

Placental Pathology Correlation to Fetal and Neonatal Diagnostic
Outcomes in a Cohort of Infants Admitted in a Newborn High Risk
Follow-up Program

By

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TABLE OF ABBREVIATIONS

(ACA)	Acute Chorioamnionitis
(CAP)	College of American Pathologists
(CMV)	Cytomegalovirus
(CNS)	Central Nervous System
(D&C)	Dilatation & Curettage
(DiDi)	Diamnionic/Dichorionic
(DiMo)	Diamnionic/Monochorionic
(DSM-AP)	Diagnostic Services of Manitoba – Anatomical Pathology
(EFAs)	Essential Fatty Acids
(FTV)	Fetal Thrombotic Vasculopathy
(GLUT)	Glucose Transporter
(hCG)	Human Chorionic Gonadotropin
(HIE)	Hypoxic Ischemic Encephalopathy
(HISCAD)	Health Information Services Coding and Abstracting Department
(HREB)	Health Research Ethics Board
(HRFU)	High Risk Follow-up
(HSC)	Health Sciences Centre
(IUFD)	Intrauterine Fetal Demise
(IUGR)	Intrauterine Growth Restriction
(LH)	Luteinizing Hormone
(MoMo)	Monoamnionic/Monochorionic
(MRN)	Medical Record Number

(NICU)	Neonatal Intensive Care Unit
(PIE)	Pulmonary Interstitial Emphysema
(PMN)	Polymorphonuclear
(POC)	Products of Conception
(PPHN)	Persistent Pulmonary Hypertension
(PVE)	Periventricular Echodensities
(PVL)	Periventricular Leukomalacia
(SBGH)	St. Boniface General Hospital
(SUA)	Single Umbilical Artery
(TTTS)	Twin-Twin Transfusion Syndrome

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ABSTRACT

Systematic examination of the placenta by some has led to the publication of few well-designed studies implicating the placenta in a variety of fetal and neonatal morbidities as well as mortality. However, the majority of data is reported in either preterm pregnancies or premature neonates where it has been found to be associated with numerous clinical outcomes. This study is designed to investigate the nature and extent of pathologically significant placentas and their corresponding fetal and neonatal outcomes in term infants. In particular, we aim to examine the correlation between specific placental pathologies and a variety of neonatal clinical conditions, including brain injury, requiring admission to a hospital's neonatal care unit and subsequent follow-up in our High Risk Newborn Follow-up Program (HRFU). A retrospective cohort study was conducted through review of maternal and neonatal charts and placenta reports. Data were examined using descriptive statistics and differences were compared using χ^2 test and correlation coefficients. Overall 48 neonates had placentas submitted for examination. Sixty-one percent of placentas in the HRFU group and 62% of placentas in the non-HRFU group had findings consistent with ischemic changes, meconium staining and calcifications. Three infants with placentas submitted for examination had hypoxic ischemic encephalopathy. No infants in the study suffered neonatal stroke. This study found no difference in placental pathology between non-HRFU infants and infants enrolled in the HRFU Program. This raises questions and warrants further study on the efficacy for placental submission as a predictive measure for neonatal outcomes.

1. INTRODUCTION

The placenta is a unique multifunctional organ and therefore unlike any other organ in the human body (Redline et al., 2004). The placenta is intimately connected to two different people: the mother and the baby. Problems that affect the placenta may affect each person in different ways. Furthermore, when problems occur, mother and child will likely come under the care of two or more different physician specialists, each of whom is interested in the implications of placental pathology for a specific - and different – individual. While all physicians interested in perinatal injury have similar goals and overlap in approach of problems, there are also differences in emphasis by different specialists with reflection on the different focus of current placental research. In this thesis, our concern was on *mechanisms* of injury and the adverse *outcomes* occurring as possible result of these mechanisms.

1.1 A Brief Functional Structure of the Placenta

In the most basic of terms, Mossman (1937) described the placenta as ‘an apposition of maternal and fetal tissue for the purpose of physiological exchange.’ (Wooding & Burton, 2008). Its absence is incompatible with life and the developing fetus is unsustainable. Not only does it provide a conduit for nutrition and gas exchange, the placenta plays a vital role in metabolite synthesis, fetal protection and hormone production (Moore et al., 2008). The placenta provides the necessary nutrients for fetal growth and development including: oxygen, water, carbohydrates, lipids, amino acids, vitamins, and

minerals while removing waste products such as carbon dioxide (Gude et al., 2004). Transfer of such products is generally achieved in four ways: simple diffusion (O_2 and CO_2), facilitated diffusion (glucose), active transport (amino acids), and pinocytosis (proteins) (Moore et al., 2008).

Collectively, glucose, amino acids, and fatty acids are crucial for fetal development and deficiencies of these nutrients have altered placental nutrient transport and reduced overall fetal birth weight. Glucose is the main carbohydrate transported from the mother to fetus via the protein-mediated family of glucose transporters (GLUTs) (Belkacemi et al., edited by Kay et al., 2011). For example, GLUT1 has been found in term placentas on both the maternal and fetal surface and is thought to be a major contributor for glucose transport (Gude et al., 2004). Furthermore, GLUT4, an insulin-dependent glucose transporter is linked to glucose to glycogen conversion in response to insulin in the fetal circulation (Gude et al., 2004).

Fetal amino acid plasma concentrations are generally higher than those of the mother, indicating both active mother-to-fetus transport and placental synthesis (Moore et al., 2008). Transport of amino acids from the mother is achieved exclusively by monomeric and heterodimeric transmembrane proteins located within the placenta (Gude et al., 2004). Certain amino acids, such as glycine have been shown to be produced by the placenta via glycolytic and citric acid cycle intermediates. For example, serine derived from the fetus is converted to glycine in the placenta and transported back to the fetus (Belkacemi et al., edited by Kay et al., 2011).

Fatty acids including essential fatty acids (EFAs) and polyunsaturated fatty acids serve an important purpose in fetal development and organogenesis. Because mammals cannot synthesize EFAs, the fetus is solely dependent on the maternal dietary intake for proper development (Xu et al., 2007). The fatty acid supply may be in the form of free fatty acids, bound to serum albumin or glycerol (Gude et al., 2004; Xu et al., 2007). In the latter two, the maternal surface of the placenta contains lipoprotein lipase that hydrolyzes the albumin-fatty acid complex into free fatty acid constituents. These then are transported to the placental cells by way of simple passive diffusion (Gude et al., 2004).

Altogether, placental nutrient transport, synthesis, and metabolism act as a proverbial life line for the developing fetus.

Not only does the placenta provide nutritional support for the fetus, it also is an essential organ in the maintenance of pregnancy and maternal health. It releases hormones both into the maternal and fetal circulations including, but not limited to: estrogen, progesterone, human chorionic gonadotropin (hCG), human placental lactogen, and human chorionic thyrotropin (Moore et al., 2008). Similar to luteinizing hormone (LH), hCG is initially secreted by the placenta up to eight weeks gestation whereby it maintains the corpus luteum and therefore, prevents the onset of menstruation (Moore et al., 2008). After atrophy of the corpus luteum, the placenta produces most of the circulating progesterone, preventing uterine contraction (Gude et al., 2004) and influences maternal glucose delivery to the fetus (Belkacemi et al., edited by Kay et al., 2011). At this time, estrogen levels also rise due to placental secretion (Gude et al., 2004). So much so, that

a bilateral oophorectomy can be performed after the first trimester without compromising the pregnancy (Villaseca et al., 2004).

Human placental lactogen or chorionic somatomammotropin, is homologous to both human growth hormone and prolactin (Porterfield, 2001). Its primary role in the fetus is to modulate fetal development, stimulate the production of insulin-like growth factors, pulmonary surfactant, and has been implicated in angiogenesis (Gude et al., 2004). In contrast, small amounts of chorionic thyrotropin are released in the maternal circulation, although the effects of which are still unclear (Porterfield, 2001), it is thought to modulate maternal metabolism and other physiological processes (Gude et al., 2004).

1.2 Embryology

1.2.1 The Blastocyst

In order to evaluate placentas for clinically significant abnormalities, one needs a thorough understanding of the basic embryology and anatomy, how it changes during development, and how it translates into organ function (Redline et al., 2004). Approximately three days after fertilization, the dividing zygote (also known as the morula at this stage) containing anywhere from 12 to 32 blastomeres enters the uterus and begins to swell, forming a fluid-filled cavity called the blastocystic space (Guyton & Hall, 1997). Most of the cells contained within this mass, called a blastocyst, line the outer surface surrounding the blastocystic cavity (See Fig 1). This outer layer of cells composed of trophoblast

cells eventually gives rise to the fetal membranes and embryonic placenta (Bernischke et al., 2006). A small group of larger inner cells (embryoblast) attached to the trophoblast layer give rise to the embryo, umbilical cord and amnion (Moore et al., 2008). Furthermore, the embryoblast differentiates into a bilaminar disc containing cuboidal hypoblast cells and columnar epiblast cells (Rampersad et al., edited by Kay et al., 2011). The hypoblast cells lining the blastocystic cavity transform into endoderm forming the primitive yolk sac and the extraembryonic mesoderm, while the fluid that collects between the epiblast cells forms the amniotic cavity. The cells which line the cavity are called amnioblasts (Martini et al., 2003). Thus, the amnioblasts line the epiblast and separate the embryo from the trophoblast.

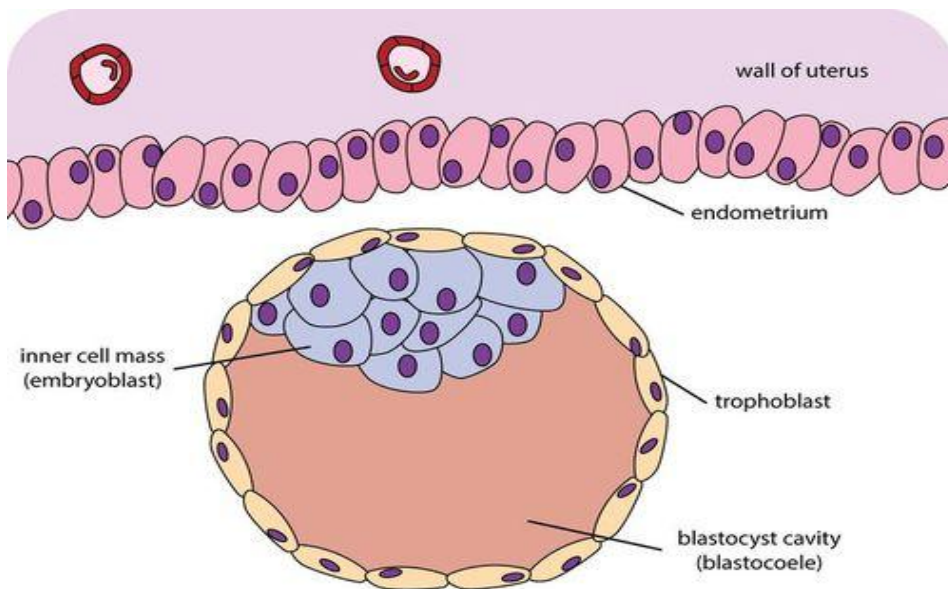


Figure 1. Blastocyst. The blastocyst consists of an outer layer of cells called the trophoblast and an inner cell mass called the embryoblast. (CK-12 Foundation, 2012).

1.2.2 Implantation

Approximately eight days after fertilization, the blastocyst consists of anywhere from 107 to 256 cells and measures approximately 0.1 X 0.3 X 0.3mm (Bernischke et al., 2006). At this time, implantation occurs and the blastocyst is usually oriented in such a way that the pole nearest the embryoblast attaches to the endometrium first (Bernischke et al., 2006). After attachment, the trophoblasts undergo rapid proliferation eventually resulting in a double layer. The outermost layer is transformed into syncytiotrophoblasts that are a result of fusion of adjacent trophoblast, while the inner layer transformed cytotrophoblasts remain unfused and act as a type of stem cell for the proliferating trophoblast (Bernischke et al., 2006).

1.2.3 Early Villous Development

As the syncytiotrophoblasts continue to multiply, they make direct contact with the maternal tissue and surround the majority of the blastocyst. Eventually, spaces within the syncytiotrophoblasts, called lacunae, develop causing a mixture of maternal blood and cellular debris from eroded uterine cells to flood the spaces, providing essential nutrients to the embryonic disc by way of diffusion (Moore et al, 2008). As more and more lacunae fuse, forming a lacunar network, the syncytiotrophoblast appear sponge-like, and this developing network will become the intervillous space of the placenta (Ramparsad et al., edited by Kay et al., 2011).

