Universidade do Minho
Escola de Medicina

Rosete Maria Amorim Novais Nogueira Cardoso

A Multicenter Study of Singleton Placentas Biometric Parameters and Fetal Weight In Function of Gestational-Age

novembro de 2019

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Tese de Doutoramento
Doutoramento em Medicina

Trabalho efetuado sob a orientação do
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novembro de 2019

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STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

TÍTULO: ESTUDO MULTICÊNTRICO DE PARÂMETROS BIOMÉTRICOS DE PLACENTAS SIMPLES E PESO FETAL EM FUNÇÃO DA IDADE-GESTACIONAL

RESUMO

Qualquer distúrbio na normal sequência do desenvolvimento e crescimento pode levar a discrepâncias dos aspetos placentares e fetais. Sabe-se que o rácio peso fetal (R-PFP) e o rácio peso placentar (R-PPF), são parâmetros mais importantes na avaliação da função da placenta e do crescimento fetal que o peso da placenta e o peso fetal (ou do recém-nascido) isoladamente. Assim, avanços na compreensão do peso placentar em função da idade-gestacional (IG) poderão expandir a utilidade do exame da placenta e o seu contributo para o "Projeto da Placenta Humana".

O *Objetivo Geral* desta tese foi a produção de curvas de percentis de peso e diâmetros da placenta e de peso ao nascimento (de fetos e/ou de recém-nascidos) em função da IG tendo por base um estudo-retrospetivo de placentas de gestações simples de grávidas portuguesas, não descrito até à data. Como *Potenciais Objetivos* estabeleceram-se valores de referência em função da IG para R-PFP, R-PPF e rácio de diâmetros placentares e espessura da placenta e volume placentar. Outros *Objetivos Adicionais* decorreram do projeto de tese, resultando em outros artigos relacionados como a classificação das lesões em produtos de aborto do primeiro trimestre e a avaliação de aspetos patológicos placentares em contextos fetais específicos.

Acredito em geral que a informação obtida pelas curvas de percentil será útil para o acompanhamento fetal e perinatal/neonatal através da melhoria da avaliação da função placentar e do equilíbrio entre o crescimento fetal e placentar. Contribuirão ainda para a prevenção de situações materno-fetais, concorrendo para o alívio psicossocial do casal individual e das famílias. Em particular, proverão para uma melhor estimativa do volume da placenta (enquanto parâmetro preditor da função placentar). Esta informação potenciará a avaliação de situações de risco associadas a desfechos adversos. Otimizará ainda as normas de orientação clínica para o seguimento fetal/perinatal, concorrendo para a diminuição da taxa de mortalidade fetal. Por último serão também relevantes para os investigadores envolvidos em estudos atualmente designados de "doenças do adulto com origem fetal - placentar".

Palavras-chave: Curvas de percentil; Gravidez simples; Idade-gestacional; Rácio peso fetal/peso placentar; Rácio peso placentar/peso fetal.

TITLE: A MULTICENTER STUDY OF SINGLETON PLACENTAS BIOMETRIC PARAMETERS AND FETAL WEIGHT IN FUNCTION OF GESTATIONAL-AGE

ABSTRACT

Any disturbance in normal sequence of development and growth may lead to discrepancy of placental and fetal features. It is known that birth weight/placental weight ratio (BPW-R) and placental/fetal weight ratio (PW-R) are more important parameters in the evaluation of placental function and fetal growth than placental weight and fetal (or newborn) weight alone. So, advances in understanding of placental growth in function of gestational-age (GA) could expand and focus the utility of placental examination and directed to the accomplishment of "Human Placenta Project" also.

The *General aim* of this thesis was to produce a Portuguese population-based, gestational-age-specific, percentile curves for placental weight (PW) and diameters, birth weight (BW), in singleton gestations, not yet described to date. *Potential objectives*: Establish reference values in function of GA for BPW-R, PW-R and ratio of Placental Diameters and Thickness and Placental Volume. *Additional objectives*, associated with the thesis project, resulted in others related manuscripts as a classification of First-trimester lesions and evaluation of placental features in fetal specific conditions.

I believe that these percentile curves information may be useful in many ways for clinicians to promote advances in placental and fetal follow up enlightening the placental function evaluation and the balance between the fetal and placental growth. These will also contribute to the prevention of maternal and fetal situations, improving the psychosocial relief of the individual couple and their families. In particular, will provide a better assessment of placental volume (as a predictor of placental function). This information will enhance the assessment (and prevention) of risk situations associated with adverse outcomes. It will further optimize the clinical guidelines for fetal/perinatal follow-up, contributing to the reduction of the fetal mortality rate. Finally, could be relevant to the research involved in the so-called "fetal and placental origins of adult diseases".

Keywords: Birth/placental weight ratio; Gestational-age; Percentile curves; Placental weight ratio; Singleton gestation.

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ABBREVIATIONS

ACho	acute chorioamnionitis
AC	abdominal circumpherence
AGA	appropriate (average) for gestational-age
AP	accreta placenta
APS	abnormal placental shape
AROM	artificial rupture of membranes
AART	assisted reproductive technology
AUIS	abnormal umbilical insertion site
β-hCG	human chorionic gonadotrophin β
BPW-R	birthweight placental weight ratio
BW	birthweight
CCho	chronic chorioamnionitis
CDKN1C	cyclin-dependent kinase inhibitor 1C
CGC	centro genética clínica
CHI	chronic histiocytic intervillositis
CHL	crown-heel length
CHM	complete hydatidiform mole
ChoCa	choriocarcinoma
CK	cytokeratin
CNS	central nervous system
CP	cerebral palsy
CPM	confined placental mosaicism
CRL	crown-rump length
CV	chronic villitis
CVS	chorionic villus sampling
DA	decidual arteriopathy
DM	diabetes mellitus
EA	early abortion
E-CH	embryo cranium-heel length
e-CHM	early complete hydatidiform mole
E-CRL	embryo-cranium-rump length
E-FD	early fetal death

EFPL	embryofetal pathology laboratory
EFW	estimated fetal weight
EGA	estimated gestational-age
EMA	epithelial membrane antigen
EP	ectopic pregnancy
EPS	exaggerated placental site
ESA	early spontaneous abortion
EVT	extra villous trophoblast
EW	embryo weight
FGR	fetal growth restriction
FL	foot lenght
FM	fetal movement
FMH	fetomaternal hemorrhage
FPW-R	fetal:placental weight ratio
FSVL	fetal stromal-vascular lesions
FTSA	First-trimester spontaneous abortion
FTSAS	First-trimester spontaneous abortion specimens
FVM	fetal vascular malperfusion
FW	fetal weight
GA	gestational-age
GD	growth-disorganized
GDM	gestational diabetes mellitus
GS	gestational sac
GTD	gestational trophoblastic disease
GTN	gestational trophoblastic neoplasia
HC	head circumpherence
HELLP	hemolysis, elevated liver enzymes, low platelets
H&E	hematoxylin-eosin staining
hCG	human chorionic gonodotrophin
HIE	hypoxic-ischemic encephalopathy
HM	hydatidiform mole
hPL	human placental lactogen
ICSI	intracytoplasmic sperm injection
IG	idade gestacional
IIL	inflammatory infectious lesions

IIIL	immune/idiopathic inflammatory lesions
IM	invasive mole
IUFD	intrauterine fetal demise
IUGR	intrauterine growth restriction (formerly “retardation”)
IUP	intrauterine pregnancy
IUPND	intrauterine pregnancy not documented
IVH	intraventricular hemorrhage
Ki-67	Ki-67 proliferation-related antigen
LA	late abortion
LGA	large for gestational-age
LPD	lymphoplasmacytic deciduitis
L-PD	largest placental diameter
MA	maternal age
MAP	morbidly adherent placentas (accreta)
MCI	massive chronic intervillitis
Mel-CAM	melanoma cell adhesion molecule
MFI	maternal floor infarction
MIR	maternal inflammatory response
MP	molar pregnancy
MPVFD	massive perivillous fibrinoid deposition (maternal floor infarction)
MSD	metabolic storage disease
MSVL	maternal stromal-vascular lesions
MVM	maternal vascular malperfusion
MSF	meconium-stained (amniotic) fluid
NIFH	nonimmune fetal hydropsis
NCV	nonspecific chronic villitis
NMP	nonmolar pregnancy
NRBC	nucleated red blood cells
OR	odds ratio
P	percentile
p57	p57 protein
PAS	periodic acid Schiff
PCK	pan-cytokeratin
PcV	plasm cell villitis
PD	placental diameter

PD>	largest placental diameter
PD<	smallest placental diameter
PEC	preeclampsia
PET	preeclampsia, toxemia
PFW-R	placental fetal weight ratio
pGTD	persistent gestational trophoblastic disease
PHM	partial hydatidiform mole
PIIP	placental inflammatory- immune-processes
PIH	pregnancy-induced hypertension
PMP	placental malperfusion
PPROM	preterm premature rupture of membranes; may be (>24hours)
PSTT	placental site trophoblastic tumor
PT	placental thickness
PVL	periventricular leukomalacia
PVP	placental vascular processes
PW	placental weight
PW-R	placental weight ratio
ROM	rupture of membranes
R-PFP	rácio peso fetal peso placentar
RPh	retroplacental hemorrhage
R-PPF	rácio peso placentar peso fetal
SA	spontaneous abortion
SAS	spontaneous abortion specimens
SCCA	suggestive change of chromosomal abnormality
SGA	small for gestational-age
S-PD	smallest placental diameter
SROM	spontaneous rupture of membranes
TA	therapeutic (or induced) abortion
TOP	termination of pregnancy
TORCH	toxoplasma others rubella cytomegalovirus herpes virus
UC	umbilical cord
US	ultrasound
ve-CHM	very early complete hydatidiform mole
VUE	villitis of unknown etiology

To António Paulo my dear husband, Pedro Luís and Filipa De Maria, our loving children.

To my mother Maria Amélia and my longing father Adelino by wisdom in teaching me the truth and its importance in all circumstances, and by their example in medicine practice.

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PREFACE

Human Developmental period is not controlled by a strict, hard-wired genetic programme. Complex interactions between genes and environmental factors during developmental period particularly in early stages when cells are differentiating and tissues are developing, play an important role not only in human behavior but also in susceptibility to diseases. Now this period is considered as a plastic phase which allows the organism to adopt to changes in the environment. Recently, studies have revealed that chronic placental insufficiency may result in fetal disorders namely neurologic diseases and increase the risks of diseases later in life.

So, placental studies can be useful in a variety of ways: (1) immediate diagnosis of important conditions affecting the mother, fetus or newborn; (2) identification of conditions that are likely to recur in subsequent pregnancies; (3) information that can guide the management of future pregnancies or influence the long-term care of the mother and infant; (4) giving a diagnosis that explains unexpected adverse outcomes; (5) as a gold standard for many epidemiologic studies and improving a real time image technics approach; (6) in recent years, understanding of aspects of placental pathology in perinatal adverse settings is widely used in medicolegal disputes.

When adequately studied, the placental findings including placental biometries have often been useful in settling of many difficult cases of perinatal mortality and neonatal diseases. Thus, recently, we have seen a greater number of requests for placental pathological study even in routine preterm or term deliveries.

The characterization and randomization of pathological placental processes were determinant for the gradual acceptance of a standardized, reproducible and biology-based classification system. (Amsterdam Placental Workshop Group Consensus Statement). This has led to advances in health care and the improvements of preventive measures in specific maternal and fetal conditions.

There are several placental conditions diagnosed only on histological examination. However, fetal morbidity and mortality related with placental gross phenotype are well-known. Also, any disturbance in normal sequence of development and growth may lead to discrepancy of placental and fetal features. Recently associations between birth weight placental weight ratio (BPW-R) and placental thickness (PT) with perinatal morbidity and mortality were described. So, advances in understanding of placental growth as a function of GA could expand and focus the utility of placental examination directed to the accomplishment of “Human Placenta Project”.

Placental pathology is related with clinical history and has significant implications in fetal, perinatal and neonatal periods, and also in maternal health.

The placental measurements namely, PW-R and BPW-R are important and common parameters in the evaluation of the placental function and for the balance between fetal and placental growth. However, the evaluation of placental parameters and measurements in relation to a Portuguese-based population has not been carried out to date.

Thus, the potential objective was to establish placental and fetal percentile curves across gestational-age (GA) in a Portuguese-based pregnant woman.

I believe that these percentile curves information may be useful in many ways for clinicians to promote advances in placental and fetal follow up enlightening the placental function evaluation, perinatal care and maternal health. , and consequently to recover psychosocial relieve of the couples and families. In particular, these may contribute to increase perinatal success and, directly the birth rate. Finally, could be relevant to the research involved in the so-called fetal and placental origins of adult disease and “Human Placenta Project”.

STATE OF ART

Initially, placental pathology focused on macroscopic abnormalities. Although distinctive, the macroscopic features do not explain all adverse outcomes.

Classification of placental lesions has evolved from being a purely descriptive exercise through a stage in which the major pathophysiological processes such as disorders of maternal implantation and amniotic fluid infection syndrome were first described to a recently proposed comprehensive classification system that includes maternal and fetal processes, infectious and idiopathic/immune inflammatory processes.

Authors demonstrated the association of microscopic features such as delayed villous maturation and fetal death; chorangiosis and neonatal morbidity and mortality; chronic villitis infections and intrauterine growth restriction (IUGR), fetal demise and neonatal morbidity or mortality; placental mesenchymal dysplasia and high rates of IUGR and fetal demise.

But new challenges appeared, in part associated with the advent of AART (Assisted Reproductive Technology) and ICSI (Intra-Cytoplasmic Sperm Injection) related both with parental infertility and also with increasing of maternal age and morbidity. There are well-known condititons associated with a distinct placental phenotype.

The understanding of placental pathology and the systematic approach to placental diagnosis have been important in expanding and focusing the usefulness of placental examination.

So, the request for placental pathologic examination (First-trimester abortions specimens (FTAS) and Second and Third trimester placenta) has increased in part by the requirements of obstetricians related with the advancements in prenatal diagnosis and with the new medically litigious challenging situations.

Even though risk factors for high and low placental weight are poorly understood we have seen a growing interest in the evaluation of PW and BPW-R as factors associated with adverse fetal or perinatal outcomes and diseases later in life.

Also, Measurements of the gestational sac (GS) and embryonic size have been used to evaluate pregnancy failure, intrauterine growth restriction (IUGR) and maternal diseases. However, in Portugal unfortunately, early abortion and placental pathology is still a subspecialty of surgical pathology undervalued, ineffectively handled and not clinically shared.

The pathological study of the placenta, serving as a gold standard, will continue to play an important role in assessing the quality of the diagnosis and in preventing potential adverse outcome in subsequent pregnancies of specific individual situations.

Placental morphology and biometric parameters adapted to the Portuguese population, was carried out to date. Knowing that placental volume is a better parameter to determine placental function, placental biometric parameters are important to evaluate placental volume. So, part of this study will include the evaluation and design human placental percentiles curves and embryo-fetal biometric parameters and birth weight as a function of gestational-age (GA) that reflect the Portuguese population. These may provide additional relevant information on fetal and placental follow-up in current maternal contexts.

Placental pathology will likely be important to validate new diagnostic test evaluation and comparative efficacy trials. Also, the goal of the recently initiated “Human Placenta Project”, are unlikely to be realized if pathological phenotype and data from the various “omics” technologies are not considered together.

"Lo que más importa a un sistema científico es que sea verdadeiro, e además de ser verdadeiro es preciso que sea compreendido"

In El Tema de Nuestro Tiempo
José Ortega Y Gasset

THESIS LAYOUT

The seeds of this thesis were sown when, about 30 years ago, at the beginning of my career as a pathologist, I began to study placentas in large numbers.

Placenta is a shared organ and the most important organ in the maintenance of a healthy pregnancy. It is a recognized diary of intrauterine life promising to explain the mysteries underlying poor pregnancy outcome.

Placental pathology in earliest stages were focused on macroscopic abnormalities. However, in itself, the macroscopic aspects do not explain all the adverse outcomes. Consequently, progresses in placental classification lesions, have been achieved through the gradual acceptance of standardized and biologically based pathophysiological processes.

Also, over the last decade, increasing research interest has been attached to the placenta weight and particularly to placental weight ratio (PW-R) and birthweight/placental weight ratio (BPW-R) has a risk factors to fetal outcome and early mortality, morbidity and even the development of diseases in adult life.

So, in order to prepare this thesis and to approach it in a systematic way, I read exhaustively theses, chapters of books and articles on the subject. Also, I prepared multidisciplinary courses with my obstetrician colleagues and trained my colleagues of surgical pathology and fellows in this specific area of pathology.

Thus, to introduce the topic of study of the thesis, in Chapter 1 I will develop a global view of the placenta, the methodology study approach and its more common pathological processes. Consequently, a comprehensive overview of the related methodology placental study and terminologies is covered in Chapter 1. In this chapter, I will emphasize the usefulness and importance of a competent evaluation of the placenta from macroscopic and microscopic examination until the production of the final report. Also, the normal morphology of the placenta and common lesions are briefly discussed and adequately illustrated. It whenever necessary, I would consider a comment heading for the understanding of the placental pathology and its clinical implications.

I believe that only a competent and standardized examination of the placenta could improve the comparison between studies of frequencies and significance of placental parameters and lesions. Also, will contribute to the appreciation of this area and will be useful to trainees in pathology, obstetrics and gynecology, cardiology, neonatology/perineonatology and other specialties such as radiology and genetics. It is certainly beneficial for clinical practice as well as could be an essential resource in litigation.

I hope that placental procedure and the percentile curves generated may serve as a guide for obstetricians and general surgical pathologists and, in the near future, as a comparative population-based percentiles curves. These may be essential for national and regional epidemiological studies on maternal, fetal or perinatal and child health.

In accordance with the standards outlined by Medical school of Minho University and Postdoctoral studies, this thesis is presented in the integrated-article format.

Therefore, this thesis consists of two distinct, yet highly dependent investigations. Both were addressed using data from the Embryo Fetal Pathology Laboratory Centro de Genética Clínica (CGC), in Porto, Portugal. The specific objectives are outlined below.

The principal goal consists in Portuguese Population-Based Placental measurements, Placental Weight Ratio (PW-R) and Birth/placental Weight Ratio (BPW-R). These results will be presented as 2 published manuscripts in Chapter 2.

Nowadays, birth weight (BW) percentiles curves are widely known and used in clinical practice to monitor fetal growth and classify the risk of some fetal conditions. Also, justify further investigation, in order to distinguish constitutionally small fetuses for gestational-age (SGA) from fetal growth restriction (FGR).

Although, studies referring to Placental percentile's curves such as PW, BPW-R and PW-R are very rare. Also, the evaluation of largest placental diameter (LPD or PD>), smallest placental diameter (SPD or PD<) and placental thickness (PT), and its relationship with fetal growth (e.g. FGR, SGA, appropriate or large for gestational-age) and others fetal adverse outcome are poorly explored yet.

So, resulting from the main investigation of the thesis – evaluation of placental biometric parameters across gestational-age (GA) – some additional investigations were generated and therefore are presented separately in Chapter 3, as well as referenced in Appendix 1, Appendix 2 and Appendix 3.

Consequently, the present Thesis is organized in 4 parts.

In Chapter 1, will cover a placental morphology from First-trimester to Second and Third trimester. The most common placental (macroscopic and microscopic) lesions will be briefly discussed and adequately illustrated. Also, some aspects of the usefulness of a competent examination of the placenta and their relevance in the litigious contexts are briefly introduced. This section will help to recognize a systematic approach to placental pathology, encompassing gross evaluation, sampling for histology, microscopic examination and pathological diagnosis.

Despite description of placental features and their maternal and fetal significance in the chapter, tables for easy and quick consultation were created. Also, a succinctly message of the utility of placental examination and placental measurements is introduced.

The subsequent Chapters 2 and 3 of this Thesis contains three manuscripts each with other contributing authors. Also, related works are referenced in Appendices 1, 2 and 3.

For the purpose of the thesis project Chapter 2, will develop a Population-Based Placental Weight across Gestational-age Pathological Study, ensuing two manuscripts: *Measurements in First-trimester Abortion Products: A Pathologic Study*, the order of authorship is a follow: Nogueira R, Sousa S; Braga AC, Azevedo A, Pereira N, Carmo O, Correia-Pinto J. and *Placental Biometric Parameters: The Usefulness of Placental Weight Ratio and Birth/Placental Weight Ratio Percentile Curves for Singleton Gestations as a Function of Gestational-age*, the order of Authorship is a follow: Nogueira R, Cardoso PL, Azevedo A, Gomes M, Almeida C, Varela C, Braga AC, Correia-Pinto J. Also, other related works are referenced in Chapter 3.

Chapter 3, integrates an approach to the usefulness of a classification of placental lesions presenting as manuscript: *Histopathological Classification of First-trimester Abortion Products Linking a Clinical Follow-up*, the order of Authorship is a follow: Nogueira R, Azevedo A, Cardoso PL, Cadillá J, Rodrigues G, Gomes M, Almeida C, Varela C, Correia-Pinto J. Others related works are referenced in Appendix 1, Appendix 2 and Appendix 3. Appendix manuscripts were published each with other contributing authors. (Appendix 1) *Macroscopic and Histological Study of the Placenta: An Essential Resource in Litigation Processes*, the order of authorship is as follows: Nogueira R, Pinto-Ribeiro F, Pereira S, Valente F. (Appendix 2) *Morphological Placental Portraits in Congenital Diaphragmatic Defects: A Pathological Study Approach*, the order of authorship is as follows: Nogueira R, Cardoso P, Braga AC, Almeida C, Nogueira-Silva C, Pinto-Correia J. (Appendix 3) *Four FATCO Syndrome Cases: Clinical, Autopsy and Placental Features with Literature Review Update*, the order of Authorship is a follows: Nogueira R, Sá J, Varela C, Amorim G, Valente F, Tavares P.

Chapter 4, will address the Thesis General Results and Discussion, and the Final Conclusions.

At each Chapter contexts, even though I followed the orientation of the Amsterdam consensus I would like to emphasize my points of view and relate them to my experience. Also, where relevant and related to my own experience, I gave my own opinions.

I hope that the readers will find the Thesis useful for clinical practice and, consequently, for maternal, placental and fetal management.

"The wisdom of the nature is such that it produces nothing superfluous or useless."

Nicolaus Copernic

CHAPTER 1

GENERAL INTRODUCTION

BACKGROUND OVERVIEW OF THE HUMAN PLACENTA ACROSS GESTATIONAL-AGE

At the beginning of my learning and introduction to placenta pathology and early miscarriage specimens, I confess that I was intimidated by the complexity of their anatomy and pathology. Today, I feel that the placental examination leads, in experienced hands and in most of situations, to a diagnosis. These allows parents and clinicians to understand the cause of the miscarriage or adverse outcome and also gives them an option of conduct in future pregnancies.

Pathological placental examination is an important step in the assessment of embryo and fetal malformation or identification of a previously unsuspected disease processes in the mother or infant that could require immediate attention.

Knowing that the goals in examination of therapeutic abortion include the goals stated for a SA, except for the increased likelihood of pathologic findings in the first and the fact that specific studies are more commonly requested at the last situation. It is important known that the First-trimester and Second and Third trimester placentas have distinct aspects and different types of lesions.

Adittionally, to be reproducible, pathological reports must adopt uniform sampling criteria and internationally accepted macroscopic and microscopic terminologies. (Khong TY, *et al.*, 2016; Turowski G, *et al.*, 2012; Redline RW, 2015). The standardization of gross and histological sampling of the placenta is important because focal lesions should not be missed, and because the comparison between studies of frequencies and significance of lesions is dependent on sampling methods.

A few research studies on the growth and development of human placenta are published to date. But placental measurements are crucial tools on pathological examination and essential for an understanding fetal outcome (e.g., small for gestational-age (SGA), large for gestational-age (LGA) or fetal growth restriction (FGR), fetal diseases and even fetal demise) and maternal disturbance.

The development of new technologies, imaging and other ways of monitoring the placental growth could be easier if it is matched with pathological competent examination. So, this is plausible that advances on pathological evaluation of the growth and development of the human placenta bring additional knowledge in this very significant field. This advances could be important implications in antedating the diagnosis of possible diseases related to problems suffered in utero and that may express later in adult life.

In routine pathology practice gross description of the placenta include the placental weight and biometry's: (i) Placental weight (PW) must be acquired trimmed of extraplacental membranes and umbilical cord. (Khong TY, *et al.,* 2016); (ii) Placental disk dimensions should include the

measurement of the placenta in three dimensions: the maximal linear dimension [length – largest placental diameter (LPD)], the greatest dimension of the axis perpendicular to this linear measurement [width – smallest placental diameter (SPD)], and the mural minimal and maximal placental thickness (PT). (Khong TY, *et al.*, 2016) Only the achievement of these measures allows percentile curves and correlation studies available.

There are evidences suggesting that placental shape and size are factors that may be statistically associated with pregnancy complications such as FGR, reduced fetal movements, and an individual's long-term health (Ericksson JG, *et al.,* 2011; Shehata F, *et al.,* 2011; Barker DJ, *et al.,* 2011; Barker DJ, *et al.,* 2012).

Placental biometric percentile curves are important predictors of fetal outcome, perinatal and neonatal morbidity or mortality and adulthood diseases. (Risnes KR, *et al.,* 2009; Yu KM, *et al.*, 1992). These are essential for comparative epidemiological national and regional studies also.

Actually, there are many studies on BW percentiles, however, the same does not happen with placental measurements parameters. I believe that additional information from placental biometric (e.g., PW, PDs, PT, PW-R) and BPW-R percentile curves studies as function of GA may be useful to clinicians and researchers in different areas. (See Chapter 2).

Such as the placental measurements, the placental phenotype and dysmorphology may explain a fetal demise or anomalies. These could be relevant to anticipate a fetal, maternal and/or placental disorders that could be monitoring in future pregnancies. (See Appendix 1, Appendix 2 and Appendix 3).

The characterization of pathological placental processes and their randomization with reproducible criteria allow the advances in health care and in appropriate preventive procedures. (Chang KT., 2014). So, the classification of placental lesions should be established as early as possible, in order to improve fetal follow-up (see Chapter 3). A competent placental examination could identify: (1) maternal or fetal disease requiring immediate medical intervention (e.g., fragmentation suggestive of placenta accrete; unusual infections such as cytomegalovirus, parvovirus or listeria; suggestive changes of aneuploidy or metabolic storage disease and mesenchymal dysplasia); (2) specific placental lesions with high risk of recurrence and relevant in the follow-up of future pregnancies (e.g., chronic histiocytic intervillitis, non-specific villitis, spontaneous preterm birth with histological chorioamnionitis between others); (3) specific conditions (e.g., umbilical cord disorders; early marginal abruption and oligohydramnios sequence; maternal floor infarction; placental hypoplasia within others) that explain an adverse outcomes as a fetal death, FGR, spontaneous

preterm birth, or central system nervous (CSN) injury; (4) placental lesions that can guide the management of future pregnancies or influence the long-term care of mother and infant (e.g., gestational trophoblastic diseases (GTD); maternal vascular malperfusion (MVP) or fetal vascular malperfusion (FVP) with neonatal sequelae; idiopathic/immune lesions, etc.) and, so, minimize these in future pregnancy. So, a competent placental examination allows the couple's monitoring and reproductive planning. (Redline RW, 2015). Furthermore, any additional information is relevant to the development of fetal or placental studies and research of the actually designated adult diseases with fetal origin.

Below, will be presented a briefly information on definitions, terminology and pathological examination approach.

1.1. PLACENTAL MORPHOLOGY

1.1.1 FIRST-TRIMESTER SPECIMENS

1.1.1.1 General Considerations

First-trimester spontaneous abortion specimens (FTSAS) are one of the most frequent specimens submitted to pathology study. They differ greatly in their composition (e.g, blood clots admixed with minimal decidua tissue, fragmented villous tissue and embryonic/fetal parts; a completely empty gestational sac (GS); an embryo; or something between). (Baergen RN, 2011; Nogueira R *et al.,* 2019).

For the pathologist, examination of FTSA generally involves several goals. The most basic is a documentation of an intrauterine pregnancy. At this instance it is important to known that the presence of decidual endometrium alone does not confirm an intrauterine pregnancy as this change is hormonally dependent and can be seen outside of pregnancy. Also, occasional trophoblastic elements or chorionic villi do not confirm an intrauterine pregnancy because rarely, a few chorionic villi or trophoblastic cells may be transported to the endometrial cavity from an ectopic pregnancy. (Baergen RN, 2011). Therefore, in these situations, communication with the submitting physician is essential in determining if an ectopic pregnancy is likely.

Potential goals of a competent FTSA products examination include the identification of previously unsuspected lesions; exclusion of gestational trophoblastic disease (GTD); estimation of

gestational-age (GA); and estimation of maceration (e.g., a change related with the time of intrauterine retention after embryonic death).

So, First-trimester pathological reports must adopt on sets of uniform gross descriptors, GS and embryonic sampling criteria, microscopic descriptors and terminologies. (Baergen RN, 2011; Middeldorp S. 2007; Freire K, *et al.*, 2009; Nogueira R, *et al.*, 2019).

1.1.1.2 Gross Descriptors

Macroscopy of FTSAS is primarily concerned with identification of different components such as decidual tissue, GS (villous tissue, amnion), yolk sac, umbilical cord and embryo.

Often the components are disrupted and intermixed with non-embryonic tissues usually more abundant than embryonic tissue (Figure 1). In general, gross examination of FTSAS does not provide diagnostic information alone, except in the cases of hydatidiform mole (HM) (Figure 2).

If genetic tests are required, embryonic and GS connective tissue samples must be sent in saline with ampicillin. Rarely, requests for molecular testing to rule out a specific disease are asked therefore, in a suspect context a special test may be requested. Nevertheless, this may not be possible on every spontaneous abortion (SA) for financial or practical reasons.

However, due to either unusual anomalies or no precise clinical information, it is prudent to freeze embryonic and GS (e.g., chorionic villi and chorionic plate) connective tissue samples in nitrogen liquid and store at -70°C. (Baergen RN, 2011). In this way, tissue will be preserved and may be used for many types of molecular testing, namely in the future. (Baergen RN, 2011).

It is known that chromosomal anomalies are present in more than 50% of spontaneous abortion (SA) (Brook *et al.*, 1984; Morton *et al.*, 1988; Philipp T, *et al.*, 2003; Kalousek, 1987; Kalousek, *et al.*, 1993; Nogueira R, *et al.*, 2019). Changes suggesting of chromosomal anomalies or metabolic diseases could be suspected on microscopy. So, oriented genetics or molecular tests (e.g., cytogenetics or flow cytometry, exome with copy number variation (CNV) or whole-exome sequencing) can be advantageous in specific microscopic findings. (Baergen RN, 2011; Lomax *et al.*, 1994; Criado *et al.*, 1997 Eiben *et al.*, 1990; Nogueira R, *et al.*, 2019).

If resources permit, fetal (e.g., muscle, lung, kidney and liver) and placental (e.g., chorionic villi and chorionic plate with amnion) connective tissue samples should be submitted for cytogenetic analysis for optimal results anticipating or minimizing in advance the possible diagnosis of confined

placental mosaicism (CPM) and the possibility of non-growing of cultures in cases of maceration namely embryo-fetal maceration. (Baergen RN, 2011).

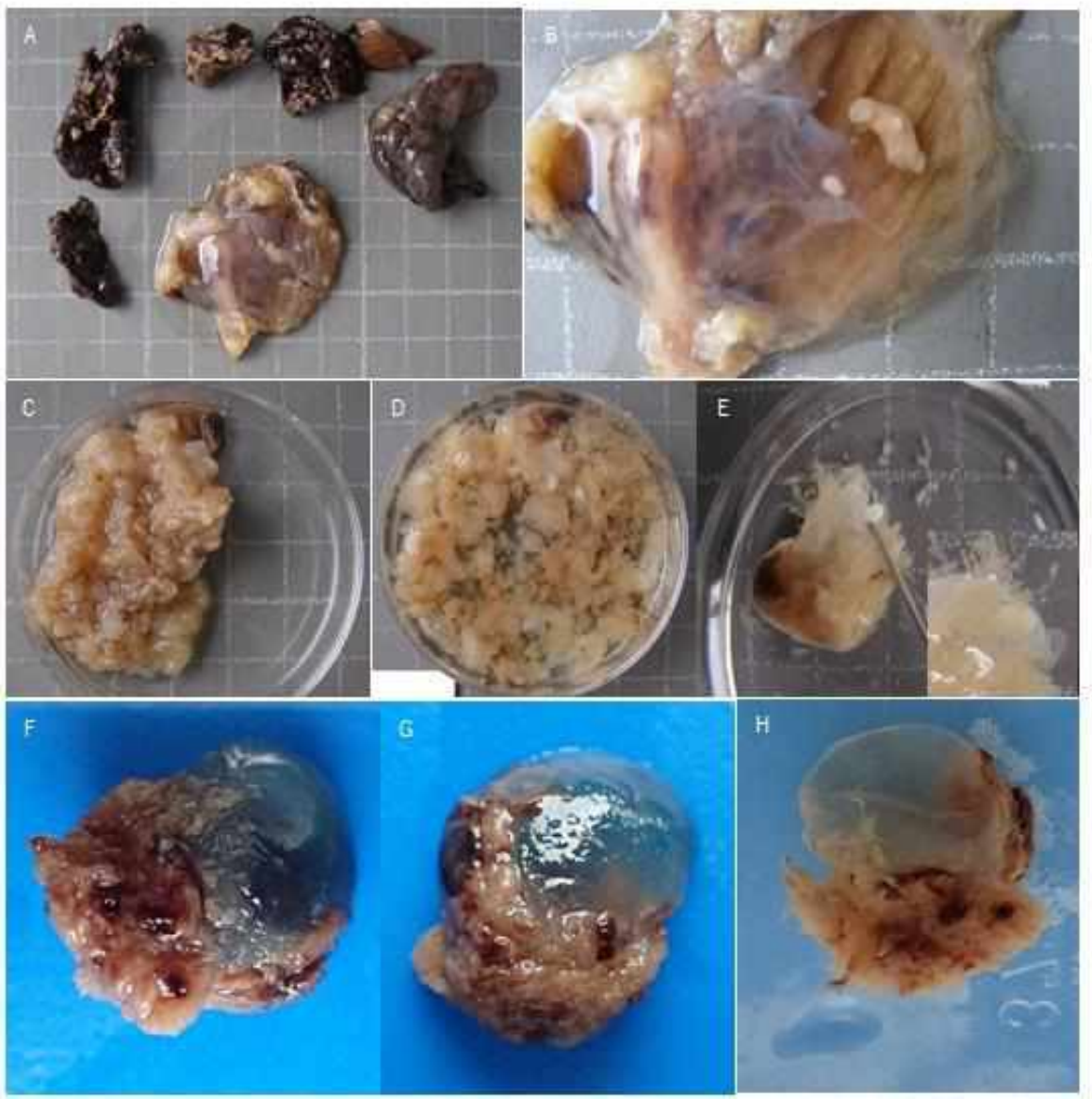


Figure 1. Macroscopic evaluation of First-trimester spontaneous abortion specimens. (A), Mixed disrupt components: decidua, blood clots, gestational sac, embryo. (B), Gestational sac with a growth disorganized embryo. (C), Fragment of decidual tissue. Note the membranous character with a granular surface. (D), Mixed disrupt components of decidual tissue and chorionic vill at center. (E), Intact gestational sac under saline with hydropic villi (inset). (F-H), Empty intact gestational sac under saline (H). Nogueira R, Embryofetal Pathology laboratory, CGC genetics.

1.1.1.3 Microscopic Descriptors and Pathological Report

Microscopic examination of FTSAS is essential to understand the underlying causes of pregnancy loss. These are often complex and with distinct pathogenic mechanisms. (Kalousek DK, *et al.*, 1987; Choi TY*, et al.*, 2014; Kalousek DK, 1998; Baergen RN, 2011; Philipp T, *et al.*, 2003).

Thus, microscopy is an essential key step that allows definitive diagnosis in most cases. (Middeldorp S, 2007; Freire K, *et al.*, 2009; Kalousek DK, 1998).

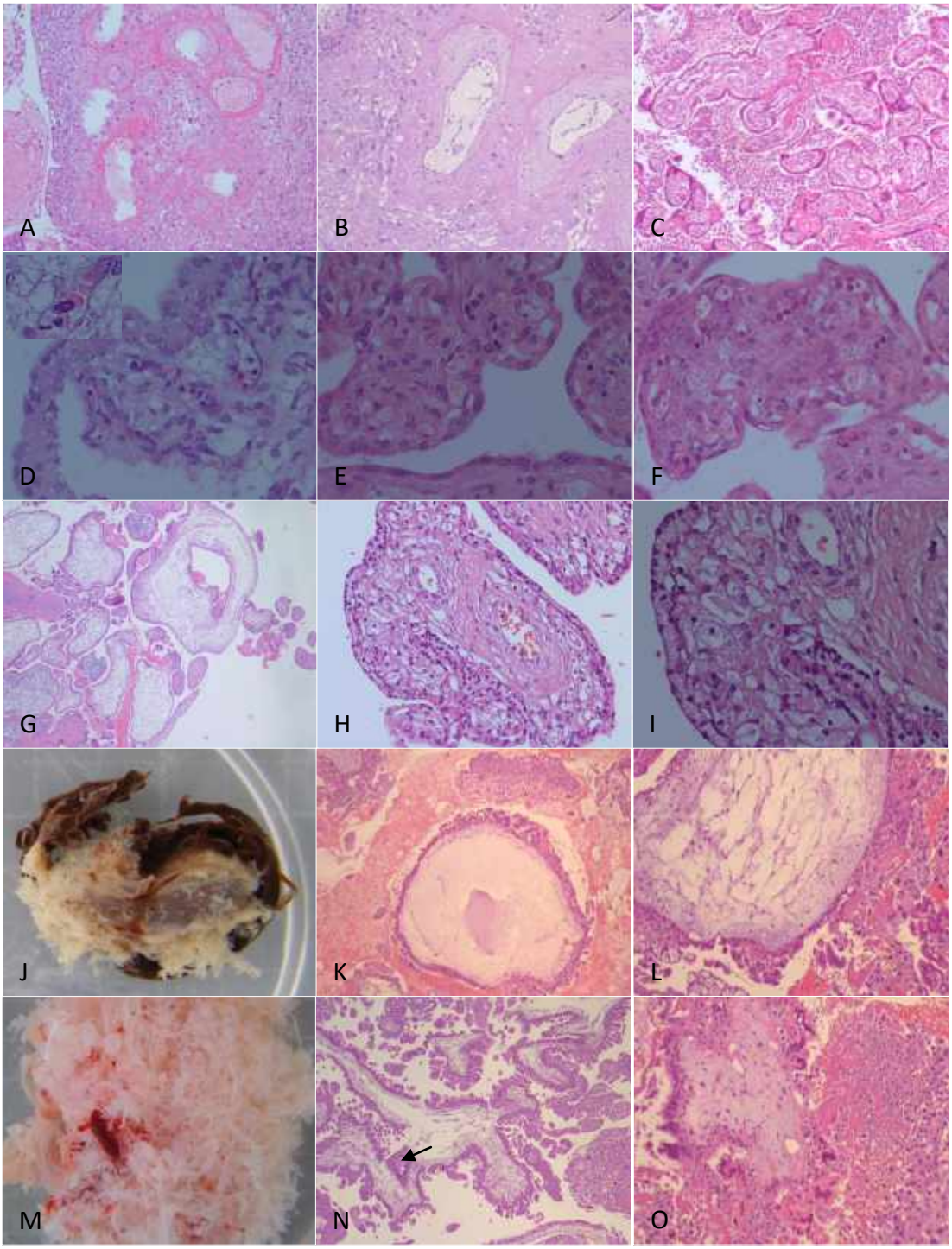
Microscopy (Figure 2) is crucial for evaluation the embryonic and GS development. It is essential to match the growth as a function of gestational-age (GA) and to diagnose maternal, placental or embryonic causes for pregnancy loss also.

Microscopically, on decidua and clots, we must analyze implantation zone, vessels lesions (see Figure 2) and inflammatory or infectious disorders (see Figure 2) even less common in First-trimester. Despite the characteristic macroscopic aspect of hydatidiform mole (HM) (see Figure 2) microscopic classification in partial hydatidiform mole (PHM) and complete hydatidiform mole (CHM) or early CHM (e-CHM) (see Figure 2) is very important for maternal follow-up and future pregnancies. So, microscopic evaluation and classification of HM is mandatory. Also, chorionic villi features suggesting change of chromosomal abnormalities (SCCA) or metabolic storage diseases (MSD), are well defined histologically (see Figure 2).

Additionally, Immunohistochemistry and genetic tests could be useful in some cases such as early complete hydatidiform mole (e-CHM) or very early complete hydatidiform mole (ve-CHM). (Gerulath AH, *et al.*, 2002). Besides, some trophoblastic neoplasia namely extravillous trophoblastic neoplasias and secondary neoplasias may be diagnosis only under microscopic examination and in addition requirements of immunohistochemistry may be crucial for diagnosis in difficult cases. (Ramilo I, *et al.*, 2014; Caldas R, *et al.*, 2017).

So, only microscopy (see Figure 2) allows the classification of FTSA lesions that could be: (1) maternal-deciduous (e.g., massive fibrinoid deposition, chronic intervillositis, maternal vascular malperfusion (MVM), and rare infections such as cytomegalovirus or parvovirus); (2) villous lesions (e.g., suggesting chromosomal abnormalities or metabolic disease and HM etc.) (See Figure 2); (3) embryonic disorders (e.g., growth disorganized (GD) embryo or specific organ defects such as neural tube defects between others) (Figure 3).

First-trimester classification lesions is an essential step on therapeutic approach and guidance in future pregnancies, allow the assessment of recurrence risk in subsequent pregnancy and helping in orientation of complementary genetic or others tests. (Kalousek DK, 1998; Philipp T, *et al.*, 2003; Nogueira R, *et al.*, 2019).



