT GROWTH: IMPACT ON HOST RESPONSE AND INTESTINAL FUNCTION

ABSTRACT	191
INTRODUCTION.....	192
MATERIAL AND METHODS	193
Subjects and sampling	193
Faecal DNA extraction.....	194
Sequencing and bioinformatics analysis	194
Bacterial quantification by quantitative PCR analysis	195
Cell culture	195
Stimulation of HT-29 and THP-1 cell line	196
Triple co-culture in Transwell plates: Long-term stimulation.....	197
Epithelial barrier function: Trans-epithelial electrical resistance (TEER) and apparent permeability.....	198
Mucus production and intestinal alkaline phosphatase (IAP) determination	198
Cytokine quantification in cell supernatant	199
Gene expression by real-time RT-qPCR	199
Statistical analysis	200

RESULTS.....	201
Study population	201
Perinatal factors related to the place and mode of delivery shape the neonatal microbiome composition at birth.....	201
Perinatal factors related to the place and mode of delivery shape neonatal microbiota development	203
Impact of the perinatal environment on the infant weight status at 18 months	206
Microbiota functionality during the first month of life is influenced by the birth mode and place.....	207
Gut microbiota shifts impact on the in vitro host response	208
Faecal supernatants induce the mRNA expression of TLR4 and IRAK4 in intestinal epithelial cells.....	209
HB faecal supernatants trigger higher immune response in macrophage-like cells than CS faecal supernatant	210
Triple co-culture system for host–microbiome interaction	210
DISCUSSION	213
CONCLUSIONS	219
SUPPLEMENTARY DATA.....	220
GENERAL DISCUSSION	229
MATERNAL MICROBIOTA AND GESTATION.....	231
MATERNAL AND NEONATAL MICROBIOTA: THE BIRTH CHALLENGE.....	237
MOLECULAR MECHANISMS BEYOND MICROBIOTA-HOST INTERACTIONS IN EARLY LIFE	241
MICROBITA AS ESSENTIAL NODE IN THE INFANT DEVELOPMENT NETWORK DURING THE FIRST 1000 DAYS OF LIFE.....	246
The developing infant: microbial influence beyond birth	248
STRATEGIES OF MICROBIOTA RESTORATION AFTER DISRUPTING EVENT	250
FUTURE PERSPECTIVES	252
CONCLUSIONS	257
REFERENCES	261

INDEX OF FIGURES

LITERATURE BACKGROUND:

- Figure 1.** Changes in maternal microbiota over the gestation..... 40
- Figure 2.** Factors that could influence the infant colonization process in early life 45
- Figure 3.** Scheme of the organization of the gut associated lymphoid tissue 57

RATIONALE OF THE THESIS:

- Figure 4.** Flow chart of sampling procedure..... 73

OBJECTIVES OF THE THEIS

- Figure 5.** Summary of the main research objectives of the present thesis 75

CHAPTER I:

- Figure I-1.** Maternal microbiota at delivery 87
- Figure I-2.** Maternal microbiota was associated with cytokines concentration in amniotic fluid at delivery 89
- Figure I-3.** Network of associations between maternal biochemical markers, amniotic fluid content and maternal microbiota at delivery 92
- Figure I-4.** Amniotic fluid was associated with neonatal microbiota at delivery 94

CHAPTER II:

- Figure II-1.** Maternal microbiota is clustered in two groups based in their composition and diversity at delivery 116
- Figure II-2.** Maternal microbiota composition is influenced by delivery mode..... 118
- Figure II-3.** Maternal microbiota at delivery was dissentingly shaped by delivery mode... 120
- Figure II-4.** Delivery mode affected the saliva cortisol concentration and weight gain recovery four-month post-partum..... 122

CHAPTER III:

- Figure III-1.** Maternal diet was associated with maternal microbiota..... 144
- Figure III-2.** Maternal diet was associated with intestinal markers 146
- Figure III-3.** Maternal diet was associated with the neonatal microbial richness and diversity 147
- Figure III-4.** Maternal diet was associated with neonatal microbiota at birth..... 149

Figure III-5. Neonatal microbiota was distinctly shaped depending on mode of birth and maternal diet.....	150
--	-----

CHAPTER IV:

Figure IV-1. Maternal gut microbial clusters and representative genera.....	173
Figure IV-2 Maternal microbial clusters characteristics and alfa and beta diversity.....	176
Figure IV- 3. Maternal diet shapes the microbial clusters.....	177
Figure IV-4. Maternal gut microbial clusters and diet drive the neonatal first pass microbiota.....	179
Figure IV-5. Maternal gut microbial clusters and mode of birth impact the infant risk of overweight and obesity.....	180

CHAPTER V:

Figure V-1. Factors affecting meconium and neonatal microbiota during the first month of life.....	203
Figure V-2. Differences in relative abundance of most important and variable genera in faecal microbiota among the first month of life.....	205
Figure V-3. Place and Mode of birth impact the infant growth.....	206
Figure V-4. Microbial functions computationally predicted present in neonatal microbiota along the first month of life.....	208
Figure V-5. Effect of 1-month infant faecal water exposure in epithelial (A) and macrophages-like (B) cell lines after 24h.....	209
Figure V-6. Effect of faecal water long-term exposure (7d) on the triple co-culture system.....	211

DISCUSSION:

Figure 6. Proposed mechanism for the observed cellular response in the simulated gut epithelium system after exposure of neonatal faecal samples.....	243
Figure 7. Summary of the main perinatal factors that affected maternal-neonatal microbiota during the first month of life and related health outcomes found in the present thesis.....	247
Figure 8. Sources of variability among microbiome studies through differences in technical and analytical procedures.....	253

INDEX OF TABLES

LITERATURE BACKGROUND

Table 1. Association studies between microbiota dysbiosis in childhood and risk of disease development later in life	35
Table 2. Summary of the most used techniques in microbiota analysis.....	61
Table 3. Terms commonly used in microbiome studies	64
Table 4. Comparison among 16S rRNA amplicon analysis tools.....	65

CHAPTER I

Table I-1. Participants characteristics (n=15).....	85
Table I-2. Associations between placenta gene expression and clinical parameters measured, amniotic fluid cytokines and maternal microbiota at delivery.....	91

CHAPTER II

Table II-1. Characteristics of population participant in the microbiota study (n=97)	114
Table II-2. Spearman correlation between salivary cortisol concentration and maternal microbiota at delivery.....	121

CHAPTER III:

Table III-1. Characteristics of study participants at delivery	141
Table III-2. Maternal diet during gestation according to mode of delivery	142

CHAPTER IV:

Table IV-1. Characteristics of mothers-neonates	174
--	-----

CHAPTER V:

Table V-1. Gene expression of cells in the apical and basal compartment in the triple co-culture system after 7 days of exposure.....	212
--	-----

INDEX OF SUPPLEMENTARY FILES

CHAPTER I:

Supplementary file I-1. Primers used in the study	101
Supplementary file I-2. Relation of the anthropometrical and clinical variables in the study	102
Supplementary file I-3. Heatmap of spearman correlations between amniotic fluid cytokines at delivery, biochemical markers measured at the last trimester of pregnancy and index of alpha diversity of maternal microbiota.....	103

CHAPTER II:

Supplementary file II-1. Characteristics of population participant in the post-partum weight retention study	129
Supplementary file II-2 Maternal microbiota composition at phylum level at delivery time	130
Supplementary file II-3. Differences in maternal microbiota at delivery time according to delivery mode	131
Supplementary file II-4. Occurrence of genera from maternal microbial core at delivery according to delivery mode	132

CHAPTER III:

Supplementary file III-1. Flow-chart of participants in the study.....	156
Supplementary file III-2. Maternal diet was related to the weight gain during pregnancy	157
Supplementary file III-3. Spearman correlations between reported nutrients intake of maternal diet during pregnancy	158
Supplementary file III-4. Maternal diet and intestinal markers were associated with the maternal microbiota at delivery	159
Supplementary file III-5. Comparison of bacterial population from maternal intestinal microbiota and infant faecal swabs microbiota	160
Supplementary file III-6. Neonatal microbiota was distinctly shaped depending on mode of birth and maternal diet.....	161
Supplementary file III-7. Maternal diet was associated with neonatal microbiota at birth in vaginal born infants	162
Supplementary file III-8. Maternal diet was associated with neonatal microbiota at birth in C-section born infants	163

CHAPTER IV:

Supplementary file IV-1. Direct acyclic graph (DAG) method for covariate selection adjustment 187

CHAPTER V:

Supplementary file V-1. Flowchart detailing the number of participants in each time point according to study group 220

Supplementary file V-2. Characteristics of studied population according to place and mode of delivery 221

Supplementary file V-3. Primers of 16s rRNA gene of prokaryotic targets and human genes tested by RT-PCR..... 222

Supplementary file V-4. Microbiota composition at phylum level over the first month of life 223

Supplementary file V-5. Neonatal faecal microbiota diversity and richness of meconium and infant faecal samples at 7 and 31 days 224

Supplementary file V-6. Taxonomic biomarkers of microbiota composition of each group depending on place and mode of delivery 224

Supplementary file V-7. Core group of neonatal microbiota composition at genus level over the first month of life..... 225

Supplementary file V-8. Quantitative analysis of intestinal microbiota from infants born at hospital (vaginal and C-section delivery) and at home across the first month of life..... 225

Supplementary file V-9. Microbial functions related to amino acids metabolism computationally predicted present in neonatal microbiota along the first month of life 226

Supplementary file V-10. Gene Expression of HT-29 and THP-1 cells after 24 h of faecal supernatant exposure 226

Supplementary file V-11. Epithelial barrier function and maturation of simulated intestinal epithelium of the triple co-culture during the long-term exposure 227