Approximately 12 to 13 days after fertilization, proliferation of the cytotrophoblasts into the syncytiotrophoblasts is seen and subsequent blind-ended

branches protrude into the lacunar space. These primary villi mark the development of the villous tree of the chorion (Bernischke et al., 2006). In turn, the primary villi become infiltrated with mesenchymal cords from the extraembryonic mesoderm forming the secondary villi. Furthermore, the innermost layer of the mesoderm gives rise to villous blood vessels, forming tertiary villi (Faye-Petersen et al., 2006). Initially, the villi cover the entire blastocyst but later regress, covering only the most deep-seeded portion eventually forming the placental disc (Cunningham et al., 2010). Thus, the trophoblast and extraembryonic mesoderm develops into the vascular chorionic plate and the villous tree.

1.2.4 The Decidua

Successful implantation and pregnancy maintenance is largely dependent upon the maternal endometrium. The decidua, or gravid endometrium, experiences significant changes in response to increased progesterone levels in the maternal blood (Moore et al., 2008). In particular, endometrial stromal cells and interstitial cells undergo marked hypertrophy due to increased lipid and glycogen accumulation within their cytoplasm (Rampersad et al., edited by Kay et al., 2011).

The decidua contains three distinct layers: (1) the decidua basalis forms deep to the implanted blastocyst and forms the maternal part of the placenta; (2) the decidua capsularis is superficial and overlies the entire blastocyst and; (3) the

decidua parietalis is the remaining non-placental lining covering the uterus (Moore et al., 2008).

1.2.5 Development of the Chorion and Amnion

Chorionic villi from villous trophoblasts and anchoring cytotrophoblasts rapidly proliferate along the decidua basalis forming the chorionic plate, or chorion frondosum (leafy chorion). As the embryonic sac continues to grow the blood supply to villi in contact with the decidua capsularis are compressed and atrophy. As a result, this avascular chorion comprises the chorion laeve (smooth chorion) and constitutes the fetal membrane that abuts the decidua parietalis (Faye-Petersen et al., 2006; Cunningham et al., 2010). By about 12 weeks gestation, the amniotic sac enlarges faster than the outer chorionic sac resulting in the fusion of the amnion and the chorion laeve (Faye-Petersen et al., 2006). This new chorioamnion membrane eventually fuses with the decidua capsularis. As the fetus continues to grow, the decidua capsularis intimately attaches with the decidua parietalis, essentially obliterating the uterine cavity (Cunningham et al., 2010). It is this chorioamnionic membrane that ruptures during labor, allowing expulsion of the fetus and placenta.

1.2.6 Spiral Artery Invasion

Formation of the utero-placental circulation is achieved by trophoblastic invasion of the uterine spiral arteries. Two populations of trophoblast exist: villous trophoblasts, which give rise to the chorionic villi, and extravillous

trophoblasts, which invade the adjacent decidua and myometrium (Wang & Zhao, 2010). The latter can be further classified into interstitial and endovascular. Although their function is not yet fully understood, interstitial trophoblasts invade the underlying decidua and surround the spiral arteries. It is thought that they prepare the arterial walls for endovascular trophoblast invasion (Cunningham et al., 2010). As endovascular trophoblasts invade the spiral arteries, they interdigitate with the arterial endothelial cells, forming cellular plugs, hence, removing the majority of endothelium and elastic tissue and replacing it with fibrinoid material, creating a low resistance, high flow vessel (Wang & Zhao, 2010; Cunningham et al., 2010).

1.3 Placental Circulation

While the embryo is nourished in the first weeks through simple diffusion, later, due to its rapid growth, it needs a more powerful gas and nutrient exchange system. This is made possible by the development of the utero-placental circulation system in which the circulation systems of the mother and of the embryo get closer together, thus allowing an exchange of gases and metabolites via diffusion. Of note, maternal and fetal blood never comes into direct contact with each other. In general terms, blood flow from the fetus travels through paired umbilical arteries and returns in a single umbilical vein and the chorionic villi act as the interface between fetal and maternal circulations (Martini et al., 2003).

1.3.1 Fetal Placental Circulation

At the site of attachment of the umbilical cord to the placenta, there exists a vast network of radially disposed chorionic arteries that arise from the paired umbilical arteries. These chorionic arteries branch further in the chorionic plate before entering the chorionic villi (Moore et al., 2008). Within the chorionic villi, the blood vessels form an extensive arteriocapillary-venous network which brings the fetal blood extremely close to the maternal blood, whereby the exchange of metabolic and gaseous products may be achieved (Moore et al., 2008). The oxygenated fetal blood in the capillaries passes into thin-walled veins that follow the chorionic arteries to the site of attachment of the umbilical cord. These veins converge to form the umbilical vein whereby oxygenated blood enters the fetus (Moore et al., 2008).

1.3.2 Maternal Placental Circulation

Maternal blood enters the placenta in the basal plate via the spiral arteries of the decidua and perfuses the intervillous space (Wang & Zhao, 2010). It is estimated that 80 to 120 spiral arteries feed the intervillous space at term (Moore et al., 2008). The blood entering the intervillous space from the spiral arteries is at a considerably higher pressure (70mmHg) than that of the intervillous space (10mmHg) (Moore et al., 2008; Wang & Zhao, 2010). This pressure gradient allows maternal blood flow to perfuse the intervillous space effectively allowing an exchange of metabolic and gaseous products with fetal blood two to three times per minute (Wang & Zhao, 2010). Maternal blood eventually drains back

through venous orifices in the basal plate and into the maternal systemic circulation via uterine veins (Wang & Zhao, 2010).

1.4 Gross Placental Anatomy

In over 90% of cases, the full-term, delivered placenta is a disk-like, flat, round to oval organ with extraplacental free membranes and attached umbilical cord (Bernischke et al., 2006; Faye-Petersen et al., 2006). The average diameter is 22 cm, 2.5 cm in thickness at the centre of the organ and weighs approximately 470 g (Bernischke et al., 2006). However, there is considerable variation in the size and shape of each placenta depending on factors such as: mode of birth, time of cord clamping, and time elapsed between delivery and examination (Bernischke et al., 2006).

1.4.1 Fetal Placental Surface

The fetal surface consists of the chorionic plate which in turn is covered by the amnion (Bernischke et al., 2006). The glossy appearance of the amnion is due the single layered epithelium and the avascular amnionic mesenchyme (Huppertz, 2008). This thin amnionic mesenchyme is only loosely attached to the chorion and is easily removed from the delivered placenta (Huppertz, 2008). Underlying the amnion, the chorionic vessels arise from the umbilical cord insertion site, branch in a star-like pattern over the fetal surface and eventually to their terminal branches, which supply the villous tree (Bernischke et al., 2006). It

is interesting to note, that where arteries and veins cross, the arteries are usually closer to the amnion (Bernischke et al., 2006).

1.4.2 Maternal Placental Surface

The maternal (uterine) surface of the placenta is essentially an artificial surface disrupted from what Bernischke et al. (2006) term the *junctional zone*. The separation of the junctional zone between the placenta and uterine wall results in (1) the basal plate, which is attached to the placenta, and represents the maternal surface, and (2) the placental bed, which remains in the uterus (Bernischke et al., 2006). The basal plate consists of a large mixture of cells including fetal trophoblasts, uterine decidua, extracellular matrix, fibrinoid, and blood clot (Huppertz, 2008). The basal surface of the placenta is subdivided by a multitude of grooves into slightly elevated areas termed cotyledons (Bernischke et al., 2006). These grooves coincide with the placental septa which project into the intervillous space. Furthermore, each cotyledon has been shown to house one to four villous trees (fetal cotyledons) arising from the chorionic plate (Bernischke et al., 2006).

At the placental margin, the chorionic and basal plates merge, forming the smooth chorion and hence, the fetal membranes, which consists of the amnion, chorion, and the decidua capsularis (Huppertz, 2008).

1.5 Umbilical Cord Structure

The umbilical cord, or funis, extends from the fetal umbilicus to the fetal surface of the placenta or chorionic plate (Cunningham et al., 2010). Although great variation in cord length exist (24 cm to 146 cm), the average length is approximately 59 cm and its diameter ranges from 0.8 to 2.0 cm thick (Bernischke et al., 2006). Its exterior surface is dull white and covered by amnionic epithelium. Proximally, it is made up of stratified squamous epithelium providing a transition between fetal abdominal wall and cord surface which eventually transforms to columnar epithelium farther from the umbilicus (Bernischke et al., 2006).

The extracellular connective tissue within the cord, or Wharton's jelly, is composed of polysaccharides, carbohydrates, collagens and microfibrils (Bernishcke et al., 2006). This jelly-like material liquefies when touched and has been likened by Reynolds (1952) to erectile tissue as it compresses with distended fetal vessels and expands as the vessels contract (Bernischke et al., 2006). Thus, the cord is a relatively firm, rigid structure and varies in turgidity and thickness according to vasculature expansion and contraction.

The umbilical cord usually has two arteries and one vein surrounded by Wharton's jelly. Oxygenated blood from the mother flows to the fetus via the umbilical vein and enters the inferior vena cava from either the ductus venosus or hepatic vein. Conversely, deoxygenated blood exits the fetus through the umbilical arteries from branches of the internal iliac artery (Cunningham et al., 2006). Since the vessels are longer than the cord itself, bending and twisting of

the vessels are common often forming loops and false knots with little clinical significance (Moore et al., 2008), unless there is a blockage of circulation (i.e. thrombus).

1.5.1 Cord Insertion Sites

The umbilical cord normally inserts on the fetal surface near or at the centre of the placenta (central or eccentric) (Faye-Petersen et al., 2006; Lester, 2010). However, velamentous (approximately 1%) and marginal (approximately 7%) insertion is possible, both associated with an increased risk of adverse outcomes (Bernischke et al., 2006; Faye-Petersen et al., 2006). Particularly in velamentous insertion, the cord vessels course the free membranes and are unprotected by Wharton's jelly (Faye-Petersen et al., 2006). These vessels are vulnerable to compression and thrombosis. Moreover, vasa previa is more common in velamentous inserted cords creating a greater risk of rupture and possible fetal exsanguination (Faye-Petersen et al., 2006). Similar to velamentous insertion, furcate inserted cords lack the protection of Wharton's jelly in which the umbilical vessels separate from the cord prior to reaching the surface of the placenta (Bernischke et al., 2006). Although the position of insertion is usually normal, furcate cords are also vulnerable to compression, rupture and thrombosis (Faye-Petersen et al., 2006).

1.6 Placental Histology

As the placenta develops, it reveals various normal histological changes, most of which can be identified by its varying villous structure (Bernischke et al., 2006). Regardless of the age, a typical full thickness section through the placenta will contain the chorionic plate, chorionic villi and intervillous space, decidua (basal) plate, and in some rare cases (i.e. placenta accreta), myometrium (Cunningham et al., 2010).

In the first trimester placenta, the villi are large and project into the intervillous space which is filled with maternal blood (Young, Lowe, Stevens, & Heath, 2006). At this stage, the core of each villous is predominately composed of loose mesenchymal tissue, fixed connective tissue, macrophages, and fetal blood vessels most of which are filled with nucleated red blood cells (Young et al., 2006) (See Fig 2). Surrounding the villi are an inner layer of cytotrophoblast and an outer layer of syncytiotrophoblast cells. Cytotrophoblasts contain a large single nucleus, are well demarcated and have a slight basophilic cytoplasm, whereas syncytiotrophoblasts are ill defined, contain multiple nuclei and stain eosinophilic (Young et al., 2006). In the first and second trimesters, these mesenchymal villi give rise to immature intermediate villi which later develop into stem villi (Ross & Pawlina, 2010). The immature intermediate villi demonstrate a bulbous contour and have a thick trophoblastic cover. In addition, the stromal core is edematous and contains Hofbauer cells (Baergen, 2005). In the early villous stages, anchoring villi can be seen at the periphery connected to the basal plate (Bernischke et al., 2006).