Fgure 2. Macroscopic and microscopic features of First-trimester unsuspected disease processes. (A), Microscopy of decidual vasculopathy: acute atherosis and fibrinoid degeneration. H&E. x200. (B), Hyalinized decidual vessels with adjacent minimal decidual necrosis. H&E. x200. (C), Microscopic features of severe chronic intervillositis consisting of infiltrates of lymphocytes. H&E. x100. (D-F), hydrops villi, erythroblastosis, parvorirus B19 infection (D, inset). H&E. x400. (G), Villous changes suggesting chromosomal abnormalities. H&E. x100. (H,I), Villi with trophoblastic and Hofbauer cells vacuolization typical of metabolic storage disorder. H&E. x200 (H) H&E. x400 (I). (J), Under saline gestational sac macroscopy. Note the hydropic villi character. (K), Marked villous edema with cistern formation. Note the acellular central region surrounded by edematous, loose stroma devoid of blood vessels and circumferential mild trophoblastic proliferation in a very early complete hydatidiform mole. H&E. x100. (L), Marked circumferential trophoblastic proliferation in a complete hydatidiform mole. H&E. x200. (M), Partial hydatidiform mole photographed under saline. Note bulbous swelling of terminal villi. (N), Enlarged irregular villi with cavitation and scalloped borders and trophoblastic pseudo-inclusions (arrow), circumferential mild trophoblastic proliferation. H&E. x100. (O), Marked circumferential trophoblastic proliferation in a complete hydatidiform mole. H&E. x200. Nogueira R, Embryofetal Pathology laboratory, CGC genetics.

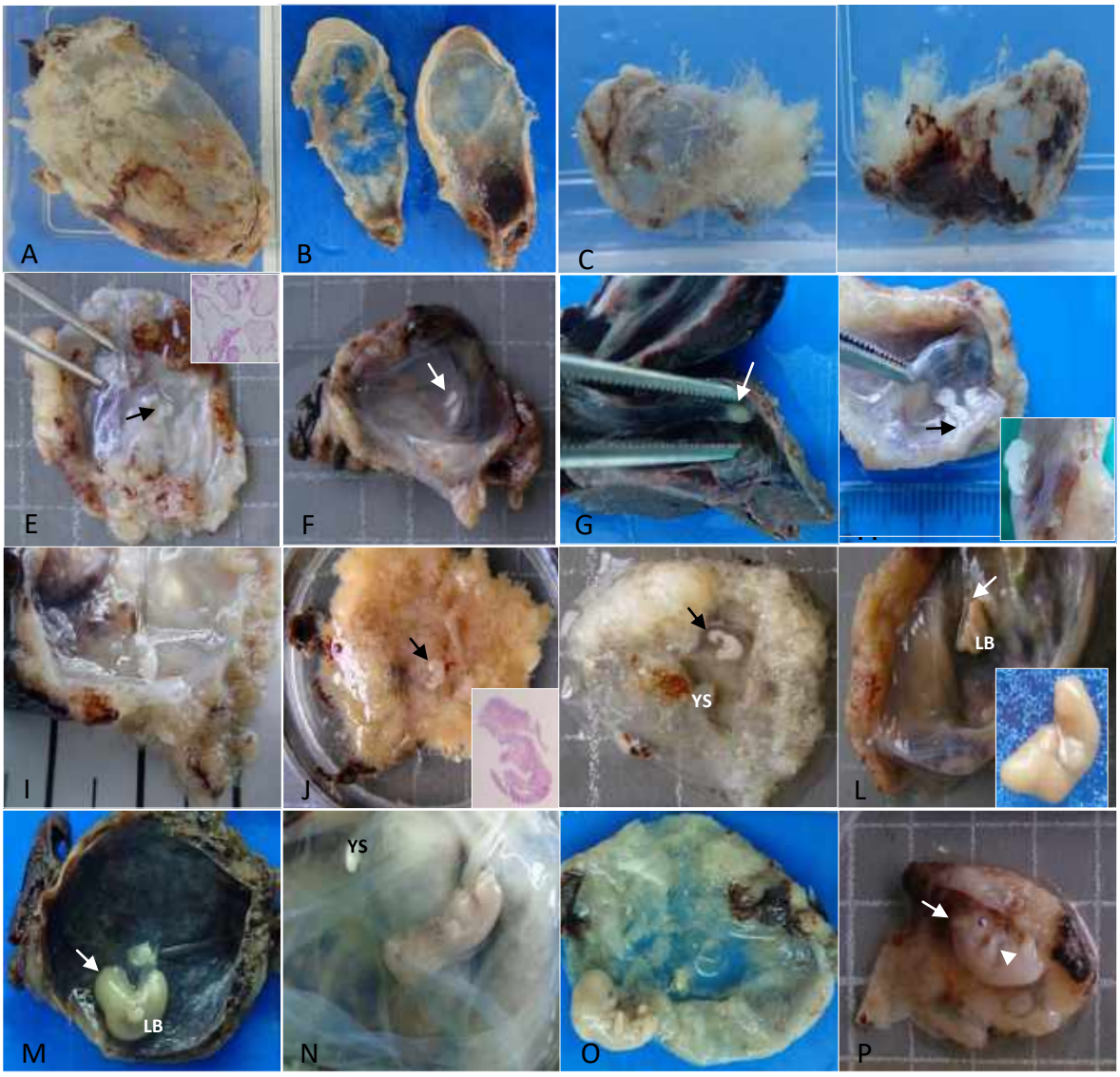


Figure 3. Macroscopic and microscopic features of normal embryo and growth disorganized (GD) embryo and sampling to histological study: (A, B), Intact gestational sac (GS) with sparse villi; B, close up opened empty GS, consistent with growth disorganized (GD) type 1 (GD1). (C), Intact GS with fine, soft and white chorionic villi with papillary fronds representative of their villous structure. (D), Empty gestational sac with chorionic hemorrhage. (E), Opened sac showing and amniotic sac that is significantly smaller than the surrounding chorionic sac (arrow), microscopy of hydropic avascular villi suggesting change of chromosomal abnormalitie (Inset). (F,G), Gestational sac containing a nodular embryo (e) consistent with a GD2, (arrows). (H), Gestational sac with an embryo with short body stalk (arrow), a GD3 embryo with no recognizable external features (Inset). (I-K), Embryos with recognizable cephalic and caudal poles (arrows). Microscopic features of a GD3 embryo (Inset). Note the yolk sac (YS) (K). (L-O), Embryos with recognizable cephalic and caudal poles. (L,M), Embryos with small and distorted head (arrow). Note the lymb buds (LB) and a cardiac prominence (Inset). (N,O), Embryos with small distorted head, absent or abnormal cervical flexion, a more develop limbs, retinal pigment and ventral prominence consistent with a GD4 embryo (N). Note the yolk sac (YS). (P), Embryo with neural tube defect: occipital encephalocele (arrow) and recognizable elbow (arrowhead). Nogueira R, Embryofetal Pathology laboratory, CGC genetics.

Microscopy is a big contribution to understand the adverse outcome including in early miscarriages. (Kalousek DK, 1998; Philipp T, *et al.*, 2003; Philipp T, *et al.*, 2002; Nogueira R, *et al.*, 1993; Nogueira R, *et al.*, 2019).

A competent First-trimester spontaneous abortion pathologic report must be biologically comprehensive, standardized and clinically easy-to-grasp to be relevant for national and international comparisons and as a platform for further research. (See Chapter 3).

1.1.2 SECOND AND THIRD TRIMESTER PLACENTA

1.1.2.1. General Considerations

Regardless placenta shows extensive growth in the Second and Third trimester, gross morphology is established early in pregnancy before the end of First-trimester.

Placental function and morphology must appropriately evaluate on the knowing of GA. These are crucial to matched fetal growth and development (Naeye RL, 1987; Thompson JMD, 2007; Pinar H, *et al.*, 1996; McNamara H, *et al.*, 2014).

The knowledge of placental phenotypes and morphology, (e.g., weight, shape, size namely diameters and thickness) have different significance at different GA, and that are related with embryo and fetal pathology allow us to provide suggestions on *what*, *when* and *how* to do, improving the interpretive context in specific maternal-placental-embryonic situations. Understanding the process of placental disease has become a challenge in assessing aspects of the placenta in new fetal and maternal situations. (Catov JM, *et al.*, 2015; McNamara H, *et al.*, 2014; Roberts DJ, *et al.*, 2006; Royal College of Pathologists, 2017).

The recently improved interest in placental study is also associated with the fact that previously unsuspected placental lesions take a major role in deciding negligence cases in obstetrics (Baergen RN, 2011; Nogueira R, *et al.*, 2015; Khong TY, 1997).

Gross and histopathological examinations of placenta have yielded insight, regarding morbidity and mortality in the fetus and offspring. Therefore, the knowledge of fetal morbidity and mortality associated with altered placental dysmorphology, structure and function had increased the demand of pathological study. (Catov JM, *et al.*, 2015).

Actually, placentas that are examined routinely provide relevant information to the management of subsequent pregnancies and maternal health. (Langston C, *et al.* 1997; Cox P, *et al.* 2011; Redline RW, 2008; Nogueira *et al.*, 2015; Khong TY, *et al.*, 2016; Turowski G, *et al.*, 2012; the Royal College of Pathologists guidelines 2017).

Although large perinatal registries have provided tremendous insight regarding risk factors and consequences of adverse pregnancy outcomes, placental data are included in only a few revisions. (Roescher AM, *et al.*, 2014; Catov JM, *et al.*, 2015; Redline RW, 2015).

Also, many reports combine placental lesions and perinatal outcome, but only some histologic lesions and its fetal consequences are partially understood (Lamont K, *et al.*, 2015; McPherson E.,

2016; Lawn JE, *et al.*, 2011; Fretts RC, 2005; Goldenberg R, 2010; Groen H, *et al.*, 2017; Stillbirth collaborative Research Network Writing Group, 2011).

1.1.2.2. Gross Descriptors

Placental Disk

Placenta is an anatomic and functionally complex organ and is ever-changing as it matures throughout gestation (Figure 4). It links the mother and fetus during pregnancy, facilitates exchanges of nutrients, oxygen and waste, and is the site of synthesis and selective transport of hormones and neurotransmitters.

Weight

As in early pregnancy, Placental weight (PW) and birth weight (BW) are associate with adverse outcomes. Placental weight can vary because it is affected by fixation, the amount of blood retained, and the intactness of the maternal surface. So, knowing that the PW is dependent on the amount of intervillous blood and blood in fetal vessel and formalin fixation it should be preferably achieved after proper washing of the placenta in current water and fixating in formaldehyde for a period of 24 hours or less, and after removing the umbilical cord and capsular membranes (see Figure 4). (Baergen RN, 2011; Benirschke K *et al.*, 2006).

Increase PW can be associated with many fetal diseases, placental tumors and infections, and maternal endocrine disorders. (Naeye RL, 1987; Thompson JMD, 2007; Pinar H, *et al.*, 1996). On the other hand, small and low weight placentas can often reflect an intrauterine growth restriction (IUGR) and may be correlated with circulatory maternal diseases (Naeye RL, 1987; Thompson JMD, 2007; Pinar H, *et al.*, 1996).

So, any deviation of normal PW and measurements can reflect fetal or maternal disorders and may be associated with disturbance in normal sequence of fetal growth and development.

Although there are few works reporting the PW and the placental weight ratio (PW-R) or birth weight placental weight ratio (BPW-R) during the grossing process. (Molteni RA, *et al.*, 1978; Perry IJ, *et al.*, 1995). Standard tables for PW in singleton placentas and BPW-R are available namely later in

pregnancy. (Thompson JMD, *et al.*, 2007; De Paepe ME, Shapiro S, Young LE, *et al.*, 2015; Asgharnia M, *et al.*, 2006).

Size and Thickness

Placenta at term has 18-cm to 20-cm diameter by 1.5-cm to 2.5-cm thick (see Figure 4). (Salafaia CM, 2009) Disturbance in normal sequence of placental development and growth may lead to disproportion of physical features. (Naeye RL, 1987; Thompson JMD, 2007; McNamara H, *et al.*, 2014). Association between morphology and chorionic thickness and early fetal growth disturbance are described previously also. (Salafaia CM, *et al.*, 2010; Salafaia CM, *et al.,* 2012).

Shape

The placental shape at Second and Third trimester generally is round to ovoid. (Salafaia CM, 2010; Salafaia CM, 2012). Any deviation in placental shape must be described since that can result from different causes (e.g., anatomic or implantation disorders, etc) as well related with adverse outcomes.

Bilobed and multilobed placentas (Figure 5) can traduce an abnormal implantation in lateral uterine sulci or be associated to uterine cavity abnormalities. Succenturiate lobes (see Figure 5), representing extra portions of placenta in the capsular membranes, are associate with a failure of atrophy of capsular villi. Unusual shape anomalies may have associated problems involving umbilical cord or membranous vessels (see Figure 5). (Salafaia CM, *et al.* 2010; Salafaia CM, *et al.* 2012).

True placenta Previa is a clinical diagnosis and means that placental villous tissue covers the cervical opening. This predispose to placenta accreta and others forms of abnormally adherent placentas (Figure 6). Associated complications can be infection, premature delivery and preterm premature rupture of membranes (PPROM).

Figure 4. Macroscopic evaluation of Second and Third trimester placenta: Placenta must be washed prior to formalin fixation (inset). Gross examination must follow a routine protocol. Evaluation of the umbilical cord color, insertion, length and diameter. Remove the cord from the placenta and check the number of umbilical vessels and lesions. Check for completeness, color and appearance of the membranes and insertion type. Remove fetal membranes. Make a rolling of membranes (inset). Placental disk weight and measures in three dimensions: diameters and thickness, and check for completeness, cotyledonary development, blood clots, retroplacental hematoma, etc. Serially section of placental tissue (chorion) at 0.5cm of intervals for evaluate the color and of villous lesions – note percentage, location and measure (inset). Sampling for histological study (paraffin blocks). Nogueira R, Embryofetal Pathology laboratory, CGC genetics.

Maternal surface and villous tissue

The placental parenchyma or chorion (villous tissue) is examined from the maternal surface, before and during the cut of serial sections (see Figure 4). (Baergen RN, 2011; Benirschke K, 2011).

Abnormalities on the color of villous tissue is largely determined by villous edema and fetal hemoglobin content related to hematocrit and with total blood volume. So, paler placentas are mostly characteristic in immature and some types of infections.

Palpation of the placenta often reveals lesions skipped by the knife and furthermore severe villous lesions show diagnostic gross morphology. Of the most common lesions are related to placental circulation disturbance related under perfusion maternal or fetal or both.

Villous infarcts (see Figure 6) are common lesions arising from maternal vascular malperfusion. Usually, old or subacute infarcts are grossly identified, through color change from red to white and sometimes cystic changes, also hemorrhage or intervillous fibrin thrombi may be seen. (Fox H, *et al.*, 1978; Katzman PJ, *et al.*, 2002; Mandsager NT, *et al.*, 1994).

Fetal Surface and Capsular Membranes

The fetal surface (surface membranes) and peripheral membranes (capsular membranes) are continuous, and most pathological processes are observed in both (see Figure 5). Capsular membranes normally insert at the margin of the villous tissue, which is usually the outer limit of the vascular plate (see Figure 5). A variable amount of decidua is attached to the membranes, this is where maternal vascular lesions are potentially found. The chorion is minimal at the periphery of the capsular membranes but is continuous on the surface membranes. So, there is a near proximity to the maternal blood of the intervillous space. This relationship permits maternal cells access to the membranes. Amnion is the layer of membranes closest to the fetus.

Figure 5. Second and Third trimester normal placenta and dysmorphology. (A), Normal fetal surface with eccentrically inserted umbilical cord. (B), Normal maternal surface, note the divisions into lobules or cotyledons. (C), Bilobed term placenta with velamentous cord insertion and velamentous vessels (inset). (D), Succentoriate lobes in a 35 weeks GA placenta and surface membrane invagination (inset). (E), Typical circumvallate placenta with prominent ridge of fibrin and degenerated blood at the periphery (inset). (F), Extramembranous or extracorial pregnancy (placenta) with circumvallation and diminutive opening, associated with early rupture of both the amnion and chorion. Note a white nodular cord lesion (inset), microscopically consistent with leiomyoma. (G), Dilatation of the umbilical cord associated with cavernous hemangioma (inset). (H), Aneurysmal dilatation of the umbilical vein with compression of arteries (inset). (I), Loose true knot in the umbilical cord and tight true knot (bottom). (J), Massive edema of umbilical cord at term pregnancy associated with fetal demise. (K), Opacity and gray-greenish color of fetal surface associated to inflammatory-infectious chorioamnionitis (inset). Note the subchorial / subamniotic hematoma. (L) Recent subamniotic diffuse hemorrhage. Fetal placental surface hemorrhage in circunmarginate placenta (bottom). Nogueira R, Embryofetal Pathology laboratory, CGC genetics.

Figure 6. Second and Third trimester placental lesions (cont.). (A), Thrombi in chorionic vessel are visible as tan-white streak (arrow); recent infarct in underlying parenchyma (inset). (B), Fetal surface of the placenta with fine granules mostly near the chorionic vessel surface (Inset) and not present on the cord which shows 3 vessels (left corner). (C), Placenta sent to pathological study with information of a simple gestation, gross capsular membranes with flattened mass of macerated tissue (arrow) consisting histologically on one fetus papyraceous (compressus). (D), Multiple subchorionic cysts and mesenchymal dysplasia demonstrating dilated and tortuous vessels surrounded by gelatinous material (arrow); parenchymal surface of the same placenta with enlarged grossly identifiable chorionic villi similar to those seen in partial hydatidiforme moles (Inset). (E), Placenta with marginal insertion type and a twisted (coiled) umbilical cord with 3 vessels (Inset). (F), Spontaneous abortion with markedly coiled cord and severe constriction (torsion) near the fetal surface (upper left corner), this was the presumed cause of death; excessively long coiled umbilical cords, note the marked congestion of the cord (upper right corner) and marked umbilical vein congestion (lower right corner). (G), Furcate insertion cord with visible thrombus in the fetal surface vasculature and subamniotic hemorrhage (arrow). (H), Severe constriction of the cord near the placental surface with layered fibrin intervillous thrombi (Inset). (I), Maternal surface of membranous placenta and fetal surface (lower Inset); parenchymal section showing degenerating chorionic villi enmeshed in fibrinoid (upper Inset). (J), Placenta with maternal surface sulci and peripheral disruption; "classic" picture with chorionic villi attached directly to the myometrium (Inset). (K), Placental maternal surface with a recent retroplacental hematoma showing recent adherent blood clots; cross section of the placenta shows subacute parenchymal infarct (Inset). (L), Cross section of the placenta showing parenchymal hematomas on the top the surrounding villous tissue present as red discoloration. Note a hematoma with cystic degeneration (left lower corner). (M), Severe retroplacental hematoma with thin underlying parenchyma presenting as white infarcted tissue (Inset). (N), Maternal surface of the placenta with "maternal floor infarction" (left) showing loss of normal cotyledonary structure; on the right, cross sections of the placenta demonstrate the typical "net-like" deposition of fibrinoid throughout the parenchyma. (O), Typical chorangioma bulging from the fetal surface (arrow); the cut surface of the same area showing a nodular red-brownish discoloration, normal placental tissue is seen underlying the tumor (Inset).

Umbilical Cord

The umbilical cord (UC) is lifeline of the fetus and contains the allantois, omphalomesenteric duct, vitelline vessels and umbilical vessels. The Wharton jelly and expanding amnion surrounds these structures and covers all cord. Most of the embryonic elements and the right umbilical vein disappear leave-taking two arteries and one vein (see Figure 6). Cord length increases throughout gestation but slowly in Third trimester. Cord accidents occur at all GA.

Length and Coil

One of the most obvious gross features of the UC is the length (see Figure 6). Most cord's length is achieved by the 28th week of pregnancy and, although growth slows after this time, it never ceases entirely (Miller ME, *et al.* 1982; Benirschke K, 1994). Although it is important to measure the entire cord length received at pathology room, the true measure is ideally done at delivery room. A minimum cord length of 32cm is necessary for normal vaginal delivery.

If we think that up to 7cm is left attached to the infant at delivery, and other fragments are discarded or used for blood gas determination or other testing we know that the cord is almost never entirety submitted to pathological study. (Baergen RN, 2011).

Also, the distinction between absolute and functional lengths is important, because a cord that is long but entangled around the fetus is functionally short.

Cord lengths seems to be determined by several factors, including gestational-age, genetics and fetal movement in utero. (Santos J, *et al.*, 2013; Semedo T, *et al.*, 2012; 2013; Nogueira R, *et al.*, 2016).

Both abnormally long and short cords have significant clinical relationship and are estimated to occur, in 4% to 6% and 1% to 2% respectively. Statistically, abnormal cord length shows more problems in neurologic development. (Catov JM, *et al.*, 2015; Zhao YJ, *et al.*, 2014; Rayburn WF, *et al.*, 1981; Miller ME, *et al.*, 1981). Long cords greater than 75cm are well associated with knots and fetal entanglements (see Figure 5 and Figure 6). By other hand short cord occurs in disorders with decrease fetal movement and are related with fetal development anomalies or syndromes also (see Figure 6). (Moessinger AC, *et al.*, 1982; Nogueira R, *et al.*, 2018). A traction on a short cord can cause fetal distress, cord tearing with hemorrhage and placental separation. (Moessinger AC, *et al.*, 1982).

The umbilical cord is usually coiled or twisted (see Figure 5). Spiral twisting of the cord is established early in development and most common twist is counterclockwise, a so-called left twist (Benirschke K, *et al.*, 2006). The degree of coiling has to be somehow uniform throughout the length of the umbilical cord and may be evaluated as left (counterclockwise) or right, hyper or hypo coiling and focal constrictions.

Coiling index has been used to evaluate the degree of twisting. This is defined as the number of coils divided by length cord, with an average coiling index of 0.21/cm or one complete spiral for approximately 5cm of cord. (Machin GA, *et al.*, 2000; Boue DR, *et al.*, 1995). The cause of twisting is unknown but seems to create more functional blood flow and helps to protect the vein from compression.

Similarly to cord length, coiling is believed related to fetal activity (Baergen RN, *et al.*, 2001; Benirschke K, *et al.*, 2006; Machin GA, *et al.*, 2000; Boue DR, *et al.* 1995; Peng HQ, *et al.*, 2006). An excessive twisting is associated with fetal morbidity and mortality (De Laat MWM, *et al.*, 2006; De Laat MWM, *et al.*, 2007; Jessop FA, 2014; Khong TY, 2010).

Cord complications can be related to length, torsion or coiling and strictures, knots, edema, velamentous vessels, entanglement and prolapse (see Figure 5). (Rayburn WF, 1981; Naeye RL, 1985; Miller ME, *et al.*, 1981; Benirschke K, 1994; Boue DR, *et al.*, 1995). Cord complications such as severe edema between others (see Figure 5) can result in an occlusion, rupture or thrombosis which quickly leads to fetal demise. (Naidu M, *et al.*, 2007; Hankins GD, *et al.*, 2002). Congestion and thrombosis in cord vessels or hemorrhagic events are helpful signs of true obstruction. Knowing that intermittent obstruction has been associated with intrauterine brain damage including hypoxic-ischemic encephalopathy (HIE), intraventricular hemorrhage (IVH) or other fetal distress as a bradycardia which can result in fetal morbidity or mortality (Machin GA, *et al.* 2000; Khong TY, *et al.* 2016; Krakowiak P, *et al.,* 2004; Luo G, *et al.*, 2013; Redline RW, 2015; Downey A, *et al.*, 2014; Proctor LK, 2013; Raio L, *et al.*, 2003; Baergen RN, *et al.*, 2001; Georgiadis L, *et al.*, 2014).

Strictures and torsion are more frequent near the fetal and placental cord insertions and are actually considered not related to post-mortem artifacts (see Figure 6). (Khong TY, *et al.*, 2016). True stricture within the mid-portion of the umbilical cord led to fetal demise also. (De Laat MWM, *et al.*, 2006; De Laat MWM, *et al.*, 2007; Jessop FA, 2014; Khong TY, 2010; Machin GA, *et al.*, 2000; Benirschke K, *et al.*, 2006; Benirschke K, 1994; Heifetz SA, 1988; Redline RW, 2015; Peng HQ, *et al.*, 2006).

Some investigators have suggested that constriction may occur in part because of a gradually diminishing amount of Wharton jelly, namely near the abdominal surface and they attest that it is not an artifact. (Redline RW, 2015; Khong TY, 2010). The pathologic changes associated with strictures support this, specifically the common finding of congestion on one side of the constriction and thrombosis in fetal vessels. (De Laat MWM, *et al.,* 2006; De Laat MWM, *et al.,* 2007; Jessop FA, 2014; Khong TY, 2010; Machin GA, *et al.,* 2000; Benirschke K, *et al.,* 2006; Benirschke K, 1994; Heifetz SA, 1988; Redline RW, 2015). Constrictions are also associated with fetal growth restriction (FGR) and preterm labor fetal intolerance to labor. (Machin GA, *et al.*, 2000; Benirschke K, *et al.*, 2006; Benirschke K, 1994; Heifetz SA, 1988).

Also low arterial PH in umbilical cord blood was strongly associated with chronic fetal hypoxia, perinatal mortality, and long-term adverse outcomes. These outcomes included HIE, PVL, IVH, cerebral palsy (CP) and death. (Machin GA, *et al.*, 2000; Peng HQ, *et al.* 2006; De Laat MWM, *et al.* 2007; De Laat MWM *et al.* 2007; Strong TH Jr, *et al.* 1993; Benirschke K, 1994; Heifetz SA, 1988; Naeye RL, 1985).

Diameter

Umbilical cord diameter varies considerably along the length and is generally thicker near the infant end. It is correlated with accumulation of fluid in Wharton jelly. The diameter may be greatly increased in cases of severe edema related to abrupt torsion and is mostly associated with fetal distress and death (see Figure 5).

Premature infants tend to have thicker UC than more mature babies, whereas in utero-placental insufficiency and placental hypoplasia the cords are usually thin. (Proctor LK, *et al.* 2013; De Laat MWM *et al.* 2006; De Laat MWM *et al.* 2007; Jessop FA, *et al.* 2014; Khong TY, 2010; Machin GA, *et al.* 2000; Cromi A, *et al.*, 2007; Nogueira R, *et al.*, 2018). Also, thinner or decrease cord diameter were described in fetal development anomalies. (Proktor LK, *et al.*, 2013; Cromi A, *et al.*, 2007; Nogueira R *et al.*, 2018).

Insertion Type

Central insertion is considered normal, it means a cord insertion more than 4cm at margin of the placental disk. Marginal and peripheral insertion is considered if lesser than 1 cm and 4 cm at

margin of placental disk respectively, and when drawstring route in the capsular membranes is considered velamentous. At interpositional insertion the surface amnion attaches to the cord as a web several centimeters before the cord reaches the placental surface (see Figure 4). (Khong TY, *et al.*, 2016; Baergen RN, 2011; Di Salvo DN, *et al.*, 1988; Semedo L, *et al.*, 2012). Any type of cord insertion, even the central one, can be a furcate or an interposition phenotype. (Baergen RN, 2011; Di Salvo DN, *et al.*, 1988).

Eccentricity may be related to unequal placental growth later in pregnancy. Velamentous and marginal insertion is more common in twins and higher multiples and in association with a single umbilical artery (SUA) and fetal anomalies. (Benirschke K *et al.* 2006; Baergen RN, 2011; Khong TY, *et al.* 2016; Luo G, *et al.* 2013; Nogueira R, *et al.*, 2018). Membranous vessels are not only confined to velamentous insertion but also are present in abnormal placental shape as occurs in bilobed and multilobed placentas and occasionally in marginal insertions. One the most serious complications of velamentous vessels is vasa previa, a clinical abnormal situation, in which membranous vessels are present over the internal cervical os and come first the presenting fetal part. (Peng HQ, *et al.*, 2006; Heifetz SA, 1988). All velamentous vessels have the same susceptibility to thrombosis, compression, disruption and trauma and hemorrhage (see Figure 5).

During delivery any disruptive event could lead to fetal death more often associated with exsanguinations or neurologic injury as IVH or HIE (see Figure 5). (Torrey WE Jr., 1952; Cordero DR, *et al.* 1993). Thrombosis, rupture and hemorrhage occurs in approximately 1 in 50 velamentous insertions with a mortality rate estimated as high as 73%. (Benirshcke K, *et al.*, 2006; Benirshcke K, *et al.*, 1994; Schreier R, *et al.*, 1962; Walker C, *et al.*, 2009; Naidu M, *et al.*, 2007; Di Salvo DN, *et al.*, 1998; Benirschke K, *et al.*, 1994; Semedo L, *et al.*, 2012; Naidu M, *et al.*, 2007; Heifetz SA, 1988). In a furcate insertion (see Figure 6), the umbilical cord divides into branch vessels above the chorionic plate and these have similar risks to other velamentous vessels. At interpositional insertion there is no loss of Wharton jelly, and is not usually associated with adverse outcome. (Benirschke K, *et al.*, 2010).

Vessels

Umbilical cord has three vessels, one vein and two arteries (see Figure 4 and Figure 6). Normally, the right umbilical vein does not develop. (Benirschke K *et al.* 2006; Baergen RN, 2011; Hua M, *et al.*, 2010; Khong TY, *et al.*, 2016).

Single umbilical artery (SUA) (see Figure 6) is a most common cord vessel anomaly. Absence of one umbilical artery may occur as aplasia or as the consequence of atrophy of one artery. (Benirschke K *et al.* 2006; Baergen RN, 2011; Hua M, *et al.*, 2010).). In 73% of the cases, the defect locates to the left artery and the associated fetal anomalies are nearly exclusively seen with left-sided absence. (Benirschke K *et al.*, 2006; Baergen RN, 2011; Hua M, *et al.*, 2010). Occasional persistence of the right umbilical vein is associated with SUA. (Benirschke K *et al.*, 2006; Baergen RN, 2011).

SUA occurs in approximately 0.5% to 1% of singletons deliveries and 8.8% in twins. (Benirschke K *et al.*, 2006; Baergen RN, 2011; Hua M, *et al.*, 2010). Placental anomalies are found in 16.4% of the cases (Heifetz SA, 1984).

SUA is higher as velamentous insertion or aberrant placental shape, FGR, maternal diabetes, antepartum hemorrhage, polyhydramnios and oligohydramnios. (Heifetz SA, 1984; Benirschke K, *et al.*, 2006; Baergen RN, 2011).

Congenital anomalies in SUA may involve any organ or system, and are present in 30% to 44.7% of infants in autopsy studies (Heifetz SA, 1984; Heifetz SA, 1988; Nogueira R, *et al.*, 2018). Renal anomalies are more frequent, with an incidence of 18.5%. So, neonatal sonography is often recommended in cases of SUA (Heifetz SA, 1984; Raio L, *et al.*, 2003; Hua M, *et al.*, 2010). Supernumerary vessels are a rare event in humans, and has been reported in association with congenital anomalies also. (Puvabanditsin S, *et al.*, 2011; Nogueira R, *et al.*, 2018).

1.1.2.3. Microscopic Descriptors and Pathological Report

Histologic examination is an essential and crucial step in pathologic study. (Redline RW, 2015; Khong TY, *et al.*, 2016). The specific diagnosis (classification) and dating evolution of some type of lesions is done only in the microscopic study. Also, only microscopy could identify conditions associated with high probability of recurrence in subsequent pregnancies, that could be important to the mother and fetal management namely in future pregnancies. (Redline RW, 2015).

Current placental lesions are nowadays grouped into a simplified categories: (1) Placental vascular processes (Figure 7); (2) Placental inflammatory-immune lesions (see Figure 7); (3) Other pathological processes that do not fit into the previous categories and some of them of uncertain pathogenesis (see Figure 5 and Figure 6), increased circulating erythroblast cells (see Figure 7), massive fibrin deposition commonly known as maternal floor infarction (see Figure 6) and effects of prolonged meconium exposure). (Redline RW, 2015).

These processes can be present at any GA and are associated with FGR, CNS injury, intrauterine fetal demise, early fetal death, recurrent miscarriage, preterm birth, perinatal death or a long-term neurodevelopment disability moreover related with HIE. Some of those condition (e.g., immune/idiopathic inflammatory processes and maternal floor infarction) have a high prevalence and a significant risk of recurrence in subsequent pregnancies.

Also, the microscopic grading and evaluation of vascular endothelial injury can predict the fetal inflammatory response (FIR) (see Figure 7) and associated risk of fetal morbidity. (Holzman C, *et al.*, 2007; Holzman C, *et al.*, 2013; Khong TY, *et al.*, 2016; Redline RW, 2005; Redline RW, 2015).

Microscopy is essential to perform a specific diagnosis or exclude potential differential diagnosis and dating the evolution of the diseases processes. Also, it is critical for interpretation of new genetic and epigenetic data, delineation of mechanistic pathways, and understanding the effects of exogenous environmental exposures on placental function and so is a gold standard to the recent "Human Placenta Project". (Redline RW, 2015).

1.2. PLACENTAL PATHOLOGY

The purpose of a competently placental and First-trimester specimens examination is: (1) identification of previously unsuspected disease processes or documentation of direct or indirect placental maternal or embryonic (fetal) conditions associated with high probability of recurrence in future pregnancies; (2) identification of any additional information that provide a specific explanation for an adverse outcome that can guide the management of future pregnancies or influence the long-term care of the mother and infant. (Redline RW, 2007; Luo G, 2013; Redline RW, 2015); (3) identification of an additional useful information that could be important for legal consideration in litigious contexts. (Nogueira R, *et al.,* 2015).

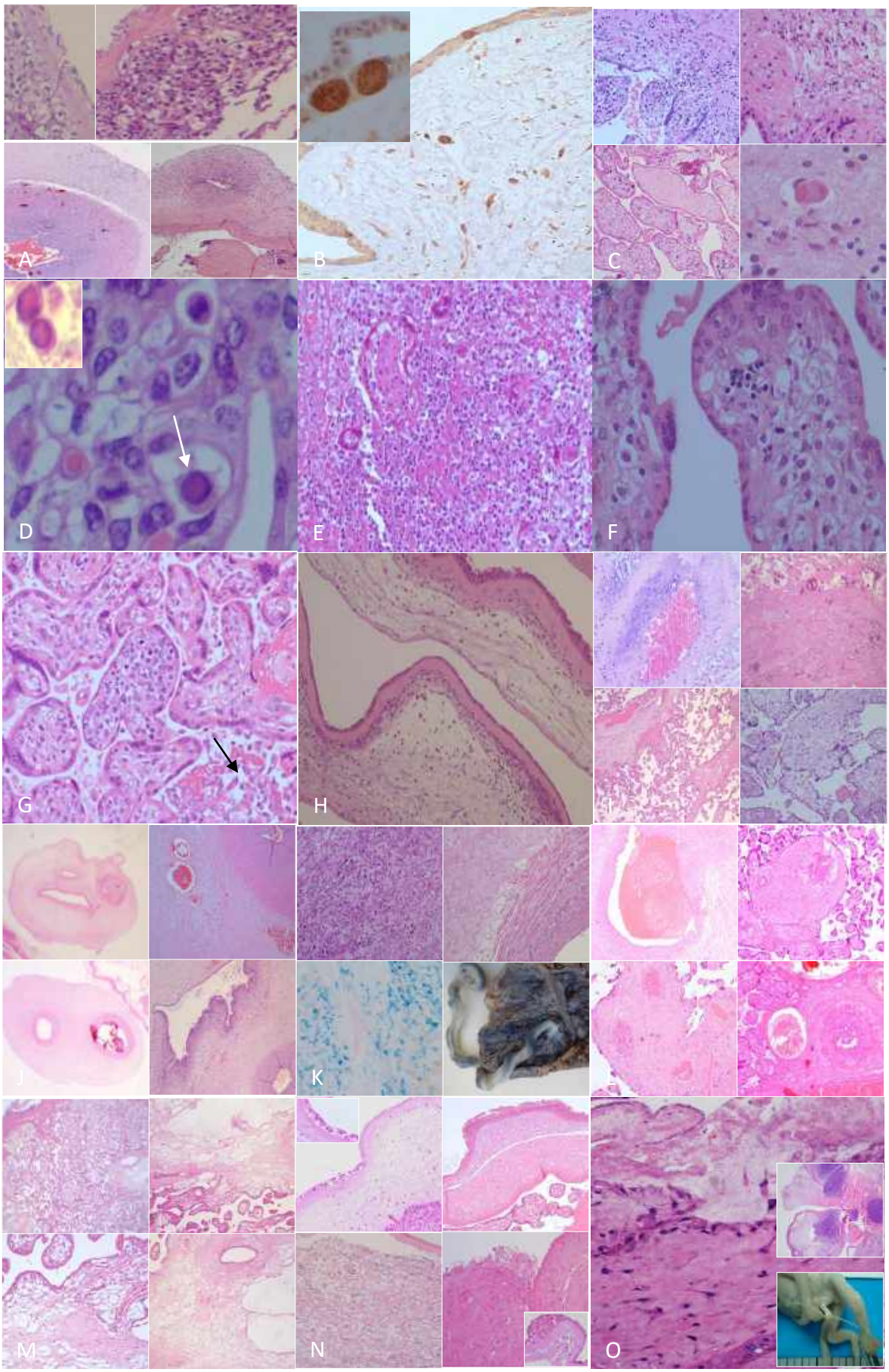


Figure 7. Microscopic features of placental disease processe

Legend of Figure 7. Microscopic features of placental disease processes: (A), Massive acute/subacute chorioamnionitis, with marked infiltration of acute and chronic inflammatory cells and necrosis is present in the amnion and chorion with fetal response and funisitis. H&E. x100. PAS stain showing pseudohyphae and yeast forms of *Candida* (upper left corner). (B), Chorioamnionitis due to *Toxoplasma*. Immunohistochemistry with antitoxoplasma marker x200. *Toxoplasma* cysts (inset). (C), Marked chronic villitis, composed almost entirely of plasma cells with focal necrosis of the trophoblast and vessel walls. H&E. x40 (upper left corner), H&E. x200 (upper right corner); Avascular villi. H&E. x100 (lower left corner); *Toxoplasma* cyst in a newborn brain with congenital infection. H&E. x200 (lower right corner). (D), Parvovirus B19 (inset) placental infection, normoblastic nuclei showing smudge intranuclear inclusion bodies (arrow). H&E. x400. (E), *Listeria* abscess, marked necrosis of villi, fibrinoid deposition and polymorphonuclear leukocytes infiltration. H&E. x100. (F), Nucleated red blood cells in placental hydrops. H&E. x200. (G), High grade chronic villitis associated with chronic intervillositis consisting of infiltrates of histiocytes and lymphocites in stromal villi. H&E. x200. (H), Chronic chorioamnionitis involving the fetal membranes. H&E. x100. (I), Maternal vascular malperfusion. Decidual vessel thrombi and vessel wall necrosis. H&E. x40 (upper left corner); Parenchymal old infarct. H&E. x100 (upper right corner); Preterm placenta with accelerated villous maturation. H&E. x40 (lower left corner); Term placenta with distal villous hypoplasia. H&E. x100 (lower right corner). (J), Segmental thinning of the umbilical vein an arterial thrombi. H&E. x20 (upper left corner); Microscopic appearance of a hemangioma of the umbilical cord. H&E. x40 (upper right corner); Single umbilical artery. H&E. x20 (lower left corner); Omphalomesenteric duct of the umbilical cord. H&E. x40 (lower right corner). (K), Old hemorrhage in the umbilical cord secondary to vein thrombosis. H&E. x40 (upper left corner) with Perl's stain positive (lower left corner); Muscle damage of the umbilical artery. H&E. x100 (upper right corner); The newborn placenta (lower right corner). (L), Fetal vascular malperfusion. Thrombosis of a fetal artery (upper left corner); Stem vessel obliteration consisting in marked thickening of the vessel wall with luminal obliteration (upper right corner); Villous stromal vascular karyorrhexis (lower left corner); Intramural fibrin deposition indicating remoteness of lesion (lower right corner). H&E. x40. (M), Mesenchymal dysplasia showing dilated and hydropic villi with no trophoblastic proliferation (top); Myxoid strome in some villi with persistence of fetal appear thick and abnormal wall (bottom). H&E. x40. (N), Pathology of the fetal membranes. Vacuolated amniotic epithelium in a case of gastroschisis (upper left corner); Squamous metaplasia typical of mature placenta (upper right corner); Vernix caseosa with squames and other debris (lower left corner); Late stage of amnion nodosum with large nodule, early stage (inset) (lower right corner). H&E. x40. (O), Placental amniotic band. H&E. x100; encircling extremities in an aborted fetus (insets).

However, different sampling and definitions between laboratories may compromises the value of placental examination in investigation of adverse pregnancy outcome and more recently, in longer-term impact on the well-being of the mother and child or both. (Rees S, *et al.,* 2005; Barker DJ, *et al.*, 2012; Amaral LM, *et al.*, 2015; DeRoo L, *et al.*, 2016). So, it was important an implementation of a unified system with reproducible sampling criteria, grading and staging of placental lesions. (Khong TY, *et al.*, 2016). These help to establish evidence-based recommendations for placental submission and play an essential role in understanding placental lesions and longer-term impact on the fetal and maternal health.

It is crucial to progress in studying the pathogenesis, diagnosis, and treatment of obstetric (fetal and maternal) disorders with an underlying placental etiology. (Redline RW, 2015; Khong TY, *et al.,* 2016).

Recognizing the importance of the placenta in understanding adverse outcomes, it is crucial to interpret disease processes in the effective development of new tools to monitor not only the size and growth of the placenta but also to measure its functional capacity.

1.2.1. FIRST-TRIMESTER SPECIMENS

1.2.1.1. Current Topics

First-trimester specimen moreover was submitted, to pathology laboratory, fresh in saline water with ampicillin or fixed in formalin.

Gross evaluation is primarily concerned with the identification of the heterogeneous components (e.g., decidual tissue, villous tissue, embryonic or fetal tissues and variable amount of blood clots). Many instances early abortion products are better examined under saline specially to detect chorionic cistern transformation such as occur in HM.

Firstly, a statement about whether or not the gestational sac (GS) and the embryo or fetus are the appropriate size for gestational-age (GA) is obligatory.

Measurements and photographs must be taken during gross examination. These are invaluable for consultation or documenting incompleteness specimens. (Gilbert-Barness E, *et al.*, 2004; Nogueira R, *et al.*, 2019).