The second trimester placenta is predominately occupied by immature intermediate villi transitioning into stem villi (See Fig 3) (Ross & Paulina, 2010). These stem villi comprise the central branches of the villous tree that connect the chorionic plate with the villous tree (Faye-Peterson et al, 2006). Similar to immature intermediate villi, stem villi contain a pronounced trophoblastic shell surrounding a stroma filled with condensed fascicles of collagen and the occasional macrophage and fibroblast (Faye-Peterson et al, 2006). In larger stem villi, a central muscular artery and vein are often present surrounded by small vessels which nourish the stem villi (Ross & Pawlina, 2010). Stem villi make up approximately 20-25% of the total villous volume of a term placenta (Faye-Petersen et al., 2006).

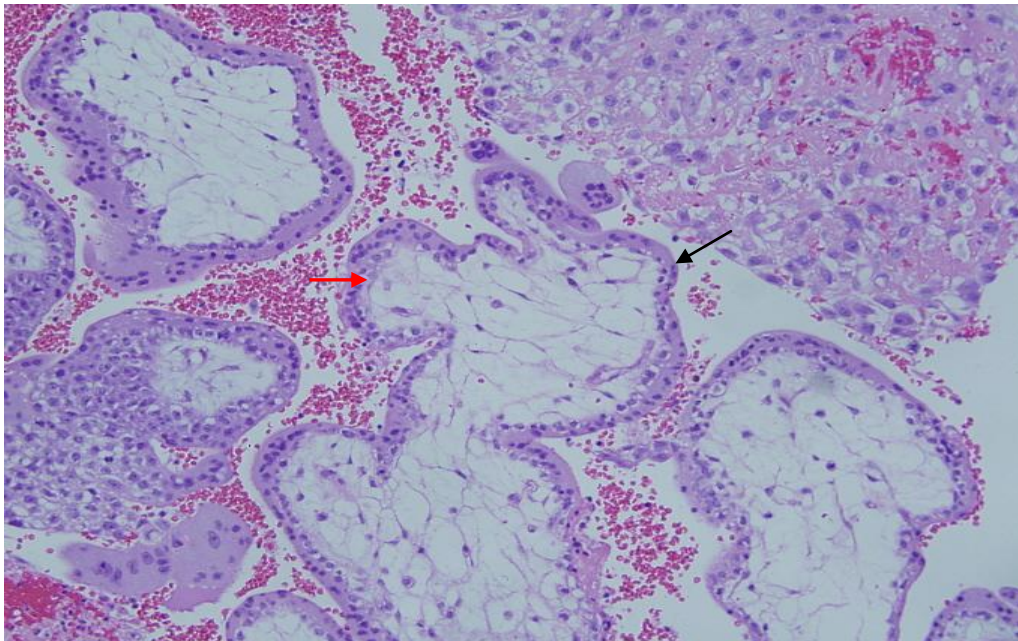


Figure 2 First trimester placenta. Early villous structure with multinucleated syncytiotrophoblasts and cytotrophoblasts. Maternal intermediate cytotrophoblasts are seen in the top right. Note the large villi absent of capillaries. Black arrow denotes syncytiotrophoblast cells; Red arrow denotes cytotrophoblast cells.

In the third trimester placenta, the villi are highly developed and the average villous diameter is much smaller as they consist largely of mature intermediate villi and terminal villi (Young et al., 2006) (See Fig 4). The predominating features are the large fetal capillaries that often extend to the periphery of the villi and the marked decrease in the number of cytotrophoblast cells (Bernischke et al., 2006). As a result, syncytiotrophoblast cells predominate and their nuclei form 'syncytial buds' that often appear as 'clumps of dirt' (Bernischke et al., 2006).

Thus, there are five main villi subtypes that are categorized based on their appearance during gestation, caliber/size, presence of fetal vasculature and hierarchy within the villous tree: (1) mesenchymal villi; (2) immature intermediate villi; (3) stem villi; (4) mature intermediate villi; and (5) terminal villi (Baergen, 2005). In the first and second trimesters mesenchymal villi give rise to immature intermediate villi and stem villi. In the third trimester mesenchymal villi give rise to mature intermediate villi which in turn develop into terminal villi (Baergen, 2005). Hence, all villi arise from mesenchymal villi. At term, the placenta is comprised of approximately 40-50% terminal villi, 25% mature intermediate villi, 20-25% stem villi, 5-10% immature intermediate villi, and less than 1% mesenchymal villi (Baergen, 2005)

The amnion, which covers the chorionic plate, consists of a single layer of cuboidal to columnar cells that cover the amnionic mesoderm. Furthermore, the amnionic layer is separated from the chorionic plate by a spongy layer (subamnionic space) (Bernischke et al., 2006). The chorionic plate is a

multilayered structure that consists of a spongy layer followed by a compact layer of chorionic mesoderm (Bernischke et al., 2006). Varying quantities of acellular, eosinophilic fibrin that invade the intervillous space may also be seen in term placentas (Bernischke et al., 2006).

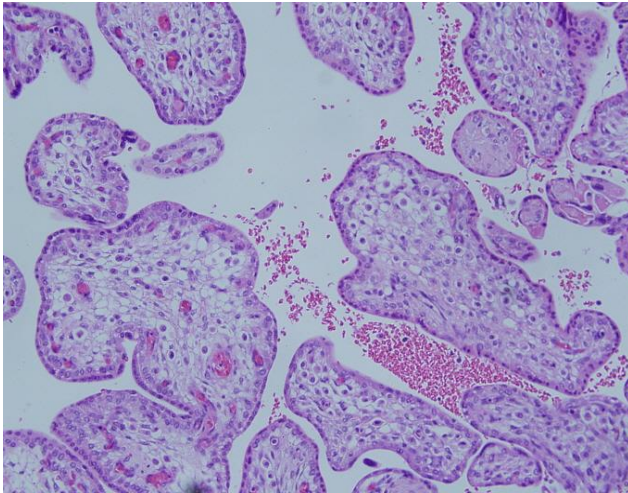


Figure 3 Second trimester placenta. Compare villi with those in first trimester. Capillaries are present and villi appear smaller.

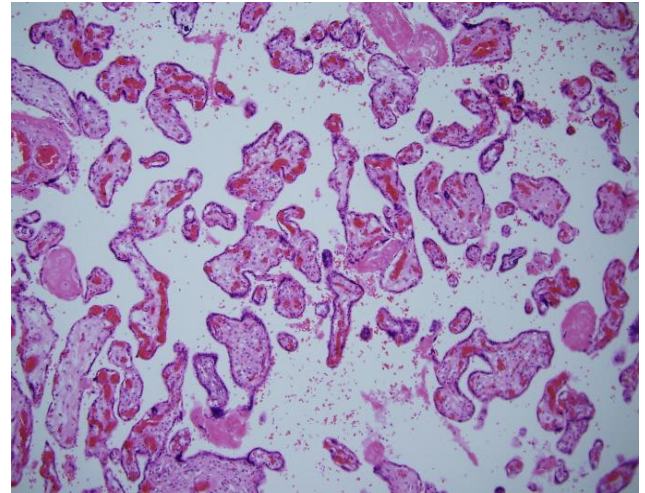


Figure 4 Third trimester villi. Villi are highly vascularized and mature.

1.7 Pathological Conditions of the Placenta

Pathologies of the placenta cover a wide spectrum of diseases that have varying effects on mother and fetus – some insignificant, others fatal. Placental pathologies may be grouped as: (1) disorders of placental development; (2) inflammation and infection; (3) circulatory problems, (4) pathology of the membranes; (5) pathology of the umbilical cord; (6) tumour like conditions and neoplasms and, (7) multiple pregnancy.

1.7.1 Disorders of Placental Development

Implantation and placentation of the developing fetus normally is at the site of the superior-posterior uterine wall (Moore et al., 2006). In cases of placenta previa however, the placenta lies in the lower uterine segment and covers the cervix either completely or partially (Faye-Petersen et al., 2006). Complete placenta previa covers the entire internal cervical os, whereas partial placenta previa has the inferior edge of the placenta partially overlapping the internal cervical os and is adjacent to or within 2 cm of the internal cervical os in marginal placenta previa (Rao et al., 2012). In all cases, there is substantial risk of severe maternal hemorrhage, and since the placenta precedes the fetus, vaginal delivery is almost always ruled out (Faye-Petersen et al., 2006).

A placenta creta - accreta, increta, or percreta - is a placenta that grows during pregnancy into or through the uterus. Having this condition is life-threatening and requires expert surgical and medical care. During pregnancy, the placenta attaches to the uterine wall and is separated from the uterus by the Nitabuch fibrinoid layer. Sometimes though, the Nitabuch fibrinoid layer is thinned or missing, and the placenta will attach itself too deeply into the uterine wall, resulting in a placenta creta. Depending on how deep the placenta invades the uterus, placenta creta presents itself in three different forms: Placenta accreta, increta and percreta.

Placenta accreta is the abnormal attachment of the placenta to the uterine wall which may involve a portion or the entire maternal placental surface (Faye-Petersen et al., 2006). It involves trophoblastic invasion beyond the decidua

basalis and hence, attachment of chorionic villi to the uterine wall due to the absence of the Nitabuch fibrin layer between the placenta and uterus (Rao et al., 2012). Invasion of chorionic villi into the myometrium is termed placenta increta, while invasion through myometrium and beyond the uterine serosa is referred to as placenta percreta (Rao et al., 2012). In placenta percreta, there is often extension to neighbouring organs such as the bladder and bowel (Faye-Petersen et al., 2006). Generally in all placental attachment disorders 75% are placenta accreta, 18% placenta increta, and 7% placenta percreta (Rao et al., 2012). Hysterectomies are usually required to prevent or control life threatening hemorrhage, up to 91% of the time (Faye-Petersen et al., 2006).

1.7.2 Inflammation and Infection

Prenatal infections are a common and important aspect to placental pathology (Bernischke et al., 2006). Different infectious agents have been implicated in producing distinct patterns of inflammation in the placenta (Varli et al., 2012). In general, bacterial agents produce acute inflammation of the placental membranes, such as acute chorioamnionitis (ACA), while viral infections are associated with chronic inflammation of the placental parenchyma, most notably chronic villitis (Varli et al., 2012).

Histologically, ACA consists of the presence of an inflammatory exudate of polymorphonuclear (PMN) leukocytes located in the amnion, subchorionic space, vessels of the chorionic plate or umbilical cord (Faye-Petersen et al., 2006; Bernischke et al., 2006) (See Fig 5). Generally, PMNs seen in the amnion,

chorionic plate, and subchorionic space are of maternal origin, while those seen in the chorionic vessels and umbilical cord are of fetal origin (sometimes referred to as vasculitis; (See Fig 6) (Faye-Petersen et al., 2006). Grossly, the placental surface appears opaque and yellow and the amnion may be roughened or lost its normal lustre (Bernischke et al., 2006). Clinically, chorioamnionitis often presents with fever, leukocytosis, uterine tenderness and foul-smelling amniotic fluid (Faye-Petersen et al., 2006). Various bacterial organisms in the maternal genital tract have been implicated in ACA, such as group B *Streptococcus*, *Escherichia coli*, and *Staphylococcus* (Faye-Peteresen et al., 2006).

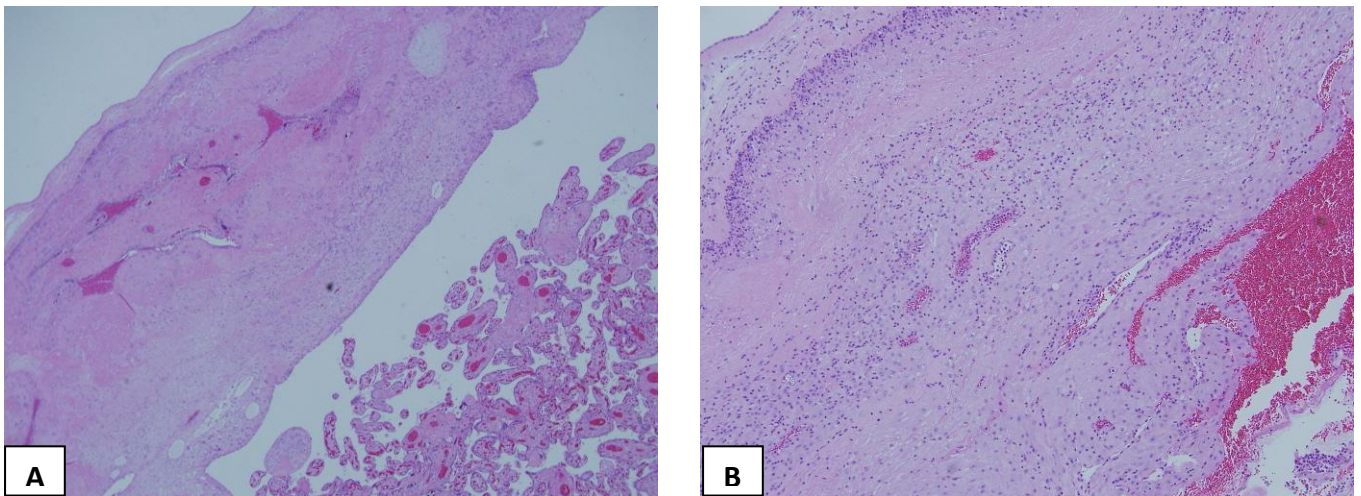


Figure 5. Severe acute chorioamnionitis. (a) Low power magnification of the chorion, sub amniotic space, and amnion. The entire thickness of the chorion is infiltrated by neutrophilic leukocytes. (b) High power microphotograph demonstrating neutrophilic infiltration of the amnion (top left).

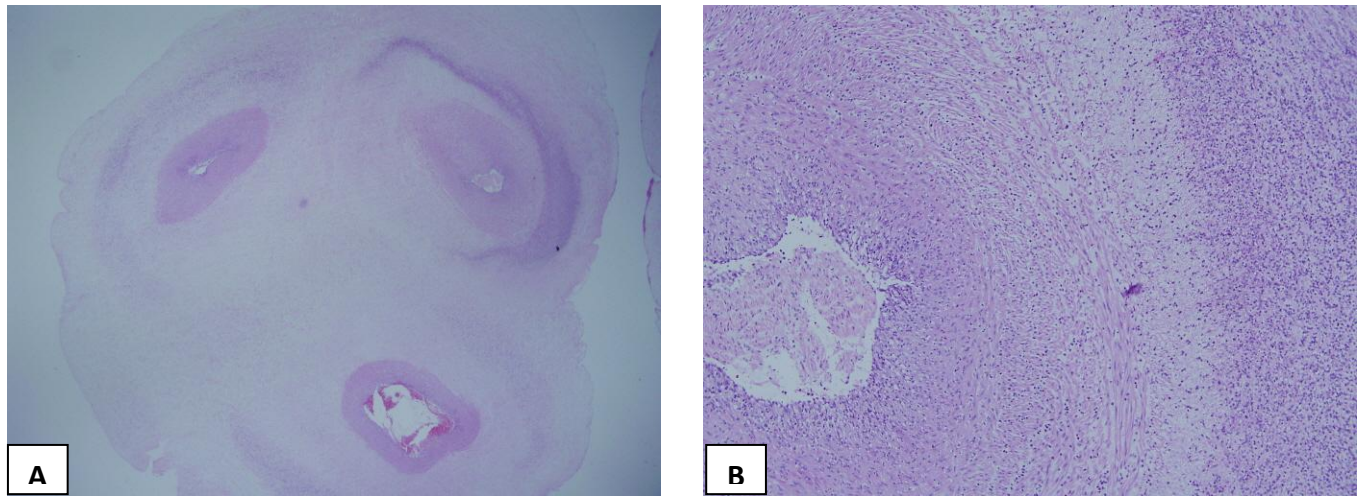


Figure 6. Severe umbilical cord vasculitis and funisitis. (a) low power magnification depicting neutrophilic infiltration of the umbilical arteries. Note the inflammation is localized near the amnion, away from the centre of the umbilical cord. (b) high power magnification featuring the umbilical artery with extensive infiltration into Wharton's jelly, a key feature in funisitis.

Unlike ACA, where transmission is primarily through ascending infections, villitis reflects hematogenous transmission of pathogens from the maternal blood to the chorionic villi (Faye-Petersen et al., 2006). Several classifications exist, depending on the infectious agent and the histological pattern: (1) proliferative villitis – contain varying populations of lymphocytes, histiocytes and plasma cells. This is usually seen in cytomegalovirus (CMV), rubella, and treponemal infections (Faye-Petersen et al., 2006) (See Fig 7); (2) necrotizing villitis – chorionic villous necrosis. Characterized by *Listeria monocytogenes* infection (Faye-Petersen et al., 2006); (3) granulomatous villitis – formation of granulomas (giant cells, epithelioid cells, and lymphohistiocytes). Infections due to *Toxoplasma*, *Mycobacterium*, and fungal infections may cause this (Faye-Petersen et al., 2006); (4) reparative villitis – characterized by the formation of

granulation tissue and fibroblast proliferation. This is often seen in later stages of villitis due to CMV and syphilis infection (Faye-Petersen et al., 2006).

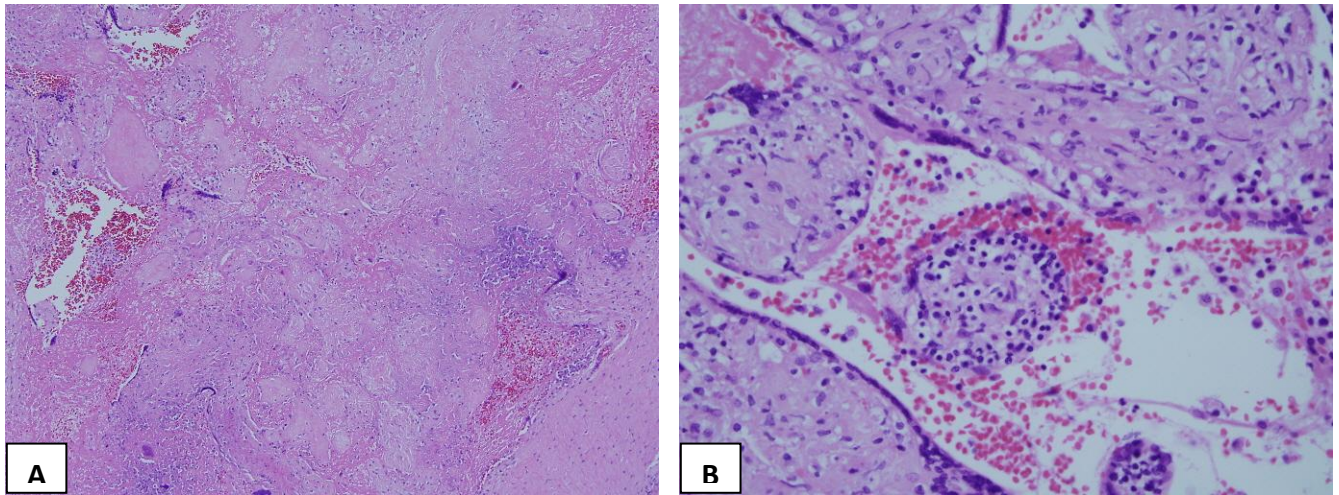


Figure 7. Villitis and intervillitis. (a) low power microphotograph demonstrating obliterated intervillous space with necrotizing inflammation and clumping of villi. (b) high power microphotograph demonstrating intervillous neutrophilic infiltration.

1.7.3 Circulatory Problems

The placenta is essentially a vascular organ that provides a conduit for the flow of maternal blood from the uterine arteries to the placenta villi in the intervillous space (Redline, 2009). For it to function properly, the placenta depends on the integrity of the uterine arteries, adequate flow of maternal blood into the intervillous space, sufficient diffusion of maternal blood through the villous trophoblast layer, integrity of larger capillaries in the villous tree, and adequate flow in the umbilical cord (Redline, 2009). Thus, placental circulatory abnormalities are too numerous to describe herein, and only those with important sequelae will be discussed further.

One of the most common maternal conditions associated with placental abnormalities is that of pre-eclampsia (Bernischke et al., 2006). Clinically, pre-

eclampsia is defined as pregnancy induced hypertension (PIH) with a systolic pressure of ≥ 140 mmHg and/or a diastolic pressure of ≥ 90 mmHg in pregnancies over 20 weeks with proteinuria of 0.3g or more in a 24 hour urine collection (Faye-Petersen et al., 2006). One of the hallmark lesions of PIH is underperfused/narrow undilated spiral arteries in the decidua that leads to ischemia and eventually regional or global infarction (Faye-Petersen et al., 2006). Thrombosis of the spiral arteries leads to the absence of blood in the intervillous space, as a result, the villi collapse on each other, undergo fibrinoid necrosis and lipid-laden macrophages (atherosis) are often present in the vessel wall (Roberts, 2008; Faye-Petersen et al., 2006) (See Fig 8). Small villi, abundant syncytial 'knots', and large intervillous spaces are characteristic of chronic ischemia (Roberts, 2008). Grossly, placentas from pre-eclamptic mothers are often of reduced size and contain infarcts involving more than 5% of the placenta (Faye-Petersen et al., 2006).

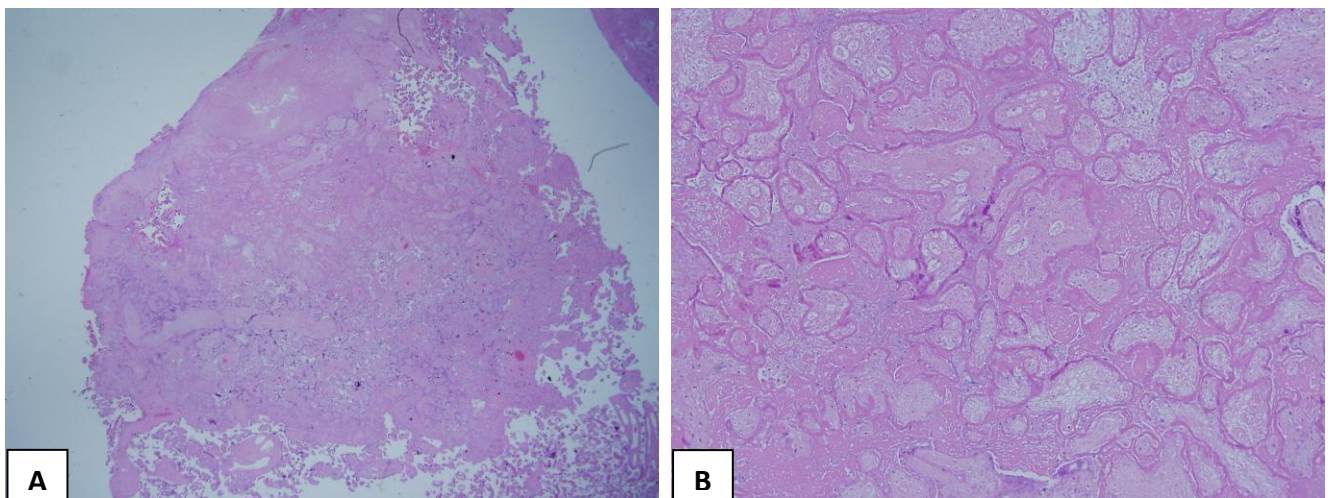


Figure 8. Chronic infarction. (a) loss of intervillous space and villous crowding can be seen. (b) increased perivillous fibrin deposition between the intervillous spaces is also evident. Nuclear chromatin of the villi clump together and eventually fade leaving an eosinophilic ghost-like outline of the villi.

Furthermore, atherosclerosis of maternal decidual arteries may often rupture causing a retroplacental hematoma (Faye-Petersen et al., 2006). Grossly, the lesions involve variable amounts of the maternal surface. New, fresh hematomas appear bright red and have a soft consistency, often they can easily be removed from the maternal surface (Faye-Petersen et al., 2006). In contrast, older hematomas (See Fig 9) have a firm, red-brown appearance and are firmly adhered to the maternal surface, often producing a depression or crater (Faye-Petersen, et al, 2006).