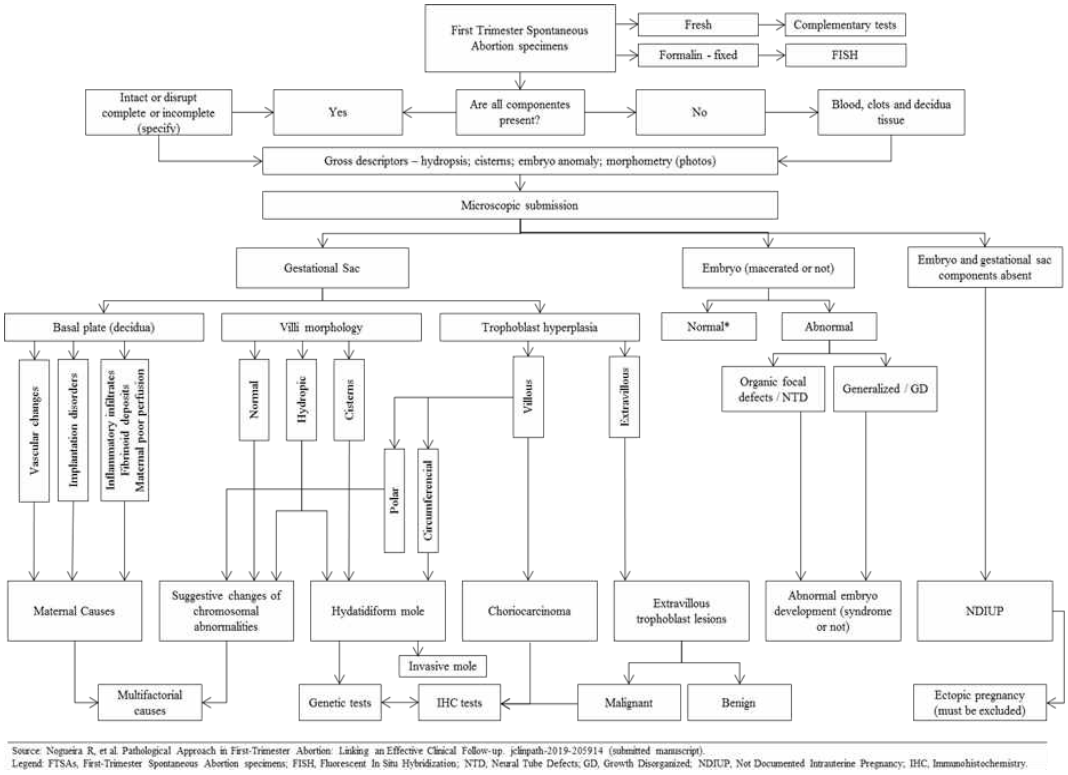
Measurements is best accomplished comparing GS-diameter and chorion-thickness, embryo crown to rump length (E-CRL), foot length (FL) and embryo weigh (EW) with standard tables. Tables of normative values with GS-weight and GS-diameter, E-CRL, embryo crown-heel (E-CH), and foot length can be used for this purpose (Nogueira R, *et al.*, 2019; Kalousek DK, *et al.*, 1992).

Hematoxylin and eosin (H&E) stain is the routine standard stain for histology which always optimizes the gross examination.

In specific context and if the diagnosis is equivocal, additional sections to microscopic examination should be submitted. If the question is not clarified or remains equivocal, additional tests (e.g., immunohistochemistry, fluorescence in situ hybridization (FISH) should be done on paraffin-embedded, fixed tissue for the identification of chromosomal aneuploidy, confined placental mosaicism (CPM) and lesser for identification of microorganisms). (Criado B, *et al.*, 1999; Lomax BL, *et al.*, 1994). Immunohistochemistry can be used in some difficult cases as an early-complete hydatidiform mole (e-CHM) (see below).

An algorithm of First-trimester specimen's was constructed (Table 1) as a quickly approach to classification of First-trimester lesions and a more developed approach could be consulted below (see Chapter 3).

Table 1. Algorithm to a classification of First-trimester lesions.



Source: Nogueira R, et al. Pathological Approach in First-Trimester Abortion: Linking an Effective Clinical Follow-up. jclinpath-2019-205914 (submitted manuscript).
Legend: FTSAs, First-Trimester Spontaneous Abortion specimens; FISH, Fluorescent In Situ Hybridization; NTD, Neural Tube Defects; GD, Growth Disorganized; NDIUP, Not Documented Intrauterine Pregnancy; IHC, Immunohistochemistry.

1.2.1.2. Gross Examination

A competent evaluation (see below) must examine all the components of the specimen and several general question should be addressed in the initial examination of First-trimester specimens (FTS) (see Table 1). Histological sample must always submit for microscopic examination to perform a classification of FTSA lesions.

Decidual tissue usually appears as a small, irregular or flattened sheet of tissue and must be always submitted for histology (see Figure 1).

Blood clots must be quantified and despite do not contain usually diagnostic material, if it is granular or firm, it may contain fragments of degenerated chorionic villi within (see Figure 1) and must be submitted for histology. This may be helpful, when villous tissue is not identified grossly.

Gestational sac (GS), chorionic villi are generally thin, soft, and white, with papillary fronds representative of their villous structure, and it is often attached to fragments of shiny, translucent membranes (see Figure 1). GS can be intact and empty or with an embryo, and disrupt or

fragmented. Weight, diameter and chorion-thickness must be achieved whenever possible. Grossly hydropic villi are usually not present except in the case of HM (see Figure 1).

Embryo (or embryo tissue components) should be evaluated and described (see Figure 3). Any abnormal embryo development or maceration should be documented.

The growth disorganized (GD) embryos (see Figure 3) were based on their degree of disorganization, usually defined in 4 types: GD1, GD2, GD3 and GD4. (Gilbert-Barness E, Debich-Spicer D, eds, 2004; Phillip T, *et al.*, 2003; Kalousek DK, 1998; Baergen RN, 2011; Nogueira R, *et al.*, 2019). The GD1 is characterized by an intact amniotic sac with no evidence of embryo; the GD2 consists of a nodular embryo; the GD3 relates to an embryo up to 10 mm long, with caudal and cephalic poles present, but without recognizable external structures, moreover retinal pigment can be present and the GD4 is an embryo with 3-17 mm long, usually with a major distortion of body shape always involving head and generally with a fusion of the chin and chest.

If a normal Embryo with the appropriate size and features for the stated GA (see Figure 3) is identified, and the overall components are present and intactness, maternal factors may be the cause of early abortion (see Table 1). So, histological study is essential for diagnosis (see Figure 3).

Histology must always complement the gross features concerning almost the cases a diagnosis (see Table 1).

Rarely, request for molecular testing of embryonic or fetal tissue are made to rule out a specific disease such as chromosomal or metabolic disorders (see Figure 3). In this case, communication with the genetic laboratory is essential.

1.2.1.3. Sampling for Histology

At least four cassettes should be submitted and must be represent of all components with completeness of the embryo. Additional sections are recommended in patients with recurrent or habitual abortions. If on initial microscopic examination an intrauterine (or ectopic pregnancy) cannot be documented, the remaining tissue or even the entire specimen should be submitted.

1.2.1.4. Microscopic Examination, Terminology and Diagnosis

An easy approach to microscopy of GS (First-trimester placenta) and embryo, is to review each part of the components (e.g., gestational sac, umbilical cord, membranes and embryo).

Microscopic sections of each component should evaluate the maturity and appropriateness for stated GA of overall components, the presence of abnormalities either in embryo or GS, as well as estimate the interval of retention after embryonic death (see Table 1).

The GS must be analyzed sequentially, that is, the basal plate (e.g., decidual tissue and decidual vessels; placental chorionic villi (septal and anchoring villi); cells islands; intervillous space; chorionic plate, fetal vessels, yolk sac; umbilical cord, number of vessels and other lesions and membranes.

Embryo must be analyzed in completeness and any abnormal deviation stated for GA.

Clots must be analyzed particularly in abortion retention or incompleteness namely associated with molar or other trophoblastic diseases.

Basal Plate

Represent the part of the maternofetal junctional zone. Is composed of an admixture of decidual cells, extra-villous trophoblast, utero-placental vessels and endometrial glands.

Utero-placental veins consist of endothelium surrounded by few medial and adventitial cells embedded in decidua and extra-villous trophoblast cells (see Figure 2).

Utero-placental arteries traverse the basal plate in spiral turns, and connect the maternal uterine arteries to the intervillous space (see Figure 2).

Decidual cells are transformed endometrial stromal cells and are round to ellipsoid phenotype (see Figure 2).

Normally, in the decidual and implantation site, we can identify lack of physiologic conversion or thrombosis and atheromatosis in decidual vessels that could be associated with SA and are most suggestive of maternal factors (e.g., preeclampsia and other conditions of decreased utero-placental perfusion) (see Figure 2).

Chorionic Villi

Chorionic villi changes can suggest possible etiologies for the pregnancy failure (see Figure 2): (1) Delayed maturation, with decreased vascularity and the presence of large atypical cells of unclear origin in the stroma (e.g., trisomies 13, 18, 21); (2). Variable cellularity and stromal fibrosis, trophoblastic hypoplasia with deficient syncytial budding (e.g., classic hydropic abortion, monosomy-X); (3) Villi edema, trophoblastic pseudo-inclusions or invaginations suggesting chromosomal anomalies; (4) epithelial trophoblastic and amniotic changes suggesting metabolic diseases; (5) mild hydropic change in the villi may be seen in embryonic death whereas markedly hydropic villi suggest a molar pregnancy.

Microscopy is essential to distinguished between hydropic abortion and HM namely e-CHM or ve-CHM (see Figure 2) and to make a differential diagnosis among some gestational trophoblastic diseases (e.g., placental site trophoblastic tumor (PSTT), choriocarcinoma (ChoCa) and mesenchymal dysplasia (Table 2). So, in difficult cases in which histology cannot make the diagnosis alone, it is recommended complementary studies such as immunohistochemistry, genetic and molecular tests.

Table 2. WHO classification of gestational trophoblastic disease.

Trophoblastic neoplasms	**Non-neoplastic lesions**	**Molar pregnancies**	**Abnormal (non-molar)**
Choriocarcinoma	Exaggerated placental site	Hydatidiform mole	Mesenchymal dysplasia
		Complete	
Placental site trophoblastic	Placental site nodule and	Partial	Confined placental
tumor	plaque	Invasive	mosaicism
Epithelioid trophoblastic tumor			

Adapted from WHO Classification of Tumours of Female Reproductive Organs, Lyon 2014, P. Hui, R. Baergen.

Immunohistochemistry is essential to differentiate some type of cells and components in some difficult lesions. Immunohistochemical studies could help to distinguish trophoblast cells from other cells and may be able to differentiate types of trophoblast cells and different types of trophoblastic lesions (Table 3). Anti-cytokeratin (CK) markers stain epithelial cells such amniotic epithelium and all trophoblast cells. It is a useful marker in distinguishing extra-villous trophoblast from decidual cells or intra-arterial trophoblast from maternal endothelium. Human chorionic gonadotropin (hCG) stains strongly syncytiotrophoblast cells, while human placental lactogen (hPL), α-inhibin (alfa-inhibin), melanoma cell adhesion molecule (Mel-CAM) also known as CD146 and placental protein 19 (PP19)

are good markers in the differential diagnosis of certain lesions including melanomas and various types of gestational trophoblastic lesions including extra-villous trophoblast disorders.

Also, a cyclin-dependent kinase (CDK) inhibitor p57KIP2 also referred to as p57 is strongly expressed in maternal tissue such as decidua and cytotrophoblast cells and villous stromal cells if a maternal component is present. So, it may be helpful in difficult cases such as hydatidiform partial mole (HPM), hydropic abortion and complete hydatidiform mole (CHM) namely early CHM (e-CHM) or very early CHM (ve-CHM). (Kurman RJ, *et al.*, 1984; Baergen RN, 2011; Fukunaga M, 2002; Genest DR, 2001). Cytokeratin and P57 are useful markers in distinguishing gestational trophoblastic neoplasia (see Table 3). (Kurman RJ, *et al.*, 1984; Baergen RN, 2011).

The antibody Ki-67, is expressed in proliferating cells and is a useful marker to distinguish proliferating stem cells population from differentiated ones. It is useful to evaluate the labeling index in specific conditions as placental site nodules (PSN) and placental site trophoblastic tumor (PSTT) (see Table 3) (Baergen RN, 2011). Also, in some cases with equivocal diagnosis, serum β-hCG levels should be requested and clinical correlation is mandatory and may be essential in the differentiation of these kind of lesions.

Other antibody studies may be helpful when abnormal or atypical cells are present in the intervillous space or fetal vessels. These may represent choriocarcinoma or metastatic tumors from the mother or fetus.

Finally, in some occasion's immunohistochemistry may be helpful in identification of rare specific organisms causing chronic infectious villitis such as cytomegalovirus, herpes virus and parvovirus B19 within others.

Table 3. Trophoblastic neoplasms and nonneoplastic, non-molar lesions.[a]

Pathologic characteristics	PSN	EPS	PSTT	ETT	ChoCa
Gross					
forms a mass	-	-	+	+	+ -
Histology					
chorionic villi presente	-	+	very rare	very rare	-[1]
Fibrinoid	+	+	+	-	-
Hemorrhage	-	+ -	+	+	+ +
Necrosis	-	+ -	+	+ +	+ +
vascular invasion	-	+	+	+	+
degenerative changes	-	-	-	-	+
extravillous trophoblast	+	+	+	+	-
syncytiotrophoblast	-	+	-[2]	-	+ +
nuclear pleomorphism	-	-	+ +	-	+ +
mitotic activity	minimal or absent	minimal or absent	+	+	+
History of previous mole	-	-	5-8%	5-8%	50%
Metastasis	none	none	occurs in 10-15%	occurs in 10-15%	Potential
Prognosis	no sequelae	no sequelae	guarded if malignant	guarded if malignant	>90% responsive to chemo-therapy
Serum βhCG	normal	appropriate for pregnancy	moderately ↑in 80%	moderately ↑in 80%	markedly ↑

[a]Adapted from Benirschke and Kaufmann's, pathology of the human placenta, Baergen. Springer, 2004 ISBN 0-387-22089.
PSN, placental site nodule; EPS, exaggerated placental site; PSTT, placental site trophoblastic tumor; ETT, epithelioid trophoblastic tumor; ChoCa, choriocarcinoma; βhCG, β human chorionic gonadotrophin; GTD, gestational trophoblastic disease; CHM, complete hydatidiform mole; PHM, partial hydatidiform mole; IM, invasive mole; hPL, human placental lactogen; EVT, extravillous trophoblast.
[1]Villi are present only in placental or "in situ" ChoCa. [2]Multinucleate cell similar to syncytiotrophoblast may be present.

Embryo

Embryo abnormalities could be related with several causes, moreover suggesting of a genetic or chromosomal disorder, and could justify the pregnancy loss. Hydrops could be associated with aneuploidy, teratogens or some lethal submicroscopic genetic events; disruption and infectious contexts could explain miscarriage also (see Figure 3).

If a focal or generalized defect was identified correlation with clinical history and genetic tests should be performed. Microscopy is essential to identify a previously unsuspected disease such as Parvovirus B19 infection or metabolic disorder (see Figure 3).

Gross and microscopic findings should be integrated with the goal of making a specific diagnosis that could be associated with a high probability of recurrence in a subsequent pregnancy. (Edmonds DK, *et al.*, 1982; Redline RW, 2015). In fact, different syndromes have markedly different recurrence risks and so have significance to the family in making decisions about future pregnancies.

Blood Clots

Usually, blood clots do not contain diagnostic material, but histology can identify fragments of degenerated chorionic villi within (see Figure 1).

1.2.2. SECOND AND THIRD TRIMESTER PLACENTA

1.2.2.1. Current Topics

Placentas may be sent to the pathology laboratory either fresh or fixed, depending on institutional practice. Fresh tissue has the added an advantage of providing material for cultures, genetic, metabolic or ultrastructural studies. Fresh placentas can be stores in a refrigerator, kept at 4° C, for at least a week.

An adequate clinical history is extremely helpful in directing placental and fetal examination, but this is not always forthcoming.

Adequate formalin fixation of Second and Third trimester placental specimen is essential to microscopy study and these is mandatory in blood-borne diseases such as hepatitis or maternal immunodeficiency virus. (Baergen RN, 2005).

If the placenta is delivered spontaneously or after labor induction and cesarean, it may be intact. Otherwise, it may be quite disrupted associated to surgical procedures related to termination of pregnancy (TOP) or implantation disorders such as placenta accreta.

Placentas must be evaluated in size (two diameters and thickness) and weight. Also, umbilical cord (UC) length and diameter and insertion type must be analyzed. In fragmented placentas diameter and weight must be achieve in aggregate. In these cases, clinician contact may be important, as this may indicate intrauterine tissue retention and could led to bleeding or infection. Cord length, and cord diameter should be ascertained if possible and a systematic macroscopic

examination of each component of the specimen must be examined to maximize the information gained.

A correlation between pathological features and clinical aspects is important and may be essential in the differentiation of placental lesions, knowing that some lesions are better identified in fresh specimens whereas others are better recognized after fixation. So, formalin fixation must be correctly and adequately performed.

Photographs are invaluable tools for later study or consultation namely in cases of fetal anomaly or incompleteness specimens.

If a cytogenetic analysis is required, it is prudent to send samples of both placenta and fetus. The rationale for this is the following: (1) the fetal tissue is optimal as it will be most representative of the fetal genetic makeup; (2) macerated fetal tissue, may not grow in tissue culture and placental tissue may be used; (3) in confined placental mosaicism (CPM) the placental tissue is not representative of the fetus. Therefore, it is best if samples of both, (e.g., fetus and placenta) are submitted.

1.2.2.2. Gross Examination

Gross examination is an important step in placental study. As like First-trimester specimen's it is crucial for measurements and weight evaluation, to determine the site and extension of chorion lesions, and to document membranes or cord abnormalities (see Figure 4).

Gross evaluation must include notation of the following features: (1) Abnormalities of placental shape or configuration (e.g., succenturiate lobe; abnormalities of placentation such as extrachorial or membranous placentation, etc); (2) Chorion should be examined for blood clots, infarcts, hematoma, fibrin deposition, abscesses or others lesions (see Figure 4). The appearance and extension of each lesion should be reported. Three-dimensional measurements of all lesions should be recorded, or an estimation of the percentage of the placenta involved in a process such as infarction or hematoma; (3) Capsular and surface (fetal) membranes should be evaluated for opacity, translucency, or discolorations, amniotic bands, amnion nodosum, fetus papyraceus (compressus), etc., (see Figure 5 and Figure 6); (4) Cord parameters (e.g., length and diameter) type insertion and number of vessels and others features such as, discoloration, edema, hemorrhage, coil and constrictions, true knots, etc should be evaluated also (see Figure 4 and Figure 5).

Also, if a fetus or fetal components is present, external examination is performed first. Measurements should be taken at this time: (1) crown-rump length (CRL); (2) Crown-heel length (CHL); (3) Head circumference (HC); (4) Abdominal circumference (AC); (5) Chest circumference (CC); (6) Hand length (HL); (7) Foot length (FL). In fetal maceration, FL have a good correlation with GA. Other measures such as philtrum, internal and external intercantal distance must be achieved.

After measuring and weighing the fetus, the external appearance of each area of the body is examined, starting with the skin. Then, evaluation of the skull, head and face, neck, chest, abdomen, pelvis and external genitalia should be performed, followed by examination of the extremities. Fetal Rx is essential and it is crucial in skeletal dysplasia. If fetus is fragmented or incomplete, the fetal tissue must be separated and an attempt to reconstruct should be made placing the fetal parts in anatomical position. Photographs may be taken at this time.

1.2.2.3. Sampling for Histology

At least a minimum of 5 or 6 blocks should be submitted: 1 block to include a roll of the extraplacental membranes from the rupture edge to the placental margin, including part of the marginal parenchyma and 2 cross section of UC, one from the fetal end and another approximately 2cm from the placental insertion end. (Khong TY, *et al.,* 2016). Also, three other full-thickness section of normal-appearing placental parenchyma should be taken from close to the umbilical cord insertion site, from within the central two-third of the placental disk. (Khong TY, *et al.,* 2016). If the transmural thickness is greater than the length of the cassette, two options are available: the upper third (chorionic plate and subjacent tissue) and lower third (basal plate) of the parenchyma can be submitted in one cassette, or the gross slice can be divide into two and submitted in two cassettes (see Figure 4). A block of the lesion (one of each type of lesion) should be sampled, with adjacent normal parenchyma if possible, in up to 3 additional blocks. (Placental workshop group consensus statement).

For histological examination the standard hematoxylin and eosin (H&E) stain is usually adequate. On many occasions, however, it is useful to employ special stains for microorganisms such as Gram, Warthin-Starry, periodic acid-Schiff (PAS), Giemsa, Ziehl-Nielsen, etc.

Immunohistochemistry may be used to differentiate trophoblast cells from other cells and may be able to differentiate different types of lesions and placental processes (see above). (Baergen RN, *et*

al., 2003; Baergen RN, *et al.*, 1997). (see Table 2 and Table 3). (Kurman RJ, *et al.*, 1984; Bargen RN, 2005; Baergen RN, 2011; Fukunaga M, 2002; Genest DR, 2001).

1.2.2.4. Microscopic Examination, Terminologies and Diagnosis

An easy approach to microscopic examination of the slides is to review each part of the placenta sequentially, that is, the fetal membranes, umbilical cord (e.g., Wharton jelly and vessels) and placental disk (e.g., basal plate including basalis decidua, and decidual vessels; chorionic villi, villous stroma, villous capillaries, stem vessels; cells islands, intervillous space and chorionic plate, chorionic vessels, amniotic epithelium).

Sections of the placenta have a complex configuration. Therefore, a low-power survey is essential for orientation and identification of the specific structures and evaluation of a whole placenta as function of GA. This is essential for interpretation of any maturity deviation also.

Chorionic Villi

The chorionic villi are the only components of the placenta that have a dual blood supply from the fetal and maternal circulations. Despite the diversification of villous types, all the chorionic villi exhibit the same basic structure (see Figure 7). They are covered by an epithelial surface layer (syncytiotrophoblast) that is in direct contact with maternal blood and functions as an endothelium. Between syncytiotrophoblast and the basement membrane are the villous cytotrophoblast, (or Langhans' cells), stem cells of the syncytium, supporting its growth and regeneration. The trophoblastic basement membrane separates the trophoblast from the villous stroma. These is composed of connective tissue cells, connective tissue fibers, ground substance, and fetal vessels (see Figure 7). In the larger stem villi, the vessels are mainly arteries and veins, while in the peripheral branches most fetal vessels are capillaries (Demir R, *et al.* 1989).

Fetal Membranes

The membranes, (capsular and surface (fetal) membranes) are usually taken to be synonymous with the amnion and the chorion leave of extraplacental membranes. These are the "bag of waters" in which the fetus is enclosed.

The structure of the membranes remains constant from the 16th weeks until term. Histologically can be seen the following layers: Amnion (amnionic epithelium, amnionic mesoderm, basal lamina or basement membrane, compact stromal layer, fibroblastic layer); intermediate spongy layer, chorionic leave (chorionic mesoderm, blood vessels, basal lamina or basement membrane); trophoblast and decidua capsular (see Figure 7).

Umbilical Cord

The umbilical cord contains two arteries and one vein suspended in Wharton's Jelly sometimes surrounded by myofibroblasts (see Figure 6) (Fox H, *et al.*, 1990). Lymphatic vessels do not exist in the cord or placenta. (Fox H, *et al.*, 1990).

The surface of the cord consists of a layer of amniotic epithelium, which is contiguous with the surface of the placenta and the fetal skin. The cord is sparsely cellular with a few macrophages and mast cells (see Figure 7). The conjunctive tissue of the cord is derived from the extraembryonic mesoblast. The jelly-like material of Wharton's jelly is composed of a ground substance (collagen, laminin, heparin sulfate, hyaluronic acid, carbohydrates with glycosol and mannosyl groups) distributed in a fine network of micro fibrils.

Placental disorders could be related with (i) gross major phenotypes or dysmorphology (e.g., placental shape abnormalities; increase or lower size and weight; implantation disorders) (see Figure 5 and Figure 6); (ii) inflammatory-immune or inflammatory-infectious processes (see Figure 7); (iii) maternal vascular lesions such as marginal venous abruption and arterial abruption placenta (see Figure 7); (iv) fetal vascular lesions (see Figure 7) and (v) other placental processes of uncertain pathogenesis or do not fit comfortably into either the vascular or inflammatory categories (e.g., floor maternal infarct; morbidly adherent placentas (see Figure 5 and Figure 6); increased circulating nucleated red blood cells (NRBC) (see Figure 7) and effects of prolonged meconium exposure).

So, a biologically comprehensive system classification of placental lesions is a pre-requisite for the definition of robust and reproducible placental phenotypes and dysmorphology (Table 4).

So, a usefulness from a competently performed placental evaluation (Table 5) falls into the main 4 categories (see above) (Redline RW, 2015). Microscopy usually leads a diagnosis that could provide an additional relevant information. For exemple metabolic diseases (e.g., gangliosidoses, mucolipidoses, Gaucher's disease, Zellweger syndrome, glycogen storage disease, etc.) could be suspected by the presence of empty vacuoles in the syncytiotrophoblast, extra-villous trophoblast,

Hofbauer cells, endothelium and fetal white blood cells in villous capillaries. But a specific categorization of metabolic disorder is not possible by placental examination alone. So, molecular, enzymatic and electron microscopy is essential to a specific diagnosis.

1.3. PLACENTAL MEASUREMENTS

Any deep knowledge involving placental and fetal parameters adapted to the understanding the placental function has been crucial for a clinical and well-founded interpretation of placental phenotypes and dysmorphology. (Wallace JM, *et al.*, 2013; Vinnars MT, *et al.*, 2015; Redline RW, 2009) Its relation with poor obstetric outcomes are well defined also. (Wallace JM, *et al.*, 2013; Vinnars MT, *et al.*, 2015; Redline RW, 2009; Redline RW, 2006; Chang KT, 2014; Adams-Chapman I, *et al.*, 2002).

Placental weight (PW) is the most common way to characterize placental growth. It is a summary of many measurements of the placenta (e.g., the laterally expanding growth and increasing thickness of chorionic disk). Placental growth reflect the ramification and vascular exchange surface of chorionic villi across gestational-age (GA). (Thame M, *et al.*, 2001; Thompson JMD, *et al.*, 2007; Almog B, *et al.*, 2011; Armitage P, *et al.*, 1967).

Fetal growth depends on placental growth (Little RE, *et al.*, 2003; Lo Y-F, *et al.*, 2002; Heinonen S, *et al.*, 2001; Lurie S, *et al.*, 1999; Dombrowski MP, *et al.*, 1995; Molteni RA, *et al.*, 1978; Yu KM, 1992; Little WA, 1960) and several studies have given emphasis to the clinical importance of placental growth and adverse fetal outcome including early mortality. (Lao TT, *et al.*, 1999; Thompson JMD, *et al.*, 2007; Naeye RL, 1987; Risnes KR, *et al.*, 2009; Zhao YJ, *et al.*, 2014). PW has been found to be lower in small for gestational-age (SGA) than in average or appropriate for gestational-age (AGA) and large for gestational-age (LGA) fetus, newborn and infants. (Heinonen S, *et al.*, 2001; Lo Y-F, *et al.*, 2002; Thame M, *et al.*, 2011; Wallace JM, *et al.*, 2004).

Despite the fact that percentile curves for birth weight (BW) are relatively common in most countries, percentile curves for placental parameters are rare, even in large series of placental studies. Also, in Portugal, there aren't population-based studies on placental biometric parameters.

The placental weight ratio (PW-R) is defined as the placental weight (PW) divided by the birth weight (BW), and it changes across gestation as the placenta matures. (Thame M, *et al.*, 2001; Thame M, *et al.*, 2004; Thompson JMD, *et al.*, 2007; Molteni RA, *et al.*, 1978; Wallace JM, *et al.*, 2013). Similarly the birth weight placental weight ratio (BPW-R) is defined as the BW divided by the

PW, and it changes across gestation also. The PW-R decreases as GA increases (Almog B, *et al.*, 2011; Thame M, *et al.*, 2004; Nogueira R, *et al.,* 2019) and the BPW-R increases as GA increases but not in reverse. (Nogueira R, *et al.*, 2019)

A significant correlation between PW and placental volume (PV) across gestational-age (GA) exist. (Almog B, *et al.*, 2011; Thame M, *et al.*, 2004) Figure 8 and Figure 9 documents this correlation as function of gestational aage (GA) also. (Nogueira R, *et al.*, 2019).

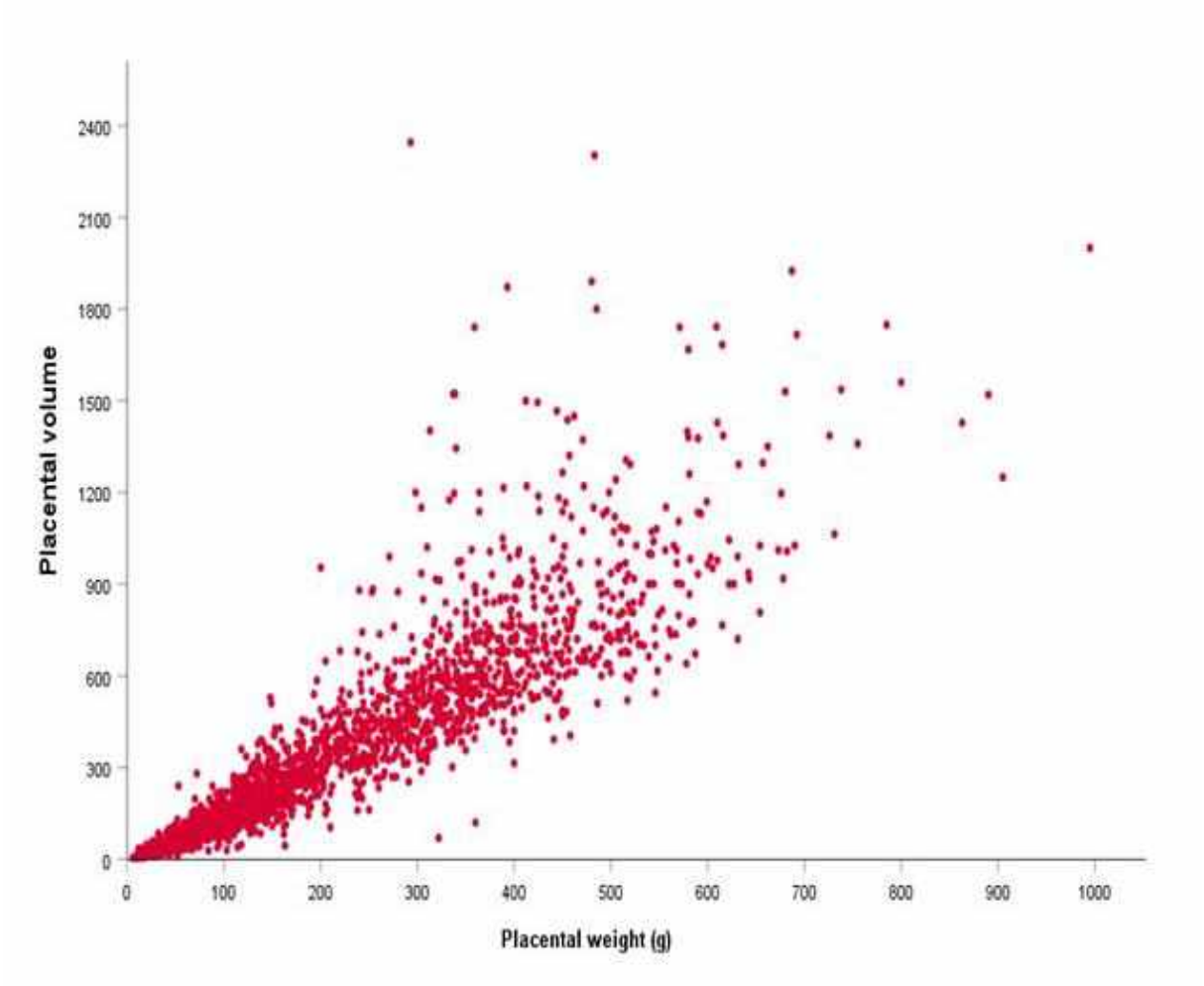


Figure 8. Relation between placental weight and placental volume. Nogueira R, *et al.*, Embryo Fetal Pathology Laboratory, CGC Genetics, Porto

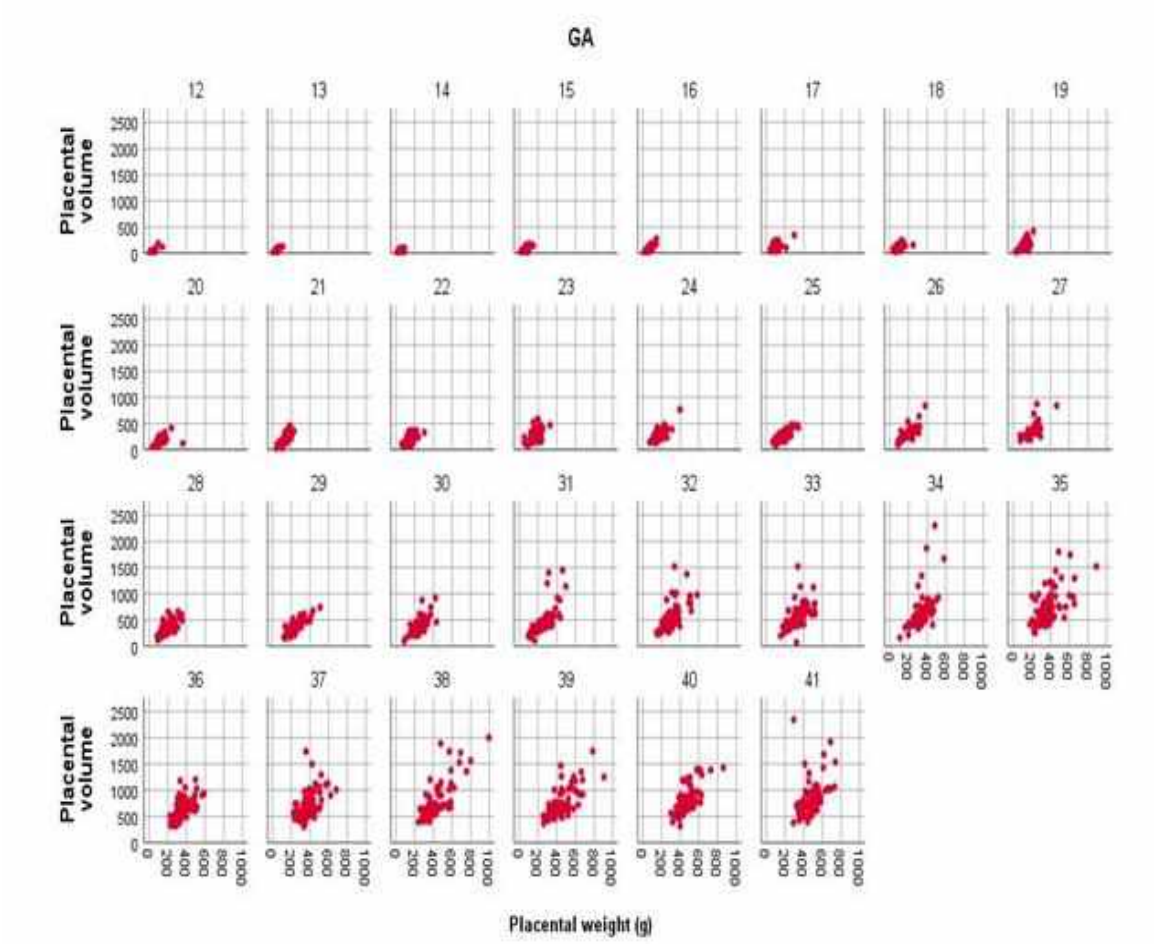


Figure 9. Relation between placental weight and placental volume by gestational-age. Nogueira R, *et al.*, Embryo Fetal Pathology Laboratory, CGC Genetics, Porto

Also, placental hypertrophy and reduced fetal growth have been postulated to be an adaptation to maintain placental function in pregnant woman with complications such as malnutrition or other environmental factors such as tobacco, endocrine disorders between others.

Recently studies documented that PW, PW-R and BPW-R are important predictors of maternal disease. (Myatt L, 2002; Wagarachchi PT, *et al.*, 2001; Brody SC, *et al.*, 2003). Also, association with poor obstetrical outcome, perinatal and neonatal morbidity or mortality and adulthood diseases were documented. (Barker DJ, *et al.*, 2013; Barker DJ, *et al.*, 2012; Barker DJ, *et al.*, 2011; Wen X, *et al.*, 2011; Winder NR, *et al.*, 2011; Redline RW, *et al.*, 2005; Redline RW, 2008; DeRoo L, *et al.*, 2016; Rees S, 2005; Risnes KR, *et al.*, 2009; Barker DJ, *et al.*, 1993; Barker DJ, *et al.*, 1989; Barker DJ, *et al.*, 1990; Nyongo AD, *et al.*, 1991; Zhao YJ, 2014; Asgharnia M, *et al.*, 2008).

Some studies documented that the BW and PW Mean significantly increase from SGA to LGA infants, yet the PW-R is significantly increased in SGA infants. (Lo Y-F, *et al.*, 2002; Thame M, *et al.*,

2004; Thame M, *et al.*, 2001; Eskild A, *et al.*, 2009; Janthanaphan M, *et al.*, 2006). Also, a high PW-R is significantly correlated with short-term adverse perinatal outcomes. (Shehata F, *et al.*, 2011).

Since PW differ between SGA, AGA and LGA infants, size distribution trajectories to determine when and how they differ across GA and percentiles will be useful for both research and clinical practice.

The knowledge of the PW-R and BPW-R as a potential determinant of health throughout life, increased the clinical and investigational relevance of the reference values for the present-day population. (Wallace JM, *et al.*, 2013; Asgharnia M, *et al.*, 2008; Thompson JMD, *et al.*, 2007; Lurie S, *et al.*, 1999; Barker DJ, *et al.*, 1993; Barker DJ, *et al.*, 1990; Molteni RA, *et al.*, 1978; Armitage P, *et al.*, 1967; Aherne W, 1966; Little WA, 1960).

Although, our-days percentile curves for PW, PW-R and BPW-R are rare comparatively to most common BW percentile curves. (Thompson JMD, *et al.*, 2007; Almog B, *et al.*, 2011; Asgharnia M, *et al.*, 2007).

So, this thesis consists of two distinct, yet dependent investigations. Both objectives were addressed using data from Embryo Fetal Pathology Laboratory, CGC Genetics in Porto.

The specific objectives are outlined before. The Principal Objective, to establish PW, BW, PW-R and BPW-R percentile curves across GA in a Portuguese-based sample, is addressed as one investigation in Chapter 2.

So, a retrospective cross-study intended to evaluate fetal weight (FW), birth weight (BW), and PW in singleton gestations, in order to design percentile curves for PW, PW-R and BPW-R between the 5th and 41st weeks of GA.

In constructing the pathological study basis on placental and fetal or live born biometric parameters in singleton gestations, a work were developed at Embryo Fetal Pathology Laboratory, Centro de Genética Clínica, Porto, Portugal over the four-year period between 1st January 2014 and 31st December 2017.

According to a standard protocol guidelines of placental examination (see above), percentile curves for PW, BW, PW-R and BPW-R in singleton gestation across GA were achieved.

The main work, see Chapter 2, covers the specific objective is presented as two manuscripts: the first one – *Measurements in First-trimester Abortion Products: A Pathological study* and the second one – *Placental Weight, Placental Weight Ratio and Birth-Placental Weight Ratio Percentile Curves across Gestational-age*.

Additional consequential work has been addressed in particular the classification of placental lesions from First-trimester gestation (see Chapter 3), as well as the usefulness of a competent placental study namely in litigious context (see Appendix 1) and placental dysmorphology associated with congenital fetal and birth defects (see Appendix 2 and Appendix 3).

1.4. PLACENTAL LESIONS CLASSIFICATION

A lack of interest in the study of the placenta compared to the fetal study is notorious in the literature.

Although, the competent examination of placenta would demonstrate important information about whatever has happened on embryo or fetus and mother. (Barker DJ, *et al.*, 2013; Barker DJ, *et al.*, 2012; Barker DJ, *et al.*, 2011; Redline RW, 2015).

As during my practice of pathologist, I always studied placentas, averaging about 1,500 per year, I began to recognize variants of common placental lesions and also to identify unusual and rare types of lesions.

The present study, was a basis for the need I felt to organize the placental disease processes that allowed a biologically and clinically comprehensive classification, since a First-trimester abortion specimens. I am pleased that my approach to classification of placental lesions is in general close and in agreement with the current Amsterdam Placental Workshop Group Consensus Statement. (Khong TY, *at al.,* 2016) and in approach to coleagues works that I very appreciate as Redline and Bernirschke.

Also, in recent times evidence has begun to accumulate that placental function has even longer-term impact on the well-being of both the mother and the child and even the adult. (Redline RW, 2015; DeRoo L, *et al.*, 2016; Rees S, *et al.*, 2005)

Similarly, recent studies have highlighted the clinical importance of placental biometries and phenotypes and its association with fetal disturbances and diseases later in life. (Barker DJ, *et al.*, 2013; Barker DJ, *et al.*, 2012; Barker DJ, *et al.*, 2011; Wen X, *et al.*, 2011; Winder NR, *et al.*, 2011; Redline RW, 2015; Khong TY, 2016; Winder NR, *et al.*, 2011; Myatt L, 2002; DeRoo L, *et al.*, 2016, Rees S *et al.*, 2005).

Even though the major advances in Second and Third trimester placentas, First-trimester abortion specimens (FTAS) are often poor described from a developmental perspective. (Edmonds DK, *et al.*, 1982; Wilcox AJ, *et al.*, 1988; Whittaker PG, *et al.*, 1983; Zinaman M, *et al.*, 1996).

So, the examination of placenta across gestational-age (GA) would be useful in a variety of ways. It could evaluate a quickly approach of weight, size and morphology deviation. Also, it could diagnose important conditions affecting the mother and infant related to pregnancy loss and identify conditions that are likely to recur in subsequent pregnancies. (Redline RW, 2015) So, placental examination could help on separating clinical syndromes and associations into distinct pathological phenotypes for further investigation and uncovering the underlying cause of unexpected adverse outcomes. (Redline RW, 2015).

Although, epidemiologic studies have reported wide geographic and temporal variations in the prevalence of some placental diseases, making reliable international comparisons difficult namely in severe disorders (e.g., gestational trophoblastic diseases such as hydatidiform mole and extra villous lesions). (Joneborg U, *et al.*, 2018).