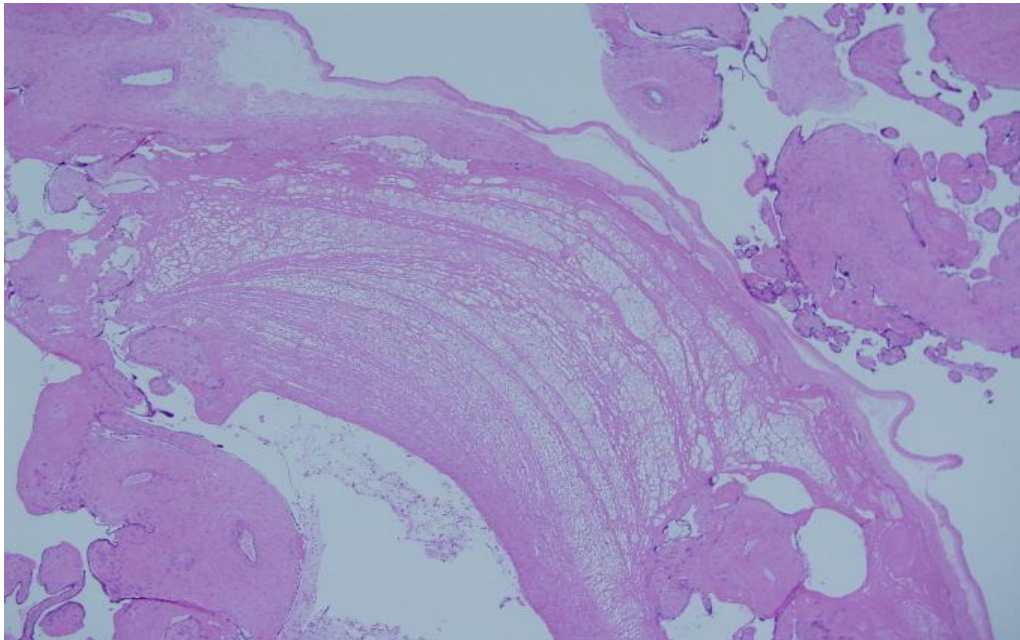


Figure 9. Subchorionic organizing hematoma. Note the presence of the lines of Zahn and multiple fibrin strands.

Interestingly, changes in the placenta due to hypoxemia do not produce the same characteristic lesions of those described due to ischemia (Roberts, 2008). Instead, hypoxemic placentas often show signs of marked hypervascularity, or chorangiosis, which is characterized by the 'rule of ten' (Faye-Petersen et al., 2006). That is, ten or more villi, that each contain ten or more capillaries are observed under a X10 power field (Bernischke et al., 2006). It is often seen in mothers with long standing diabetes mellitus, high altitude pregnancies and Rh incompatibility (Faye-Petersen et al., 2006).

Other lesions that impede conductance of blood and nutrients from the intervillous space to the trophoblast epithelium include fibrin or fibrinoid deposition on the surface of trophoblast cells (Redline, 2009). Known as perivillous fibrin deposition; these lesions are often idiopathic and are associated with adverse fetal outcomes including, stillbirth, miscarriage, intrauterine growth restriction, prematurity, and neurological disturbances (Redline, 2009). Grossly, these placentas are characterized by a mature, firm, marbled appearance that, under microscopic examination demonstrate significant areas of chorionic villi that are entrapped with fibrin, with obliteration of the intervillous space (See Fig 10). Massive perivillous fibrin deposition signifies that more than 70% of the placental parenchyma is involved by this process.

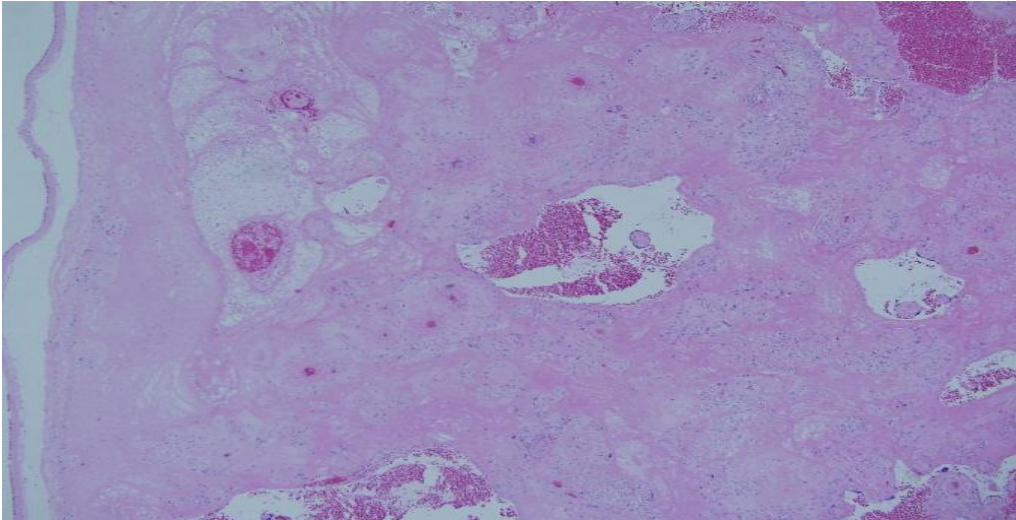


Figure 10. Massive perivillous fibrin deposition. Villi are involuted and clumped together. The villi are outlined by a scant eosinophilic fibrin material.

Fetal circulatory lesions possess characteristic pathologies that differ from maternal vascular abnormalities mainly by the type of vessels that are affected; and almost all are thromboinflammatory (Roberts, 2008). That is, large fetal vessels in the chorionic plate and stem villi are the most common site of injury of which, the intervillous spaces are patent but the surrounding villi are devoid of fetal blood and functioning vessels (Roberts, 2008). This perfusion pathology, known as fetal thrombotic vasculopathy (FTV), is defined by extensive avascular villi downstream from a vascular occlusion (Redline, 2009) (See Fig 11). The diagnostic criteria of such lesions are made when an average of 15 or more avascular villi are observed per slide with associated upstream thrombotic vessels in one third of cases (Redline, 2009). Several risk factors including thrombophilic mutations and diabetes have been suggested, however, only abnormalities of the umbilical cord have been directly associated with this disease (Redline, 2009). Another common thromboinflammatory lesion is

meconium-associated vascular necrosis (see Pathology of the Membranes). Here, prolonged exposure to meconium leads to apoptosis of vascular smooth muscle cells and vasospasm. As a result, the blood vessels are often rendered flaccid, resulting in vessel collapse and interrupted blood flow (Roberts, 2008; Redline, 2009). Other fetal thromboinflammatory lesions include those with infectious elements such as chorioamnionitis, vasculitis, and villitis (see Inflammation and Infection).

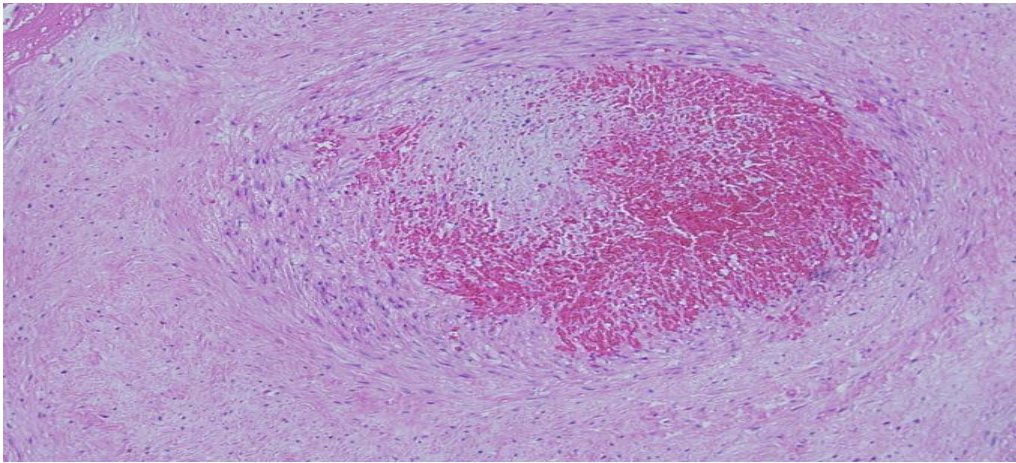


Figure 11. Fetal thrombotic vasculopathy. The chorionic plate vessel is distended and the vessel wall contains fibrinoid necrosis. The lumen is largely obliterated.

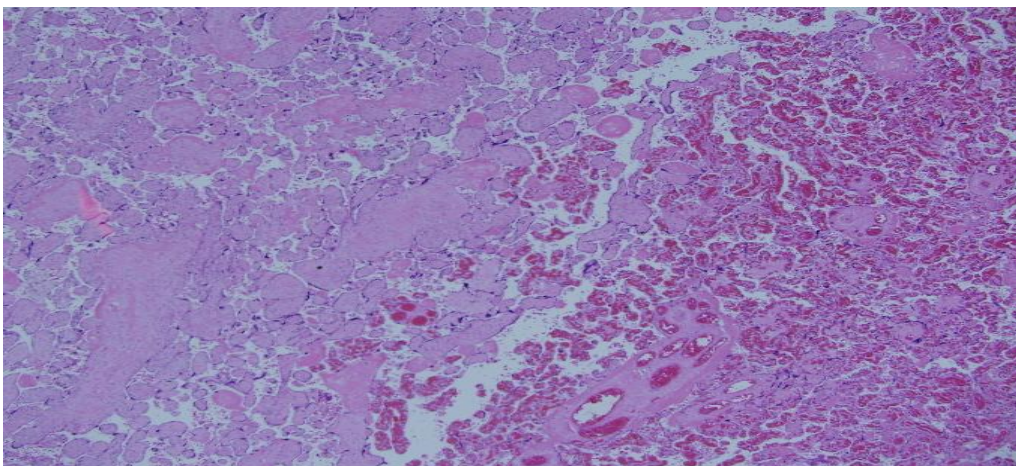


Figure 12. Late stage fetal thrombotic vasculopathy. A large focus of fibrotic, avascular villi can be seen to the left of hypervascularized villi distinct to chorangiosis due to upstream arterial occlusion.

1.7.4 Pathology of the Membranes

The placental membranes, including the amnion and chorion laeve serve as a watery containment zone for the developing fetus. Although the amniotic fluid volume changes dramatically during pregnancy, as much as 770mL of amniotic fluid surrounds the fetus at one time (Beall & Ross, edited by Kay et al, 2011).

Several lesions of the membranes exist including: squamous metaplasia, amniotic web, amniotic cysts, embryonic rests, polyps, and amnion nodosum. Although all have varying degrees of clinical importance, only the later will be discussed in the present paper. Amnion nodosum is usually indicative of fetal genitourinary anomalies and renal agenesis (Bernischke et al., 2006) (See Fig 13). Most commonly though, it is seen in cases complicated with oligohydramnios due to premature membrane rupture.

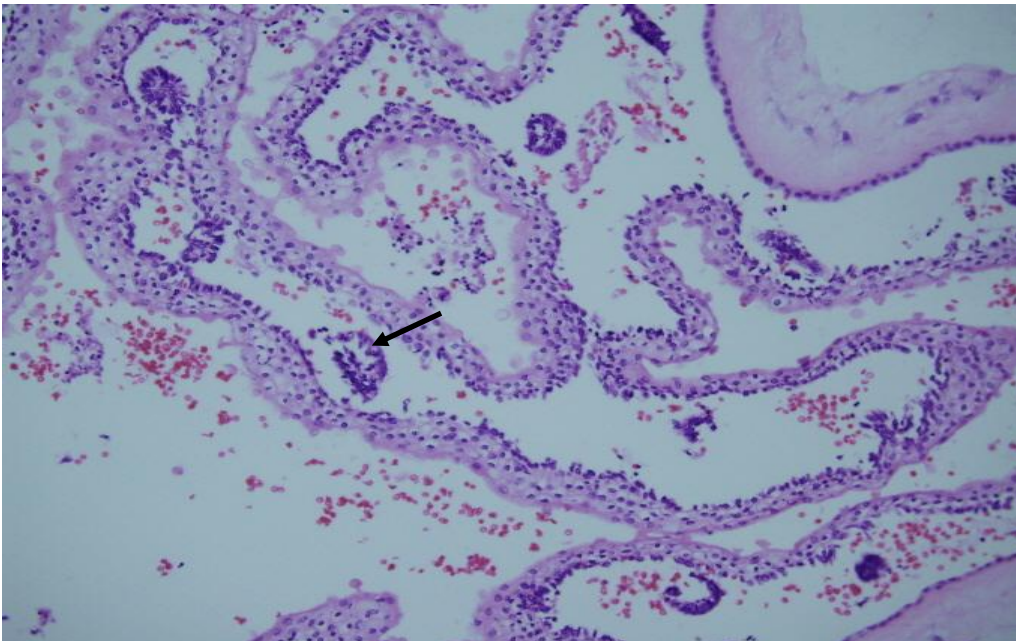


Figure 13. Squamous metaplasia and amnion nodosum. The cuboidal amniotic layer is replaced by a multi layer of keratinized squamous epithelium. Small nodular blebs can be seen (arrow) consistent with amnion nodosum.

Grossly, the placenta contains multiple small, yellow-white nodules (1-5mm) that are usually detected on the fetal surface but may also extend on to the free membranes and rarely, on to the umbilical cord (Faye-Petersen et al., 2006). These lesions generally develop during late fetal life and are composed of squames (flakes of skin), sebum and hair (Bernischke et al., 2006).

During gestation, the fetus may pass meconium, a bile-stained intestinal substance usually admixed with mucus contained within the small bowel of the fetus. Normally, the meconium is eliminated after birth but occasionally may be passed in utero but usually not before 30 weeks gestation (Faye-Petersen et al., 2006). Recent meconium discharge is often evidenced by a dark pea-green thick or thin amniotic fluid or as staining of the amnionic surface of the placenta, while old meconium is paler in colour (Faye-Petersen et al., 2006). An increased duration of exposure of the amnion to the meconium spillage leads to the progression of amnion and chorionic plate pigmentation and eventually infiltration of the large fetal vessels (Redline, 2006) (See Fig 14). It is estimated that when meconium is evident across the placental chorion and deep within the umbilical cord, fetal intestinal discharge took place at least two and a half hours prior to delivery (Altshuler & Hyde, 1989). Furthermore, Altshuler & Hyde (1989) demonstrated that meconium-washed umbilical veins showed substantial vasoconstriction within five minutes of exposure. In addition, they surmised that these changes in fetal vascular tissue aided in fetal cerebral hypoperfusion and fetal hypoxia. In some cases, the noxious effects of meconium substances, such as bile acids, can lead to degenerative changes of the epithelium and apoptosis

of the vascular smooth muscle cells of the chorionic and umbilical vessels (Bernischke et al., 2006). These findings have been strongly implicated with adverse fetal outcomes including cerebral palsy (Redline, 2006).

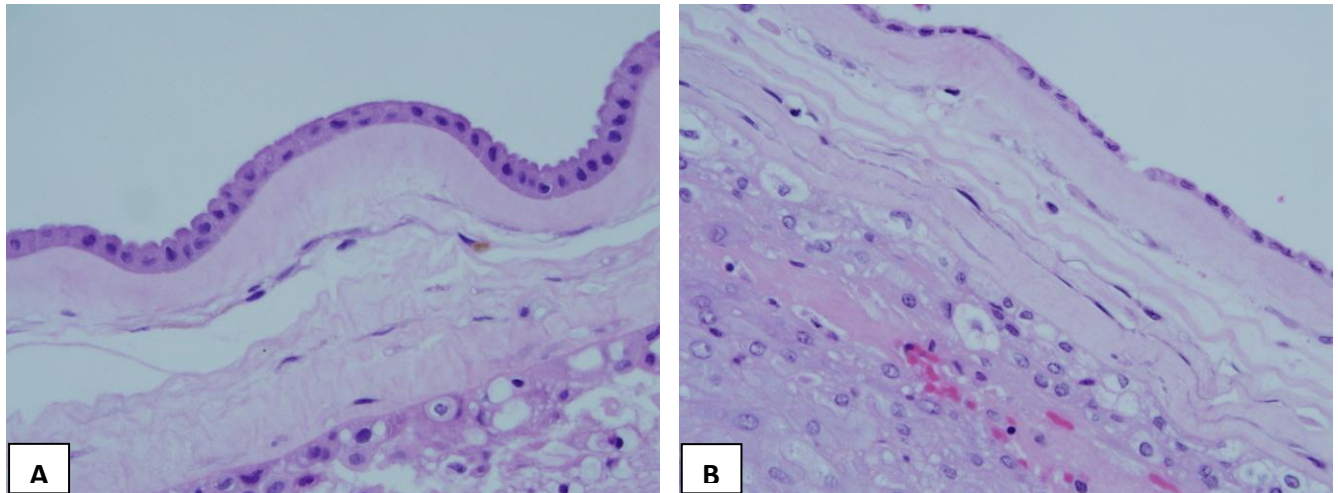


Figure 14. Recent meconium exposure of the amnion. (a) meconium-laden macrophage, stained brown-red. (b) macrophages are plump and elongated from meconium exposure. Prolonged meconium exposure would involve the appearance of meconium pigmented macrophages in the superficial and deep layers of the chorion.

1.7.5 Pathology of the Umbilical Cord

The most common pathologies associated with the umbilical cord are those that involve mechanical factors leading to cord compression, such as cord length, cord insertion (see Cord Insertion Sites), knots, entanglement, and coiling (see Umbilical Cord Structure) (Faye-Petersen et al., 2006). Any one of these factors can lead to diminished blood flow in the umbilical vessels and hence, hypoxia in the developing fetus (Tantbirojn et al., 2009). Abnormally long cords (>70cm), often lead to cord entanglement, nuchal cords, coiling and knot formation, that all favour stricture or complete occlusion of the cord and thus, reduced blood flow (Faye-Petersen et al., 2006). In contrast, short cords

(<40cm) may avulse from the placenta resulting in partial or complete cord rupture or abruption leading to substantial hemorrhage or hematoma formation (Bernischke et al., 2006). Moreover, velamentous insertion (see Cord Insertion Sites) also poses a significant risk of hematoma development (Faye-Petersen et al., 2006).

Recall that the normal umbilical cord contains two arteries and one vein (see Umbilical Cord Structure). The most common congenital anomaly of the umbilical cord is a single umbilical artery (SUA), having an incidence rate of 0.5-1% of all deliveries (Redline, edited by Kay et al., 2011). Commonly, it is due to the absence of one of the two umbilical branches of the internal iliac arteries, and it is usually the left branch that is missing (Redline, edited by Kay et al., 2011). The most common fetal abnormalities associated with SUA are imperforate anus, renal agenesis, cardiac anomalies, and vertebral defects (Redline, edited by Kay et al., 2011).

Similar to the placenta, the umbilical cord is also subject to bacterial and viral infections. Of importance, is that of acute and necrotizing funisitis, which is often seen in association with ACA (Faye-Petersen et al., 2006). Histologically, neutrophils are often seen migrating into Wharton's jelly from the fetal vessels producing arcs of inflammatory debris near the amnionic surface (Faye-Petersen et al., 2006). Grossly, the umbilical cord surface contains multiple tan-yellow gritty nodules (Faye-Petersen et al., 2006). Necrotizing funisitis represents a chronic form of inflammation that is usually characterized by calcification surrounding the umbilical cord vessels (Bernischke et al., 2006). In more severe

cases, mural thrombosis of the vessels can occur and documented cases have been reported where cord clamping was difficult to perform (Bernischke et al., 2006).

1.7.6 Tumour like Conditions and Neoplasms

Neoplasms of the placenta are an uncommon entity that typically can be classified into two histological groups: (1) vascular lesions, and (2) trophoblastic neoplasms (Murtoniemi et al., 2009). It can be said that vascular lesions (with rare exceptions) are the only benign lesions of the placenta (Bernischke et al., 2006). Although a number of tumours with vascular designations have been described including: chorangiomas/chorioangiomas, fibromas, and fibroangiomyxomas, all are essentially similar (Bernischke et al., 2006) and only the former will be discussed here.

Chorangiomas are the most common benign tumour of the placenta, having an overall occurrence rate of approximately 1% in term placentas and are akin to a hemangioma elsewhere in the body (Faye-Petersen et al., 2006). They appear to have a greater preponderance in mothers with advancing age (>30 years), pre-eclampsia, and gestations with female fetuses (Faye-Petersen et al., 2006). Grossly, these lesions are small (≤ 5 mm), however, Kodandapani et al. (2012) reported a case of a 32 week placental chorangioma measuring 12cm in dimension. They are relatively well circumscribed, nodular lesions localized near the fetal plate. Their cut surface demonstrates a fleshy, deep red appearance, and may be variegated with a tan-yellow discoloration (Faye-

Petersen et al., 2006). Histologically, these lesions are predominately composed of fetal blood vessels with minimal amounts of connective tissue (Bernischke et al., 2006). Typically, they are surrounded by a trophoblast layer due to the expansion of the villous structure caused by the proliferation of fetal capillaries (Bernischke et al., 2006) (See Fig 15). Moreover, tumours that arise near the umbilical cord base often involve Wharton's jelly, and thus demonstrate a myxomatous appearance (Bernischke et al., 2006). Placentas with multiple chorangiomas are said to have chorangiomas (Bernischke et al., 2006) (See Fig 16). Admittedly, there is considerable confusion and overlap in the literature involving chorangiomas, chorangiomas, and chorangiomas (see Circulatory Problems). However, Ogino and Redline (2000) defined chorangiomas as having similar histology to that of chorangiomas but instead of forming an expansile nodular lesion within the villi, it invades the normal villous structures.

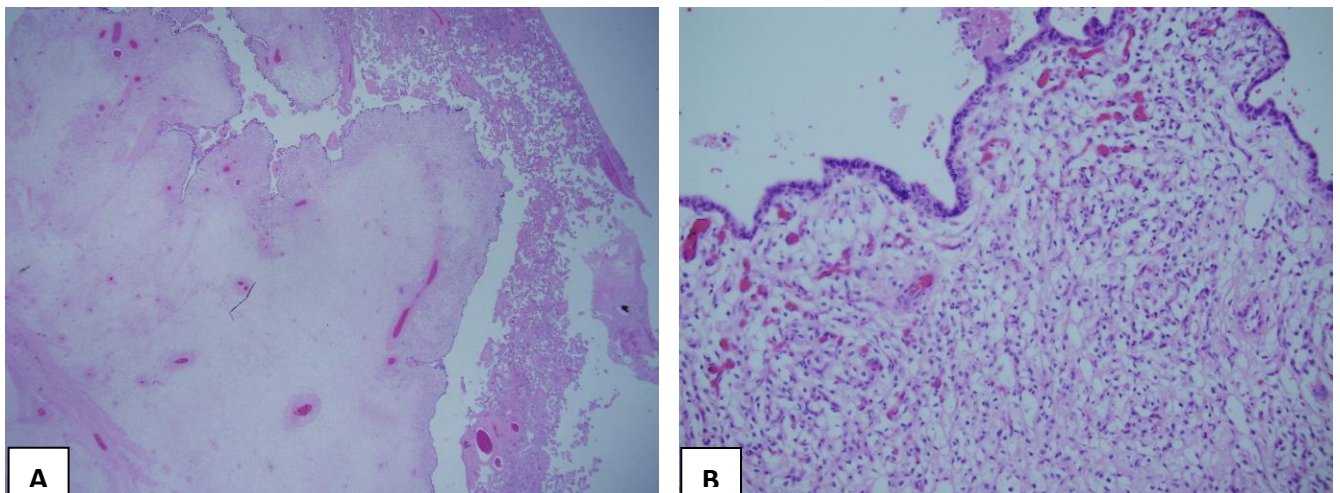


Figure 15. Chorangioma. (a) low power view of an infarcted chorangioma with expanded villi and abundant fibrous and eosinophilic stroma. (b) large number of fetal vessels can be seen around the periphery of the villous.