So, recently, a international comprehensive classification of Second and Third trimester placental lesions has been proposed. (Khong TY, *et al.*, 2016)

The implementation of a unified system with reproducible criteria helps to establish evidence-based recommendations and terminologies for the pathological study of the Second and Third trimester placentas and First-trimester abortion specimens too. (Redline RW, 2015; Turowski G, *et al.*, 2012) Also, a competent placental examination improves the advances in the knowledge of the pathogenesis, diagnosis and treatment of obstetric disorders.

Knowing that placental lesions have a heterogeneous and complex etiologies associated with distinct placental phenotype, these understanding could improve both an effective clinical follow-up in future and on new emergent diagnostic “omics” approach and target therapies.

So, the Chapter 3 covers my approach to a comprehensive classification of First-trimester abortion lesions.

1.5. UTILITY OF PLACENTAL EXAMINATION

Useful information from a competently performed placental examination allows a specific diagnosis and serves for the purposes of quality assurance, risk process disease management of the mother or infant and patient health care education. (Redline RW, 2015).

Depending on their clinical relevance (Table 6), placental lesions can be broadly classified into four types: 1. Lesions responsible for fetal or neonatal morbidity and/or mortality (e.g., infarction, unusual infection, abruption, etc.) (see Table 6); 2. Lesions related to premature delivery (e.g., chorioamnionitis, abruption, etc.) (see Table 6); 3. Lesions that are likely to modify immediate management of the mother (e.g., HM, accreta) 4. Placental lesions that explain specific adverse outcomes (e.g., abruption, MVM, FVM, etc).

A comprehensive classification of placental lesions (see Table 4) have significant implications for fetal, infant and maternal health, and may provide valuable information to the clinicians and families (see Table 6). Also, could prevent the risk of recurrent lesions (Table 7). (Redline RW, 2015; Salafaia CM, *et al.*, 2009; Roberts DJ, *et al.*, 2006; Langston C, *et al.*, 1997; Khong TY, *et al.*, 2003; Fox H, *et al.*, 2007; Turoswski G, *et al.*, 2102; Vinnars MT, *et al.*, 2015, Nogueira R, *et al.*, 2015). In addition, it is important in litigation processes related to adverse pregnancy outcomes, sometimes associated with inflammatory-immune infectious diseases (IIID) (Table 8) (Chang KT, 2014; Nogueira R, *et al.*, 2015; Khong TY, *et al.*, 1997).

Table 4. Comparative classification of placental lesions.

Redline (2015)	Amsterdam Placental Workshop Group Criteria (2016)
Placental vascular processes	
a. Maternal stromal-vascular lesions	**a. Maternal vascular malperfusion (MVM)**
Developmental	
Superficial implantation/ decidual arteriopathy	Decidual arteriopathy
Increased immature extravillous trophoblast	Other findings
Malperfusion	
Global/Partial	Placental hypoplasia (PW<P_{10} or UC Θ< 8mm or <P_{10})
Early: distal villous hypoplasia	Distal villous hypoplasia
Late: accelerated villous maturation	Accelerated villous maturation
Segmental/Complete	
Villous infarcts	Infarcts
Loss of Integrity	Infarction hematoma
Abruptio placenta (arterial)	Retroplacental hemorrhage/Retroplacental hematoma (RPH) [6]
Marginal abruption (venous)	
Acute	
Chronic	
b. Fetal stromal-vascular lesions	**b. Fetal vascular malperfusion (FVM)**
Developmental	
Villous capillary lesions	
Delayed villous maturation*	Delayed villous maturation*
Dysmorphic villi	
Malperfusion	
Global/partial	High grade(severe form)
Segmental/complete	Low grade
Loss of integrity	
Large vessel rupture (fetal hemorrhage)	
Small vessel rupture (feto-maternal hemorrhage)	
Villous edema	
Placental inflammatory-immune processes	
a. Infectious–Inflammatory lesions	**a. Infectious inflammatory lesions**
Acute	**Ascending Intrauterine infection**
Maternal inflammatory response:	Stage and grading (see table 8)
Fetal inflammatory response:	
Chronic	**Chronic plasma cell villitis**
Plasma cells villitis (CMV, others)	Plasma cells villitis (CMV, others)
Intervillositis (malaria, others)	
b. Immune/Idiopathic–Inflammatory lesions	**b. Immune/idiopathic-inflammatory Lesions**
VUE[9] and related/associated lesions	Nonspecific chronic villitis[10]
Chronic villitis	Chronic usually lymphohistiocytic villitis
	Other inflammatory lesions
Chronic chorioamnionitis	Chronic choriomanionitis
Lymphoplasmacytic deciduitis	Chronic deciduitis
Eosinophil T-cell fetal vasculitis	Eosinophilic/T-cell vasculitis
Chronic histiocytic intervillositis	Chronic intervillositis or chronic histiocytic intervillositis
Other placental processes	
Massive perivillous fibrin deposition (MFI)	Massive perivillous fibrin deposition (MFI)[11]
Abnormal placental shape or UC insertion site	Abnormal placental shape or UC insertion site
Morbidity adherent placentas (accreta)	Morbidity adherent placentas (accreta)
Meconium-associated changes	Meconium-associated changes
Increased circulating nucleated red blood cells	Increased circulating nucleated red blood cells

Legend of Table 4. [1]For gestational-age, delayed villous maturation, as being the opposite of accelerated villous maturation, usually seen after 36 weeks and rarely before 34 weeks. MVM, maternal vascular malperfusion: Infarcts must be qualified as recent or remote and central or peripheric, the central one should be preferentially sampled for histology. When hemorrhage is encased by infarction, the term infarction hematoma should be used. Placental abruption is a clinical diagnosis and the correct description is retroplacental hematoma (RPH).FVM: The lesions described under this umbrella are due to obstruction in fetal blood flow (e.g., umbilical cord lesions with partially obstructed blood flow, hypercoagulability, complications of fetal cardiac dysfunction, hypoxia, etc.). Histopathological findings are thrombosis; recent intramural fibrin in large vessels; segmental avascular villi (more than one focus), and villous stromal-vascular karyorrhexis. FVM must be classified classified: Low grade: and High grade. Thrombosis would be considered to be a pre-mortem process.
Inflammatory-infectious process – The topography and constituents of the inflammatory response should be documented. Poor fetal outcomes are more often associated with a fetal inflammatory response (FIR). Histologic chorioamnionitis may not be equivalent to clinical chorioamnionitis. Chronicity of response may have different clinical implications from purely acute response.
VUE, villitis of unknown etiology is a histologic diagnosis and it is usually a lymphohistiocytic villitis, although rare plasma cells may be present. Must be classified as: high grade or low grade. An infectious etiology, such cytomegalovirus (CMV) herpes virus must be suspect when frequent plasma cells are present. High grade nonspecific villitis has significant association with fetal growth restriction, neurodevelopmental impairment and recurrence risk in subsequent pregnancies.
MFI, maternal floor infarction; a kind of lesion with high recurrence risk and association with adverse pregnancy outcomes.

Table 5. Usefulness from a placental examination.*

Identification of unsuspected disease	Placenta accrete Unusual infection (eg. CMV, Listeria, Parvovirus) Findings suggestive of aneuploidy or metabolic storage disease
Conditions associated with a high recurrence in future pregnancies	Idiopathic/Immune inflammatory lesions (chronic histiocytic intervillositis) Massive fibrin deposition (maternal floor infarction) Non-specific chronic villitis Placenta accrete Maternal vascular malperfusion[1] Spontaneous preterm birth with histological chorioamnionitis
Information that guide the management of future pregnancies	Maternal vascular malperfusion Spontaneous preterm birth with histological chorioamnionitis Idiopathic/immune inflammatory lesions Fetal vascular malperfusion Delayed villous maturation
Diagnosis that provide a specific explanation for an adverse outcome[2]	Umbilical cord severe edema, stenosis, thrombosis

*Adapted from Redline RW 2015. Terminology consensus Khong TY, 2016.
Legend: CMV, cytomegalovirus; FD, fetal death; FGR, fetal growth restriction; CNS, central nervous system.
[1] Distal villous hypoplasia is associated with early-onset fetal growth restriction. Accelerated villous maturation is associated with FGR, preeclampsia, preterm labor.
[2]FD, FGR, Preterm birth, CNS injury

Table 6. Clinical relevance of placental lesions.

	Placental causes of specific adverse outcomes
Preterm fetal death	Maternal vascular malperfusion
	High grade / Global fetal vascular malperfusion
Spontaneous preterm birth	Accute chorioamnionitis[1]
	Marginal abruptio
	Maternal vascular malperfusion
	Idiopathic/Immune inflammatory lesions (non-specific chronic villitis)
	High grade / Global fetal vascular malperfusion
Term fetal death	Abruptia placenta (RPH)
	High grade / Global fetal vascular malperfusion
	Umbilical cord accident
	Fetomaternal hemorrhage
	Delayed villous maturation
CNS injury at term	Global fetal vascular malperfusion
	Global fetal vascular malperfusion (UC accident)
	Idiopathic/Immune inflammatory lesions (non-specific chronic villitis)
	Accute chorioamnionitis with severe fetal response
	Multiple placental lesions
Growth fetal restriction	Maternal vascular malperfusion[2]
	Idiopathic/Immune inflammatory lesions

Modified from Redline RW 2015. Terminology consensus Khong TY, 2016.
Legend: UC, umbilical cord; CNS, central nervous system; RPH, retroplacental hemorrhage/hematoma.
[1] Staging and grading of the maternal and inflammatory responses is essential.
[2] Distal villous hypoplasia is associated with early-onset fetal growth restriction.

Table 7. Placental lesions with recurrent risk in subsequent pregnancies.*

Rare	Chronic histiocytic intervillositis
	Massive perivillous fibrin deposition
More common	Placenta accrete
	Non-specific chronic villitis
	Idiopathic/Immune inflammatory lesions (Maternal vascular malperfusion)
	Fetal vascular malperfusion
	Spontaneous preterm birth with histological chorioamnionitis
	Aneuploidy
	Hydatidiform mole

*Modified from Redline RW 2015.

Detailed study of the placenta after delivery contributes to the understanding of placental function towards the identification of specific primary (intrinsic) or secondary lesions that can reflect an adverse intrauterine environment and explain an adverse fetal or perinatal outcome (see Table 6 and Table 7). (Vinnars MT, *et al.*, 2015; Redline RW, 2015; Khong TY, *et al.*, 2016).

Recently, evidence has begun to accumulate that placental function has even longer-term impact on the well-being of both the woman and the child – and even the adult – who results from the pregnancy. (Rees S, *et al.*, 2005; Barker DJ, *et al.*, 2012; Amaral LM, *et al.*, 2015; DeRoo, *et al.*, 2016).

As an emergent topic I alert to the importance of a competent placental examination in litigation against hospitals and clinicians (see Appendix 1).

Finally, considering the inclusion and exclusion criteria for the selection of percentile placental sample and in order to exclude twin pregnancies, fetal conditions (e.g., congenital fetal malformation), placental disorders (e.g., tumors) and maternal disorders that could affect placental growth and consequently PW and PW-R a global retrospective placental and fetal pathological reports was performed at the CGC Embryo Fetal Pathology Laboratory. As a result, others studies were consequent and secondarily developed such as, an overview of fetal congenital malformation that could be directly related to PW and dysmorphology were developed as additional studies which results in two manuscripts (see Appendix 2 and Appendix 3).

Table 8. Histopathological chorioamnionitis classification.

		MATERNAL RESPONSE	FETAL RESPONSE
ACUTE		Neutrophils between amnion and chorionic plate	–
STAGE	1	Neutrophils in subchorionic fibrin (subchorionitis/chorionitis)	Vasculitis (chorionic vessel or umbilical phlebitis)
STAGE	2	Neutrophils within mesenchymal chorioamniotic tissue	Umbilical phlebitis and arteritis
STAGE	3	Karyorrexis, necrosis of amniocytis (necrotizing chorioamnionitis)	Funisitis necrotizing
GRADE	1	Not severe (mild)	Not severe (mild)
GRADE	2	Severe: Confluent neutrophils in chorion or micro abscess	Severe: Intramural neutrophils with destructing smooth muscle cells
SUBACUTE		Neutrophils and mononucleates cells between amnion and chorion, necrosis	–
CHRONIC		Lymphocytes between amnion and chorion	–
STAGE	1	Lymphocytes on chorionic	–
STAGE	2	Lymphocytes on mesenchymal chorioamniotic tissue	–
GRADE	1	≥ 3 foci or patchy inflammatory	–
GRADE	2	Diffuse inflammatory process	–

*Modified from Khong TY, *et al.*, 2016

1.6. ETHICAL ISSUES

The increase of litigation processes towards hospitals and obstetricians is a reality, and in most cases, the litigant part claims negligence (see Appendix 1).

In litigation processes, the pathologist is frequently called upon and questioned to clarify specific pathological processes and dating their evolution (see Table 5 and 6). The evaluation of the real retention-time after fetal death is frequently asked also.

Several placental lesions of previously unsuspected disease process in the mother or fetus are identified only on microscopic placental examination. So, placental examination including the membranes and umbilical cord, is crucial in specific maternal and fetal conditions. (Table 9).

A useful information from a competently placental evaluation must combine a macroscopic and microscopic examination.

A competent gross and microscopic placental examination is an important issue with strong association with adverse outcomes and high recurrence rate in future pregnancies (see Table 7). However, these issues are sometimes challenging to assess due to the coexistence of competing injuries, requiring a confident understanding of its pathogenesis and implications (see Appendix 1).

Microscopy is essential for classification, evaluation and dating of the placental vascular maternal or fetal malperfusion and inflammatory-immune processes (either infectious or idiopathic) (see Table 4 and Table 8).

Table 9. Placental, membranes and umbilical cord abnormalities in fetal conditions.[a]

	Placental Processes	Fetal Process
Umbilical Cord		
Tumor / masses	Teratoma	may represent an acardiac twin
	Hemangioma	may associate rupture or hematoma
Vessels abnormalities	Single umbilical artery	associated with other fetal anomalies
	Supernumerary vessel	rare, may be associated w/ fetal anomalies
Membranes		
Circunvallate	Fibrin/fibrinoid	preterm delivery, fetal anomalies
Chorionic Plate		
Miscellaneous	Yolk sac remanants	no clinical significance
	Fetus papyraceus	
Hematoma	Subchorionic	
	Subamnionic	if large, may be associated with stillbirth
Vascular mass	Chorangioma	if large may associated with fetal hydrops
Chorionic Villi and		
Blood clot	Intervillous thrombus	may be associated with FMH
Fibrin / fibrinoid	Maternal floor infarction often involves full	may be associated with IUGR/FGR
Infarcts	related to MVM	often associated with IUGR
Abscess	related to infection	often associated with fetal sepsis
Edema or hydropic	Mesenchymal dysplasia	may be associated with placental mosaicism and
Vesicles	Hydatidiform mole	may be partial or complete including ve-CHM[b]
Basal Plate		
Increased fibrinoid	Maternal floor infarction	may be associated with IUGR
Hemorrhage	Retroplacental hematoma	may be associated with other changes of
Adherent myometrium	Placenta accrete	may be associated with maternal and fetal

[a]Adapted from the Manual of Benirschke and Kaufmann's Pathology of the Human Placenta.
IUGR, intrauterine growth restriction; FGR, fetal growth restriction; MVM, maternal vascular malperfusion; FMH, fetomaternal hemorrhage; ve-CHM, very early complete hydatidiform mole.
[b] microscopy is essential to classification and may be complemented with immunohistochemistry and genetic tests.

1.7. SUMMARY AND FUTURE DIRECTIONS

The major processes frequently found in the placenta have been outlined previously. The availability of clinical information and reason for pathological submission study is critically important to all examinations because some processes, such as premature placental separation, may have little recognizable gross change (see above).

In experienced hands macroscopic examination of placenta optimizes the histological interpretation through appropriate choice of areas to submitting to microscopy (see Figure 4).

Macroscopy allows assessment of placental biometry and morphology which could related with several maternal and fetal conditions (see Appendix 2 and Appendix 3).

Placental measures are crucial tools to evaluate prenatal PW, placental size and PV. PW is the most common way to characterize placental growth, and it is a summary of many measurements of the placenta. Actually, several studies have given emphasis to the clinical importance of placental growth and placental function and adverse fetal outcome including fetal death and perinatal mortality.

Microscopy is essential to define placental underlying maternal or fetal disease processes. A simple, comprehensive, and widely accepted classification system including in First-trimester abortion specimens (see Chapter 3) is a pre-requisite for definition of a robust and reproducible placental phenotypes and terminologies. (Khong TY, *et al.,* 2016; Redline RW, 2015).

Once a clinical context and significance of the placental phenotypes across GA are better understood (see Chapter 3), it should be possible to refine submission guidelines and decrease the number of unhelpful placental examinations, thereby reducing costs to health care system. (Redline RW, 2015).

The goals of the "Human Placenta Project", to develop new biomarkers and imaging techniques that allow the prospective diagnostic evaluation and the development of new target therapies will be greater if the contribution of placental biometry, dysmorphology and phenotype are considered together. (Redline RW, 2015).

Like as other authors I believe that placental pathology will likely continue to play an important role, serving as a gold standard for diagnosis, quality assessment, diagnostic test evaluation and comparative effectiveness trials, and guiding the management of individual patients to prevent adverse outcomes in subsequent pregnancies. (Redline RW, 2015).

AIMS

The primary aim of this thesis was to produce a Portuguese-based population percentile curves for placental weight (PW), placental diameters (PDs), fetal weight (FW) / birth weight (BW), placental weight ratio (PW-R) and birth weight placental weight ratio (BPW-R) in singleton gestations across gestational-age (GA) .

Additional relevant goals were: (1) to develop a classification of placental lesions namely in early abortion products. That helps to establish evidence-based recommendations for complementary tests and allow advances in the understanding early abortion disease processes which can guide the management of future pregnancies, particularly in maternal disorders; (2) to evaluate macroscopic placental dysmorphology and biometric parameters associated with fetal diseases.

Future directions: Related to critical placental measurements across GA and the goals of the "Human Placenta Project".

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"Measure what is measurable, and make measurable what is not so."

Galileo Galilei

CHAPTER 2

PLACENTAL PERCENTILE CURVES

2.1. MEASUREMENTS IN FIRST-TRIMESTER SPECIMENS

Original Article

Measurements in First-Trimester Abortion Products

A Pathologic Study

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• *Context.*—Related to the advances in prenatal diagnosis and the emergence of medically challenging situations, there has been an increased interest in conducting a pathologic study of first-trimester abortion products.

Objective.—To evaluate measurements across a large group of first-trimester spontaneous abortion specimens. Potential goals include a validation of prenatal embryo and gestational-sac measurements as a function of gestational age (GA).

Design.—A retrospective case study of first-trimester spontaneous abortions between June 2015 and April 2017 in Centro de Genética Clínica Embryo-Fetal Pathology Laboratory, Porto, Portugal. Considering the inclusion criteria, 585 complete gestational sacs, 182 embryos, and 116 umbilical cords were selected. We recorded the weight of the gestational sacs and embryos and measurements of gestational sacs, umbilical cords, and embryo crown-rump length. Models were computed using regression techniques.

Results.—Gestational-sac diameter percentiles 5, 25, 50, 75 and 95 were calculated according to GA, and at each 1-week interval the diameter increased an average of 3 mm. Umbilical cord length percentiles 5, 25, 50, 75 and 95 were calculated according to GA, and at each 1-week interval, the length increased an average of 1.35 mm. Embryo crown-rump length estimated mean ± SD values were GA 6 weeks, 5.3 ± 2.3 mm; GA 7 weeks, 9.4 ± 4.8 mm; GA 8 weeks, 13.7 ± 8.2 mm; GA 9 weeks, 20.8 ± 9.1 mm; GA 10 weeks, 22.6 ± 13.4 mm; GA 11 weeks, 29.4 ± 12.9 mm; and GA 12 weeks, 52 mm.

Conclusions.—Pathologic measurements obtained should be compared to expected measurements and correlated with ultrasound findings, clinical information, and microscopic findings. Deviations from expected values could lead to an understanding of early pregnancy loss.

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Human embryogenesis is divided into 2 major periods, blastogenesis and organogenesis, happening from conception to the eighth week of development.[1]

Spontaneous abortion (SA) refers to spontaneous delivery before fetal viability. Clinically, SA is classified as early if it occurs at or before the 12th week of gestational age (GA).[2]

Prenatal growth evaluation is done from the first day of the last menstrual period. This is termed GA (2 weeks longer than embryonic age).[3] A gestational sac (GS) can usually be identified at the fifth week and is an early indication of an intrauterine pregnancy.[4]

A precise incidence of first-trimester SA (FTSA) is not well established. Currently, it is accepted that up to 60% of all conceptions will be miscarried, most of these not being noticed by the woman.[5,6]

Risk(s) of SA is a complex process. Several causes, from maternal, paternal, or biparental to multifactorial, placental, and embryonic factors may be associated in the pathogenesis of SA.[7] Are well known, although the most common causes of early or very early pregnancy loss are aneuploidies or chromosomal aberrations. These often result in a grossly disorganized embryo and sectary or multiple malformations indicative of specific syndromes or associations.[8] Seventy percent of SAs at less than 8 weeks of GA exhibit an

abnormal karyotype; the presence of an abnormal karyotype decreases at higher GA.[illegible]

Pathologic examination of FTSA specimens is important for diagnosis. Combined gross and microscopic findings provide crucial information for the management of subsequent pregnancies and maternal health in specific conditions and help guide the need for complementary genetic studies or others tests.[illegible]

A complete gross examination in FTSA specimens is the first step to correctly identify different constituents.[illegible] In general, gross examination of nonembryonic tissue does not provide diagnostic information except in the case of gestational trophoblastic disease. The success of a gross approach is partially dependent on the skill and experience of the examiner. As with examination of any specimen, it is wise to follow a routine protocol that includes measurements and weight of various components of FTSA specimens and guides the choice and number of histologic sections submitted.[illegible] A complete and well-oriented gross examination of the first-trimester placenta must include the chorionic plate, villi and intervillous space, and basal plate. Gross vesicles are usually not present except for complete hydatidiform mole and partial hydatidiform mole. Early complete and partial hydatidiform moles may need to be examined under a dissecting scope to see the abnormal villi. Usually hydatidiform moles are more voluminous products and translucent vesicles are better identified in fresh specimens and under water, particularly in early moles (especially those less than 10 weeks).[illegible] Grossly, FTSAs are heterogeneous. Decidual tissue usually appears as a small, flattened sheet that is relatively smooth on one surface and granular or nodular on the opposite surface.[13] Microscopically, it is composed of an admixture of extravillous trophoblast, decidual cells, uteroplacental vessels, and endometrial glands usually embedded in fibrinoid material. The basal plate lesions as vascular, inflammatory, or implantation disorders are only evaluated on microscopic study.[13] Blood clot usually does not contain diagnostic material[13]; however, it should be submitted for histologic examination, particularly if it is granular or firm.[13] Many embryo and umbilical cord (UC) characteristics are diagnosed solely by gross examination.[illegible] Gross examination is crucial to achieve biometric parameters and to document suspected (or not) development anomalies and potential abnormal chorionic villi features, such as are seen in gestational trophoblastic disease.[illegible] Moreover, along with a histologic study, it can identify potential diseases and causes of early abortion. Together, these are crucial steps in assessing and predicting recurrent risk in future pregnancies as well as its impact for the mother and fetus.[illegible]

Growth is a highly complex process. It takes place in a completely ordered fashion in the biological system.[illegible] Knowledge of distinct intrauterine phases in which the growth and body composition of the fetus are related to the mode of nutrition are well documented.[illegible] Studies of human intrauterine growth usually are based on anthropometric measurements of infants born at various gestational periods. Weight is a nonspecific measurement of growth; however, it is still the most widely used single clinical measurement of growth in intrauterine and postnatal life.[illegible] Measurements of growth after birth at all ages are mainly longitudinal.[illegible] However, growth measurements of body composition are not longitudinal in early development.[illegible] Numerous percentile charts have been constructed that relate embryo-fetal measurements to GA.[illegible] Recent studies have sought to demonstrate the importance of embryo crown-rump length (CRL) and placental/GS parameters as potential predictors of early pregnancy loss or maternal risk diseases.[illegible] However, these studies must be validated with embryonic and GS histologic features seen in embryonic and placental disorders, such as chronic massive intervillositis and gestational trophoblastic disease, among others. The accurate measurement of embryo/fetal crown-heel length provides the best clinical measurement of skeletal growth.[illegible] Some studies investigated efficacy of first trimester ultrasound parameters such as GS thickness for prediction of maternal risk disorders such as preeclampsia and/or the delivery of small for gestational age neonates; however, there were no specific pathologic aspects reported.[illegible] Others have documented such parameters as median GS diameter to CRL ratio as better predictors of pregnancy loss than GS diameter and embryo CRL alone.[illegible]

Four types of growth disorganization have been established.[illegible] Type 1 consists of an intact chorionic or amniotic sac with no evidence of an embryo or body stalk.[illegible] Type 2 consists of a chorionic sac containing a nodular embryonic tissue 1 to 4 mm long, usually attached to the amnion.[illegible] Type 3 consists of a chorionic sac containing a disorganized embryo up to 10 mm long with recognizable cephalic and caudal poles, retinal pigment and a short body stalk may be present.[illegible] Type 4 consists of an embryo that has a CR length from 3 to 17 mm with major distortion of the body shape, always involving the head, which usually is small; cervical flexion is absent or abnormal. These embryos have a recognizable head, trunk, and limb buds, and the morphologic characteristics are not consistent with any one stage of development.[illegible]

Placenta, especially in the second and third trimester, loses some weight during storage by evaporation but predominantly by leakage of blood and serum, although weight loss is most significant in hydropic or edematous placentas.[illegible] On the other hand, the placenta gains approximately 5% in weight after formalin fixation, but small FTSA specimens are little or not affected.[illegible] Knowing the GA and growth of the embryo and GS are important to appropriately evaluate the measured parameters. The value of this paper is in the gross pathologic measurements and expected growth during GAs of 4 to 12 weeks. Our objective was to obtain measurements in a large group of FTSA specimens and compare them with published studies.

MATERIALS AND METHODS

Sample Definition

We conducted a retrospective case study of [illegible] FTSA specimens sent to Centro de Genética Clínica Genetics Embryo-Fetal Pathology Laboratory (Porto, Portugal). The specimens had been sent for pathologic examination to determine pregnancy loss etiology between June 2013 and April 2017. Inclusion criteria were complete intrauterine FTSA until 12th week of GA and gross parameters appropriately available for at least one component: GS, UC, or embryo. Exclusion criteria were gestational trophoblastic diseases, abortion specimens relating to a medical termination of pregnancy, twin pregnancy, ectopic pregnancy, assisted reproductive technology pregnancy loss abortion products, known and well documented maternal disorders, incomplete specimens, and unknown GA.

All the samples used in the present study were unlinked and unidentified from their donors. Because of the retrospective nature of the study, the local ethical review committees of the involved

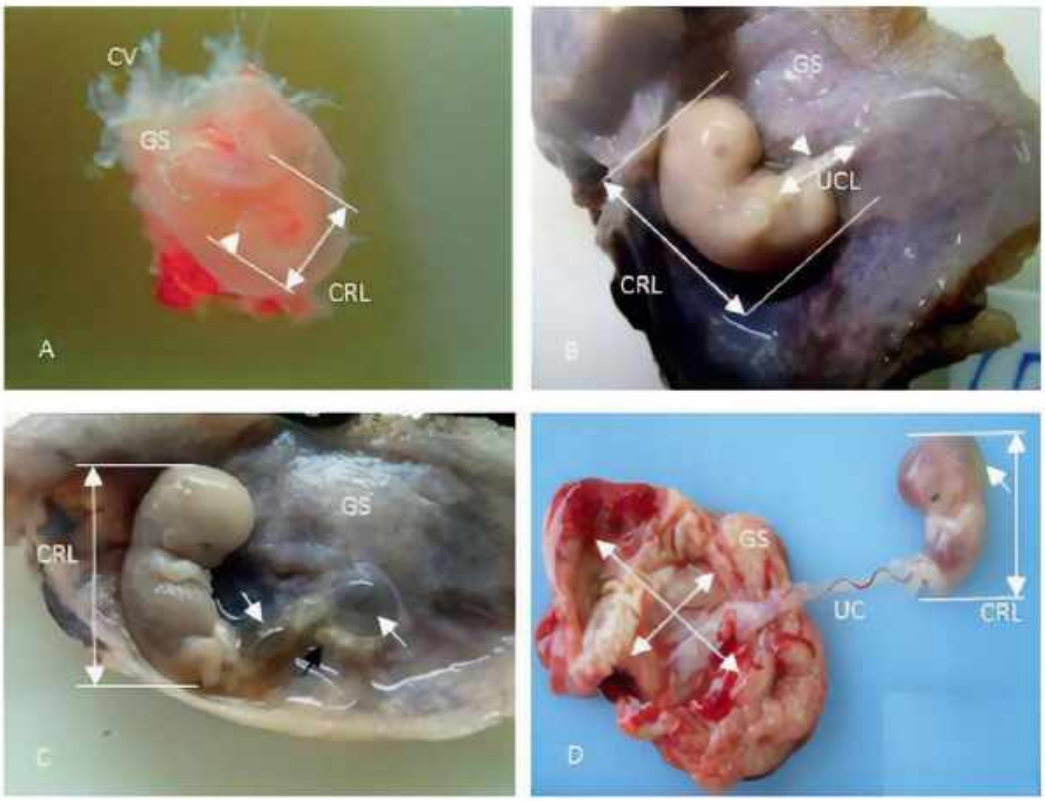

Figure 1. *Examples of complete first-trimester spontaneous abortion pathologic specimens. Gross parameters were taken of each individual component: gestational sac (GS), embryo crown-rump length (CRL), and umbilical cord (UC). A, Intact GS with sparse chorionic villi (CV) containing an early embryo (development age 32 days) with recognizable cephalic pole (arrowhead) and caudal pole without other recognizable external features. B, Opened GS containing an embryo with a small head and retinal pigment, chin fused to chest, paddle-shaped hand plate and lower limb bud showing inconsistent development, short UC length (UCL) (arrowhead). This embryo is a growth-disorganized type IV with 47,XY,+16 karyotype. C, Opened GS with a normal embryo at week 7, with pigmented eye, auricular hillocks, elbow, and free fingers. Umbilical cord cysts (white arrows) and yolk sac remnant (black arrow). D, Opened GS showing a normal coiling UC and an intact fetus at beginning of the fetal period (9th development weeks). Nuchal thickening translucency (white arrow).*

institutions and Minho University Medicine School (Braga, Portugal) approved the work and waived the need for written informed consent.

Collecting Data

General maternal parameters were collected: mother's age, clinical data, obstetric history, and pregnancy GA. Gross parameters were taken of each individual component: gestational sac diameter and weight, embryo CRL and weight, and umbilical cord length and diameter. Measurements were acquired using a digital caliper with measuring range 0 to 150 mm (0–6 inches; Würth International AG). Gestational sac diameter was evaluated by measuring distance between the curved membranes in the chorionic plate. Embryo CRL was measured in its natural position, from the outer edge of the cephalic pole to the outer edge of the embryo rump in younger embryos and from the crown to the rump in older embryos as they began to straighten. Any potential gross embryo malformations were identified as growth disorganized. Umbilical cord length was measured only in those cases where the embryo remained attached to the GS. After removal of the clots and decidua and formalin fixation during 24 hours, the weights of GS and embryo separately were acquired using a GS620 balance with measuring range 0.01 to 620 g (serial number 12105085, Kern).

Statistical Analysis

Data analysis was performed using descriptive statistics and linear regression techniques, determining the mean, median, standard deviation, minimum, and maximum as well as the percentiles of the different parameters analyzed (diameter and weight of the GS, UC length and diameter, embryo CRL and weight). Data tabulation and graphical construction were performed using the statistical software IBM SPSS Statistics version 24.0. According to the nature of the variables, we conducted a descriptive study using charts and/or tables; an analytical study (95% confidence intervals for the mean values); a causation study (regression models to estimate parameters according to GA); or a distribution adjustment to verify the normality assumption (Shapiro-Wilk test).

Table 1. Summary Statistics for Gestational Sac (GS) Diameter

		GS Diameter, mm								
							Percentile			
GA, wk	No. Valid	Mean	SD	Median	Min	Max	5th	25th	75th	95th
4	3	7		7	7	7				
5	17	11.9	6.1	10	4	25	4	6	15	25
6	89	20.7	9.4	20	2	50	10	15	25	40
7	91	31.5	9	35	6	60	10	30	30	40
8	159	38.4	8.9	40	6	60	15	30	40	40
9	93	43.9	11.9	45	6	60	15	35	40	60
10	83	51	11.1	50	30	90	15	45	55	70
11	46	57	11	55	30	80	15	45	60	80
12	20	60.6	15	60	30	80	15	50	65	80

Abbreviations: GA, gestational age; Min, minimum; Max, Maximum.

RESULTS

Sample Characterization

One thousand three hundred ninety-one specimens were sent to pathology, and clinical and pathologic exclusion criteria (883 cases remained with 1, 2, or 3 of the inclusion criteria: 585 with complete GS, 182 with embryo, and 116 with UC). Of the 585 GSs, diameter was available for 577 (98.6%) and weight for 478 (81.7%). Of the 182 embryos, weight was available for 61 (33.5%) and CRL for 109 (59.8%). Of the 116 UCs, length was available for all, and diameter was available for 114 (98.1%) (Figure 1, Table 1 through 10).

Descriptive Parameter Analysis

Maternal Age. The mean maternal age was 32.9 years with a standard deviation of 6 years. It was also found that 50% of mothers were younger than or equal to 32 years.

Gestational Sac. Gestational sac parameters were calculated according to the GA. For GS diameter and weight, the mean, SD, and percentile curves are shown in Tables 1 and 2 and Figures 2 and 3. At 10 weeks' gestation, the mean diameter of the GS was 50 mm, and it increased 3 mm each week.

Umbilical Cord. After evaluation of UC length and diameter, the mean, SD, and percentile curves were calculated according to the GA (Tables 3 and 4 and Figure 4 explain these values). At 10 weeks' gestation, the mean UC length was 19.5 mm, and it increased 1.26 mm each week.

Embryo. A crown-to-rump (CR) was present in 182 cases, with parameters obtained from GA weeks 5 to 12. Table 5 and Figure 5 explain embryo weight values. At 10 weeks' gestation, the mean embryo weight was 2 g, and it increased 0.2 g each week.

Table 6 and Figure 6 explain embryo CRL. The estimated mean ± SD values obtained were GA 6 weeks, 5.3 ± 2.3 mm; GA 7 weeks, 9.1 ± 3.8 mm; GA 8 weeks, 13.7 ± 8.7 mm; GA 9 weeks, 20.8 ± 9.1 mm; GA 10 weeks, 27.6 ± 15.1 mm; GA 11 weeks, 29.1 ± 12.9 mm; and GA 12 weeks, 52 mm. At 10 weeks' gestation, the mean embryo CRL was 22.8 mm, and it increased 2.26 mm each week.

DISCUSSION

Evaluation of these specimens is enhanced by an understanding of the purpose of pathologic examination and how it may be helpful to both patients and clinicians.

First trimester abortion samples are a common pathologic specimen. The specimens have a varied composition and are often discarded and "terminived" [5,6]. A complete gross examination will allow appropriate assessment of the maternal, placental, and embryonal components and guide sampling of tissue for histologic examination. Microscopic study may show abnormalities, which suggest possible etiologies for the pregnancy failure [7–10].

Table 2. Summary Statistics for Gestational Sac (GS) Weight

		GS Weight, g								
							Percentile			
GA, wk	No. Valid	Mean	SD	Median	Min	Max	5th	25th	75th	95th
4	1				0.8	0.8				
5	17	4.7	6.7	1.5	0.1	26	0.1	0.7	6	26
6	71	7.7	7.9	5	0.4	31	1	3	10	29
7	70	9.1	7.7	6.5	0.4	34	1.8	4	10	33
8	103	13.6	11.1	7	1	62	2	4	22	32
9	65	11.9	12.8	10	1.7	50	3	6	30	47
10	70	14	11.1	10.5	1	56	4	7	26	35
11	43	19.4	13.4	18	2	49	3.7	7	38	46
12	20	18.1	13.8	14.5	1	49	1	7	31	46

Abbreviations: GA, Gestational age; Min, minimum; Max, maximum.

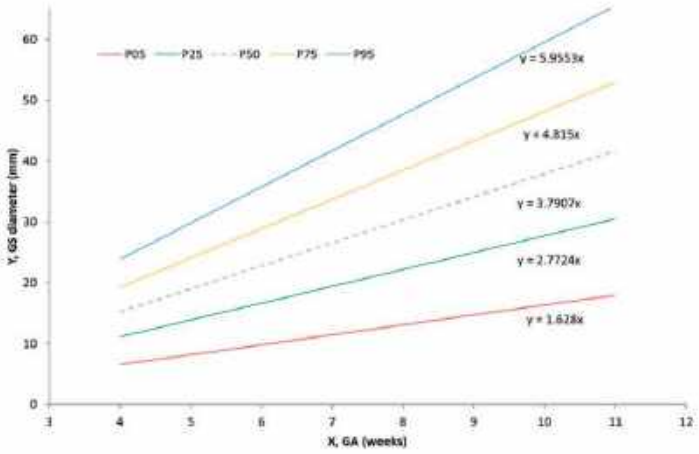

Figure 2. *Percentiles (P) for gestational sac (GS) diameter according to gestational age (GA).*

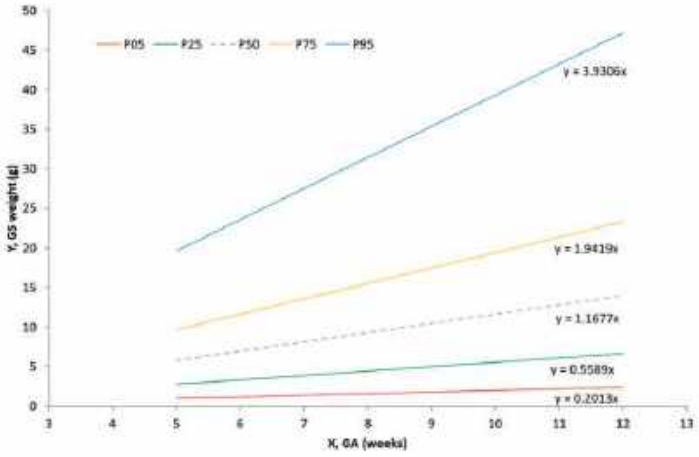

Figure 3. *Percentiles (P) for gestational sac (GS) weight according to gestational age (GA).*

Table 3. Summary Statistics for Umbilical Cord (UC) Length

		UC Length, mm								
							Percentile			
GA, wk	No. Valid	Mean	SD	Median	Min	Max	5th	25th	75th	95th
6	2	10.5	—	10.5	6	15	—	—	—	—
7	7	7	2.9	6	3	10	3	5	10	10
8	19	12.5	8.4	10	2	40	2	7	15	40
9	31	10.8	8.1	10	3	43	3	5	15	24
10	26	19.6	17	13.5	2	80	4	10	25	45
11	23	20.9	22.1	10	2	80	3	6	30	70
12	8	26.5	25.3	16	5	80	5	10	37.5	80

Abbreviations: GA, gestational age; Min, minimum; Max, maximum.

Table 4. Summary Statistics for Umbilical Cord (UC) Diameter

		UC Diameter, mm								
							Percentile			
GA, wk	No. Valid	Mean	SD	Median	Min	Max	5th	25th	75th	95th
8	2	4	—	—	3	5	—	—	—	—
9	5	3	1	3	2	4	2	2	4	4
10	8	3.3	1.3	3	2	5	2	2	4.5	5
11	2	4.5	—	—	4	5	—	—	—	—
12	2	3	—	—	2	4	—	—	—	—

Abbreviations: GA, gestational age; Min, minimum; Max, maximum.

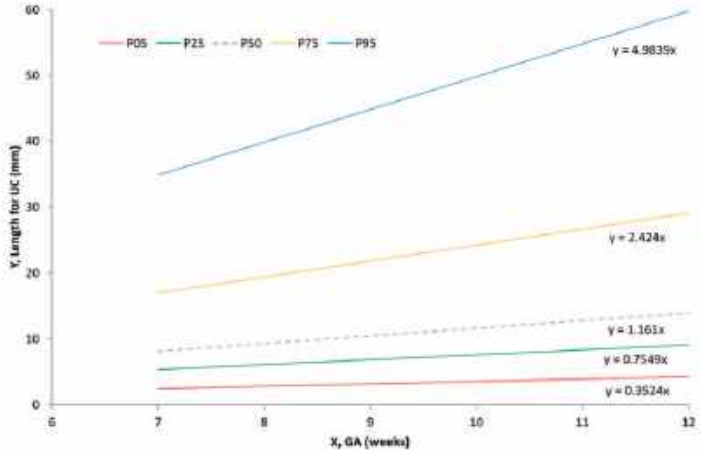

Figure 4. *Percentiles (P) for umbilical cord (UC) length according to gestational age (GA).*

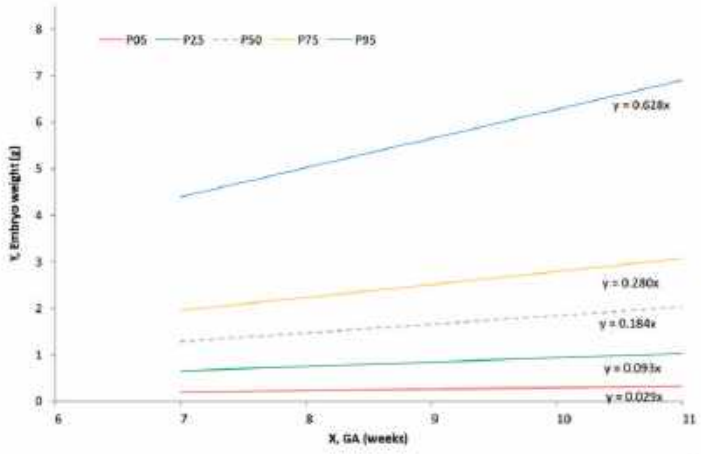

Figure 5. *Percentiles (P) for embryo weight according to gestational age (GA).*

Table 5. Summary Statistics for Embryo Weight

		Embryo Weight, g								
							Percentile			
GA, wk	No. Valid	Mean	SD	Median	Min	Max	5th	25th	75th	95th
6	1	—	—	—	0.1	0.1	—	—	—	—
7	3	0.3	0.3	0.1	0.1	0.6	0.1	0.1	0.6	0.6
8	8	1	0.7	0.7	0.1	2	0.1	0.5	1.8	2
9	22	2.5	3.5	1.3	0.5	14	0.6	1	2	12
10	13	2.2	1.1	2	0.3	4	0.3	1.4	3	4
11	16	3.2	2.4	3.5	0.2	8.4	0.2	1	4.5	8.4
12	1	—	—	—	10	10	—	—	—	—

Abbreviations: GA, Gestational age; Min, minimum; Max, Maximum.

Table 6. Summary Statistics for Embryo Crown-Rump Length (CRL)

		Embryo CRL, mm								
							Percentile			
GA, wk	No. Valid	Mean	SD	Median	Min	Max	5th	25th	75th	95th
5	4	9.8	7.6	8	3	20	3	4	15.5	20
6	8	5.3	2.3	5	2.3	10	2.3	4	6	10
7	7	9.4	4.8	11	3	15	3	4	14	15
8	19	13.7	8.2	13	2	30	2	6	20	30
9	27	20.8	9.1	20	2	42	6	15	27	35
10	23	22.6	13.4	28	2	40	2.8	6	35	40
11	19	29.4	12.9	30	12	58	12	20	40	58
12	2	52	—	52	44	60	—	—	—	—

Abbreviations: GA, gestational age; Min, minimum; Max, maximum.

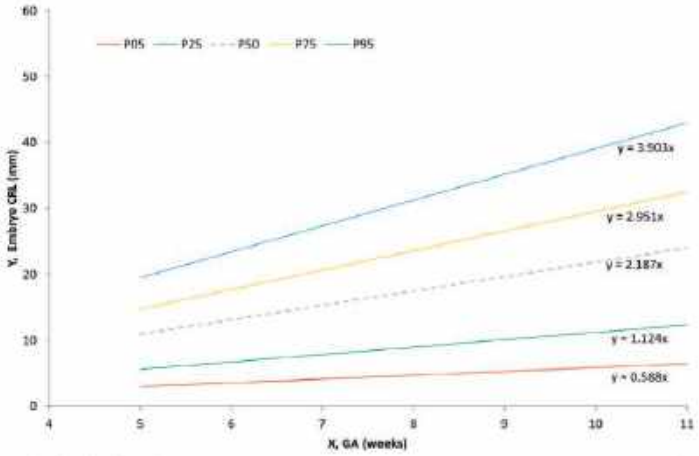

Figure 6. *Percentiles (P) for embryo crown-rump length (CRL) according to gestational age (GA).*

Given the GS and embryo measurements between weeks 5 and 12 that were performed in this study, a comparison of these measurements with prenatal ultrasound findings would be helpful to identify the accuracy of the latter modality.[illegible] Pathologic study is important for understanding causes of early pregnancy loss and counseling and treatment of patients during subsequent pregnancies. Multidisciplinary studies on early abortion have been increasing.[illegible] However, the correlation studies, especially on biometrics, are not yet well established.

Pathologic examination of FTSA is critical to validate ultrasound findings, including measurements, and also to understand the etiology of early abortion. Any major deviations in ultrasound and pathologic features may be important in determining embryonic, placental, or maternal disorders.[illegible]

CONCLUSIONS

The pathologic study of first trimester abortion samples adds information to prenatal ultrasound, which may be helpful to parents and clinicians. Deviation from normal expected growth of the embryo, GS, or UC may be the etiology of first trimester abortion.

The extension of the series with a greater number of cases is important for a sample validation and to determine a table of biometric values in early pregnancy loss. As a future perspective, it would be critical to continue the exploration of this topic, looking for the precise percentage of early SA worldwide and a shortage of pathologic studies evaluating the gross and microscopic etiologies of FTSA.

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2.2. SECOND AND THIRD TRIMESTER PLACENTAL MEASUREMENTS

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Placental Biometric Parameters: The Usefulness of Placental Weight Ratio and Birth/Placental Weight Ratio Percentile Curves for Singleton Gestations as a Function of Gestational age

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Abstract

Objective: To produce reference values for the placental weight (PW), Placental diameters (PDs), Placental thickness (PT), placental weight ratio (PW-R) and birth/placental weight ratio (BPW-R) in singleton gestations as a function of gestational age (GA).

Study Design and Setting: A retrospective 4-years case study of singleton placentas reports between, 1st of January 2014 to 31st of December 2017. The placentas were sent for histopathological diagnosis to Embryofetal Pathology Laboratory, Centro de Genetica Clínica (CGC), Porto, Portugal. In a cohort of singleton placentas, PW, PDs, PT, PW-R, and BPW-R were analyzed to produce percentile curves. Considering the inclusion criteria, 1,951 singleton placentas were selected from a sample of 7,321 placentas. We recorded the PW, PDs, PT, PW-R, and BPW-R between 12th and 41st GA.

Results: PW, PDs, PW-R and BPW-R tables and percentiles curves for singleton placentas across GA were produced.

Conclusions: Placental percentile curves may act as a reference for other populations as well until population-specific curves can be produced. PDs could predict placental volume and could help to estimate the prenatal PW-R and BPW-R.

Keywords: Placental weight; placental diameter; placental weight ratio; birth/placental weight ratio; percentiles; singleton gestation.

Introduction

Recently we have seen an increasing interest on the evaluation of biometric parameters of the placenta and its relation with the obstetric outcome. However, the relative lack of interest in the study of the placenta when compared to the fetal study was responsible for the existence of a great gap in the understanding of the biological significance of the placental lesions related to perinatal and neonatal context [1-5].

Macroscopic placental evaluation in the delivery room may improve a selection of placentas to histopathological study and, on the other hand, allow the evaluation of the placental weight (PW) and consequently the placental weight ratio (PW-R) and birth/placental weight ratio (BPW-R). Knowing these are factors that may be associated with pregnancy complications [1-5].

While birthweight (BW) percentile curves are relatively common in most countries, percentile curves for PW are rare, even in large series of placental studies [6,7]. At present we have available some fetal and placental percentiles curves which the majority refers to gestational age (GA) above 24 weeks [6,7]. However, some of the existing information may be out of date, as documented for the BW percentile curves [6,7]. Thus, the updating of percentile curves and their comparison between regions and even between countries are important to manage the pregnancy risks and to enhance the mother education and healthcare [1-4].

Although additional evidence is needed, the percentile curves are useful in evaluating fetal follow-up and maternal and child diseases. The percentile curves comprehension can optimize a targeted intervention in fetal adverse contexts such as intrauterine growth restriction(IUGR) and maternal diseases such as hypertension and diabetes also.

Objective

To produce gestational age-specific percentile curves for PW, placental diameters (PDs), placental thickness (PT), PW-R and BPW-R.

Material and methods

Sample and Definition

We conducted a retrospective case-study of 7,321 placentas sent to Embryo-fetal Pathology Laboratory, Centro de Genética Clínica (CGC), Unilabs, Porto, Portugal. The specimens had been sent for histopathological examination to confirm or determine suspected or unsuspected lesions that explain the obstetric outcome such as fetal demise and perinatal morbidity and mortality.

We collected information of 4-years placental pathological report performed between 1st of January of 2014 to 31st of December of 2017. The GA range of 12 to 41 weeks. Biometric parameters were collected from placentas and fetal deaths autopsy reports. Also, biometric parameters of newborns were obtained through the information contained in the clinical requisition of the placental pathological study. The registry involved data on maternal age and parity; GA; pathological placental reports; fetal autopsy reports and newborns clinical data. Placental parameters biometry's: PW, placental shape and diameter, umbilical cord length, diameters, and type insertion. Fetal deaths parameters acquired: weight and gender. Newborns parameters: weight and gender. Inclusion criteria: – 1. Known GA – 2. Maternal: i. Portuguese population-based woman; ii. Singleton deliveries ≥ 12 weeks of gestation. – 3. Placental: i. Formalin fixation equal or inferior lesser than 24 hours; ii. Absence of macroscopic lesions before 36th weeks of GA; iii. Macroscopic peripheral parenchymal lesion < 5% at ≥37 weeks of GA. – 4. Fetal deaths: i. Maceration is lesser than 12 hours; – 5. Newborns: i. Known birthweight. Exclusion criteria – 1. Maternal: i. Non-Portuguese woman; ii. Multiple pregnancies; iii. Singleton gestation relating to assisted reproductive technology. iv. Known chronic maternal disease (e.g., diabetes, hypertension with or without preeclampsia).–2. Placental: i. Macroscopic lesions more than 5% at any GA; ii. Gestational trophoblastic diseases; iii. Tumors; iv. Disease processes with high-grade histopathological lesions; v. Hydrops. vi. Incomplete, fragmented or disrupted placenta; vii. Placental curettage.–3. Fetal: i. maceration≥12h; ii. Hydrops; iii. intrauterine growth restriction (IUGR).–4. Newborn: i. Unknown BW; ii. IUGR.

The PW and FW were acquired using a balance GS6202 with measuring range 0.01g-620g – scale 0.01g (serial number 12105085, Kern); a balance MOD 470 with measuring range 0.5g-2,000g – scale 0.1g (serial number 42770096, Kern) and balance MOD 734 with measuring range 0g-20,000g – scale 0.1g (serie 1/1, Seca). The placental measures were acquired with a visual scale linear millmetric graduation ruler. Also, to smallest specimens, a comparative measures study achieved with a two linear scale ruler and a digital Vernier gauge 0-150mm scale Würth® was performed with similar results. BW was achieved in the delivery room care unit.

To produce percentile curves, 1,951 placentas were selected from a sample of 7,321 placental histopathological reports. Corresponding fetal gender, BW and FW registry were analyzed. We exclude non-native Portuguese woman (701); Newborn cases with missing data (846); Maceration traducing fetal demise with retention ≥ 12h (438); congenital abnormality was recorded as: no abnormalities, minor abnormalities, and major abnormalities (e.g., neural tube defects, such as, anencephaly, cranium-rachischisis, exencephaly and holoprosencephaly, skeletal dysplasia; limb body stalk complex). So, major defects were excluded (108); Fetal hydrops, or placental findings suggesting aneuploidy or metabolic storage diseases (306); IUGR (190); Multiple pregnancy (621); Partial hydatidiform moles (14); Extravillous trophoblastic diseases (4); Giant chorioangioma (7); Placental maternal vascular processes (652); Placental fetal vascular processes (314); histopathological pattern consistent with high grade immune / idiopathic inflammatory lesions (257) and infectious inflammatory lesions such as chronic plasma cell villitis (CMV, Parvovirus B19, Herpesvirus, Toxoplasma, listeria) (80) and chronic histiocytic intervillositis (CHI) (36); Other placental processes as massive fibrin deposition and maternal floor infarction (98); Single placenta gestation relating to assisted reproductive pregnancy technology (135); Incomplete, fragmented or disrupted placenta (474); Placenta accreta (37); and Placental curettage associated with retention (52). Knowing that PW increase approximately 5% after formal in fixation and the weight loss is little and most significant in hydropic or edematous placentas [6-9]. Initially, placentas were fixed in formalin for 24 hours. Then, after remoting the capsular membrane and umbilical cord, the PW, PDs and placental thickness (PT) were achieved in accordance with international guidelines. [8-9]. Placental disk dimensions include the measurements of the placenta in three dimensions at manual macroscopic examination in embryofetal pathology laboratory, and were achieved as: The maximum linear dimension (largest diameter = length) and the minimum linear dimension (smallest diameter = width) always acquired through the insertion point and perpendicular to each other. The maximum thickness was acquired in the central two-thirds of the disc, in accordance with international guidelines [8-9]. In addition, newborns were weighted in the delivery room care unit and fetus were weighted in the autopsy room. Placental macroscopic examination, sampling, and classification of placental lesions were performed in accordance with international guidelines [6-9].

All the samples used in the present study were unlinked and unidentified from their donors. Due to the retrospective nature of the study, the Local Ethical Review Committees of the involved institutions and Minho University Medicine School (Braga, Portugal) approved the work and waived the need for written informed consent.

Statistical Analysis

The percentiles curves for PW, PDs, PT, PW-R, and BPW-R were based on the same observations.The statistical analysis was conducted in IBMSPSS Statistics version 25 using the most appropriate tests according to the nature of the variables involved. To evaluate the normality, we used the Q-Q plots due to the sample size.

Results

The final sample was 1,951 singleton placentas. PW, PDs, PT, BPW-R, and PW-R mean, standard deviation (SD), median, minimum and maximum to maternal, placental and fetal or newborn quantitative and qualitative variables are summarized in (Table 1) and (Table 2). Maternal age range from 15 to 48 years. Sex was defined as either: female, male and ambiguous or unknown if the data was missing. So, the gender distribution was female in 818 (47.7%) cases, male in 884 (51.5%) cases and ambiguous in 13 (.8%) cases (Table 2). GA was a key variable for this research and played an integral role in establishing BPW-R and PW-R. For the purpose of this study, GA remained as a continuous integer variable, but only the gestational week was used, not the number of days. According to clinical practice, GA estimation was derived from the first day of the last menstrual period. Otherwise, GA was corrected on the basis of ultrasound measurements that are routinely obtained for all pregnant woman in Portuguese hospitals. Placental weight, Fetal and newborn BW was recorded in grams as a continuous variable.

Measures of interest for this study were PW, PDs [e.g., largest placental diameter (LPD or PD>) smallest placental diameter (SPD or PD<) and placental thickness (PT)], BW, BPW-R, and PW-R. The t-student test was used to compare the mean value of PW at each GA according to gender and likewise for the BW or FW. According to gender, with the exception of 27 weeks ($p = .033$), there were no statistically significant differences between mean PW for male and female fetuses ($p > .05$). These results are summarized in the graph of Figure 1. Also, except for 16 weeks ($p = .021$) and 40 weeks ($p = .018$), there were no statistically significant differences between mean BW for male and female fetuses ($p > .05$). These results are shown in the graph in Figure 2. Taking into account these results, it was decided to draw tables for percentiles, a number of observations, mean and standard deviation, minimum and maximum for the PW, BW (e.g., fetal weight and newborn weight). These results are shown in (Table 3) and (Table 4) respectively. The same analysis was

These results are shown in (Table 5) and (Table 6) respectively.

Table 1. Summary statistics for some important fetal, placental and maternal quantitative variables

	Valid (N)	Mean	SD	Median	Min	Max
MA (y)	1947	31.8	6.1	32.1	15.1	48.2
GA (w)	1951	27	9	26	12	41
PW (g)	1951	233.25	159.25	195.00	6.00	995
PD (cm)	1949	13.5	5.0	13.0	1.7	32.0
PT (cm)	1947	2.11	.70	2.00	.30	5.50
FW (g)	1951	1248.70	1153.93	766.00	5.40	4880

Legend: SD, standard deviation; Min, minimum; Max, maximum; MA, maternal age; GA, gestational age; PW, placental weight; PD, placental diameter; PT, placental thickness; FW, fetal weight; y, years; w, weeks; g, grams; cm, centimeters.

Table 2. Summary statistics for some important fetal, placental and maternal qualitative variables

		N	Percent
Mother Parity	1	820	45.2%
	2	595	32.8%
	3+	400	22.0%
	Total	**1815**	**100.0%**
Fetal Gender	F	818	47.7%
	M	884	51.5%
	A	13	.8%
	Total	**1715**	**100.0%**
Placental Shape	Normal	1731	88.7%
	Bilobed	113	5.8%
	Circumvallate	104	5.3%
	Membranacea	3	.2%
	Total	**1951**	**100,0%**

Legend: F, female; M, male; A, ambiguous.

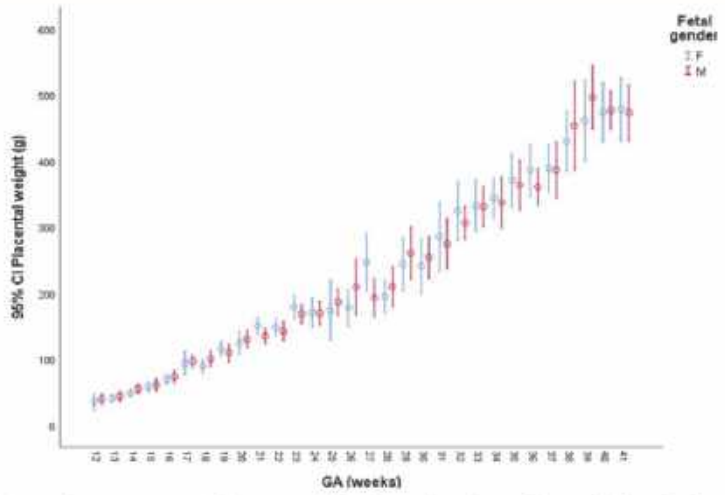

Figure1.–Meanandrespective95%confidenceintervalsforplacentalweightaccording to fetal gender for each GA.

Legend: CI, confidence interval; g, grams; GA, gestational age; F, female; M, male.

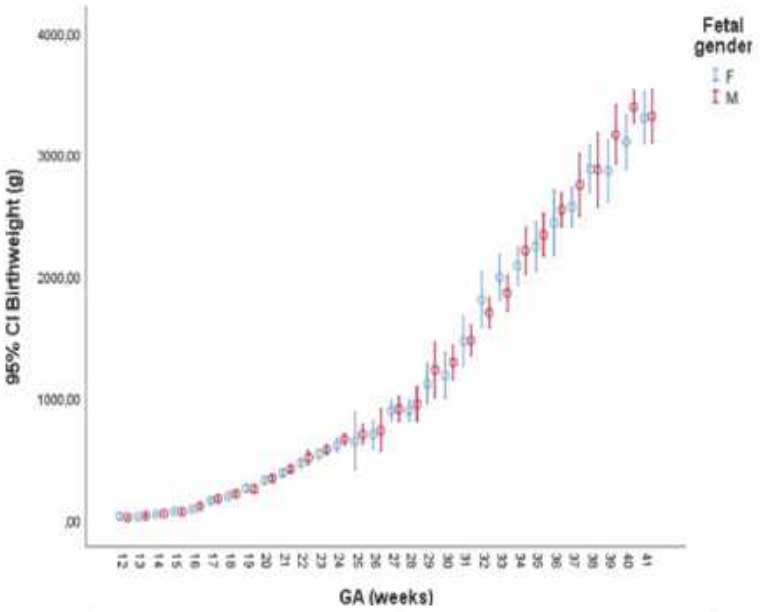

Figure 2. – Mean and respective 95% confidence intervals, for the birth weight according to the fetal gender for each GA.

Legend: CI, confidence interval; g, grams; GA, gestational age; F, female; M, male.

Table 3. Percentiles, number of observations, the mean and standard deviation for placental weight as a function of GA

	Percentile						Placental weight (g)	
GA	3rd	10th	50th	90th	97th	Number	Mean	SD
12	10	13	33	67.3	96	53	36.72	23.93
13	10	21	38	60	74	61	40.79	17.29
14	20.6	30	49.5	75	88	68	51.22	17.96
15	16	36	58	90	108	72	60.03	23.53
16	23.8	43	71	100	108	72	71.36	25.04
17	44	57	89	126	160	69	95.39	38.05
18	51.3	60	95	132	149	73	95	32.49
19	41	68	114	160	169	72	113.39	35.67
20	57	83	120	166	190	69	126.69	45.79
21	68	103	143.3	184	200	71	141.89	35.44
22	74	104	141	191	220	71	145.28	39.37
23	88	123	174	223	243	72	172.19	44.78
24	97	107	153.5	241	260	68	170.6	55.81
25	74	82	184	270	330	48	181.94	69.42
26	88	100	190	288	319	43	190.67	71.35
27	76	131	238.65	289	291	35	220.3	73.23
28	96	123	202	307	351	61	203.38	67.66
29	134	154	240	365	434	43	254.84	89.83
30	99.6	151	247	345	423	47	249.1	77
31	114	145	293.5	405	462	56	280.46	90.97
32	182	203	307	471	512	67	314.61	89.16
33	190	222	334.5	442	515	72	336.67	88.37
34	195	253	344	448	496	72	341.94	84.01
35	200	237	356	546	654	75	374.79	123.68
36	237	267	350	490	515	77	361.16	80.6
37	242	275.3	364.5	507	593	72	380.02	91.21
38	273	296	405	590	755	72	433.61	136.89
39	259	312	444	610	678	71	450.38	128.09
40	335	378	459	580	632	77	475.44	90.66
41	327	353	470	615	690	72	478.18	105.99

Legend: GA, gestational age; g, grams; SD, standard deviation. Nogueira R, *et al.*, Portugal, Porto, Embryofetal Pathology Laboratory.

Table 4. Percentiles, number of observations, the mean and standard deviation for birthweight as a function of GA

	Percentile						Birthweight (g)	
GA	3rd	10th	50th	90th	97th	Number	Mean	SD
12	5.7	9.3	16	29	111	53	23.83	38.99
13	6.7	16	27	41	46	61	28.22	20.96
14	16	24	46	70	77	68	44.72	17.3
15	15	24	70.5	100	120	72	67.86	32.27
16	25	44	99.35	164	185	72	99.8	44.33
17	75	89	166	226	266	69	165.63	51.58
18	66.5	122	209	261	309	73	201.52	56.84
19	56	127	261	333	402	72	253.83	86.96
20	141	242	325	430	455	69	330.38	81.85
21	190	296	409	497	535	71	400.56	86.21
22	241	353	495	589	648	71	486.48	141.64
23	325	411	565.5	700	772	72	560.09	109.35
24	343	444	642.5	786	854	68	637.79	143.32
25	179	280	731	870	1175	48	678.61	332.26
26	364	460	640	925	1670	43	710.84	293.23
27	488	620	958	1150	1200	35	911.78	192.51
28	300	549	960	1240	1400	61	928.62	270.84
29	497	638	1075	1720	2160	43	1203.65	488.27
30	660	845	1275	1730	1860	47	1267.34	349.7
31	796	920	1469.5	1930	2180	56	1479.11	358.7
32	937	1219	1750	2250	2500	67	1759.27	487.78
33	1177	1500	1960	2360	2490	72	1931.08	391.98
34	1331	1515	2095	2690	2860	72	2136.57	443.36
35	1530	1720	2270	2850	3640	75	2337.69	561.11
36	1640	1860	2440	3175	3390	77	2454.43	457.96
37	2000	2140	2450	2995	3690	72	2570.04	513.11
38	2090	2200	2737.5	3580	4120	72	2846.26	587.45
39	1930	2230	2780	3660	3840	71	2882.54	590.53
40	2400	2580	3230	3860	3940	77	3253.27	477.65
41	2500	2600	3312.5	3900	4210	72	3308.19	507.44

Legend: GA, gestational age; g, grams; SD, standard deviation. Nogueira R, *et al.*, Portugal, Porto, Embryofetal Pathology Laboratory.

Table 5. Percentiles, number of observations, the mean and standard deviation for birth/placental weight ratio (BPW-R) as a function of GA

	Percentile						BPW-R	
GA	3rd	10th	50th	90th	97th	Number	Mean	SD
12	.21	.28	.51	1.22	1.97	53	.68	.47
13	.32	.41	.63	1.06	2.5	61	.76	.46
14	.35	.51	.94	1.32	1.38	68	.92	.32
15	.34	.53	1.1	1.92	2.48	72	1.19	.55
16	.54	.68	1.41	2.11	2.32	72	1.43	.52
17	.83	1	1.78	2.71	3.16	69	1.86	.68
18	1	1.37	2.18	3.04	3.66	73	2.25	.76
19	.93	1.47	2.28	3.06	3.77	72	2.28	.69
20	1.41	1.92	2.59	4	4.39	69	2.78	.81
21	1.6	2.13	2.84	3.99	4.5	71	2.95	.82
22	1.97	2.34	3.41	4.5	5.57	71	3.45	.88
23	2.12	2.38	3.36	4.69	5.21	72	3.43	.91
24	2.35	2.67	3.82	5.15	6.1	68	3.94	1
25	1.82	2.23	3.56	5.22	7	48	3.83	1.57
26	2.16	2.55	3.9	5.14	6.44	43	3.93	1.19
27	2.66	3.39	4.09	6.42	7.08	35	4.45	1.2
28	2.45	3.09	4.82	6.45	7.7	61	4.77	1.35
29	2.91	3.19	4.63	6.27	7.74	43	4.85	1.49
30	3.52	3.73	5.42	6.74	7.68	47	5.28	1.16
31	3.58	4.17	5.42	6.99	8.07	56	5.54	1.17
32	3.78	4.12	5.67	7.53	7.95	67	5.74	1.28
33	3.82	4.33	5.96	7.37	8.63	72	5.96	1.26
34	4.59	4.91	6.45	7.98	8.91	72	6.49	1.78
35	4.73	5.04	6.45	8.1	8.53	75	6.48	1.18
36	4.58	5.3	6.93	8.89	9.77	77	6.96	1.31
37	4.96	5.46	6.76	8.89	9.69	72	6.95	1.27
38	4.64	5.29	6.93	8.56	9.51	72	6.86	1.38
39	3.96	4.96	6.53	8.5	8.88	71	6.65	1.34
40	4.82	5.68	6.92	8.57	8.98	77	6.97	1.07
41	5.35	5.95	7	8	9.16	72	7.07	.94

Legend: GA, gestational age; SD, standard deviation. Nogueira R, *et al.*, Portugal, Porto, Embryofetal Pathology Laboratory.

Table 6. Percentiles, number of observations, mean and standard deviation for placental weight ratio (PW-R) as a function of GA

	Percentile						PW-R	
GA	3rd	10th	50th	90th	97th	Number	Mean	SD
12	.51	.82	1.96	3.55	4.84	53	2.22	1.77
13	.40	.94	1.59	2.45	3.10	61	1.63	.67
14	.72	.76	1.07	1.96	2.83	68	1.27	.60
15	.40	.52	.91	1.89	2.90	72	1.08	.72
16	.43	.47	.71	1.47	1.86	72	.84	.47
17	.32	.37	.56	1.00	1.20	69	.62	.26
18	.27	.33	.46	.73	1.00	73	.50	.20
19	.27	.33	.44	.68	1.07	72	.50	.28
20	.23	.25	.39	.52	.71	69	.40	.15
21	.22	.25	.35	.47	.63	71	.37	.10
22	.18	.22	.29	.43	.51	71	.31	.08
23	.19	.21	.30	.42	.47	72	.31	.08
24	.16	.19	.26	.37	.43	68	.27	.07
25	.14	.19	.28	.45	.55	48	.29	.10
26	.16	.19	.26	.39	.46	43	.28	.09
27	.14	.16	.24	.30	.38	35	.24	.07
28	.13	.16	.21	.32	.41	61	.23	.07
29	.13	.16	.22	.31	.34	43	.22	.06
30	.13	.15	.18	.27	.28	47	.20	.05
31	.12	.14	.18	.24	.28	56	.19	.04
32	.13	.13	.18	.24	.26	67	.19	.07
33	.12	.14	.17	.23	.26	72	.18	.05
34	.11	.13	.15	.20	.22	72	.16	.03
35	.12	.12	.16	.20	.21	75	.16	.03
36	.10	.11	.14	.19	.22	77	.15	.03
37	.10	.11	.15	.18	.20	72	.15	.03
38	.11	.12	.14	.19	.22	72	.15	.03
39	.11	.12	.15	.20	.25	71	.16	.04
40	.11	.12	.14	.18	.21	77	.15	.02
41	.11	.13	.14	.17	.19	72	.14	.02

Legend: GA, gestational age; SD, standard deviation. Nogueira R, et al., Portugal, Porto, Embryofetal Pathology Laboratory.

Percentiles curves for PW, BW, PBW-R, and PW-R, between 12th and 41st weeks of GA, were produced. These results are shown in (Figure 3), (Figure 4), (Figure 5) and (Figure 6) respectively. An approach to placental volume (PV) was determined using the calculation [LPD x SPD x PT]. So, graphs to evaluate PV – PW, and PDs – PW correspondences were produced. These results are shown in (Figure 7).

To assess whether there was an association between PW and PV a Pearson correlation test was performed to evaluate the linear association between variables. The results obtained are found in the matrix (Table 7). It is verified that there is significant linear association with: Positive Very Strong association between: LPD and SPD ($r=.918$, $p<.01$); Positive Strong association between: PW and PV ($r=.833$, $p<.01$); PW and LPD ($r=.826$, $p<.01$); PW and SPD ($r=.829$, $p<.01$); PV and LPD ($r=.833$, $p<.01$), and PV and SPD ($r=.877$, $p<.01$); Moderate Positive association between: PW and PT ($r=.619$, $p<.01$) and PV and PT ($r=.621$, $p<.01$); Weak Positive association between: LPD and PT ($r=.396$, $p<.01$) and SPD and PT ($r=.398$, $p<.01$).

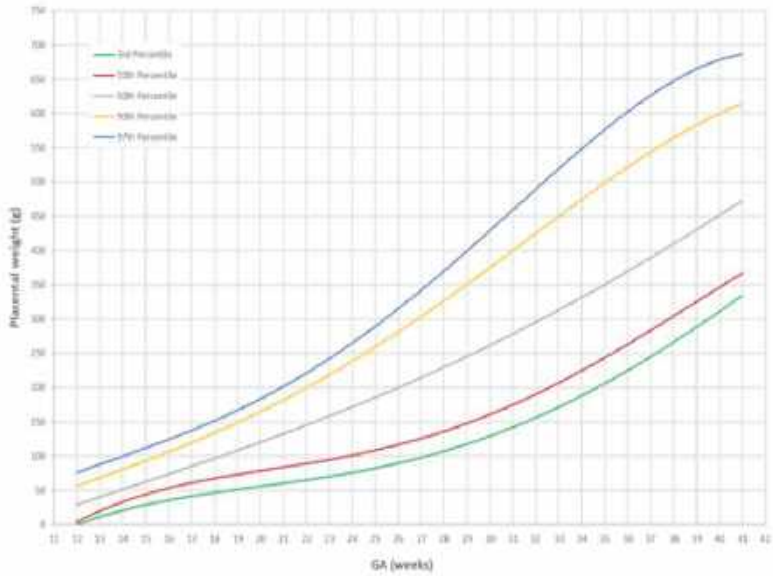

Figure 3. – Placental weight percentile curves by gestational age, Nogueira R, et al., Portugal, Porto, Embryofetal Pathology Laboratory.

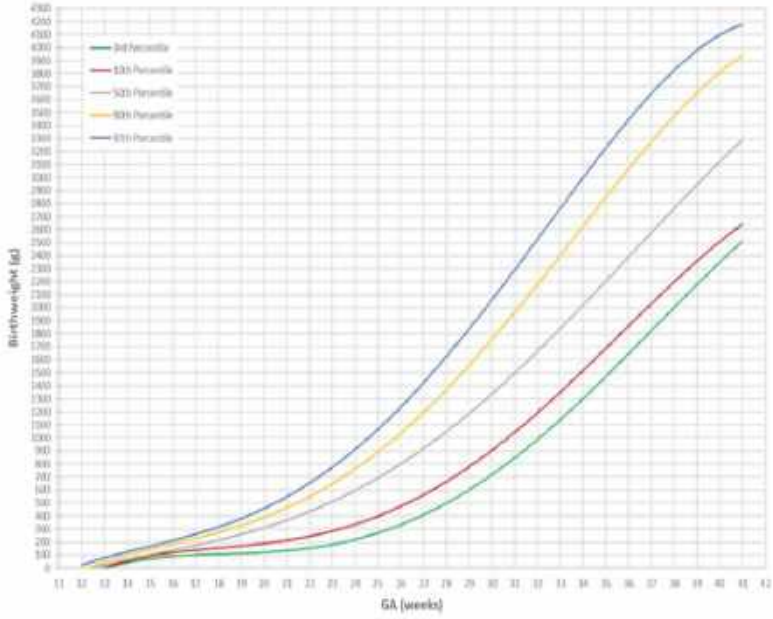

Figure 4.–Birthweight percentile curves by gestational age, Nogueira R, et al. Portugal, Porto, Embryofetal Pathology-Laboratory.

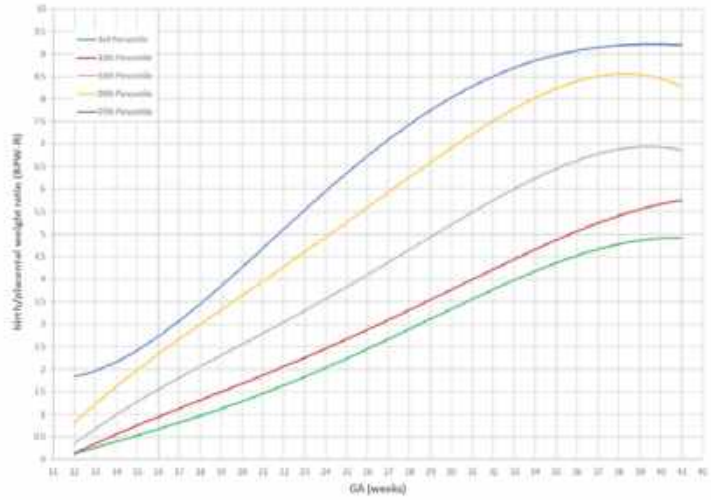

Figure 5. – Birth/placental weight ratio percentile curves by gestational age, Nogueira R, et al., Portugal, Porto, Embryofetal Pathology Laboratory.

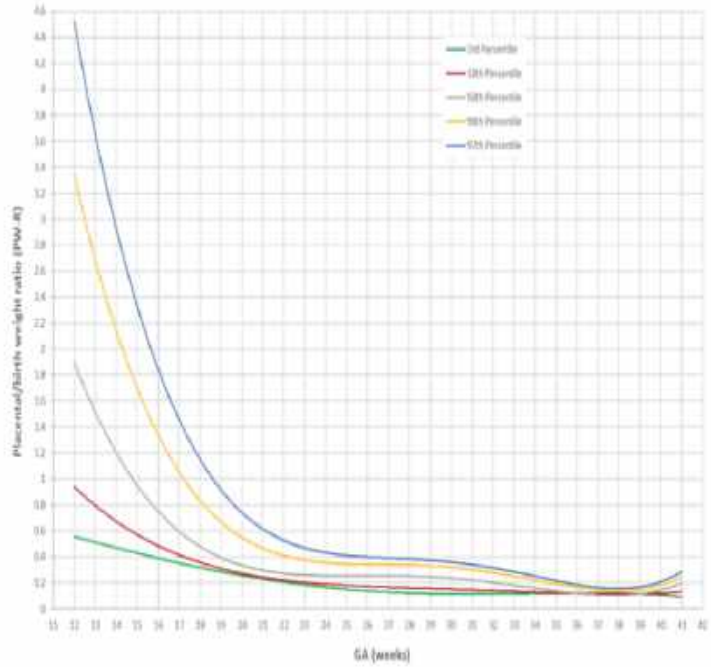

Figure 6. – Placental/birth weight ratio (PW-R) percentile curves by gestational age, Nogueira R, et al., Portugal, Porto, Embryofetal Pathology Laboratory.

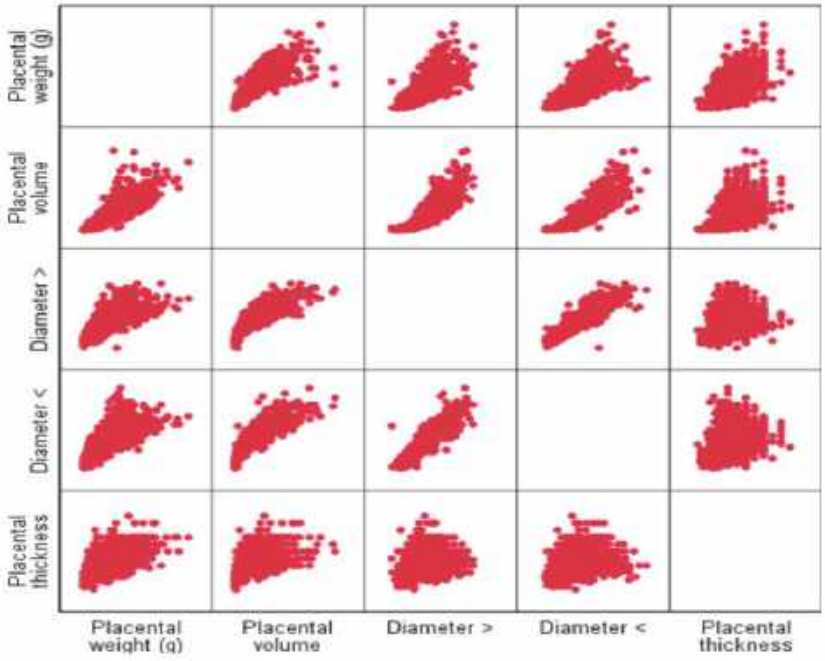

Figure 7. – Relation between placental measures.
Legend: g, grams; Diameter >, largest placental diameter; Diameter <, smallest placental diameter.

Discussion

The placental examination has been important in documenting a pathophysiological complex process associated with poor obstetric outcomes such as fetal and neonatal morbidity and mortality and chronic diseases in later life [1-5].

Over the years there has been the production of percentile curves for BW as a function of GA to guide physicians and parents about fetal and newborn growth [5-7]. Those mostly charts are restricted to 3rd trimester gestation [5-7]. Also, some of these studies address specific contexts such as fetal gender, parity, and ethnicity [10-12]. Beings a positive association between PW and BW with ethnicity and parity [10-12]. Moreover multiparous increases the odds of having a PW-R≥90th percentile, and the effect is most pronounced in the infants born at ≤32 weeks [10]. Knowing that fetal gender shows association with PW, the categorization into male and female-specific curves is important because male weigh more than female at each GA [11-18]. Unlikely, the present study discloses non-statistically significant differences between gender for PW, FW, and BW, except for the PW at 27 weeks of GA (p=.033) and BW at 25 weeks (p=.021) and 40 weeks (p=.018). This suggest that the association between or BW and gender will not be relevant at early GA.

There is some evidence that the shape and size of the placenta are factors that may be statistically associated with pregnancy complications (e.g, IUGR, reduced fetal movements) and an individual's long-term health [19-24].

Besides PW has been described as an independent predictor of BW and a good predictor for chronic diseases in later life [2,3,11,13,15,17-24]. PW percentile curves are rare and mostly refer to GA ≥ 24 weeks [14-18,21,22]. BPW-R (e.g. the BW over the PW) and PW-R (e.g. PW over the BW) percentile curves were a significant contribution to the literature and medicine practice [14-18,21,22] However, rare population curves to date have looked at an early GA such as 12th weeks or earliest [25].

Although reversed, PW-R percentile curves are more specific to the purpose of the present study see (Figure 5) and (Figure 6). Also, a significant linear association with a very strong

Table 7. Correlation between placental measures, placental weight and placental volume

Correlations

		Placental Weight (g)	Placental Volume	Largest Placental Diameter	Smallest Placental Diameter	Placental Thickness
Placental Weight (g)	Pearson Correlation	1	,883**	,826**	,829**	,619**
	Sig. (2-tailed)		,000	,000	,000	,000
	N	1951	1944	1949	1948	1947
Placental Volume	Pearson Correlation	,883**	1	,883**	,877**	,621**
	Sig. (2-tailed)	,000		,000	,000	,000
	N	1944	1944	1944	1944	1944
Largest Placental Diameter	Pearson Correlation	,826**	,883**	1	,918**	,396**
	Sig. (2-tailed)	,000	,000		,000	,000
	N	1949	1944	1949	1948	1945
Smallest Placental Diameter	Pearson Correlation	,829**	,877**	,918**	1	,398**
	Sig. (2-tailed)	,000	,000	,000		,000
	N	1948	1944	1948	1948	1944
Placental Thickness	Pearson Correlation	,619**	,621**	,396**	,398**	1
	Sig. (2-tailed)	,000	,000	,000	,000	
	N	1947	1944	1945	1944	1947

**Correlation is significant at the 0.01 level (2-tailed). Nogueira R, *et al.*, Portugal, Porto, Embryofetal Pathology Laboratory.

or strong positive association between placental biometries may improve the charaterization of the PW and PV and consequently the placental function evaluation.

Knowing that BPW-R and PW-R are important parameters for the balance between fetal and placental growth and considering the functional reserve capacity of the placenta, those may be the greatest predictors of IUGR and diseases in later life than PW and BW alone [1,12,15-18,21,22]. PW-R appears to reflect differences in growth pattern and placental efficiency and correlates significantly with fetal morbidity and short-term adverse perinatal outcomes also [19-24].

Thus, the existence of a linear correlation between placental measurements and a good association with placental volume demonstrated in the present study, may improve prenatal diagnosis and anticipate measures in specific placental and/or fetal situations to prevent the adverse outcome of pregnancy.

Conclusions

Gestational-age-specific placental percentile curves for PW, BPW-R, and PW-R for singleton delivery between 12th and 41st weeks of gestation are available to liken results between countries and regions. The significant association between placental measurements contributes to the assessment of placental function (related to size and volume) and its implication in fetal growth, assisting clinicians in preventing fetal life risks and improving maternal and child health.

Acknowledgments

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"The natural condition of the bodies is not the rest, but the movement."

Nicolaus Copernic

CHAPTER 3

CLASSIFICATION OF PLACENTAL LESIONS

3.1. FIRST-TRIMESTER ABORTION LESIONS

Heliyon

Histopathological Classification of First-Trimester Abortion Products Linking a Clinical Follow-up

--Manuscript Draft--

Manuscript Number:	HELIYON-D-19-01603
Article Type:	Original Research Article
Section/Category:	Clinical Research
Keywords:	First-trimester abortion; Histopathology; Chorionic villi morphology; Embryo pathology; Classification system; Clinical relevance.
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Abstract:	BACKGROUND The histopathological study of first-trimester abortion products (FTAp) may be useful in document an intrauterine pregnancy, identifying an important pathology affecting the mother or the embryo and diagnosing conditions that are likely to reccur in future pregnancies or that warrant the adverse outcome. Relevant information provided by a systematic histopathologic study is essential to determine the cause and to guide the patients with early pregnancy failure. AIMS A histopathological classification of FTAp. METHODS International pathologic published criteria in first-trimester abortion were collected, standardized and focused into a comprehensive diagnosis. The idea was to create a comphrensive classification related to major pathophysiological processes. So, 7 categories were created: i. Changes suggesting aneuploidy (SCA) or metabolic storage disease; ii. Embryo anomaly (EA); iii. Multifactorial (MF) causes; iv. Maternal causes (MC); v. Gestational trophoblastic disease, such as hydatidiform mole and non-neoplastic lesions and neoplasms; vi. Ectopic pregnancy; vii. Other. A 5-years retrospective study of FTAp < 12 th weeks of gestational age (GA). Two groups were created: i. A study group of FTAp with a pathological diagnosis; ii. A control group FTAp with pathological diagnosis and cytogenetic result. RESULTS Histopathological criteria concordance between inter-observers was generally good, with an excellent correlation in the diagnostic categories of EA and HM. In the CSA and MC categories there was greater disagreement even though the correlation with the cytogenetic and molecular result were very positive. CONCLUSION A standardized, reproducible and biologically comprehensive FTAp classification based on histopathological criteria may improve fetal follow-up and couple's management.
Suggested Reviewers:	
Opposed Reviewers:	

Title: Usefulness of Histopathological Classification in First-trimester Abortion Products - Linking a Clinical Follow-up

Running heads: First-trimester Abortion Histopathology

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Conflict of interest disclosure

Authors have no conflict of interest to declare.