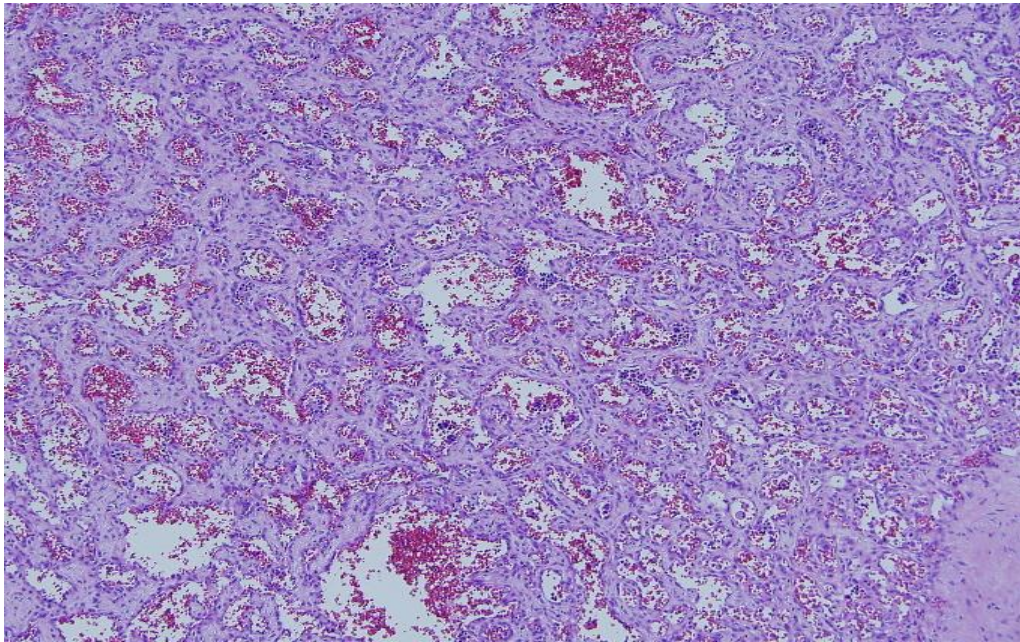


Figure 16. Multifocal chorangiomas. Villi show increased stromal proliferation and abundant capillaries. Increased capillarization in fetal stem and intermediate villi determines chorangiomas.

Gestational trophoblastic diseases are exceedingly rare and encompass several non-neoplastic and neoplastic lesions, one of which is choriocarcinoma (Bernischke et al., 2006). Its incidence is one in 25,000 – 40,000 pregnancies and is often preceded by a hydatidiform mole (see below), abortion, normal pregnancy, or ectopic pregnancy (Baergen, 2011). It is a malignant neoplasm composed exclusively of cytotrophoblast and syncytiotrophoblast cells and is rapidly invasive (Bernischke et al., 2006). Grossly, it produces a soft, yellow-white tumour with marked areas of hemorrhage. It readily invades the myometrium and has a propensity for vasculature and lymphatic invasion (Kumar et al., 2010). Histologically, the tumour consists of solid sheets of cytotrophoblast and syncytiotrophoblast cells with absent chorionic villi (Kumar et al., 2010).

It has been estimated that approximately 50% of choriocarcinomas are preceded by a hydatidiform mole (Baergen, 2011). Hydatidiform moles are often classified as complete or partial (Baergen, 2011). Complete moles are edematous, enlarged placentas with enlarged villi that generally lack blood vessels and contain fluid-filled cavities that are almost always devoid of embryo and chorionic tissue (Bernischke et al., 2006). Complete moles often result from the fertilization of an 'empty egg' devoid of its nucleus, resulting in strictly paternally derived genetic material (Baergen, 2011). Commonly, the complete mole will have a 46XX, or less commonly, a 46XY genotype (Baergen, 2011). In contrast, a partial mole is usually triploid, having two sets of paternal genes and one set of maternal genes, thus, they exhibit a 69XXX, 69XXY, or rarely 69XYY genotype (Baergen, 2011). They appear similar to those of complete moles but are less voluminous and contain a mixture of normal villi, admixed with distended hydropic villi (Baergen, 2011). Embryonic and chorionic tissue is often present and villous capillaries are usually identified (Baergen, 2011).

1.7.7 Multiple Pregnancies

The pattern of placentation in multiple gestation pregnancies is dependent upon the zygosity of the twins and ultimately the implantation of the blastocyst. The incidence of twinning is approximately 1 in 90 pregnancies with a slight increase due the rise in practice of assisted reproductive technologies (Faye-Petersen et al., 2006). Dizygotic twins originate from the release of two ova within the same menstrual cycle that are subsequently fertilized by two sperms,

resulting in a diamnionic/dichorionic (DiDi) placenta (Papanna & Moise, edited by Kay et al., 2011). These comprise of more than two thirds of all twin births and are considered 'low-risk' (Papanna & Moise, edited by Kay et al., 2011). Grossly, they may be entirely separate (similar to a singleton placenta) or may be fused together (Faye-Petersen, et al, 2006). The dividing amnionic membranes between the two chorions are generally thick and opaque (Lester, 2010). In contrast, diamnionic/monochorionic (DiMo) placentas consist of a single placental disc with two amnionic sacs (Faye-Petersen et al., 2006). The dividing membrane consists of two amnions that are thin and translucent which can be easily peeled apart (Lester, 2010). DiMo placentas arise from the division of a fertilized ovum (monozygotic) into two embryos between the fourth and eighth day of life, whereas monoamnionic/monochorionic (MoMo) placentas result from the division of the embryo between the 9th and 12th day of life (Papanna & Moise, edited by Kay et al., 2011). MoMo placentas have a high rate of complications, often due to cord entanglement and have no dividing membrane (Lester, 2010).

The relationship of placental fetal vasculature in twin pregnancies is one of the most important determinants of fetal outcome. For example, in monochorionic placentas, the frequency of arterio-venous anastomoses associated with twin-twin transfusion syndrome (TTTS) is estimated to reach as high as 30% (Baergen, 2011). The unidirectional flow of blood from the 'donor' twin results in deprivation of nutrients, rendering the 'donor' much smaller in size, anemic, dehydrated, and pale, whereas the 'recipient' is larger, often

polycythemic, and afflicted with congestive heart failure (Baergen, 2011). The placenta is also markedly different, showing signs of congestion and edema on the recipient aspect, while the donor side is often pale and shrunken (Baergen, 2011).

2. LITERATURE REVIEW

Much can be gained by the thorough examination of placentas in understanding neonatal illness. Booth, Nelson, Dambrosia & Grether (1997) and Redline, Hellber, Keating, & Kingdom (2005) concluded that current practices in placental submissions for pathological examination are inadequate and suggest that policies determining placental examinations be re-evaluated to provide an increased yield of potentially useful information. The College of American Pathologists subsequently endorsed this in 1997. The most recent guidelines published by the College of American Pathologists (CAP) recommend that placentas from all pregnancies be examined grossly and triaged appropriately for formal placental histopathological examination. The indications for complete placental examination as per the CAP guidelines include the following: maternal indications: systemic disorders with clinical concerns for mother and infant, premature delivery < 34 weeks gestation, peripartum fever and/or infection, unexplained third trimester bleeding or excessive bleeding, clinical concern for infection during pregnancy, severe oligohydramnios, unexplained or recurrent pregnancy complication, invasive procedures with suspected placental injury, abruption, non-elective pregnancy termination, thick meconium, premature

delivery from >34 weeks to <37 weeks gestation, severe unexplained polyhydramnios, history of substance abuse, gestational age >42 weeks, severe maternal trauma, or prolonged rupture of membranes; fetal/neonatal indications: admission to other than Level 1 nursery, stillbirth/perinatal death, cord blood pH <7.0, Apgar score <6 at five minutes, ventilator assistance >10 minutes, severe anemia (Hct <35%), hydrops fetalis, birth weight <10th percentile, major congenital anomalies, dysmorphic phenotype, or abnormal karyotype, discordant twin growth (>20% weight difference), multiple gestation with like-sex infants and fused placentas, birth weight >95th percentile, asymmetric growth, multiple gestation without other indication, or vanishing twin beyond the first trimester; placental indications: any gross anomalies or abnormal appearance of the placenta (e.g. infarct, mass, vascular thrombosis, retroplacental hematoma, amnion nodosum, abnormal coloration, bad odour, umbilical cord thrombosis, torsion, true knot, single artery, absence of Wharton's jelly), small or large placental size for gestational age (<350g or >750g), short cord length (<32cm), abnormalities of placental shape, long cord length (>100cm), or marginal/velamentous cord insertion (Langston et al, 1997). Based on the CAP recommendations, most, but not all, centres adopted protocols and strategies to be able to perform placental examination, at least for most of the above-mentioned fetal, maternal and neonatal indications.

Systematic examination of placenta by some led to the publication of few well-designed studies implicating placenta to a variety of fetal and neonatal morbidities as well as mortality. However, the majority of data is reported in either

preterm pregnancies or premature neonates where placental abnormalities have been found to be associated with fetal demise, still birth, neonatal death, necrotizing enterocolitis, and intraventricular hemorrhage \geq grade 3 bronchopulmonary dysplasia, fetal thrombotic vasculopathy, and intrauterine growth retardation (IUGR) (Baschat et al., 2007; Beaudet et al., 2007; Resnik, 2002; Wintermark et al., 2010).

In term neonates, data correlating placental pathologies to a certain diagnostic condition or outcome is scarce (Redline, 2006; Redline & O’Riordan, 2000; Booth et al., 1997). In addition, in these infants association of placental pathology to long-term neurodevelopmental outcomes has not been systematically studied. The relative lack of data in term neonates is due to several reasons: the cost associated with conducting large cohort studies for placental examination, relative shortage of placental pathologists and physician’s lack of interest in the placental submission for examination mainly due to the scarcity of evidence linking placental abnormalities with neonatal morbidities and long term outcomes in term infants. The reported clinical consequences of fetomaternal placental abnormalities in term neonates include fetal hydrops, stillbirth, IUGR, fetal anemia and thrombocytopenia, fetal congestive heart failure, disseminated intravascular coagulation, cerebral palsy and other neurologic impairment in term infants. Some recent studies in term infants have reported that inflammation or infarction of the placenta may be related to certain neonatal conditions and outcomes mainly hypoxic ischemic encephalopathy, (Blair, de Groot, & Nelson, 2011; Redline, 2006) cerebral palsy and perinatal stroke (Wu,

Lynch, & Nelson, 2005; Lee et al., 2005; Elbers, Viero, MacGregor, deVeber, & Moore, 2010) but the number of cases with placentas studied was quite small. Umbilical cord vascular thrombi typically associated with cord abnormalities (velamentous insertion, true knot, inflammation, entanglement, hypercoiling, amniotic bands and others) reportedly may cause grave clinical consequences including death, IUGR, and neurologic injury including neonatal stroke (Chan & Baergen, 2012; Tantbirojn et al., 2009).

Although these data indicate that the placenta plays a vital role in neonatal outcome, given the small number of placentas examined in these studies, further study is warranted. The present study is determined to provide clearer insight into abnormalities of the placenta that may be predictive of certain neonatal diagnostic outcomes.

3. PURPOSE

The present study is designed to investigate the nature and extent of pathologically significant placentas and their corresponding fetal and neonatal outcomes. In particular, we aim to examine the correlation between specific placental pathologies and a variety of neonatal clinical conditions, including brain injury, requiring admission to hospital's neonatal care unit and subsequent follow-up in our High Risk Newborn Follow-up Program.

The aim of the present study is twofold. One, we hope to provide greater impetus for the submission of placental examinations based on potential indications of adverse placental anomalies and subsequent neonatal outcomes. Second, by correlating placental pathology with neonatal brain injury, we will hopefully provide better predictive measures in aiding a timely diagnosis.

4. MATERIALS AND METHODS

After approval by the Health Research Ethics Board (HREB) of the University of Manitoba (Ethics # H2013:150), the Diagnostic Services of Manitoba Delphic Anatomical Pathology (DSM-AP) database was searched for placenta cases received for examination between January 1, 2009 and December 31, 2010 at two tertiary hospitals in Winnipeg, Manitoba – Health Sciences Centre (HSC) and St. Boniface General Hospital (SBGH). Six hundred and six placental pathology reports from 2009 (43.2% HSC; 56.8% SBGH) and 633 reports from 2010 (40.7% HSC; 59.3% SBGH) were reviewed by the author. Only those placentas submitted to HSC were included in the present study. The placentas were submitted for detailed histopathologic examination to our pathology laboratory when one or more of the following clinical indications were present: (1) placental factors such as adherence, tumour, cord abnormalities or accidents, or unusual placental abnormalities; (2) maternal factors including abruptio placentae, peripartum hemorrhage, high risk pregnancy, prolonged rupture of membranes or prolonged labour, recurrent obstetrical catastrophe, radiation, drug or toxin exposure, maternal disease, spontaneous abortion, or polyhydramnios/oligohydramnios; and (3) fetal/neonatal factors such as fetal distress, malformations, sepsis, prematurity, multiple gestation, stillbirth, and growth disturbances (IUGR). The clinical data from these reports were examined and those placentas that: (1) were submitted but not for microscopic examination; (2) came from products of conception (POC); (3) miscarriages; (4) intrauterine fetal demise (IUFD); (5) placental tissue from dilatation and curettage

(D&C) procedures; (6) preterm infants (<37 wks); and (7) stillborn infants, were excluded from the study population (see Table 1).