ABSTRACT

BACKGROUND The histopathological study of First-trimester abortion products (FTAp) may be useful in document an intrauterine pregnancy, identifying an important pathology affecting the mother or the embryo and diagnosing conditions that are likely to reccur in future pregnancies or that warrant the adverse outcome. Relevant information provided by a systematic histopathologic study is essential to determine the cause and to guide the patients with early pregnancy failure.

AIMS A histopathological classification of FTAp.

METHODS International pathologic published criteria in First-trimester abortion were collected, standardized and focused into a comprehensive diagnosis. The idea was to create a comphrensive classification related to major pathophysiological processes. So, 7 categories were created: i. Changes suggesting aneuploidy (SCA) or metabolic storage disease; ii. Embryo anomaly (EA); iii. Multifactorial (MF) causes; iv. Maternal causes (MC); v. Gestational trophoblastic disease, such as hydatidiform mole and nonneoplastic lesions and neoplasms; vi. Ectopic pregnancy; vii. Other. A 5-years retrospective study of FTAp < 12th weeks of gestational age (GA). Two groups were created: i. A study group of FTAp with a pathological diagnosis; ii. A control group FTAp with pathological diagnosis and cytogenetic result.

RESULTS Histopathological criteria concordance between inter-observers was generally good, with an excellent correlation in the diagnostic categories of EA and HM. In the CSA and MC categories there was greater disagreement even though the correlation with the cytogenetic and molecular result were very positive.

CONCLUSION A standardized, reproducible and biologically comprehensive FTAp classification based on histopathological criteria may improve fetal follow-up and couple's management.

Keywords: First-trimester abortion; Histopathology; Chorionic villi morphology; Embryo pathology; Classification system; Clinical relevance.

INTRODUCTION

Spontaneous abortion (SA) it's one of the most common First-trimester complications affecting over 15% of pregnant women in the childbearing age and can rise to 45%.[1-4] A precise incidence of First-trimester spontaneous abortion (FTSA) is not well-established[1-4] In Portugal, there are no statistical data in FTSA.

Regardless of the high incidence of early abortion, the First-trimester abortion products (FTAp) are often poor described from a developmental perspective, which contributes to the low number of pathology examination requests.

A competente FTAp examination is essential in identification of previously unsuspected disease.[5-10] Also, the success of pathological examination is partially dependent on the skill and experience of the examiner.[5] As such as examination of any specimen, it is wise to follow a routine protocol.[5-12] FTAp differ greatly in their composition. So, grossly, they may consist of blood clot admixed with minimal decidua tissue and fragmented villous tissue and embryonic/fetal parts, a complete (or not) gestational sac (GS) and an embryo; or something between.[5,11] Histopathological examination is an integral and a routine component of the management of patients with sporadic and recurrent early pregnancy failure.[5-9]

Useful information from a competently performed FTAp evaluation falls into the following categories: (1) Document an intrauterine pregnancy; (2) Identification of previously unsuspected disease affecting the mother or the embryo (fetus) that require immediate attention (eg, unusual infections, hydatidiforme mole; changes suggesting aneuploidy or metabolic storage disease); (3) Identification of conditions that are likely to reccur in future pregnancies or that warrant the adverse outcome (eg, unusual infections, chronic histiocytic intervillositis, maternal malperfusion, hereditary settings); (4) Conditions that can guide management of future pregnancies (e.g., hydatidiform mole, extravillous trophoblastic lesions).[8-13] Also, routine histopathologic examination of FTAp, either spontaneous or recurrent, and surgically or medically evacuated, is beneficial in protecting obstetrician and gynecologist from medico-legal recrimination. At our institution, these are routinely subjected to histopathological examination.

Etiology of SA is a complex and heterogeneous process correlated with gestational age (GA).[12-16] The most common causes of early or very early spontaneous abortion (ESA) are aneuploidies or chromosomal aberrations often resulting in abnormal embryo and solitary or multiple malformations

indicative of specific syndromes or associations.[12,15-24] Embryo anomaly (EA), maternal causes (MC), multifactorial (MF) or environmental contexts and teratogens are often adverse causes of miscarriage. These interfere with normal embryogenesis resulting in distint embryonic abnormalities related on timing of the errors occurs.[6-8,14,18,20-22,27-29] Gestational trophoblastic diseases (GTD) comprise a complex and challenging group of lesions that are classified as into several groups.[16,30-34] Molar pregnancies, e.g., hydatidiform moles (HM) are an abnormal placenta with variable degrees of trophoblastic hyperplasia and villous hydrops.[33] HM morphologicaly and genetically are classified as complete hydatidiform mole [CHM, very early complete hydatidiform mole (VECHM)], partial hydatidiform mole (PHM) and invasive type.[30-37] In Portugal, the incidence of HM is one in 570 pregnancies (0.2%).[38] Non-neoplastic gestational trophoblastic lesions [e.g., exaggerated placental site (EPS) and placental site nodule (PSN)], and gestational trophoblast neoplasms [e.g., choriocarcinoma (ChoCa), placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT)] are an heterogeneous group of tumors consisting of a extravillous trophoblastic cell proliferation.[30-37] EPS represents part of the spectrum of the normal impantation-site changes. PSN is a benign, well circumscribed nodule consisting of trophoblastic cell proliferation. ChoCa, is a malignant trophoblastic tumor consisting of a trimorphic proliferation of extravillous trophoblastic cells, syncytiotrophoblast and cytotrophoblast, in the absence of chorionic villi.[31,37,39,40] PSTT, is a trophoblastic tumor consisting of neoplastic implantation site-type extravillous trophoblast, while the ETT is a trophoblastic tumor consisting of neoplastic chorionic-type extravillous trophoblast.[31,37,39-41]

In a muldisciplinary practice, a biologically comprehensive, histopathological-based classification of FTp allows a guide treatment decision and management in sporadic and recurrent early miscarriage. [5,7-11,12,29]. Also, a competent FTAp histopathological examination could improve a selection-case to complementary genetic or molecular tests.[5,12,13,29] Finally, genetic correlation (control group) make available evidence-based histopathological criteria for future investigation in this area.

OBJECTIVE

A biologically comprehensive histopathologically-based classification of FTAp.

MATERIALS AND METHODS

A retrospective crossy-study of FTAp reports of 3,228 products sent to histopathological diagnosis to Embryofetal Pathology Laboratory, centro de genética clínica (CGC) unilabs, Porto, Portugal, between January 2013 and December 2018. Histopathological diagnosis was based on routine histopathologcial examination [hematoxylin and eosine (H&E)-stained slides of paraffin-embedded material]. Additional complementary study (e.g., immunohistochemistry) had been performed when applicable. Guidelines to FTAp examination were used.[5,9,10,12,28] The full text of FTAs reports were screened to identify those potentially fulfilling the sample selection criteria. Study sample was categorized into two groups: – 1. Study Group: i. Intrauterine FTp < 12th week of GA. – 2. Control Group: intrauterie FTp < 12th week of GA with concurrent genetic study (n=223). Inclusion criteria: i. histopathological diagnosis classification into one of the defined categories; ii. Interobserver agreement. Exclusion criteria: i. Degenerative changes associated with retention; ii. FTp relating to a medical or social termination of pregnancy (TOP); iii. Twin pregnancy.

A competent gross examination allowed adequate evaluation of maternal (decidua), placental and embryonic components, and submission for microscopy (see above). For the present study the original hematoxylin and eosin (H&E) sections were used for the review. Gestational age (GA) dated from the first day of the last menstrual period was the only available information. Discrepant criteria were revised by two experienced embryofetal pathologists and an interobserver consensus was achieved together with a senior embryofetal Pathologist. The histopathological interobserver agreement was calculated by un-weighted kappa analysis as a measurement of interobserver reproducibility. So, a diagnostic algorithm in FTAp was created. (Figure 1).

In the interdisciplinary discussions we used the international guidelines for the FTAp examination with the objective of evaluate histopathological criteria like seem to an algorithm approach to FTAp classification (see Figure1). Also, an additional recommendation in simplified comment mode, could summarize an explanation in difficult cases. This system has been routinely used in our laboratory for several years with very positive feedback from obstetricians.

The sum of the histopathological features fell preferentially in a single diagnostic category integrating all the findings, e.g., when CSA were histopathologically identified and a growth disorganized (GD) embryo coexists, the classification was in the EA category. (see Figure 1).

So, a simplified classification was constructed, consisting into 7 main diagnostic categories, including one category for multifactorial or debated etiology (Table 1).

Table 2, summarizes the histopathological FTAp classification at each Pathological category. CSA, concerns a gestational sac (GS) and chorionic villi dysmorphic features for the GA and rare specific conditions (e.g., metabolic storage dieseases). EA category encodes 5 embryo phenotypes-based on the embryonic developmental defects; MC category covered microscopic features of decidual arteriopathy and impaired trophoblast invasion like as a feature related in antiphospholipid antibody (aPL)-associated loss. Also, unusual infections, and rare specific conditions such as chronic histiocytic intervillositis (CHI) and fibrin(oid) deposition were categorized under the MC. MF category enclosed mixed histopathological villous dysmorphic features and other chorion-decidual anomalies considering hindering a single unequivocal etiology. Ectopic pregnancy means a pregnancy outside the uterine cavity, which can occur in different localizations. Gestational trophoblastic diseases (GTD) classification were summarized in Table 3. So, GTD were classified as hydatidiform mole (HM), and extravillous trophoblastic (EVT) lesions (e.g., non-neoplastic lesions such as EPS and PSN, and neoplasms such as ChoCa and PSTT).

In order to validate the histopathological diagnosis previously performed, a control group of 223/3,169 (7%) cases with concurrent cytogenetic study were compared. (See Table 4). Genetic studies was conducted by CGC Cytogenetics and Molecular Laboratory, Porto, Portugal. It consisted of conventional cytogenetic techniques by culturing fibroblasts in separate bottles harvested tissue of embryo and chorionic villi samples. Whenever necessary, the exclusion of maternal contamination, from samples of chorionic villi and maternal blood, was performed by amplification (Polymerase Chain Reaction - multiplex PCR) of polymorphic genetic markers. Also, the results of molecular tests for thrombophilia were evaluated.

All the samples enrolled in the present study were unlinked and unidentified from their donors. The study was approved by the Local and Regional Ethical Committees for Medical Research. Due the retrospective nature of the study, the Local Ethical Review Committees of the involved institutions and Minho University Medicine School, Braga, Portugal, approved the work and waived the need for written informed consent.

RESULTS

The pathological criteria considered in each category allows a conclusive diagnosis in 98.2% (3,169/3,228) cases (See Table 1 and Table 2). The category "other" includes 59/3,228 (1.6%) cases of not documented intrauterine pregnancy (NDIUP) and severe degenerative changes related with retention.

Histopathological CSA criteria (Figure 2) concern a gestational sac (GS) and chorionic villi dysmorphic features for the GA (e.g., enlarged scalloped, clubbed, round villi; multifocal and polar trophoblastic proliferation/hyperplasia or hypoplasia; stromal pseudo-inclusion and decreased stromal vessels).[5,14,30]

EA encodes 5 embryo phenotypes, (Figure 3) based on the embryonic developmental defects, since individual organ malformation such as neural tube defects, to growth disorganized embryo (GD). So, GD1, was characterized by an intact GS with no evidence of embryo (Figure 3A). [5,14] GD2, consists of a nodular embryo moreover attached to chorionic plate (Figure 3B).[5,14] GD3, relates to an embryo up to 10 mm long, with caudal and cephalic poles without others recognizable external structures, moreover retinal pigment may be present (Figure 3C-G).[5,14] GD4, consists of an embryo with 3-17 mm long usually with a major distortion of body shape always involving head and generally with a fusion of the chin and chest (Figure 3H-I).[5,14]

Maternal causes (MC) (Figure 4) were diagnosed hystologicaly as impaired trophoblastic invasion, decidual vessels pathology, decidual lymphocyte cell infiltration or fibrinoid deposition.[5,30] Also, unusual infections (Figure 4A); massive perivillous fibrin deposition, or maternal poor perfusion (eg, increase number of tertiary villi, fibrotic villi and decidual arteriopathy) (Figure 4B); chronic histiocytic intervillositis (CHI) (Figure 4C) were included under this category.[5,30] Multifactorial (MF) category covering mixed histopathological chorionic villi pattern between MC and CSA considering hindering a single unequivocal etiology. Hydatidiform moles (HM) (Figure 5) traditionally, have been subdivided into complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM). CHM macroscopically, have abundant tissue with grossly identifiable translucent vesicles (Figure 5A-C). On microscopic examination, the villi, primarily in the terminal villi, are diffusely hydropic with a central acellular space and trophoblast hyperplasia (Figure 5A). Circumferential, trophoblast hyperplasia is universally present and is a requirement for diagnosis (Figure 5B-C).[30] Extravillous trophoblast (EVT) lesions and intraplacental ChoCA are rare with 0.25% and 0.05% of cases respectively.[17,24,40] In our serie ectopic pregnancy (EP) occurrence was 48% (155/3,228) cases.

CSA and EA groups are the most frequent pathological diagnosis with 1,279 (39.6%) cases and 953 (29.5%) cases respectively (Table 1). CSA and EA groups did have a good correlation with genetic results (Table 4). In CSA category, 71% (59/83) cases did had an abnormal karyotype. Trisomy 16 and trisomy 21 were the most prevalent with 16.9% (10/59) cases each, followed by trisomy 22, trisomy 15, monosomy X and triploidy. EA group showed a high incidence of chromosomal abnormalities with 66.2% (51/77) cases, being monosomy X and triploidy the most frequent 13.7% followed by trisomy 16 and then by trisomy 15 and trisomy 21. Structural chromosomal changes was detected in 5.5% (6/110) cases, of CSA and EA groups. MC and MF categories encompass 337 (10.4%) cases and 317 (9.8%) cases respectively. These categories were a good correlation with cytogenetic results (see Table 4). In MC category 82.6% (19/23) cases did had a normal karyotype. Also, molecular tests identified thrombophilic defects in 21/337 cases of MC category. In MF category, 40% (10/25) cases did had a normal karyotype. The incidence of overall HM was 3.8% (122/3,228) cases (see Table 4). HM matched to cytogenetic results, occurs in 13.1% (16/122) cases. Furthermore, the classical pathological study showed PHM and CHM criteria in 12 and 4 cases respectively. Cytogenetic study documented triploidy, triploidy and tetrasomy and tetraploidy in overall PM (Table 4). One case of CHM did had a 46,X,+18 karyotype and another one had a mosaicism (Table 4). EVT lesions and ChoCa occur in 0.25% and 0.05 cases respectively. EP occured in 4,8% (155/3,228) cases, mostly (98.7%) on the Fallopian tube wall and one case on the ovarian surface, and one-other on the abdominal cavity.

DISCUSSION

SA is an event with great impact on the couple who want a child. Also, FTSA are a common and clinically significant problem. Although, there are a few reports addressing the clinical value of routine histopathological examination of FTAp. A competent and systematic approach to macroscopic and microscopic study of FTAp allows a conclusive histopathological diagnosis in majority of the cases .[5-11] However, despite these general findings, about 2-12% of the cases in our serie remains without a specific histopathological feature or an obvious etiology of the miscarriage. Although, an useful information from a competent histopathological study are relate to: (1) confirmation of the presence of an intrauterine pregnancy; (2) identification of previously unsuspected disease process in the mother or embryo; (3) exclusion of a gestational trophoblastic disease, namely in the form of CHM or PHM; (4) identification of conditions with a high probability of recurrence in future pregnancies.[14,16,21,27,28,31,32]

Besides, FTAp histopathology could estimate the GA and the interval of retention after embryonic death.[5,7,11] Also, it is beneficial in protecting obstetrician and gynecologist from medico-legal recrimination. So, a competent examination of FTAp must take into account the confirmation of an intrauterine pregnancy, the validation of ultrasound features and an effective clinical follow-up toward parental orientation and additional complementary tests.[14,16,21,27,28,31-36,38]

A systematic performed histopathological examination of FTAp demonstrate that CSA and EA criteria had a good correlation with genetic tests results.[5-7,21,26] Trisomy 16, 15, 21 and monosomy X were the most prevalent. Several studies indicate that more common trisomy is the trisomy 16, followed by trisomies involving chromosomes 15, 21, 22, and 13.[6,7,21,26] Trisomy occurrence was associated with advanced maternal age, which is a growing problem our-days, seeing that the average of maternal age has increased in most developed countries. Monosomy X is one of the most common chromosome anomaly detected in cytogenetics tests of FTp and occurs in most cases as a result of paternal nondisjunction errors. Structural chromosoamal changes frequency is identical to others studies, in which the presence of structural changes occurs in about 4% of the cases of First-trimester abortion with abnormal karyotype.[6,7,21,26] EA category, showed a high incidence of chromosomal abnormalities when matched the morphological criteria and cytogenetic results. However, the existence of EA with a diploid normal karyotype, may suggest the interference of teratogenic agents (eg, drugs, viruses, alchol, tobacco, hyperthermia) or other lethal disruptive events during normal embryogenesis.[18,21,26,27,28]

Also, the frequency of normal karyotype in MC and MF categories supports and validates the histopathological criteria. Maternal or embryonic nongenetic factors including environmental contexts and teratogens are well-known causes associated with failure of protective mechanisms inherent to normal gestation or severe embryo malformation.[17-19,21,26,28,29] Immunological disorders, thrombophilia and antiphospholipid antibody syndrome, especially in aPL-associated loss have been associated with early miscarriage. Presently available data indicate that a small subgroup of women with recurrent FTSA may show evidence of CHI or massive perivillous fibrin deposition. Both conditions are histopathological diagnosis which may influence future reproductive management. These conditions are moreover related with maternal homeostasis disturbance.[17,18,20,27] Also, it is currently known that celiac disease have an increased risk of FTSA, in fact related to deficience absorption of essential factors to organogenesis, such as iron, folic acid and vitamin K.[17,18,20] Despite that molecular tests identified thrombophilic defects in 21/337 (6.2%) cases. Also maternal age (MA) at the time of

abortion shows a significant effect on the incidence of FTSA moreover associated with SCA and EA, namely growth disorganized (GD) embryo.[10,12,18,19,21,26,28]

An additional minority of FTAp will be due to HM. The incidence of HM in our study was 3.8% (122/3,169), of which 53.3% (65/122) cases were not previously suspected. Pathological evaluation improves the diagnosis of GTD. So, histopathological study has been a major clinical significance because these patients require surveillance for detection of persistent gestational trophoblastic disease and also for the risk for recurrent mole in future pregnancy. Also, a very early complete hydatidiform mole (VECHM) represent a greate challenge in diagnosis, as the pathologic features are more subtle and less developed.[32] PHM features are similar to the CHM but are usually less striking and have an admixture of relatively normal immature villi and irregularly distended and scalloped hydropic villi (Figure 4). Invasive mole is a rare entity composed of trophoblastic cells and molar villi, which invade the uterus and have potential invasion of adjacent structures.[17,24,31-37] In Portugal, the incidence of HM is one in 570 pregnancies (0.18%).[38] However our study document a higher incidence. HM tend to have macroscopic and microscopic characterisitcs features but in difficult cases the use of immunohistochemistry and genetic tests may be useful.[14,28-37] Although both forms of HM include excessive expression of paternally derived genes. But, CHM and PHM are slightly different conditions with different clinical implications. CHM presents an increased risk of developing ChoCa and is generally diploid, while the PHM is mostly triploid. In our serie, the higher incidence of overall HM could reflect the under pathological examination of FTSAp and the difficult diagnosis of VECHM cases.[16,30,33,35,36] In HM group, a concurrent cytogenetic result validates the pathologic diagnosis in overall cases of PHM. Also, EVT lesions are a complex and heterogeous group of lesions, since a benign nonneoplastc lesions (eg, placental site nodule and exaggerated placental site) to a potentialy malignant conditions such as placental site trophoblastic tumor and intraplacental ChoCa. A routine histhopatological examination of all the products of early miscarriage can increase the real incidence of HM, EVT lesions and intraplacental ChoCa and consequently improve maternal management.[32,33-37,40-42]

Ectopic pregnancy can occur in different localizations, mostly in the fallopian tube and rarely in the ovary or in the abdomen.[41] Pelvic inflammatory disease, tubal surgery, oophoritis and in vitro fertilization therapy have been implicated as risk factors, being a HM, a rare complication described.[43]

CONCLUSIONS

A comprehensive histopathological classification of FTAp is a prerequisite for the definition of reproducible embryonic and placental phenotypes. Understanding its clinical significance can serve as a gold standard for additional complementary test and comparative effectiveness trials. Together could guiding the management of individual patients improving maternal and fetal monitoring in future pregnancies.

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Conflict of interest statement

None declared.

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Figure Legends

Figure 1. Algorithm Diagnosis in First-trimester Spontaneous Abortion specimens.
Legend: FTSAs, First-trimester Spontaneous Abortion specimens; FISH, Fluorescent In Situ Hybridization; NTD, Neural Tube Defects; GD, Growth Disorganized; NDIUP, Not Documented Intrauterine Pregnancy; IHC, Immunohistochemistry.

Figure 2. Spontaneous abortion from 6th week GA age concerning pathological features to the category *changes suggesting aneuploidy*. A, Macroscopic features of a shrunken GS, chorion hemorrhage and clots and 47,XX,+22 karyotype. B, Histhopatological section, hydropic avascular villi (V). H&E stain, x100. C, fibrotic villi (V), and disorganized embryonic tissue (E). H&E stain, x100. D, Intact GS photographed under saline, with bulbous swelling villi. E, Hydropic villi with hypoplastic trophoblast and diploid karyotype. H&E stain, x100. F, amniotic epithelium (A) and yolk sac (Y). H&E stain, x100.
Legend: GA, gestational age; GS, gestational sac; H&E, hematoxylin eosin.

Figure 3. Etiologic significance of macroscopic and microscopic features concerning to the *embryonic development anomaly* category. The following detailed pictures, refer an abortion specimens with 7 to 9 weeks GA dated from first day of last menstrual period. A, an Empty GS consistent with GD1 and normal karyotype (46,XY). B and C, histological features of a nodular embryo without any differentiation consistent with GD2 and a 47,XX,+13 and 48,XX,+2,+22 karyotype respectively; amniotic epithelium and villi at the left superior corner. H&E stain, x40. D and E, macroscopic features GS containing a GD3 embryo with 47,XX,+15 and 47,XX,+16 karyotype respectively. F, GS with hyperplastic chorion and GD4 embryo, with a major distortion of body shape (arrow) and 47,XY,+16 karyotype. G, GD4 embryo with major distortion of the body and fusion of the chin to the chest (black arrowhead). H and I, embryo with a parietal encephalocele (white arrowhead) and umbilical cord cyst (double arrowhead) with a normal 46,XX, karyotype. J, embryo with acrania and diploid 46,XX karyotype. K, macroscopic and microscopic normal embryo with diploid karyotype.
Legend: GA, gestational age; GS, gestational sac; GD, growth disorganized; H&E, hematoxylin eosin.

Figure 4. Etiologic significance of macroscopic and microscopic features concerning *maternal cause* category. The following detailed pictures, refers a 10th week's GA specimens dated of first day of last menstrual period. A, macroscopy: hydropic embryo and placental hydropsis; microscopy: marked swelling of the villi and erytroblasts with parvovirus inclusion (inset). H&E stain, x200. B, microscopy: decidual vascular changes with adjacent decidual necrosis. H&E stain, x100. C, macerated embryo with disruption of abdominal wall (iatrogenic); microscopy: massive chronic intervillitis, consisting of histiocytic and lymphocytic infiltrates in maternal space. H&E stain, x100.
Legend: GA, gestational age; H&E, hematoxylin eosin.

Figure 5. Macroscopic and microscopic features of *hydatidiform mole* category. Specimens corresponding to abortions with a GA range between 6th and 10th week dated from first day of last menstrual period. A, B, C, macroscopic features of a HM photographed under saline, note bulbous swelling of villi. Microscopic features: A, hydropic villi intermixed with translucent vesicles with mild trophoblastic hyperplasia consistente with PHM and 69,XXX,+7,+14 karyotype, H&E stain, x40; B, hydropic villi with central cistern and circumferential trophoblastic hyperplasia and absence of fetally-derived tissue in a case of VECHM with a 46,X,+18 karyotype, H&E stain, x100; C, scalloping hydropic villi with central cisterns and circumferential (apolar) trophoblastic hyperplasia in a case of PHM with 69,XXY, karyotype H&E stain, x40.
Legend: GA, gestatonal age; HM, Hydatidiforme mole; PHM, partial hydatidiforme mole; VECHM, very early complete hydatdiforme mole; H&E, hematoxylin eosin.

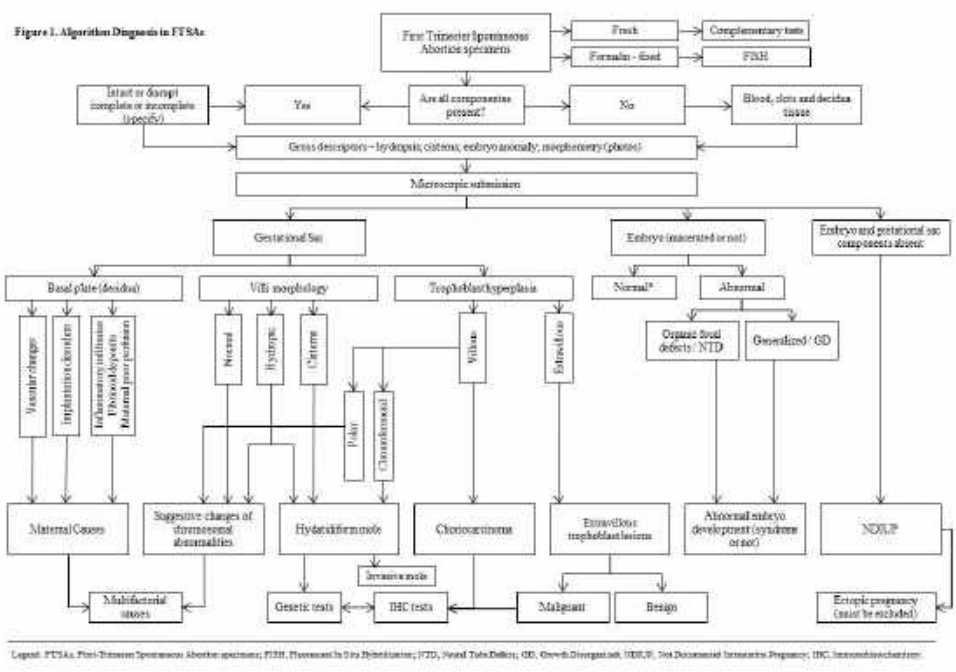

Figure 1. Nogueira R., *et al.*, 2019

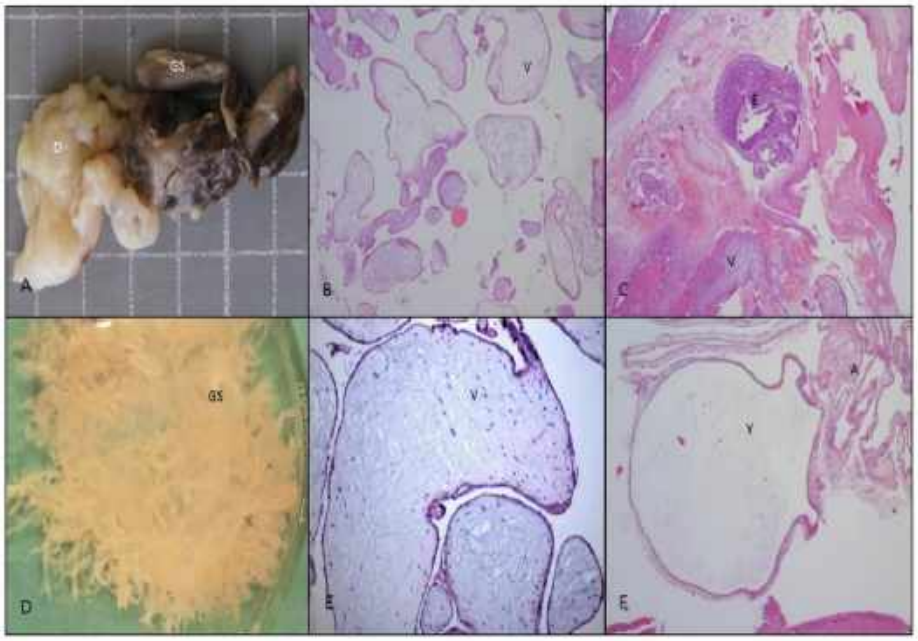

Figure 2. Nogueira R., *et al.*, 2019

Figure 3. Nogueira R., *et al.*, 2019

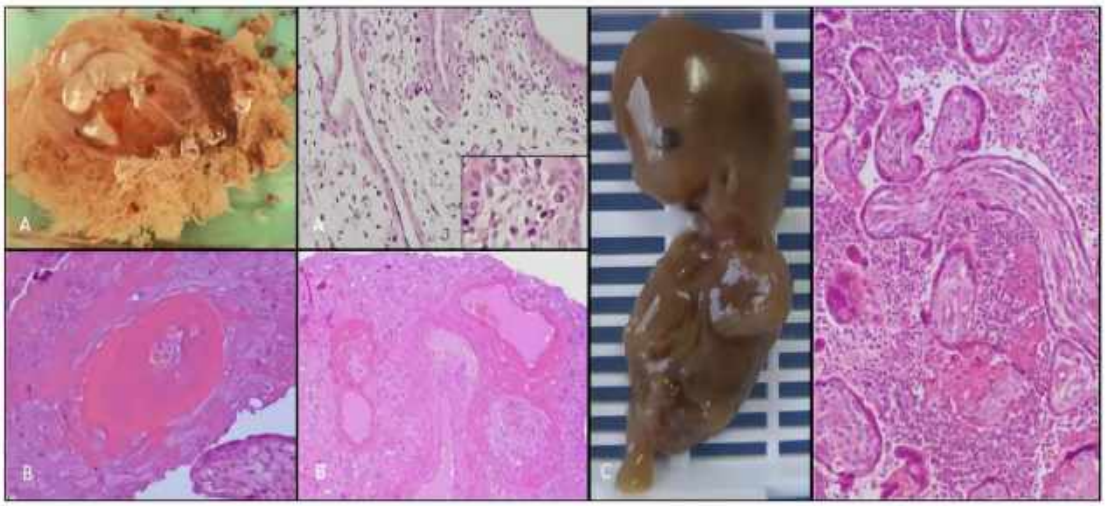

Figure 4. Nogueira R., *et al.*, 2019

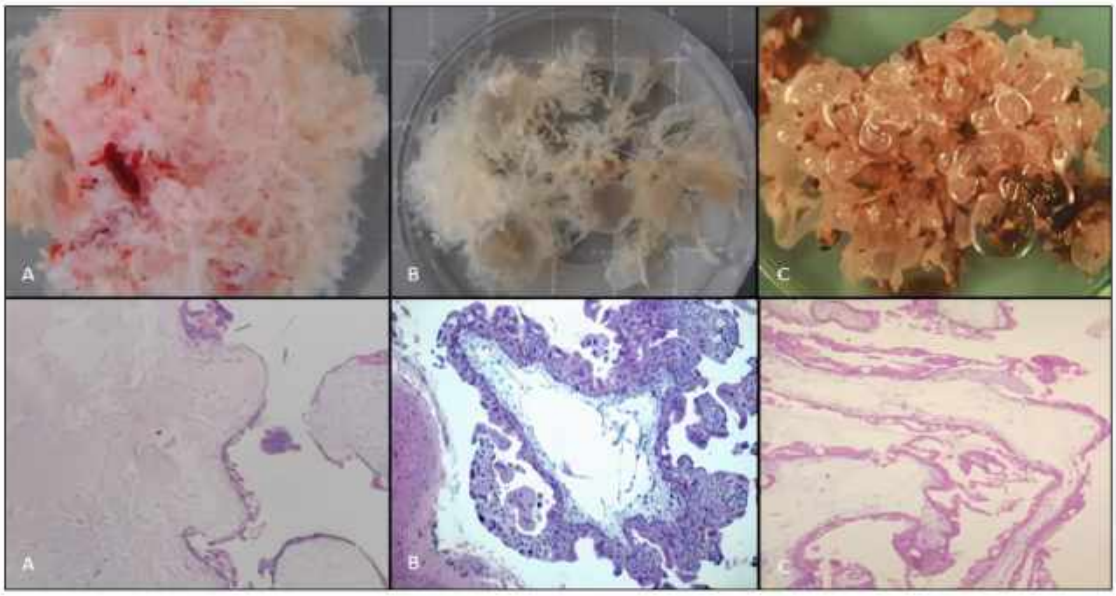

Figure 5. Nogueira R., *et al.*, 2019

Table 1. Distribution of the cases according to the main seven histopathological categories given in absolute value (n) and percentage (%).

	Cases (3,228)	
Classification of First Trimester Abortion Specimens Lesions[a]	***n***	**%**
i. Changes Suggesting Aneuploidy	1,279	39.6
ii. Embryo Anomaly	953	29.5
iii. Maternal Causes[1,2]	337	10.4
iv. Multifactorial Causes	317	9.8
v. Gestational Trophoblastic Disease		
Hydatidiform Mole	122	3.8
Extravillous Trophoblastic Lesions	5	0.25
Choriocarcinoma	1	0.05
vi. Ectopic Pregnancy	155	4.8
vii. Other [a]	59	1.8
Total	3,228	100

[1] molecular thrombophilic defects identified in 21 cases. [2] Cytogenetic study was performed in 23 cases

[a] other (e.g., undocumented intrauterine pregnancy, retention abortion components and degenerative changes associared with intrauterine retention).
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Table 2. First-trimester Abortion Products Pathological Categories

Histopathological Categories of First-trimester Spontaneous Abortion Products [a]

Characteristics [b]	CSA	EA	MC	MF	HM		EP[c]
					Complete	Partial	
Embryo/Fetus	may be present	usually abnormal	may be present	may be present	rarely present	often presente	may be present
Gestational sac histology							
- villous enlargement	mild to moderate	mild to moderate	rare	mild	marked [1]	mild to moderate	may be present
- villous population	range of villi from small to hydropic[2]	range of villi from small to hydropic[2]	admixture of villi, to tertiary villi	admixture of villi from fibrotic to clubbed or round	relatively uniform; hydropic villi	Partly normal, partly hydropic	admixture of villi from fibrotic to clubbed or round
- villous shape	clubbed or round and scalloping	clubbed or round and scalloping	elongated to small	admixture from elongated to clubbed or round and scalloping	usually round	scalloping with inclusions	Variable[c]
- cisterns	usually absent	usually absent	usually absent	usually absent	common[3]	rare to mild	usually absent[c]
- trophoblastic poliferation	rare to mild (focal) and polar	rare to mild (focal) and polar	usually absent; increase syncytial knots	rare to mild (focal); increase syncytial knots	common; apolar,circumferential hyperplasia[4]	mild to moderate (focal); apolar, circumferential hyperplasia	usually absent[c]
- trophoblastic atypia	none or rare	none or rare	none or rare	none or rare	common[5]	Minimal	none or minimal
- fetal blood vessels/nucleate red blood cells	absent or rare[6]	moderate	mild to moderate	mild to moderate	rare usually absent[7]	Common	common
- intervillous inflammatory infiltrates	usually absent	usually absent	may be present[d]	may be present	usually absent	usually absent	none or rare
Plate basal							
- utero-placental vessels lesions	may be present	may be present	usually present	usually present	may be present	may be present	absent
Persistent GTD	no	no	no	no	>20%, may develop ChoCa	<5%, usually not requiring chemotherapy	none or rare

[a] Adapted from Benirschke and Kaufmann's, pathology of the human placenta, Baergen. Springer,2004 ISBN 0-387-2208

CSA, changes suggesting of aneuploidy or metabolic storage disease; EA, embryo anomaly; MC, maternal causes; MF, multifactorial; HM, Hydatidiform Mole; EP, ectopic pregnancy; GTD, gestational trophoblastic disease; ChoCa, choriocarcinoma;

[b] most common presentation, but variation may occur. [c] molar disease may exist. [d] viral inclusion may be identified.

[c] one case with Very Early Complete Hydatidiform Mole (VECHM) included in HM category.

[1] may be mild in VEHCM; [2] namely in hydropic abortion; [3] rare in VECHM; [4] moderate in VECHM; [5] including in VECHM; [6] particularly in hydropic abortion; [7] may be present in VECHM

Table 3. Histopathological classification of Extra Villous Trophoblastic Lesions

	Extra Villous Trophoblastic Lesions [a]				
Characteristics	**PSN**	**EPS**	**PSTT**	**ETT**	**ChoCa**
Macroscopy					
- forms a mass	-	-	+	+	+ -
Histopathology					
- chorionic villi present	-	+	very rare	very rare	-[1]
- fibrinoid	+	+	+	-	-
- hemorrhage	-	+ -	+	+	+ +
- necrosis	-	+ -	+	+ +	+ +
- vascular invasion	-	+	+	+	+
- degenerative changes	-	-	-	-	+
- extra villous trophoblast	+	+	+	+	-
- syncytiotrophoblast	-	+	-[2]	-	+ +
- nuclear pleomorphism	-	-	+ +	-	+ +
- mitotic activity	minimal or absent	minimal or absent	+	+	+
History of previous Mole	-	-	5-8%	5-8%	50%
Metastasis	none	none	occurs in 10-15%	occurs in 10-15%	potential
Prognosis	no sequelae	no sequelae	reserved if malignant	reserved if malignant	>90% responsive to chemo-therapy
Serum β-hCG	normal	appropriate for pregnancy	moderately ↑ in 80%	moderately ↑ in 80%	markedly ↑

[a] Adapted from Benirschke and Kaufmann's, pathology of the human placenta, Baergen. Springer,2004 ISBN 0-387-22089

PSN, placental site nodule; EPS, exaggerated placental site; PSTT, placental site trophoblastic tumor; ETT, epithelioid trophoblastic tumor; ChoCa – choriocarcinoma.; β-hCG, β- human chorionic gonadotrophin

[1] Villi are present only in placental or "in situ" ChoCa.

[2] Multinucleate cells similar to syncytiotrophoblast may be present.

Table 4. Correlation of Histopathological Categories (CSA, EA, MC, MF and HM) and Control Group (genetic results) in absolute values (n).

		Histopathological Classification of FTSAp Lesions					
		CSA	EA	MC[1]	MF	HM	Total
Control Group: Cytogenetic Results	Normal	24	26	19	10	3[a]	82
	Trisomy 2	0	1	0	0	0	1
	Trisomy 4	0	1	0	0	0	1
	Trisomy 5	1	1	0	0	0	2
	Trisomy 7	2	1	0	0	0	3
	Trisomy 8	0	2	0	0	0	2
	Trisomy 9	2	0	0	0	0	2
	Trisomy 10	2	0	0	0	0	2
	Trisomy 13	0	1	0	0	0	1
	Trisomy 14	0	1	1	0	0	2
	Trisomy 15	4	6	0	0	0	10
	Trisomy 16	10	3	0	0	0	13
	Trisomy 17	0	1	0	0	0	1
	Trisomy 18	4	2	0	1	0	7
	Trisomy 20	1	0	0	0	0	1
	Trisomy 21	10	4	0	0	1[b]	15
	Trisomy 22	5	3	0	3	0	11
	Monosomy X	4	7	2	8	0	21
	Triploidy	4	7	0	2	8[b]	21
	Tetraploidy	3	2	0	0	1[b]	5
	Mosaicism	3	1	0	1	1[a]	6
	Structural aberration	1	0	0	0	0	1
	Hypertriploidy	1	0	0	0	0	1
	Additional material of unknown origin	0	1	0	0	0	1
	Monosomy 21	0	1	0	0	0	1
	Monosomy X + Trisomy 18	0	0	0	0	1[a]	1
	Robertsonian translocation	1	3	1	0	0	5
	Triploidy + two additional Chr. 7 and one additional Chr. 9	0	0	0	0	1[b]	1
	Triploidy + Tetrasomy 7 and 14	0	0	0	0	1[b]	1
	Trisomy 2 + Trisomy 22	0	1	0	0	0	1
	Trisomy 7 + Trisomy 18	1	0	0	0	0	1
	Trisomy X + Trisomy 22	0	1	0	0	0	1
	Total	83	77	23	25	16	223

Legend: FTSAp, First-trimester spontaneous abortion products; CSA, changes suggesting aneuploidy; EA, Embryo anomaly; MC, maternal causes; MF, multifactorial; HM, hydatidiform mole; PHM, partial hydatidiform mole; CHM, complete hydatidiform mole; VECHM, very-early complete hydatidiform mole; Chr. Chromosome.

[1] molecular thrombophilic defects identified in 21/337 cases of MC category.

[a] all CHM, 2 VECHM;. [b] all PM.

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"Quality is not an act, it is a habit."

Aristotle

CHAPTER 4

GENERAL DISCUSSION

4.1. THE IMPACT OF SPONTANEOUS ABORTION

In Portugal, the main birth and fertility indicators show that after two years of recovery, birth numbers have fallen in 2017 and the ongoing postponement of childbearing persists. Although, the crude birth rate remained, 8.4 live birth per 1,000 inhabitants. The mean age of women at first pregnancy remained at 29.6 years of age, and the mean age of women at childbirth rose from 31.1 to 31.2 years of age. (INE, IP, Lisboa, Portugal 2018).

As recorded by the statistics Portugal Register, in 2017 there were 86,154 live births from women living in Portugal, 972 less than registered in 2016, corresponding to a decrease of 1.1%. (INE, IP, Lisboa, Portugal 2018).

Up to 60% of all conceptions will be lost, most of them not being noticed by the woman, so-called "occult abortion". (Roberts CJ, *et al.*, 1975; Miller JF, *et al.*, 1980; Edmonds DK, *et al.*, 1982; Rolfe BE, 1982; Whittaker PG, *et al.*, 1983; Kline J, *et al.*, 1985; Wilcox AJ, *et al.*, 1988) It is generally known, that 1 out 6 clinically recognized pregnancies end in spontaneous abortion (SA) (Kline J, *et al.*, 1985; Wilcox AJ, *et al.*, 1988; Harlap S, *et al.*, 1980; Miller JF, *et al.*, 1980). Similarly, it is estimated that about 12-15% of all clinically recognized pregnancies result in miscarriage most of them (60-70%) taking place before 12th weeks of gestation, commonly between 6th and 12th weeks.

Accordingly, in Portugal nearly 10,338 to 12,923 SA occurs every year resulting in a common phenomenon. Also, the infant mortality until 1 year of age was 2.7 deaths per thousand live births. There are no statistical data about fetal death namely early and late fetal death.

The divisions in early and late abortion almost equals the definitions of SA. In Portugal, First-trimester spontaneous abortion (FTSA) is defined as loss before the end of the 11w+6d of pregnancy and late abortion as occurring at Second and Third trimester pregnancy. This makes sense as the causes and morphology are different for both periods. (Lawn JE, *et al.*, 2011; McPherson E, 2016; Lamont K, *et al.*, 2015).

Pregnancy is a very complicated "process" where the participation of mother, embryo/fetus and gestational sac/placenta are essential. Problems in one of these "compartments" might lead to disturbances in the others "compartments". However frequently, the primary site of deterioration cannot be discerned.

The cause of early miscarriage can be related to several factors, knowing that there are certain factors and uncertain factors dependent on gestational-age (GA) and related to maternal (parental), embryonic or placental conditions.

So, as other authors, in this thesis I attest, the human placenta is a multitalented, but still a mysterious shared organ. (Guttmacher AE, *et al.*, 2015; Burton G, *et al.*, 2015; Nelson DM, 2015). Solo, it performs at intrauterine life functions that, after birth, will require numerous "vital" organs that will include lungs, liver and kidneys. (Guttmacher AE, *et al.*, 2015) Recognizing the vital importance of the placenta, there are more and more focused studies on it. (Guttmacher AE, *et al.*, 2015).

The placental features may explain some fetal anomalies that could be relevant to understanding the miscarriage and may also be crucial to monitoring the newborn in early neonatal care. The characterization of pathological placental processes and their randomization allow the advance in health care, as well as, taking the appropriate preventive procedures to specific maternal, placental and fetal conditions.

Pathologic categories in First-trimester spontaneous abortion specimens and Second and Third trimester singleton (and multiple) placentas, permit monitoring the couple and reproductive planning. This can provide data to evaluate fetal risks including of neurologic effect on the child and its recurrence, and could minimize the maternal risk diseases in future pregnancy also. Moreover, any additional information is relevant to studies of fetal and placental growth and development, and to research involved in the so-called fetal origins of adult disease.

4.2. BRIEF SUMMARY OF RESULTS

Percentile curves for placental and embryo-fetal parameters across gestational-age (GA) between 5th and 41st weeks were produced.

First-trimester Products

Gestational sac (GS), umbilical cord (UC) and Embryo cranio to rump length (CRL) percentile curves between 5th and 11th weeks were generated.

GS diameter percentiles 5, 25, 50 75 and 95 were calculated according to the gestational-age (GA) and at each 1 week interval it increases an average or 3.7907 mm. UC length percentiles 5, 25, 50, 75 and 95 were also calculated and at each 1 week interval it increases an average of 1.161 mm. Embryo-CRL estimated mean±SD (standard deviation) values were: GA 6: 5.3±2.3 mm; GA 7: 9.4±4.8 mm; GA 8: 13.7±8.2 mm; GA 9: 20.8±9.1 mm; GA 10: 22.6±13.4 mm; and GA 11: 29. 4±12.9 mm; GA 12: 52 mm.

Second and Third Trimester Placenta

In a cohort of singleton placentas, placental weight (PW), birth weight (BW), placental weight ratio (PW-R) and birth-placental weight ratio (BPW-R) were analyzed to produce percentile curves. Considering the inclusion criteria 1,951 singleton placentas (form a sample of 7,321 placental reports) between 12th and 41st weeks of gestation were selected. The percentiles curves for PW, fetal or birthweight (BW) and BPW-R and PW-R were based on the same observations. Mean, standard deviation (SD), median, minimum and maximum for maternal, placental and fetal quantitative and qualitative variables are summarized in the second manuscript of the Chapter 2. With the exception for PW for 27 weeks ($p = .033$), and BW for 16 weeks ($p = .021$) and 40 weeks ($p = .018$), in our study there were no statistically significant differences between male and female fetuses ($p > .05$) unlike previous studies. So, gender freely percentile curves for PW, PW-R, BPW-R and PW-R were produced between 12th and 41st weeks of GA.

4.3. STUDY IMPLICATIONS, STRENGTHS AND LIMITATIONS

Placental characteristics and morphology has been reported to influences placental weight (PW), placental weight ratio (PW-R), birth weight (BW) and birth weight placental weight ratio (BPW-R) and placental thickness (PT). (Little WA, 1960; Aherne W, 1966; Armitage P, *et al.*, 1967; Molteni RA, *et al.*, 1978; Lurie S, *et al.*, 1999; Wallace JM, *et al.*, 2013; Salafaia CM, *et al.*, 2010; Yampolsky, *et al.*, 2009; Salafaia CM, *et al.* 2008; Salafaia CM, *et al.*, 2007).

Although, placental size has been described as an independent predictor of BW. (Asgharnia M, *et al.*, 2008; Promboon S, *et al.*, 1983; Naeye RL, 1987). Previous studies have been postulated that placental hypertrophy and fetal growth restriction (FGR) are an adaptation to maintain placental function in pregnant woman with several conditions (e.g., malnutrition, gestational diabetes, hypertension and others endocrine or metabolic maternal disorders). (Nelson DM, 2015; Yu KM, 1992; Myatt L, 2002).

This heightened interest in PW and its relationship with BW has related to a crucial role of the placenta as a potential factor of maternal diseases and even long-term impact on the well-being of the mother. (Amaral LM, *et al.*, 2015; DeRoo L, *et al.*, 2016; Barker DJ, *et al.*, 2012; Nelson DM, 2015; Heinonen S, *et al.*, 2001; Lo Y-F, *et al.*, 2002; Naeye RL, 1987; Barker DJ, *et al.*, 1997; Barker DJ, *et al.* al., 1993; Myatt L, 2002; Dombrowski MP, *et al.*, 1994).

Also, fetal or neonatal morbidity and mortality, congenital fetal defects (see Appendices 2 and 3) and longer-term impact on the well-being of the child lifelong health are related with PW and size. (Risnes KR, *et al.*, 2009; Hasegawa J, *et al.*, 2011; Barker DJ, *et al.*, 2013; Barker DJ, *et al.* 2012; Barker DJ *et al.*, 2011; Wen X, *et al.*, 2011; Heinonen S, *et al.*, 2001).

Although, several studies has been shown that maternal diseases (e.g., gestational diabetes, severe anemia and hypertension, preeclampsia) and fetal diseases (e.g., hydrops, FGR and small for gestational-age) influence PW and fetal weight, PW-R and BPW-R. (Heinonen S, *et al.*, 2001; Hindmarsh PC, *et al.*, 2000; Thame M, *et al.*, 2000; Myatt L, 2002; Lao TT, *et al.*, 1999).

Thus, the selection of singleton placentas sample for biometric (e.g., weight and size) assessment with a view to the creation of percentile curves requires the exclusion of maternal or/and fetal disorders (e.g., hypertension, diabetes, FGR, SGA, LGA) related with gross placental lesions that may be directly implicated in the deviation of those parameters. Also some placental conditions such as large tumors and hydrops must be exclude.

Percentile curves for BW are relatively common in most countries but percentile curves for PW, and PW-R and BPW-R still rare. (Wallace JM, *et al.*, 2013; Thompson JMD, *et al.*, 2007; Almog B, *et al.*, 2011). However, recently some countries have required the placental weight be notified as a part of the medical birth registry. (Thompson JMD, *et al.*, 2007).

The present Thesis established a gestational-age-specific reference values for PW, BW, PW-R and BPW-R for singleton gestation between 5 and 41 weeks of gestational-age (GA) in a single institution over the 4-year period (see Chapter 2).

This is the first time that Portuguese population-based percentile curves have been produced for PW and hence for the PW-R and BPW-R. The percentile curves have been made possibly by including these important items of data in medical registry request and pathological reports of placental examination and fetal autopsy.

The PW, PW-R and BPW-R percentile curves achieved across GA (see Chapter 2) may act as a reference curves for Portuguese population. Those should be used with caution elsewhere knowing that BW often differ between countries and multiethnic communities. So, percentile curves should be population specific when possible.

Perhaps more important than BW and PW percentile curves alone are the PW-R and BPW-R percentile curves (see Chapter 2).

PW-R is defined as the placental weight over the birth weight and BPW-R is defined as the birth weight over the placental weight. The PW-R and BPW-R are a common indication for the balance

between fetal and placental growth. Probably, these ratios would not differ in any significant way between populations, unless there is a difference in placental function between different populations, which under normal conditions would seem less likely. So, for the meantime, these percentile curves may act as a reference for other populations as well.

Still, for the most populations in which BW percentile curves are available, it would be possible to derive suitable PW percentile curves from the ratio curves presented in this thesis.

Nevertheless, in other populations, I would recommend caution using, either placental weight and ratio curves and particularly at the extremes of the gestational range.

Because of growing evidence for a correlation of PW with chronic diseases in late-life, we suggest attention and correct examination of placenta and recording all of the observation in patients' files as an important evidence for future.

A competent placental examination is an important tool concerning the principal goal of the recently created "Human Placenta Project" also. (Guttmacher AE, *et al.*, 2014).

The selection of placentas macroscopically normal or near-normal, drastically decreased the sample for the purpose of constructing percentile curves for PW, PPW-R. Also, the lack of detailed clinical information about BW, particularly in cases of live born contributed to the decrease in the number of cases gone from the sample.

Also, knowing that the PW is dependent on the amount of intervillous blood and blood in fetal vessel and formalin fixation (see Chapter 1) it should be preferably achieved after proper washing of the placenta in current water and fixating in formaldehyde for a period of 24 hours, and after remoting the umbilical cord and capsular membranes. (Baergen RN, *et al.*, 2001; Bernischke K *et al.*, 2006).

4.4. FINAL CONCLUSIONS

The Thesis project has generated gestational-age-specific percentile curves between 5th and 41st week of GA in singleton gestation for gestational sac and placental biometry's and (embryo) fetal and BW also. Equally Percentile curves for PW-R and BPW-R were achieved.

I believe that the information provided in the thesis will be useful in various ways to medical community, in particular for obstetrics and general clinicians. It is particularly relevant to researchers with interest in embryo-fetal and placental development and to research related with so-called adult diseases with fetal origin.

In our-days the low birth rate and the advanced maternal age should lead us to bet on improvement of placental, fetal and infant health. The percentile curves show how the PW-R and BPW-R changes across gestation. Also, the overall curves offer populations standards that may have particular interest in fetal outcome.

Knowing that placental size is an independent predictor of BW, PW, PW-R and BPW-R. These may be crucial in fetal follow-up and may be an important predictor of fetal diseases and long-term childhood and maternal diseases. Thus, the assessment of the placental growth should be as early as possible and should begin in early pregnancy.

A potential interest of the data thesis could imply an upgrade of the clinical guidelines in obstetrics considering that, the Portuguese Society of Obstetrics and Gynecology has clinical guidelines which not include instructions for the weighing of the placenta registry.

These are all issues that modern clinician scientist cannot ignore, especially if we are to move to an era of precision medicine with the potential for anticipatory approaches long before disease is clinically apparent.

This work may be important to explore the challenges of the Portuguese pregnant woman in a future taking into account the placental factors that increase these challenges, and the contribution that research can make anticipating the beginning of them. We must recognize the importance of a healthy start to life for future results.

4.5. FUTURE REFLEXING

For many, the term personalized medicine is related to the uniqueness of an individual's genetic code. However, considering the health of the individual as a whole, it is the non-genomic side of personalized medicine that helps us reorganize the term *art of medicine*. This integrates the objective synthesis, which can be improved by computer tools, and the subjective global view requirement of a conscious medical researcher who interprets heterogeneous and often complex processes. Tomorrow's clinical scientist is likely to be at the forefront of this philosophy, combining the science and *art of medicine* with genuinely shared decisions to take medical practice to new heights.

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APPENDICES

APPENDIX 1

Cliniclal Research & Bioethics

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Short Communication Open acess Journal

Macroscopic and Histopathological Study of the Placenta - An Essential Resource in Litigation Processes

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Abstract

The pathological study of the placenta is of upmost importance in cases of unexplained fetal/perinatal loss and often these carry litigation implications. Integrating pathological findings and the underlying pathophysiological processes, leading to placental lesions, is fundamental for the evaluation of poor fetal and perinatal outcomes and to distinguish from cases of true negligence.

Keywords: Placenta; Litigation process; Pathology study; Histopathology

Overall Perspective

Understanding the placental function has been crucial for a clinical and well-founded interpretation of pathological alterations associated with poor obstetric outcomes. The anatomopathological study of the placenta allows the investigation of unforeseen cases of fetal/perinatal loss, while further developing our knowledge of some neonatal diseases. The significance of this study is even greater if one considers that about 3/1,000 children will suffer of some sort of cerebral palsy [1-4]. The increase of litigation processes towards hospitals and obstetricians is a reality and in most cases, the litigant part claims negligence. Setting aside, well documented cases of true negligence, one must consciously and responsibly select cases with pathology associated with a poor obstetric outcome. For this purpose, the study of the placental is fundamental [5]. It is important to note that, the aim of this article is not to describe any epidemiological study of specific cases of placental disorders but, to emphasize that the study of the placenta may reveal undiagnosed or unsuspected pathologies in cases of poor obstetric outcomes that explains and justifies fetal/perinatal loss.

Placental pathological features and adverse fetal outcome

Implantation anomalies can lead to velamentous cord insertion with or without vasa previa, to placental accrete and/or to placenta previa and it is well documented its relationship with neurological damage and increased risk of fetal loss [6,7].

Placental infarction is a pathological condition of unknown etiology with distinctive features and has been associated with fetal intrauterine growth retardation (IUGR), microcephaly and neurological complications [1,8].

Decidual arteriopathy is a type of injury diagnosed only histologically and usually occurs as result of maternal circulatory disorders, such as pre-eclampsia, hypertension, antiphospholipidic syndrome and thrombophilia. It is also associated with placental poor perfusion and low weight, leading to IUGR [6,9]. The period of time that, placental lesions take to evolve and extend, and how they become installed are determining factors for fetal complications. The most common example is the abrupt placenta, a serious condition in which, the time period taken to develop a retroplacental hematoma is crucial for the macroscopic interpretation of the lesion and is critical to understand fetal complications. When fetal death does not occur, neurological damage is usually frequent [2,3]. Similarly, placental infarctions have consequent fetal complications according to the time of occurrence, location, extension and its progression. Massive perivillous fibrin deposition is the main diagnostic features of maternal floor infarction, a placental lesion reported to be as high as 1 of 200 placentas. The cause is unknown but, congenital infection, immune-mediated rejection and abnormal extravillous trophoblastic proliferation have been suggested etiologies in part because of its recurrent nature.

Fetal vessel thrombosis is a type of lesion singularly diagnosed with histology and greatly related to prematurity and increased incidence on fetal thrombosis and neonatal neurological complications [4,8,10].

Infections are common and highly contagious situations, with infra-clinical rates in 10% to 15%. It may reach the placenta and fetus in several ways: by ascension from tract vaginal infections, by hematogenous transmission from maternal blood by direct introduction via amniocentesis, chorionic villus sampling and other invasive diagnostic procedure or by direct extensions from infection in the endometrium. It is well known an association with fetal loss and cerebral palsy [7]. Hematogenous infection can result in fetal loss due to anemia following *Parvovirus B19* infection or lead to severe fetal disease due to toxoplasmosis and/or cytomelovirus (CMV) infection [11,12]. Definitive diagnosis of an infectious disease can be established by histological observation of a characteristic inflammatory infiltrate

and of pathognomonic viral inclusions associated with CMV and parvovirus infection. When, morphological criteria are not fully present, the study should be completed with immunohistochemistry or other laboratory techniques, such as molecular analysis. The ascending/adjacent infection is a subclinical condition that is present in about 20% of cases. Of these, histology analysis allows the diagnosis of acute/subacute chorioamnionitis and should always be classified according to the stage, degree and as possible with dating time evolution by the presence of hemosiderin. Chronic chorioamnionitis is a medical condition with pathogenesis not yet determined and diagnosed only with histological examination. This condition has been demonstrated to be associated with maternal hypertension, diabetes, IUGR, oligohydramnios, hydrops fetalis and poor perinatal evolution [13,14].

Massive chronic intervillositis is a medical condition also singularly diagnosed with histology and is associated with IUGR and fetal death and commonly described in recurrent abortions. In 70% of cases, this condition is present simultaneously with chronic villitis and is related to prematurity and neurological injury [15]. Chronic villitis occurs in 5% to 10% of all placentas and higher percentage is well documented when placenta of complicated pregnancies are studied. At times infectious etiology is apparent however, in majority of cases no specific etiology is elicited and the term villitis of unknown etiology (VUE) is used. There are no doubts that VUE is a recurrent lesion with occurrence rates of 10% to 25%. Two theories are postulated with respect to the etiology of VUE. One suggest that is an infectious disease caused by a yet unrecognized agent and other suggest an immune reaction supported by the preferential localization of these lesion and type of inflammatory cells. VUE is also associated with prematurity, abnormal neurologic development and intra-uterine fetal demise [16].

The etiology of "ADAM complex" (amniotic deformities, adhesions and mutilations) is widely discussed as its incidence is difficult to document and studies suggest a rate of 1/2,500 to 1/10,000 newborns. Amnion rupture will cause, by mechanical way, amniotic bands or sheets, leading to fetal amputations, anomalies or even demise. The amnion rupture etiology is unknown however, it is important to note that this is usually nonrecurring but the fetal pathological condition is presented with a varied pattern related with the time of occurrence and can result in severe malformations if it occurs prematurely, mimicking trisomy 13 phenotype, or minor malformations sometimes not prenatally detected.

On the other hand, the color change in the placenta has a distinct pathogenesis. About 20% of newborns present a green placenta related to meconium and it is believed that fetal distress is due to vasoconstrictor effect, inducing vascular muscle cell necrosis and compromising the return of the venous oxygenated placental blood [17,18]. In premature newborns, the same color can translate in hemosiderin deposits associated with retromembranous hematoma, with risk of fetal-maternal transfusion, fetal hemolysis and thrombocytopenia [19]. In turn, the alteration in parenchyma color reflects the hemoglobin content of the villi and, when pale, usually translates in fetal hydrops/anemia, with heterogeneous etiology and regularly in neurological damage.

Villi morphology when histologically evaluated is correlated with gestational age and deviation from normal development is usually classified as: (i) maturation arrest/delay, reflecting maternal or fetal pathology and (ii) advanced maturity, reflecting poor perfusion and low weight with fetal/neonatal hypoxia and death[15,20]. When fetal death occurs, determining time of death *in utero* is always important and is based in macroscopic and histological changes of the placenta and fetus [21,22].

Chorangiosis is an alteration that results from an abnormal proliferation and capillary neovascularization process associated with prolonged fetal hypoxia and even though the significance and integration into the fetal-placental unit is not completely understood, recent studies have shown its correlation with perinatal complications [23].

Erythroblastosis usually correlates with hypoxia, infections, bleeding and hemolytic anemia and it can be associated with genetic or epigenetic disorders, being also described as a secondary effect of trauma [19,24].

Fetal injuries in cases of mesenchymal dysplasia are well reported and it is known that about 1-2% of this change, often unsuspected, is associated with placental mosaicism [25].

The intrinsic placental lesions correspond mostly to primary tumors, where chorangioma is the most frequent and have an increased risk of fetal morbidity/mortality. Gestational trophoblastic diseases are not true neoplasm. However, they are associated with an increased risk for the development of neoplasm, specifically choriocarcinoma. Other gestational trophoblastic disease like placental site nodule and exaggerated placental site, are not grossly identifiable, unlike placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT) are generally evident as infiltrative masses. Usually these situations do not pose any problem in litigation process. Chorangioma and gestational trophoblastic disease require an appropriate clinical follow-up due to the risk of recurrence and malignancy, or even more aggressive disease like can occurs in PSTT and ETT.

In twin pregnancies, in order to understand the origin of fetal lesions and repercussions, it is essential that the physician provides adequate a referencing of fetal umbilical cord. It is the pathologist's responsibility to document: (i) the relationship between membranes/division septum and their correlation with the chorionic/vascular equator, and chorionicity; (ii) the presence of vascular anastomosis (VA) with or without criteria for twin-to-twin transfusion syndrome (TTTS) and (iii) the relationship and insertion type of umbilical cords. Both cord's pathology and pathology related to the presence of VA are well documented and understood. Secondary vasculogenic organ damage and destructive lesions can occur in the survivor after fetal death of one twin, particularly in monochorionic twins, while polyhydramnios and oligohydramnios are most frequently found in chronic TTTS and yet justify the discrepancy of fetal growth [25].

What type of placentas should one select for the anatomopathological study?

Selecting a cohort of placentas to study it is not a consensual matter and may be based on specific clinical criteria, consist in macroscopic examination of all placentas and microscopic study of specific cases or be restricted to cases of fetal/perinatal complications with/without diagnosed or suspected maternal problems [26,27].

It is essential that the selection of placentas is accompanied by the complete clinical information for the diagnostic to be integrated and useful. The obstetrician should gather/provide all relevant clinical information and a list of issues to clarify; while the pathologist is responsible for studying the placenta to establish a diagnosis that

integrates pathological findings with clinical implications [27-30]. The pathological diagnosis should be integrated considering: (i) maternal age and invasive diagnostic and therapeutic procedures, including medically assisted reproduction techniques; (ii) gestational age and maturation-related deviations and (iii) traumatic injuries, including road traffic accidents, which many times present clinicopathological features similar to other conditions such as abrupt placenta, infarctions, hemorrhagic villitis and/or intra-amniotic hemorrhage [6,27,31].

Conclusion

The development of standard guidelines and multidisciplinary clinical consensus would allow the establishment of an accurate protocol and reproductive methodologies that would assist in estimating adequate criteria for subsequent actions. Table 1 reports some situations with clinical indication for the anatomopathological study of the placenta [32] (Table 1).

Maternal Diabetes	Fetal/Neonatal	Placenta, membranes and umbilical cords
Pregnancy-induced or chronic hypertension	Fetal or neonatal death	Infarctions, including infarction of the placental bed
Premature Rupture of Membranes	Twin pregnancy	Placental abruption
Preterm birth (<36 weeks)	Prematurity	Vasa previa
Post-term birth (>42 weeks)	Intrauterine growth retardation (IUGR)	Placenta previa
Poor obstetric history	Hydrops	Any abnormal appearance of the umbilical cord membranes or of the placenta
Unexplained fever or infection	Congenital anomalies	
Oligohydramnios	Fetal/neonatal erythroblastosis	
History of drug use	Not reassuring fetal status / transfer to neonatal care unit	
	Ominous heart rate	
	Meconium discharge	
	Low Apgar score (<5 at 1' and <7 at 5')	

Table 1: Indications for anatomopathological study of the placenta.

In litigation processes, the pathologist is frequently called upon and questioned to clarify specific pathological processes and their evolution. The progress of infectious, thrombotic/ischemic events and the establishment of fetal time of death are questions frequently requested. These issues are sometimes challenging to assess due to the coexistence of competing injuries, requiring a confident understanding of its pathogenesis and implications. A cautious pathologist should minimize possible errors, always integrating the complexity of processes, thus avoiding an unfounded opinion that may be disputed.

It is essential to establish a good communication conduct between obstetrician and pathologist for the production of a clinically integrated and consistent report.

Keypoints

The progress on the knowledge and understanding placenta's function and its associated pathophysiological processes has allowed the placenta to become a reflection of intrauterine life.

The placenta's anatomopathological study is fundamental for the integration and comprehension of poor obstetric outcomes and plays an important role in litigation processes.

A functionally and macroscopically normal placenta can coexist with an adverse intrauterine environment and be the cause of fetal loss.

The anatomopathological diagnosis of the placenta should integrate lesions and its progression with consequent clinical implications.

Conflicts of Interest

The authors declare no conflict of interest.

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APPENDIX 2

Clinics in Surgery

Research Article
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Morphological Placental Portraits in Congenital Diaphragmatic Defects: A Pathological Study Approach

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Abstract

Introduction: The pathogenesis of Congenital Diaphragmatic Defects (CDD) is not clear. Placental factors have been implicated in the pathogenesis of congenital anomalies.

Objective: To assess the placental measurements on CDD and to compare with a group of fetuses without CDD.

Material and Methods: In this retrospective cross-study, 30 placentas of fetuses with CDD sent to Embryo fetal Pathology Laboratory, Centro de Genética Clínica (CGC), Porto, was evaluated. To compare the placental parameters in CDD group, 71 placentas from fetuses without CDD, matched by Gestational Age (GA) were selected.

Conclusion: Placental size and umbilical cord length and diameter have an impact on CDD.

Keywords: Congenital diaphragmatic defects; Diaphragmatic agenesis; Diaphragmatic eventration; Placenta size; Umbilical cord length; Umbilical cord diameter

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Introduction

Congenital Diaphragmatic Defects (CDD) are rare anomalies, which includes a spectrum of defects such as Diaphragmatic Eventration (DE) and Diaphragmatic Hernia (DH). Besides the existence of a defect in the diaphragm, the abdominal contents protrude in to the thoracic cavity [1,2]. Development of the diaphragm starts during the 4th week of gestation and is completed by the 8th week. However, in the case of fetuses with diaphragmatic defects, the diaphragm doesn't close completely during this period [3,4]. Congenital Diaphragmatic Hernia (CDH) is a developmental anomaly, with a mean incidence of 1:2.500 births [1,2]. It is considered as the major life-threatening cause of respiratory failure in newborns. Therefore, prenatal and postnatal clinical management remains a challenge [1-4]. About 5% of CDD cases represent Diaphragmatic Eventration (DE), a condition resulting in a reduced muscle thickness that allows tenting of the diaphragm in to the thoracic cavity. Usually, it is characterized by a diaphragmatic defect that allows intra thoracic herniation of abdominal organs and consequently lung hypoplasia, or lung alveoli and pulmonary vessels deviations. At birth, this leads to respiratory insufficiency and persistent pulmonary hypertension [1-5].

CDH more over is associated with diaphragmatic agenesis or large diaphragmatic muscle defect. About 85% of cases of CDH area left-sided posterolateral location through foramen of Bochdalek and less than 2% take place in other locations [2,6]. Clinically classified as either isolated, syndromic or associated with other anomalies [2]. Infact, the CDH prognosis depend on the presence of other associated fetal anomalies including central nervous system, gastrointestinal tract, skeletal system, genitor urinary and heart defects [2,3,6,7].

The processes involved in CDH pathogenesis are still poorly understood [7]. Some studies suggest that these processes are determined by abnormal gene expression that recapitulates important event sin embryonic lung and pleuroperitoneal membrane development [7-9]. A few familiar cases described suggest a possible autosomal recessive transmission in this pathogenesis [7]. Aneuploidy, usually trisomy 21 or 18, occurs in 4% of the cases of CDH and many CDH

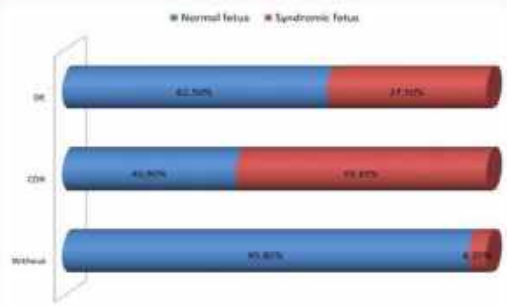

Figure 1: Distribution of fetal cases according to presence or absence of congenital diaphragmatic defects and fetal syndrome. Congenital Diaphragmatic Hernia (CDH). Diaphragmatic Eventration (DE).

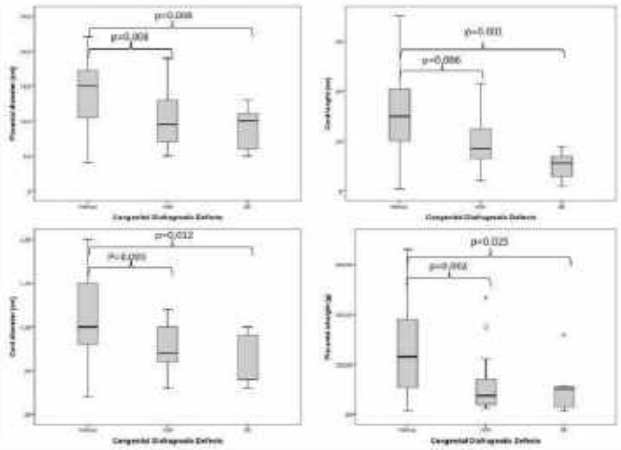

Figure 2: Quantitative parameters distribution according to the group and significance.

may be part of a syndrome, such as Beckwith-Wiedemann, Fryns syndrome, Ivemark or Goltz syndrome [6,7]. Other studies suggest that mesenchymal dysplasia, hypoxic embryonic and placental environments are involved also [8-10].

CDD can be accurately diagnosed during the second trimester routine ultrasound examination [1,6]. The optimal antenatal risk stratification of CDD is necessary for earlier prenatal counseling, personalized prognosis and appropriate perinatal and postnatal management [2,4,5,7].

CDH is a malformation which arises during the organogenesis period that affects to the development of pleuroperitoneal membrane and consequently thoracic abdominal wall. So, it is possible that umbilical cord maldevelopment can be a biologically plausible hypothesis involved in CDD [7,11-14].

Hence, the aim of this study is to investigate placental features in CDD and predict a potential target in early prenatal diagnosis.

Materials and Methods

We carried out are trospective pathological study in CGC Genetics embryofetal pathology laboratory between January 01, 2011 and December 31, 2016 in order to select placental pathological reported related with a fetal diaphragmatic defects cases. In 2,903 fetal/perinatal autopsy reports, we identified 30 CDD cases (1.03%) which 22 cases correspond a CDH and 8 cases a DE. Gestational age ranged from the 11th and the 40th week. Seventy-one placentas (1.6%), from fetus without CDD, matched by GA were selected from a sample of 4,492 placentas cases in the same period. Two Edwards's syndrome without CDD and one case of cardiac anomaly were included in group without CDD for comparison with the similar cases of CDD group.

Statistical analysis of the data was done using the statistical software IBM® SPSS® Statistics version 24.0. Given the nature of the variables involved, we opted for the use of statistical tools most appropriate to the measurement scales used. Therefore, in the descriptive study of the data-qualitative and quantitative variables (bar and pie charts, frequency tables, mean, median, standard deviations, minimum, maximum and box plot graphs) were used. In the analytical study of the data as relationship between two variables, the chi-square test (qualitative variables) was used. In the comparative study to evaluate differences between groups, one way ANOVA techniques was used

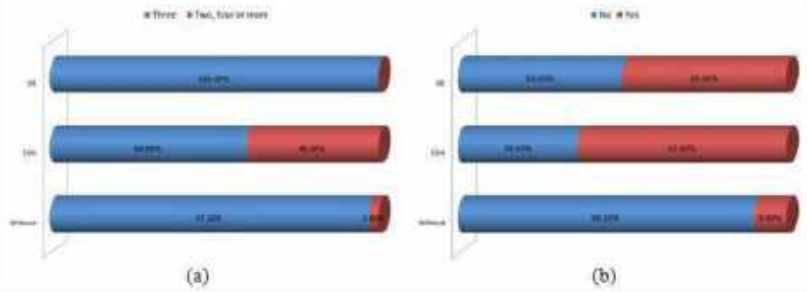

Figure 3: Significant association results of congenital diaphragmatic defects: Congenital Diaphragmatic Hernia (CDH) and Diaphragmatic Eventration (DE) vs (a) Umbilical vessels and (b) Other defects.

when the variables presented normal behavioral their equivalent and Kruskal-Wallis when the normality assumption is not verified he Shapiro-Wilk test was used to evaluate the adjustment to normality. The decision of significant statistical evidence for probability values (p-value) was less than 0.05.

Pathological studies were conducted according to published recommendations [15,16]. Placentas were initially examined macroscopically, for photos acquisition and gross parameters evaluation. Placental disk measures were achieved in all groups: placenta weight (g), disk diameters and thickness and size (cm)-umbilical cord length and diameter (cm)-cord insertion type and number of umbilical vessels. Cord insertion type was classified as normal, where there was a central or eccentric insertion. Pathologic insertion was considered when it was marginal (<1 cm from the nearest margin) and velamentous (when drawstring route in the membranes) [16].

In CDH and DE groups several fetal defects were studied for each case individually: lung malformation, congenital heart disease, thoracic-abdominal wall defect, urogenital anomaly, endocrine anomaly and neural tube defect and in the same way with the 3 syndromic cases of the group of placentas without CDD. The number of defects was compared in the three groups, and the differences were evaluated by analysis of variance (ANOVA test).

Selected fragments for histological study based on the macroscopic data, clinical information and following pathological protocols, were sampled, prepared, sectioned at 5 µm and stained with Hematoxylin-Eosin (H & E) to allow the pathological diagnosis in placenta and fetal samples [16].

All the samples enrolled in the present study were unlinked and unidentified from their donors, and fulfilled the international ethics recommendations for Medical Research in Humans of the Declaration of Helsinki, the World Health Organization and the European Community, to be used in this study. The Ethical Review Committees of the involved institutions and Medicine School of Minho University approved the work and waived the need for written informed consent. Patients authorized the realization of pathological study in CGC Genetics, Porto, as well as all the procedures needed for pathologic diagnosis education and scientific research.

Results

Of the 101 cases evaluated, Table 1 summarizes the distribution cases in the groups according to the evaluated placental and cord parameters, type of delivery, syndromic or non-syndromic cases. Thirty cases presented CDD, 8 (7.9%) corresponding a DE and 22 (21.8%) a CDH of which 16 were on the left side. Fetal gender in 31 (34.4%) cases were female and in 40 (44.4%) cases were male and in 30 cases is unknown. In order to evaluate the association of CDD with fetal syndrome, a chi-square independence test was performed, with a statistically significant association (χ^2=35.090, DF=2, p<0.001) in that the presence of syndrome is associated with CDH. This result is illustrated in the graph of Figure 1. For the comparison of the quantitative measures (placenta diameter, cord length, placenta weight and cord diameter) according to the group, the data normality was assured by the analytical test. In the placental weight parameter, the data normality was done through Kruskal Wallis. After completing the multiple comparison tests, and given the existence of a control group, Dunnett's test revealed that: 1. There were significant differences in the mean placental diameter value when we compared the CDH group with the control (p=0.008) in that the mean-value of the CDH group was significantly lower; 2. There were significant differences in the mean-value of the placent a diameter when we compared the DE group with the control (p=0.008) in that the mean-value of the DE group was significantly; 3. There were significant differences in the mean-value of the umbilical cord length when we compared the CDH group with the control (p =0.006) in that the mean-value of the CDH group is significantly lower; 4. There were significant differences in the mean-value of the umbilical cord length when we compared the DE group with the control (p=0.001) in that the mean-value of the DE group is significantly lower; 5. There were significant differences in the mean-value of umbilical cord diameter when we compared the CDH group with the control (p=0.005) in that the mean-value of the CDH group is significantly lower; 6. There were significant differences in the mean umbilical cord diameter when we compare the DE group with the control (p=0.012) in that the mean-value of the DE group is significantly lower.

To evaluate placental weight on the groups, the Kruskal-Wallis test was performed. There were significant differences in the mean-value of the placenta weight due to the group to which it belongs (χ^2=16.694, df=2, p<0.001). Multiple comparison tests revealed that 1. There were as significant differences in the mean-value of placental weight when we compared the CDH group with the control (p=0.002) in the sense that the mean-value of the CDH group was significantly lower; 2. There were significant differences in mean placental weight when we compared the DE group with the control (p=0.025) in the sense that the mean-value of the DE group was significantly lower. All

Table 1: Characterization of clinical and pathological evaluated parameters in congenital diaphragmatic defects and control groups, given in absolute evaluate (n) and percentage (%).

		CDD							
		Without		CDH		DE		Total	
Delivery type, N (%)	MT	9	(12.7)	16	(72.7)	7	(87.5)	32	(31.7)
	FD	38	(53.5)	4	(18.2)	1	(12.5)	43	(42.6)
	NND	0	(0)	1	(4.5)	0	(0)	1	(1)
	NB	24	(33.8)	1	(4.5)	0	(0)	25	(24.8)
Syndrome, N (%)	No	68	(95.8)	9	(40.9)	5	(62.5)	82	(81.2)
	Yes	3	(4.2)	13	(59.1)	3	(37.5)	19	(18.8)
Cord insertion, N (%)	Central	57	(80.3)	13	(72.2)	4	(57.1)	74	(77.1)
	Marginal	14	(19.7)	4	(22.2)	2	(28.6)	20	(20.8)
	Velamentous	0	(0)	1	(5.6)	1	(14.3)	2	(2.1)
Umbilical vessels, N (%)	Three	69	(97.2)	12	(60)	8	(100)	89	(89.9)
	Two, 4+	2	(2.8)	8	(40)	0	(0)	10	(10.1)
Umbilical knots, N (%)	Absent	62	(87.3)	18	(100)	7	(100)	87	(90.6)
	False Knots	9	(12.7)	0	(0)	0	(0)	9	(9.4)
	True Knots	0	(0)	0	(0)	0	(0)	0	(0)
Membranes insertion, N (%)	Normal	66	(93)	18	(85.7)	6	(85.7)	90	(90.9)
	Circumvallate	5	(7)	3	(14.3)	1	(14.3)	9	(9.1)
GA (weeks), (SD)		27.1	(9.6)	20	(8.4)	15.9	(4.3)	24.7	(9.8)
Placenta diameter (cm), M (SD)		13.6	(4.4)	10.5	(4.2)	8.9	(3.2)	12.8	(4.6)
Cord length (cm), M (SD)		30.5	(14.6)	19.2	(11.2)	10.1	(5.6)	26.9	(14.9)
Cord diameter (cm), M (SD)		1.1	(0.5)	0.7	(0.3)	0.6	(0.3)	1	(0.5)

N: Count, %: Percentage; M: Mean; SD: Standard Deviation; CDD: Congenital Diaphragmatic Defects; CDH: Congenital Diaphragmatic Hernia; DE: Diaphragmatic Eventration; MT: Medical Termination of pregnancy; FD: Fetal Death; NND: Neo Natal Death; NB: Newborn; GA: Gestational Age dated of the 1st day of last menstrual period

these results are illustrated in the graph of (Figure 2).

It was detected significant association between CDD and umbilical vessels (χ^2=24.736, p<0.001), other congenital defects (χ^2=29.052, p<0.001). There were no significant associations between CDD and cord insertion, umbilical knots and membranes insertion type (p>0.05).

Figure 3 illustrate the statistically significant association between CDD and umbilical vessels. In that the existence of 2 vessels was associated with the CDH group. Also there was a statistically significant association between CDD and the existence of other defects, in that other defects was associated with the CDH group.

Discussion

In the present study oneway ANOVA verified significant differences in the mean-value of umbilical cord lengths and umbilical cord diameters in CDD and control groups, (F (2,92)=10.464, p<0.001) and (F (2,93) =7.710, p=0.001), respectively. Umbilical cord characteristics are well determined [15,16]. Also, umbilical cord anomalies are well described as well as the association with fetal anomalies or genetic defects [11,12,17,18].

Excessively thin umbilical cords are associated with fetal problems, as fetal growth restriction. A thick umbilical cord are associated with maternal diabetes and fetal macrosomia and fetal hydrops [17,18]. Nevertheless, to the best of our knowledge, no previous association has been described between decreased diameter of the umbilical cord and CDD. Our study documented-significant differences in the mean-value of umbilical cord diameter when we compared the CDH and DE groups with the control (p= 0.005) and (p=0.012) respectively. In fact, the mean-value of cord diameter in CHD and DE groups are significantly lower when compared to the control group.

There are many studies documenting associations between umbilical cord length and fetal congenital malformation, namely CDH [11-26]. In our study, the mean-value of umbilical cord length was significantly lower when we compared the CDH and DE groups with the control (p=0,006) and (p=0.001) respectively. UC length showed a greater difference in the DE group possibly related with abdominal and/or thoracic-abdominal wall defects as omphalocele, ectopia cordis, and limb body stalk complex seen in these groups [11-14]. Single Umbilical Artery (SUA) occurred in 40% of CDH group and 2.8% in control group. Prevalence of SUA is higher in congenital and structural fetal anomalies and chromosomal defects compared with 0.5% to 1% incidence in normal singleton. Higher prevalence of SUA in CDH group may be related to fetal pathology associated in this group. Pathologic cord insertion is associated with congenital anomalies when compared with normal pregnancies in which velamentous and marginal cord insertion in general occurs around 1% and 7% correspondingly [25,27]. Our results documented a higher number of pathologic cord insertion in DE and CDH groups with 14.3% and 5.6% respectively.

Normal placental function is critical to optimize fetal growth and development. Some authors documented a decrease in placental size with a hypoxic environment and placental hypoxic injury, namely fetal vessels lesions [16,28-32]. Placental size deviation has

been related with morbidity and mortality in the fetus [16]. This may provide relevant information to the management of fetal and perinatal outcomes [16,32,33]. Our study documented a significantly lower mean of placent a diameter value in CDH and DE groups when compared with the control group (p=0.008). Placenta weight is well established as well as its correlation with fetal abnormalities [16]. The mean-value of placental weight is significantly lower in CDH group and DE group when compared with the control (p=0.002) and (p=0.025).

Conclusion

The results herein reported suggest that placental disk and umbilical cord measurements could be a risk predictor of fetal anomalies that must rule out the presence of congenital diaphragmatic defects.

The pathological measurements, could improve the ultra sound parameters accuracy after an appropriated protocol and serial statistical approach.

Acknowledgment

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CGC genetics, Porto, Portugal.

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APPENDIX 3

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Four FATCO syndrome cases: clinical, autopsy and placental features with literature review update

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ABSTRACT

Fibular Aplasia-Tibial Campomelia-Oligosyndactyly, or FATCO, is a rare syndromic condition reported in 18 cases so far, from which only 3 were diagnosed at prenatal stages. In this study we report comprehensive clinical, placental and autopsy findings of four additional prenatal cases of FATCO, with the aim of further delineating this syndromic condition. Understanding this disorder at prenatal stages will allow for an earlier diagnosis through the identification of key features, thus permitting an adequate parental counselling about the pregnancy development.

INTRODUCTION

Limb malformations in neonates occurs in approximately 1 in 1,000 [1]. Fibular aplasia is the most common malformation amongst long bone deficiency syndromes [2]. FATCO (OMIM 246570) is an extremely rare syndromic condition involving **F**ibular **A**plasia-**T**ibial **C**ampomelia-**O**ligosyndactyly, first designated by Courtens *et al.* (2005). Individuals with this syndrome present a remarkable shortening and anterior bowing of one or both lower limbs and oligosyndactyly of at least one foot. Frequently, involvement of upper extremities also occurs. Nevertheless, psychomotor development was always described as normal [3-11] and heart defects were rarely reported [7,11,12]. An update of the literature review describing major malformations reported so far is displayed in Supplementary data 1. Up to now, three FATCO subjects have been diagnosed at a prenatal stage [5,12,13]. From these, a single case was described where FATCO syndrome was diagnosed during ultrasound examination and where parents decided to continue with their pregnancy [5]. In the other two reports, skeletal dysplasia was identified during ultrasound imaging but FATCO syndrome was only diagnosed during fetal autopsy [12,13]. Further cases are needed in order to have a comprehensive understanding of this disorder prenatally, allowing parents to make an informed decision about their pregnancy development. In the present study, we report four unrelated cases of FATCO syndrome, of which three of them were diagnosed during fetal autopsy and one diagnosed during ultrasound examination. Prenatal, autopsy and placental findings are detailed in full for a comprehensive FATCO diagnosis.

All the cases enrolled in the present study were unlinked and unidentified from their donors. Due the retrospective nature of the study, no written informed consent from patients was obtained. The Ethical Review Committees of the involved institutions approved the work and waived the need for written informed consent.

CASE REPORT

Fetus 1. Fetus 1 was a product of the first pregnancy of a non-consanguineous Caucasian healthy couple, with no reported family history of skeletal dysplasia. The mother was 34 years of age at time of pregnancy and claims to not have taken any drugs nor been exposed to radiation during pregnancy. First trimester screening revealed no ultrasound anomaly and nuchal translucency (NT) and crown to rump length (CRL) measurements at

13 weeks (w) + 1 day (d) gestational age (GA) were 1.9 mm and 68.1 mm, respectively (>50th percentile, >P_{50}). Standard biometric measurements were appropriate for GA with free ß-hCG: 1.18 MoM and PAPP-A: 0.37 MoM, and thus overall result was of reduced risk for combined prenatal screening of trisomy 21, 18 and 13. During second trimester ultrasound screening, at 21w + 6d GA, abnormal skeletal ultrasound findings were found, showing skeletal malformations on right lower limb with shortening and bowing of the tibia, fibular aplasia and Rocker-Bottom feet with only four toes (Figure 1, A1-A2). At the same GA, fetal echocardiogram was performed with normal outcome. Amniocentesis was performed at 22w of GA and revealed a normal karyotype for a male fetus (46,XY). Parents requested medical termination of pregnancy (MTP) which was granted with permission from the Prenatal Diagnosis Unit Ethics Committee. MTP was performed at 23w + 2d GA via vaginal misoprostol. X-ray analysis confirmed prenatal findings (Figure 1, B1-B2). Fetal autopsy reported a male fetus with minor cranio-facial anomalies and oligosyndactyly, with absence of the 5th toe and the 5th metatarsal, associated with fibular aplasia and tibial campomelia. Autopsy diagnosis was concluded as FATCO syndrome (Figure 1, C; Table 1).

Fetus 2. Pregnant was referred to the Centre at 20w+3d GA due to suspected anomalies on the lower left limb of the fetus. Fetus 2 was the first pregnancy of Caucasian healthy parents. Family history of skeletal dysplasia, drug intake or exposure to radiation during pregnancy was denied. Mother was 33 years old at time of current pregnancy. Combined first trimester screening test was performed at 11w + 4d of GA with standard results with NT: 1.3 mm and CRL: 48.4 mm (P_{50}); free ß-hCG: 0.69 MoM and PAPP-A: 1.20 MoM and, therefore, result was concluded as reduced risk for trisomy 21, 18 and 13. An ultrasound at 17w + 2d GA lead to suspicion of an anomaly present on the lower left limb. No heart defects were found by fetal echocardiogram. Amniocentesis was performed and chromosomal analysis revealed a male fetus with normal karyotype (46,XY). Alpha-fetoprotein was measured in the amniotic fluid and levels (1.1 MoM) were within reference values for GA.

At 20w + 3d GA, ultrasonography showed normal right lower limb (tibia/fibula complex 16/18 mm) and left lower limb with skeletal anomaly: single bone with a remarkable shortening (10 mm), overlying soft tissue dimpling (Figure 1, D) and clubfoot. MTP was requested by the parents and performed with permission from the Prenatal Diagnosis Unit Ethics Committee, via vaginal misoprostol. Fetal X-Ray confirmed fibular aplasia and tibial campomelia (Figure 1, E1-E2). Besides the latter two features, fetal autopsy also reported bilateral oligosyndactyly (3-ray feet), indicating FATCO syndrome as diagnosis (Figure 1, F).

Fetus 3. Pregnant was referred to the Centre at 23w+2d GA due to suspected anomalies on the lower right limb. Fetus 3 was the second pregnancy of Caucasian healthy parents. Family history of skeletal dysplasia, drug intake or exposure to radiation during pregnancy was denied. Mother was 24 years old at time of current pregnancy. Combined first trimester screening test was performed at 11w + 4d of GA with standard results with NT: 1.3 mm and CRL: 48.3 mm. Alpha-fetoprotein was measured in the amniotic fluid and levels (1.1 MoM) were within reference values for GA. Result was concluded as reduced risk for trisomy 21, 18 and 13. No heart defects were found by fetal echocardiogram. Amniocentesis was performed and chromosomal analysis revealed a female fetus with normal karyotype (46,XX).

At 20w + 4d GA, ultrasonography showed normal left lower limb (tibia/fibula complex 16/18 mm) and right lower limb with skeletal anomaly: remarkable short femur and fibular aplasia and tibial campomelia (Figure 1, G). MTP was requested by the parents and performed at 23w + 2d with permission from the Prenatal Diagnosis Unit Ethics Committee, via vaginal misoprostol. Fetal X-Ray confirmed fibular aplasia and tibial campomelia (Figure 1, H1-H2). Besides the latter two features, fetal autopsy also reported a typical overlying soft tissue dimpling and bilateral oligosyndactyly (3-ray feet), indicating FATCO syndrome as diagnosis (Figure 1, I). Esophageal atresia with tracheal fistula were also identified without others anomalies.

Fetus 4. Fetus 4 was a product of the second pregnancy of a non-consanguineous Caucasian couple, with no reported family history of skeletal dysplasia. The mother was 38 years old at time of pregnancy and also presented previous arterial hypertension with taking 2.5 mg of bisoprolol per day until 9w of GA. A first trimester pregnancy-induced gestational diabetes are also diagnosed. Family history of skeletal dysplasia or exposure to radiation during the pregnancy was denied. No heart defects were found by fetal echocardiogram. Combined first trimester screening test was performed at 11w of GA with standard results of NT: 1.7mm and, therefore, result was concluded as reduced risk for trisomy 21, 18 and 13. Due to a poor ultrasound window suspicion rudimentary right leg and feet are noted. Amniocentesis was performed and array CGH study revealed a male fetus with a normal karyotype (46,XY). Second trimester echography screening at 21w+3d of GA showed a major defect in left leg, with fibular agenesis, tibial campomelia and rudimentary feet (ultrasound data not available). MTP was requested by the parents, which was granted with permission from the Prenatal Diagnosis Unit Ethics Committee, and it was performed via vaginal misoprostol at 21w+5d of GA. Fetal X-Ray confirmed fibular aplasia (unilateral) and tibial campomelia and it also revealed hypoplasia of the tarsal bones and oligosyndactyly (Figure 1, J1-J2). Fetal autopsy further reported shortening of right lower limb consistent with FATCO syndrome as diagnosis (Figure 1, K).

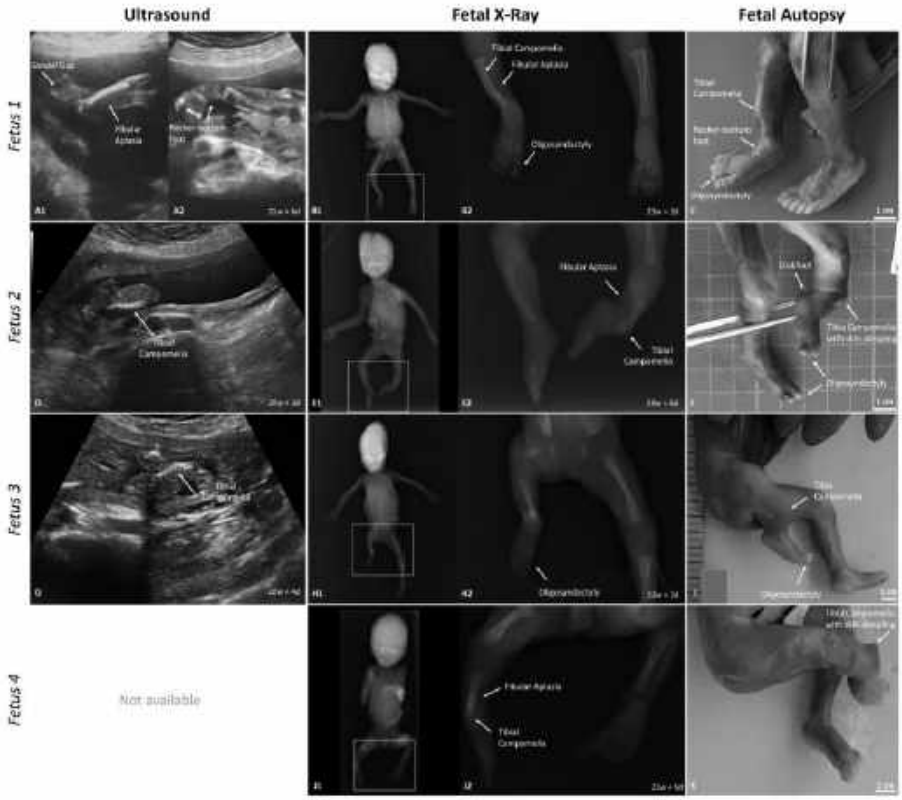

Figure 1. Image documentation of ultrasound (A1-A2, D and G), fetal X-Ray (B1-B2, E1-E2, H1-H2 and J1-J2) and fetal autopsy features (C, F, I and K) of the four fetuses presented in this study, demonstrating key features of FATCO syndrome, such as fibular aplasia, tibial campomelia and oligosyndactyly. Ultrasound imaging of the right lower limb of fetus 4 demonstrating key features of FATCO was not available.

Clinical detect anomalies and detailed autopsy description of all four fetuses and correspondent placental units are described in Table 1.

Table 1. Clinical indications and detailed macro and microscopic examination during fetal autopsy and placental study of fetuses with FATCO syndrome. (GA) gestational age.

	Fetus 1	*Fetus 2*	*Fetus 3*	*Fetus 4*
MEDICAL TERMINATION OF PREGNANCY (MTP)				
Gestational Age (GA)	23w + 2d	20w + 6d	23w + 2d	21w + 5d
Medical Information	Skeletal malformation of the right lower limb (Rocker-Bottom feet) leading to a shortening and bowing of the tibia, fibular aplasia, 4 toes	Absence of fibula in left lower limb	Right lower limb with short femur, short and bowed tibia, fibular aplasia and axial deviation of the foot	Right lower limb with fibular aplasia, short tibia and undeveloped right foot
Karyotype	46,XY	46,XY	46,XX	46,XY
FAMILY HISTORY		Arterial Hypertension		Gestational diabetes; pregnancy-induced hypertension
FETAL AUTOPSY				
Macroscopic Examination				
Fetal gender (GA)	Male (GA 23 weeks)	Male (GA 21 weeks)	Female (GA 23 weeks)	Male (GA 21' weeks)
Biometric parameters	Normal	Lower	Normal	Normal
Body weight (total, g)	Normal – 480	Normal – 330	Normal – 490	Higher – 495 (normal = 389 ± 72)
Internal organs (weight, g)	Normal	Normal	Normal, except:	Normal, except:

			. Heart: Lower – 2 (normal = 3.81 ± 0.96) . Liver: Lower – 15.1 (normal = 24.3 ± 6.5)	. Cerebrum: Higher – 78.9 (normal = 54.6 ± 10.4)
Growth and bone maturity	Normal	Normal	Normal	Normal
Visceral maturity	Normal	Normal	Normal	Normal
Thoracic circumference (TC, cm)	Normal	Lower – 24.6 (normal = 26)	Normal	Higher – 16.6 (normal = 15.2)
Cranio-caudal length (CCL, cm)	Normal	Lower – 16.5 (normal = 18)	Normal	Higher – 20.3 (normal = 18.4 – 19.8)
Occipital Frontal Circumference (OFC, cm)	Normal	Lower – 18.2 (normal = 18.5 – 21.1)	Normal	Higher – 20.1 (normal = 18.5 – 19.8)
Femur length (cm)	Normal – 3.5 (P_{50})	Normal – 3.27 (P_{50})	Short – 1.12 ($<P_{50}$)	Normal – 3.66 (P_{50})
Biparietal diameter (BPD, cm)	Normal	Lower – 4.5 (normal = 4.8 – 5.7)	Lower – 5.13 ($<P_{10}$)	Normal – 5.02 (P_{50})
Foot length (cm)	Normal	Lower – 3.1 (normal = 3.3 – 3.8)	Normal	Higher – 4.1 (normal = 3.72 – 3.91)
Transverse cerebellar diameter (TCD, cm)	Normal	Normal – 2.1 (normal = 1.9 – 2.0)	Normal – 2.3 (normal=2.2-2.8)	Normal – 2.3 (normal = 1.9 – 2.4)
Brain-liver weight relationship (g	Normal – 3.8 : 1	Higher – 4.9 : 1	Higher – 4.8 : 1	Normal – 3.6 : 1
Development anomalies				
Cranio-facial dysmorphisms	Infra-orbital grooves Micrognathia (moderated)		Micrognathia	
Cerebrum	Normal	Normal	Normal	Normal
Upper limbs	Ulnar deviation of the hands	Normal	Normal	Arachnodactyly
Lower limbs	Asymmetric limbs (shorter right) Rocker-Bottom feet Absence of the 5th toe on right foot (oligosyndactyly) Bilateral *Sandal Gap*	Asymmetric and distinct limbs (shorter left) Skin dimpling on left leg Bilateral oligosyndactyly: absence of the 4th and 5th toes Left clubfoot	Asymmetric limbs (rhizomelic shortening) Skin dimpling and spiculated on right leg Oligosyndactyly Right tight with short femur	Asymmetric and distinct limbs (shorter right) Skin dimpling on right leg Oligosyndactyly
Fetal X-Ray	Dysplasia of the 5th finger of both hands Right lower limb with shortened. Absence of fibula and angulated tibia and absence of fibula, of the 5th metatarsal bone and 5th toe	Left convex thoracic scoliosis; 11 ribs to the left Agenesis of left fibula and short and angulated tibia; hypoplasia of the tarsal bones	Rizomelic Shortening of right lower limb Fibular agenesis, tibia campomelia. Oligosyndactyly	Right lower limb shortening Fibular agenesis, tibial campomelia. Absence of 5th and 4th toes
External Genitalia	Normal	Big phallus	Normal	Normal
Cardio-pulmonary system	*Situs solitus*, levocardia, levoapex persistence of fetal circulation. Bifurcated cardiac apex due to the left ventricle	*Situs solitus*, levocardia, levoapex persistence of fetal circulation	*Situs solitus*, levocardia, levoapex persistence of fetal circulation	*Situs solitus*, levocardia, levoapex, persistence of fetal circulation
Microscopic Examination				
Lung	Ectasia of the lymphatic lobular septum. Without pulmonary hypoplasia	Without pulmonary hypoplasia	Without pulmonary hypoplasia	Without pulmonary hypoplasia
Fetal Autopsy Report/Diagnosis	Male fetus with 23 ws GA. Lower right limb localized developmental skeletal anomalies with fibular aplasia and tibial campomelia, and oligosyndactyly without other anomalies supporting the diagnosis of FATCO syndrome.	Male fetus with 21ws GA, intrauterine growth restriction (IUGR). Lower left limb localized developmental skeletal anomalies with fibular aplasia and tibial campomelia; bilateral oligosyndactyly, without other anomalies, supporting the diagnosis of FATCO syndrome.	Female fetus with 23'ws GA. Lower right limb localized developmental skeletal anomalies with fibular aplasia and tibial campomelia, oligosyndactyly. Short right femur, esophageal atresia with tracheoesophageal fistula without lung anomalies, supporting the diagnosis of FATCO syndrome.	Macerated male fetus with 21'ws GA. Right lower limbs developmental skeletal anomalies with fibular aplasia, tibial campomelia and oligosyndactyly supporting the diagnosis of FATCO syndrome.
PLACENTAL STUDY				
Macroscopic Examination				
Appearance	Single and complete	Single and complete	Single and complete	Single and complete
Weight (g)	107	103	177	108
Size (cm)	10x9x2	11x10x1.5	12x9x1.7	11x8x0.5
Membranes	Normal	Normal	Normal	Normal
Umbilical Cord	3 vessels and marginal insertion	3 vessels and central insertion	2 vessels and velamentous insertion	3 vessels and central insertion
Size (length x diameter) (cm)	18x1	25x1	15x1	31x1.2
Fetal surface vessels	Eccentric	Radial	Peripheral	Radial

Chorion	Without gross anomalies	Without gross anomalies	Without gross anomalies	Hydrops
Microscopic Examination				
Placental maturity	Normal	Normal	Hypermaturity	Imature villi
Villi				
Hofbauer cells	Vacuolated Hofbauer cells	Vacuolated Hofbauer cells		
Hydropsia	Absent	Absent	Absent	Present
Vessels	Thrombosis	Normal	Normal	Decreased
Ischemia	Increased fibrin deposits	-	Increased tertiary villi	-
	Extravillous trophoblast hyperplasia	-	Extravillous trophoblast hyperplasia	
Infection	-	-	-	Acute chorioamnionitis stage 1 grade 1
Placental Report	Low weight. Fetal vessels thrombo-inflammatory events. Atypical vacuolated Hofbauer cells. Umbilical cord marginal insertion.	Atypical vacuolated Hofbauer cells.	Placentomegaly. Umbilical cord velamentous insertion.	Mesenchymal dysplasia Acute chorioamnionitis without fetal answer

DISCUSSION

Including the four fetuses described in the present study, there are now 22 cases of FATCO syndrome reported to date [3-16] (Supplementary data 1). All authors consistently report the main features characteristic FATCO syndrome in at the least one lower limb, namely, fibular aplasia/hypoplasia, tibial campomelia and oligosyndactyly of the feet. Frequently, one or both upper limbs are also involved [3,5,7,9-11,13]. Psychomotor development was always described as normal in live FATCO syndrome individuals [3-11]. Interestingly, out of 21 cases in which patient gender was reported, only 3 females were described (male to female ratio, 18:3), thus being clearly biased towards male preponderance. One of the female cases reported had a diseased half-sibling male, from the mother side, with severe symptoms also involving upper and lower extremities: absent hands, absent left leg and absent right foot, such as observed in phocomelia features [11]. Additionally, another author reported that the mother of a patient diagnosed at 11 years of age (patient 3, [7]), presented bilateral partial skin syndactyly of toes 2-3, extending to the proximal interphalangeal joints. These two examples suggest that this disorder possibly has an X-linked inheritance with variable penetrance, which is also in agreement with a suggestion from Bieganski and colleagues [7]. An interesting feature noted from literature review on prenatal diagnosed FATCO cases was that nuchal translucency (NT) measurements, between week 12 and 13, were slightly increased with 3.6 mm for both fetuses [5,12]. Fetal NT thickness above 3.5 mm (>P_{95}), between week 11 and 14, is known to be involved in congenital cardiac defects, chromosomal abnormalities and fetal malformations, including skeletal dysplasia, deformations, disruptions and genetic syndromes [17]. In the present study, all four fetuses had standard NT measurements, with percentile above 50.

Therefore, even though it is very important to take note of NT measurements, our evidence cannot support that FATCO syndrome might be related with increased NT. Nevertheless, within reported FATCO cases, increase in NT measurement has been described in association with a membranous ventricular septal defect [12]. Also, Bieganski et al., (2012) noted that FATCO patient 1 was born with the same membranous ventricular septal defect, even though no NT value between week 11-14 was reported [7]. Furthermore, in patient 1 of Hecht and Scott (1981), a congenital cyanotic heart defect has been reported which later was identified as the cause of death at day 11 [11]. If this disorder is found to be X-linked, it would explain the severity in the male patient reported by Hecht and Scott (1981) (patient 1) in relation to his female half-sib (patient 2)[11]. Nevertheless, to this date, the etiology of FATCO syndrome remains unknown and because clinical symptoms show some degree of heterogeneity, it is very likely that many other patients were misdiagnosed and not reported.

CONCLUSION

FATCO syndrome must be considered in prenatal diagnosis, by the presence of fibular aplasia, tibial campomelia and oligosyndactyly. Shorter and asymmetric limbs can translate into a single dysplastic bone. The current study further delineates FATCO syndrome at a prenatal stage and supports that correct diagnosis during early prenatal stages is of upmost importance for adequate parental counselling on the current pregnancy and its recurrence risk for future pregnancies.

ACKNOWLEDGMENTS

We are grateful to the parents for their collaboration in this study. We also thank Dr. Paula Rendeiro for her constructive scientific comments regarding this work.

CONFLICT OF INTEREST DISCLOSURE

Authors have no conflict of interest to declare.

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APPENDIX 4

Table 3.7. Normative values.

Pregnancy week postmenstrual	Crown–rump length (mm)	Foot length (cm)	Embryonic/fetal weight (g)	Placental weight (g)	Fetal/placental weight ratio	Placental thickness (cm)	Placental diameter (cm)	Umbilical cord length (cm)
3								
4								0.2
5	2.5							0.4
6	5							0.7
7	9							1.2
8	14		1.1	6	0.18			2.0
9	20		2	8	0.25			3.3
10	26		5	13	0.38			5.5
11	33		11	19	0.58			9.2
12	40		17	26	0.65			12.6
13	48	1.2	23	32	0.72		5.0	15.8
14	56	1.7	30	41	0.73	1.0	5.6	18.8
15	65	1.9	40	50	0.80	1.1	6.2	21.5
16	75	2.2	60	60	1.00	1.2	6.9	24.0
17	88	2.5	90	70	1.29	1.2	7.5	26.4
18	99	2.8	130	80	1.63	1.3	8.1	28.7
19	112	2.9	180	101	1.78	1.4	8.7	30.9
20	125	3.3	250	112	2.23	1.5	9.4	33.0
21	137	3.6	320	126	2.54	1.5	10.0	35.0
22	150	3.9	400	144	2.78	1.6	10.6	36.9
23	163	4.2	480	162	2.96	1.7	11.2	38.7
24	176	4.5	560	180	3.11	1.8	11.9	40.4
25	188	4.7	650	198	3.28	1.8	12.5	42.0
26	200	5.0	750	216	3.47	1.9	13.1	43.5
27	213	5.3	870	234	3.72	1.9	13.7	45.0
28	226	5.5	1,000	252	3.97	2.0	14.4	46.4
29	236	5.8	1,130	270	4.19	2.0	15.0	47.7
30	250	6.0	1,260	288	4.38	2.1	15.6	49.0
31	263	6.2	1,400	306	4.58	2.1	16.2	50.2
32	276	6.5	1,550	324	4.78	2.2	16.9	52.0
33	289	6.7	1,700	342	4.97	2.2	17.5	53.0
34	302	6.9	1,900	360	5.28	2.3	18.1	54.0
35	315	7.1	2,100	378	5.56	2.3	18.7	54.9
36	328	7.4	2,300	396	5.81	2.4	19.4	55.7
37	341	7.6	2,500	414	6.04	2.4	20.0	56.5
38	354	7.8	2,750	432	6.37	2.4	20.6	57.2
39	367	8.0	3,000	451	6.65	2.5	21.3	57.9
40	380	8.1	3,400	470	7.23	2.5	22.0	58.5

Portions of this table were modified from Kalousek et al. (1992)

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APPENDIX 5

Requerimento

Excelentíssima Diretora do Centro de Genética Clínica, Porto, Portugal

Eu, Rosete Maria Amorim Novais Nogueira Cardoso, aluna de Doutoramento em Medicina, Escola de Medicina da Universidade do Minho, com o número de aluno ID5281, pretendo realizar um projeto de investigação no Laboratório de Patologia Embriofetal do CGC Genetics, sob supervisão do Prof. Dr. Jorge Correia Pinto e da Prof. Dra. Maria Purificação Tavares. O Título do Doutoramento é: "A Multicenter Study of Singleton Placentas Biometric Parameters and Fetal Weight In Function of Gestational-Age" e tem como objetivo principal produzir curvas de percentis para o peso placentar e rácio peso fetal/peso placentar em função da idade gestacional.

Assim, venho por este meio requerer a autorização de Vª Exª para a realização deste estudo.

Pede deferimento,

Com os meus mais sinceros cumprimentos,

Rosete Maria Amorim Novais Nogueira Cardoso

(Rosete Maria Amorim Novais Nogueira Cardoso, Aluna de Doutoramento em Medicina, Escola de Medicina da Universidade do Minho)

Nº aluno: ID5281

Correio eletrónico: id5281@alunos.uminho.pt

O CGC Genetics autoriza com muito gosto a participação no estudo e colabora com o que for necessário.
11.2.19

Autorização n.º 7687/ 2016

Rosete Nogueira notificou à Comissão Nacional de Protecção de Dados (CNPD) um tratamento de dados pessoais com a finalidade de realizar um Estudo Clínico sem Intervenção, denominado Estudo Multicêntrico de Parâmetros Biométricos de Fetos e Placentas em Diferentes Idades Gestacionais .

A investigação é multicêntrica, decorrendo, em Portugal, nos centros de investigação identificados na notificação.

O participante é identificado por um código especificamente criado para este estudo, constituído de modo a não permitir a imediata identificação do titular dos dados; designadamente, não são utilizados códigos que coincidam com os números de identificação, iniciais do nome, data de nascimento, número de telefone, ou resultem de uma composição simples desse tipo de dados. A chave da codificação só é conhecida do(s) investigador(es).

É recolhido o consentimento expresso do participante ou do seu representante legal.

A informação é recolhida indiretamente do processo clínico.

As eventuais transmissões de informação são efetuadas por referência ao código do participante, sendo, nessa medida, anónimas para o destinatário.

A CNPD já se pronunciou na Deliberação n.º 1704/2015 sobre o enquadramento legal, os fundamentos de legitimidade, os princípios aplicáveis para o correto cumprimento da Lei n.º 67/98, de 26 de outubro, alterada pela Lei n.º 103/2015, de 24 de agosto, doravante LPD, bem como sobre as condições e limites aplicáveis ao tratamento de dados efetuados para a finalidade de investigação clínica.

No caso em apreço, o tratamento objeto da notificação enquadra-se no âmbito daquela deliberação e o responsável declara expressamente que cumpre os limites e condições aplicáveis por força da LPD e da Lei n.º 21/2014, de 16 de abril, alterada pela Lei n.º 73/2015, de 27 de junho – Lei da Investigação Clínica –, explicitados na Deliberação n.º 1704/2015.

O fundamento de legitimidade é o consentimento do titular.

A informação tratada é recolhida de forma lícita, para finalidade determinada, explícita e legítima e não é excessiva – cf. alíneas a), b) e c) do n.º 1 do artigo 5.º da LPD.

Assim, nos termos das disposições conjugadas do n.º 2 do artigo 7.º, da alínea a) do n.º 1 do artigo 28.º e do artigo 30.º da LPD, bem como do n.º 3 do artigo 1.º e do n.º 9 do artigo 16.º ambos da Lei de Investigação Clínica, com as condições e limites explicitados na Deliberação da CNPD n.º 1704/2015, que aqui se dão por reproduzidos, autoriza-se o presente tratamento de dados pessoais nos seguintes termos:

Responsável – Rosete Nogueira

Finalidade – Estudo Clínico sem Intervenção, denominado Estudo Multicêntrico de Parâmetros Biométricos de Fetos e Placentas em Diferentes Idades Gestacionais

Categoria de dados pessoais tratados – Código do participante; dados da história clínica; dados de meios complementares de diagnóstico

Exercício do direito de acesso – Através dos investigadores, por escrito

Comunicações, interconexões e fluxos transfronteiriços de dados pessoais identificáveis no destinatário – Não existem

Prazo máximo de conservação dos dados – A chave que produziu o código que permite a identificação indireta do titular dos dados deve ser eliminada 5 anos após o fim do estudo.

Da LPD e da Lei de Investigação Clínica, nos termos e condições fixados na presente Autorização e desenvolvidos na Deliberação da CNPD n.º 1704/2015, resultam obrigações que o responsável tem de cumprir. Destas deve dar conhecimento a todos os que intervenham no tratamento de dados pessoais.

Lisboa, 09-08-2016

A Presidente

ARS NORTE
Administração Regional de Saúde do Norte, IP

ADMINISTRAÇÃO REGIONAL DE SAÚDE DO NORTE, IP
EXARADO NA ACTA Nº 26
REUNIÃO DE 28.07.2016

DELIBERADO CONCORDAR
28/07/16

Dr. Pimenta Marinho
Presidente do C.D.

José Carlos Pedro
Vogal C.D.

Dr. Pérpetuo Oliveira
Vogal C.D.

☐ ☐ ☐ DATA: 27 de Nº 2016

INFORMAÇÃO Nº 287/2016 Nº «Processo» «Registo»

PARA: Conselho Diretivo da ARS Norte

DE: Comissão de Ética para a Saúde da ARS Norte

ASSUNTO: Estudo multicêntrico de parâmetros biométricos de fetos e placentas em diferentes idades gestacionais

Levo ao conhecimento desse Conselho Diretivo a Informação sobre o "Estudo multicêntrico de parâmetros biométricos de fetos e placentas em diferentes idades gestacionais", aprovado na reunião de 26 de julho de 2016, por unanimidade, para deliberação.

À consideração superior

Ana Paula Capela

Ana Paula Capela
(Assessoria CES/UIC)

ARS NORTE
Administração Regional de Saúde do Norte, I.P.

Comissão de Ética para a Saúde
Administração Regional de Saúde do Norte, IP

Sobre o estudo T557 - "*Estudo multicêntrico de parâmetros biométricos de fetos e placentas em diferentes idades gestacionais*"

A – Relatório

1. O processo **T557** respeita a uma investigação com o título supra, no âmbito Doutoramento pela Universidade do Minho a desenvolver pela investigadora Rosete Maria Martim Novais Nogueira Cardoso, licenciada em Medicina. Em inglês o título apresentado é *"Placental weight and umbilical cord measurements and autopsy standards of body parameters and organ weights in nonmacerated and macerated human fetuses at different gestational age"*, o qual foi assim apresentado à Comissão Nacional de Proteção de Dados.
   A investigadora propõe que este trabalho de investigação seja realizado no *Centro de Genética Clínica Genetics*, no âmbito do protocolo que este detém com hospitais públicos de Portugal como prestador de serviço na área da Patologia Embrio-Fetal e da placenta. De referir que a investigadora é diretor a do laboratório de anatomia patológica / embriofetopatologia do referido Centro, sendo uma das orientadoras a Diretora do referido laboratório.
   Segundo informa a investigadora serão instituições participantes, além do referido laboratório, unidades de diagnóstico pré-natal de hospitais públicos do norte, centro e sul de Portugal.
   O objetivo geral é o de contribuir para alargar o conhecimento sobre indicadores de eficácia do diagnóstico pré-natal para deteção precoce de alterações patológicas que conduzam à possibilidade de terapêutica prevenção de complicações e/ ou de mortalidade fetal ou neonatal, contribuindo também dessa forma para a melhoria contínua dos serviços prestados pelas Instituições à população de gestantes em Portugal
   Uma parte dos objetivos envolve avaliação de parâmetros de crescimento para a idade gestacional que possibilitem o desenho de tabelas de crescimento e peso da placenta e feto para a idade gestacional e avaliem a realidade portuguesa. Por essa via também se propõe contribuir para alargar o conhecimento sobre indicadores de eficácia em diagnóstico pré-natal de patologia da placenta que permitam a deteção precoce de aspetos patológicos associados a morbilidade fetal / neonatal, ou materna e / ou a maus desfechos obstétricos.
   Os objetivos específicos envolvem estudos em patologia da placenta (de aspetos macroscópicos e microscópicos do disco corial, membranas e cordão umbilical para a idade gestacional) e estudos em patologia fetal e perinatal (de parâmetros biométricos corporais e peso dos órgãos em autópsia fetal em fetos não macerados e macerados para a idade gestacional), que possam vir a dar resposta às perguntas de investigação, de entre as quais:

- Quais as implicações dos desvios de crescimento e de desenvolvimento?
- Quais as causas dos desvios de crescimento e desenvolvimento?
- Que parâmetros se associam a maior risco de morbilidade embrio-fetal / perinatal?
- Critérios "de novo" preditivos do prognóstico de situações fetais e / ou maternas?
- Correlação anátomo-clínica. o estudo patológico e ausência / não suspeição de anomalias ultrassonográficas?

2. O estudo, do tupo observacional e descritivo, terá como população as amostras de placentas e autópsias embrio-fetais-perinatais enviadas ao Laboratório de Anatomia Patológica / Laboratório de patologia Embrio-Fetal do Centro de Genética Clínica (CGC Genetics) Porto, de acordo com o pedido clínico a solicitar o respetivo estudo patológico e mediante requisição clínica e consentimento informado para o efeito.
   Proceder-se-á à avaliação dos registo dos parâmetros clínicos referidos e dos registo dos parâmetros anatomopatológicos macroscópicos e microscópicos. Será também desenvolvido um sistema de classificação patológica das lesões clinicamente orientada.
   Não é indicada a dimensão da amostra, que se informa estar relacionada com o número de casos em arquivo, estando o período de tempo do projeto dependente do número de casos e de outros fatores.
   Para a população alvo será realizada uma recolha de daos biográficos gerais e aspetos considerados relevantes da história clínica (mas não são remetidos os formulários de recolha dessa informação). A histónia familiar e pessoal bem como os achados ultrassonográficos serão os que constam na requisição clínica associada ao processo patológico. As imagens ecográficas ou patológicas, tais como os restantes dados recolhidos serão avaliados em formato digital e codificadas de forma a garantir a sua anonimização.
   Todos os casos incluídos para estudo serão codificados através de numeração árabe atribuída de um modo crescente de acordo com a inclusão dos mesmos na amostra de estudo.

3. Segundo resulta do descrito, não haverá acesso direto aos titulares das amostras estudadas, sendo o consentimento informado conferido apenas para a realização do exame anatomo-patológico e apenas os casos com doação para estudo de investigação farão parte da amostra fetal/perinatal.

B – Conclusões

1. **Em face do que ficou exposto, a CES delibera não emitir parecer, por não se tratar de projeto com intervenção das unidades de cuidados de saúde primários, nem haver indicações de intervenção de profissionais destas.**

2. **Pelo contrário, os produtos biológicos que serão investigados são colhidos em unidades de cuidados de saúde hospitalares, tanto quanto é informado, pelo que serão competentes para a emissão do respetivo parecer as CES dessas mesmas unidades.**

Aprovado em reunião do dia 26/7/2016, por unanimidade

O relator,

Mestre Sandra Ferreira

O Presidente da Comissão de Ética para a Saúde da ARS Norte IP

Professor Doutor Alberto Pinto Hespanhol

GOVERNO DE PORTUGAL

ARSC ADMINISTRAÇÃO REGIONAL DE SAÚDE DO CENTRO, I.P.

COMISSÃO DE ÉTICA PARA A SAÚDE

PARECER FINAL:	DESPACHO:
FAVORÁVEL (A autora deve enviar o relatório final, quando for oportuno)	Homologo 16.07.15 Presidente do Conselho Diretivo da A.R.S. Centro, I.P.

ASSUNTO:

Estudo: "Estudo multicêntrico de parâmetros biométricos de fetos e placentas em diferentes idades gestacionais (Proc. nº 39/2016).

Autores: Dra Rosete Nogueira (diretora do Laboratório de Anatomia Patológica da Universidade do Minho).

É um trabalho de doutoramento a realizar pela Dra Rosete Nogueira, sob a orientação do Prof. Doutor Jorge Correia Pinto, da Universidade do Minho, e da Profª Doutora Purificação Tavares, da Faculdade de Medicina Dentária da Universidade do Porto, e que pretende incluir diversos hospitais mediante protocolo.

Este trabalho pretende analisar embriões, fetos, placentas e cordões umbilicais, de modo a correlacionar aspetos micro e macroscópicos com a idade gestacional e a causa do desfecho obstétrico adverso. Também correlaciona alguns factos clínicos maternos, incluindo ultrassonografia (anonimizadas através de codificação) com as alterações observadas.

A amostra é composta de placentas e autópsias embriofetais perinatais, enviadas ao Lab de Anatomia Patológica / Laboratório de Patologia Embrio-Fetal do Centro de Genética Clínica no Porto. É feita a requisição pelo clínico respetivo e há o consentimento informado do doente/gestante.

Todos os dados recolhidos serão confidenciais, "salvaguardando-se sempre a identidade e anonimato dos seus participantes, ficando limitado o seu acesso aos investigadores responsáveis". Para isso os cadernos de recolha de dados serão anonimizados e codificados.

Foi enviado o projeto do estudo, bem como as folhas de preenchimento de dados e questionário, que nos parecem corretamente elaborados.

Os custos serão suportados pela estudante de doutoramento, através de bolsa.

Há o consentimento informado em "boa e devida forma".

Já há autorização da Comissão de Ética da Universidade do Minho.

O Relator e Presidente da Comissão de Ética,

Carlos A. Fontes Ribeiro

Exma. Senhora
Dr.ª Rosete Nogueira
Rosete.Nogueira@cgcgenetics.com

C/C:

Sua Referência	Sua Comunicação de	Nossa Referência	Data
		5688/CES/2016	19.05.2016

Assunto: "Estudo Multicêntrico de Parâmetros Biométricos de Fetos e Placentas em Diferentes Idades Gestacionais."

A Comissão de Ética para a Saúde da ARSLVT, apreciou o projecto mencionado em epígrafe, tna reunião do dia 13-05-2016, tendo sido deliberado que o mesmo preenche critérios de isenção de parecer.

Declaração de conflito de interesses: Nada a declarar

O Conselho Directivo, atento ao teor do parecer emitido por aquela Comissão, entende que o presente projecto, cumpre critérios de isenção de apreciação.

Com os melhores cumprimentos,

O Vice - Presidente do Conselho Directivo

Luis Pisco

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Parecer

Proc.033/CES/INV/2016

Título: Estudo Multicêntrico de Parâmetros Biométricos de Fetos e Placentas em Diferentes Idades Gestacionais

Âmbito do estudo: doutoramento
Enquadramento institucional do proponente: Universidade do Minho

Investigador (es): Rosete Nogueira (Centro de Genética Clínica e Patologia)
Orientador(es): Purificação Tavares (Genética Médica FMDPorto) e Jorge Correia Pinto (Escola de Ciências da Saúde da Universidade do Minho)

Financiamento : sem financiamentos externos

Fundamentação do estudo:

"Não se conhece até à data qualquer estudo avaliando parâmetros de crescimento da placenta e anexos fetais adaptados à população portuguesa, desconhecendo-se ainda as implicações fetais e maternas que lhes possam estar associadas. Assim, parte do objetivo deste estudo é avaliar parâmetros de crescimento para a idade gestacional, que possibilitem o desenho de tabelas de crescimento e peso da placenta e feto para a idade gestacional e avaliem a realidade portuguesa.

O diagnóstico ultrassonográfico precoce de determinadas patologias da placenta e do feto estão dependentes do reconhecimento das mesmas. Assim, é determinante e útil a sua caraterização patológica (macroscópica e microscópica), a correlação com os achados pré-natais e as implicações clínicas maternas e fetais. Só assim o médico obstetra poderá desenvolver critérios ultrassonográficos para a sua deteção que resultem em marcadores de avaliação de risco de morbilidade e mortalidade fetal/neonatal, e que por outro lado permitam também a sua prevenção em futuras gestações e consequentemente resultam em melhoria da saúde materna.

Assim, propomo-nos contribuir para alargar o conhecimento sobre indicadores de eficácia em diagnóstico pré-natal de patologia da placenta que permitam a deteção precoce de aspetos patológicos associados a morbilidade fetal/neonatal ou materno, e/ou a maus desfechos obstétricos.

A deteção precoce daqueles aspetos e a atitude médica consequente poderão minimizar as perdas e otimizar ainda medidas de prevenção de riscos associados a contextos adversos em futuras gravidezes."

Apreciação

Trata-se de um estudo com potencial interesse e valor social que procura caracterizar alargar o conhecimento sobre indicadores de eficácia em diagnóstico pré-natal de patologia da placenta e poderá vir a cumprir no que concerne à sua fundamentação, critérios de valor social, interesse e relevância (clínica e de saúde pública).

Conclusão:

O presente protocolo não cumpre critérios para apreciação por esta comissão, pelos seguintes motivos :
1.- Não envolve Profissionais de Saúde das Unidades de Saúde abrangidas pelo âmbito geográfico e institucional desta Comissão;
2.- Envolve amostras e exames de utentes que foram referenciados à unidade de Saúde que pretende realizar este estudo por instituições do Serviço Nacional que dispõem de Comissões de Ética para a Saúde competentes para a sua apreciação;
Da apreciação deste projecto salientam-se alguns aspectos a merecer melhor fundamentação .

Nestas circunstâncias consideramos que não estarem cumpridos os quesitos para apreciação deste estudo por esta Comissão, devendo a investigadora referenciar o pedido de apreciação a cada uma das instituições de Saúde que referenciaram utentes ao Centro de Investigação.

06 de Maio de 2016
Declaração de conflito de interesses: Nada a declarar