Table 1. Reviewed Placenta Reports

	2009	2010
Total # Reports Reviewed	606 (n=262 HSC; n=344 SBGH)	633 (n=257 HSC; n=376 SBGH)
Preterm Infants (<37 wks)	323	361
Miscarriage	19	3
Stillbirths	53	71
POC	7	20
Other†	28	18
Term Infants (≥ 37 wks)	283	272

†includes IUFD, placental tissue from D&C, uterine contents, and retained placental tissue.

††total numbers exceed total # reports reviewed - miscarriage, stillbirths, POC, and others are included in preterm infants total.

4.1 Placental Pathology Selection

Abstracted information included specific placental, neonatal and maternal features and birth characteristics. Pathological placenta features were divided into four categories: (1) thromboinflammatory processes affecting the fetal circulation including, chronic villitis, meconium staining, thrombosis, calcification, villous ischemia and infarction, and chorioamnionitis and vasculitis; (2) decreased placental reserve such as, placental abruption and perivillous fibrin deposition; (3) adaptive responses including, villous chorangiomas and immaturity; (4) umbilical cord features including, thrombosis, calcifications, vasculitis/funisitis, and malformations (e.g. true/false knots and single umbilical artery), after Redline (2006) and Beebe, Cowan, & Altshuler (1996). The pathological definitions were

based on guidelines outlined by the College of American Pathologists (Langston et al, 1997). The pathologies were documented as present or absent only, and no attempt was made to quantify the lesions. Placentas demonstrating one or more of the above pathologies were considered abnormal/pathological. A total of 174 term placentas: abnormal (n= 133) and normal (n=41) examined at HSC were identified between 2009 and 2010.

4.2 Neonatal Medical Records Number Retrieval

Placentas received in the pathology department were given a unique Anatomical Pathology (AP) number that was registered under the maternal Medical Records Number (MRN) only and not under the corresponding infant (DSM Protocol). All term placental AP and maternal MRN numbers were subsequently inputted into the Health Information System at HSC's Health Information Services Coding and Abstracting Department (HISCAD) and cross referenced to the corresponding neonatal/infant MRN. An HSC Research Impact Approval Form (RIC#: RI2013:058) and a Study/Data Request Form were submitted to the Research Protocol Officer and the Manager of Coding and Abstracting to obtain the neonatal/infant MRNs.

4.3 Newborn High Risk Follow-up Program (HRFU) Enrollment Criteria

The HRFU program routinely enrolls and longitudinally follows high risk neonates requiring neonatal care at the Children’s Hospital (HSC) – Winnipeg, and SBGH. These are the only two hospitals in the city of Winnipeg that provide neonatal care, including neonatal intensive care units (NICU). The criteria for enrollment to our HRFU program include: birth weight <1500g, hypoxic brain or other hypoxic injury, neonatal seizures, neonatal stroke, intraventricular hemorrhage, meningitis, hyperbilirubinemia (>428µmol/L or need for exchange transfusion), congenital infections, central nervous system abnormalities, and congenital anomalies. A total of 179 and 166 neonates were enrolled in the HRFU in 2009 and 2010, respectively (see Table 2).

Table 2. Neonatal Enrollment in the HRFU Database

	HRFU		HRFU CONTROLS		TOTAL
	2009	2010	2009	2010	
Preterm Infants (<37 wks)	108	105	--	--	213
Term Infants (≥ 37 wks)	64	48	7	13	132
Abnormal placentas	14	5	--	--	19
Normal placentas	3	5	--	--	8
Total term infants enrolled with placental submission	17	10	--	--	27
Age-Matched Controls without Placental Submission	--	--	7	13	20

4.4 HRFU Database Search

The infant MRNs retrieved from HISCAD were used to search the HRFU database at HSC – Children’s Hospital Newborn HRFU Clinic. A total of 27 infants that had their placentas submitted to the pathology department were identified in the database (see Table 2). A query was established and pertinent clinical data was extracted including birth weight, head circumference, length, gestational age, 1 and 5 minute Apgar score, reason for follow up, and outcome. Maternal medical history was extracted from the database including diabetes, hypertension, PIH, vaginal bleeding, oligohydramnios, polyhydramnios, chorioamnionitis, other illnesses, maternal drug use including alcohol, smoking, and cocaine exposure. Labour characteristics, fetal presentation, delivery method, and cord gases were also recorded. Neonatal diseases including, meconium aspiration, pulmonary hemorrhage, pneumothorax, pulmonary interstitial emphysema (PIE), pneumopericardium, pneumomediastinum, number of days on ventilator, neonatal congenital heart disease and persistent pulmonary hypertension (PPHN) were documented along with neonatal central nervous system (CNS) diseases including seizures, intraventricular hemorrhage (IVH), periventricular echodensities (PVE), periventricular leukomalacia (PVL), porencephalic cyst, ventriculomegaly, hydrocephalus, neonatal stroke, hypoxic ischemic encephalopathy (HIE), and hypothermia protocol. We also included infections such as meningitis, and other disorders like acute renal failure, thrombus, and congenital anomalies. Neonatal discharge information including, weight, head circumference, length, and discharge diagnosis were also recorded.

4.5 Study Population

All term neonates that were enrolled in the HRFU program between January 1, 2009 and December 31, 2010 that had their placentas examined in our pathology department at HSC and met our inclusion criteria were included in the study. A total of 19 infants with pathological placentas submitted and 20 age-matched controls that did not have their placentas submitted for examination were reviewed. (see Table 2). We included 29 age-matched infants with similar neonatal indications including fetal distress, meconium staining, cord entanglement, and congenital anomalies and maternal indications including oligohydramnios and polyhydramnios that were not in the HRFU database but had their placentas submitted for examination (see Table 3). The microscopic slides of submitted placentas were retrieved and independently reviewed blindly by Dr. Stefanovici.

4.6 Statistical Analysis

All statistical measures of central tendency were calculated using Microsoft Office Excel 2007. Dichotomous variables were compared by using χ^2 with Yates correction 2 X 2 contingency tables using GraphPad QuickCalcs online software. Pearson correlation coefficients were calculated using GraphPad Prism statistical software.

5. RESULTS

5.1 Population Sample Statistics

A total of 519 placentas were submitted for examination at HSC between January 1, 2009 and December 31, 2010 (see Table 1). After reviewing the clinical data, 174 term infants (≥ 37 weeks) with placental submission were identified: 133 placentas contained one or more histological abnormality and 41 were absent of any histologic pathology. A total of 132 infants were enrolled in the HRFU between 2009 and 2010 (See Appendix 9.1)

5.2 HRFU Demographics

The maternal characteristics whose infants were enrolled in the HRFU program are summarized in Table 3.

The mean maternal age that had infants enrolled in the HRFU program was 27.74 ± 4.63 years with a gestational mean age of 39.35 ± 1.84 weeks. 37% of mothers delivered via Caesarean section ($n=7$), of these 4 were performed due to fetal indications, and the remaining were due to maternal factors. One mother delivered with vacuum assistance and the remaining had a spontaneous vaginal birth. All infants in the HRFU had a vertex presentation (one case was unknown).

Ten percent of mothers had a history of diabetes that was present throughout their pregnancy but none had gestational diabetes. Of note, 10% of

mothers (n=2) reported occasional alcohol use and 10% reported smoking during pregnancy.

5.3 Non-HRFU Demographics

The mean gestational age for infants that were not enrolled in the HRFU program was 40.17 ± 1.77 weeks and a mean maternal age of 28.14 ± 7.11 years (Table 3). Sixteen mothers (55%) had a Caesarean section with one infant presenting breech.

Seventeen percent of mothers reported alcohol/drug abuse during pregnancy and 14% were obese (BMI ≥ 30). One mother tested positive for herpes, while another tested positive for chlamydia.

5.4 HRFU Control Demographics

The mean birth weight of infants included in our HRFU control group was $2847.7g \pm 961.8g$ with a mean gestational age of 38.05 ± 1.35 weeks (Table 3). Eleven mothers (55%) underwent Caesarean section due to fetal indications (n=7) and maternal factors (n=4). Two infants in the group required vacuum assisted delivery.

Three mothers in the group reported smoking during pregnancy and 10% reported alcohol use. No mothers in the group suffered from essential hypertension, however, two mothers reported PIH. Furthermore, 10% of mothers in the group had a history of diabetes that was present throughout their pregnancy.

Table 3. Demographic characteristics of abnormal placentas in HRFU and non-HRFU infants.

	HRFU (n=19)	Non-HRFU (n=29)	HRFU Control (n=20)
<i>Maternal Characteristics</i>			
Gestational age	39.35 ± 1.84	40.17 ± 1.77	38.05± 1.35*
Maternal age	27.74 ± 4.63	28.14 ± 7.11	27.36 ± 5.24
Gravidity	2.70 ± 1.31	2.55 ± 2.15	--
Caesarean delivery	37% (7)	55% (16)	55%(11)
Prior term infants	1.08 ± 0.86	1.0 ± 1.41	--
<i>Prenatal Characteristics</i>			
Obesity	10%	14%	--
Diabetes	10%	3.7%	10%
Essential Hypertension	0%	10%	0%
Anemia	5%	7.41%	--
Smoking	5%	7.41%	15%
Alcohol Abuse	10%	17%	10%
<i>Infant Characteristics</i>			
Birth weight (g)	3242.2	2876.4	2847.7
Apgar score (1min)	5.7 ± 3.2	4.6 ± 3.2	6.1 ± 2.8
Apgar score (5min)	7 ± 2.2	7.2 ± 2.0	7.2 ± 1.9

*p=0.0160 (see Table 4)

5.5 HRFU Histological Features

A total of 27 placental histological abnormalities were reported in infants enrolled in the HRFU program. Twelve placentas contained only one abnormality; five placentas had two histological abnormalities; one placenta had three abnormalities; and one had four abnormalities. Meconium staining was the most prevalent histological abnormality in the HRFU group (see Fig. 17).

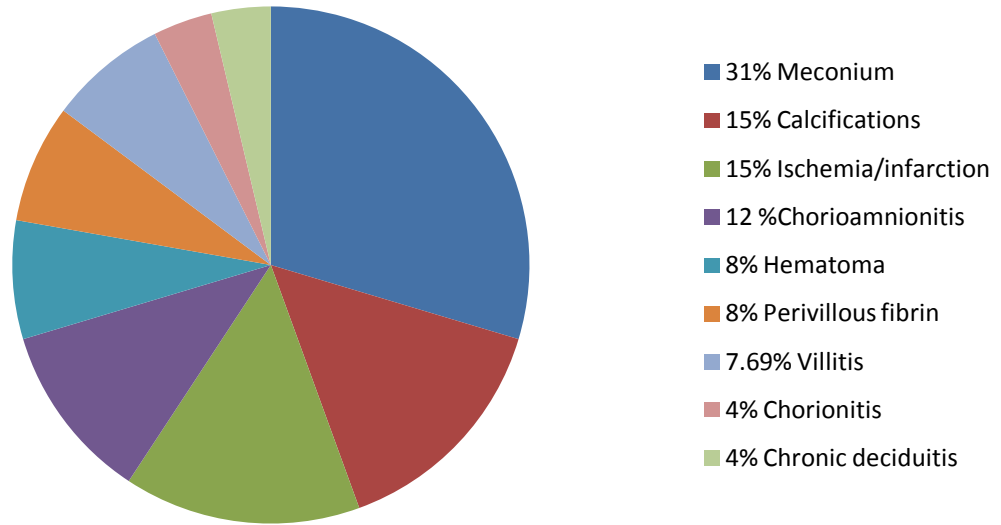


Figure 17. Frequency of pathological features in infants enrolled in the HRFU program

5.6 Non-HRFU Histological Features

Forty-five placental abnormalities were reported in infants who were not enrolled in the HRFU program. Ischemia, calcifications, and meconium staining were present in 22%, 20%, and 20% of all placentas, respectively (see Fig. 18). One placenta in the group contained four abnormalities; five contained three abnormalities; nine contained two histological abnormalities; and twelve placentas reported one abnormality.

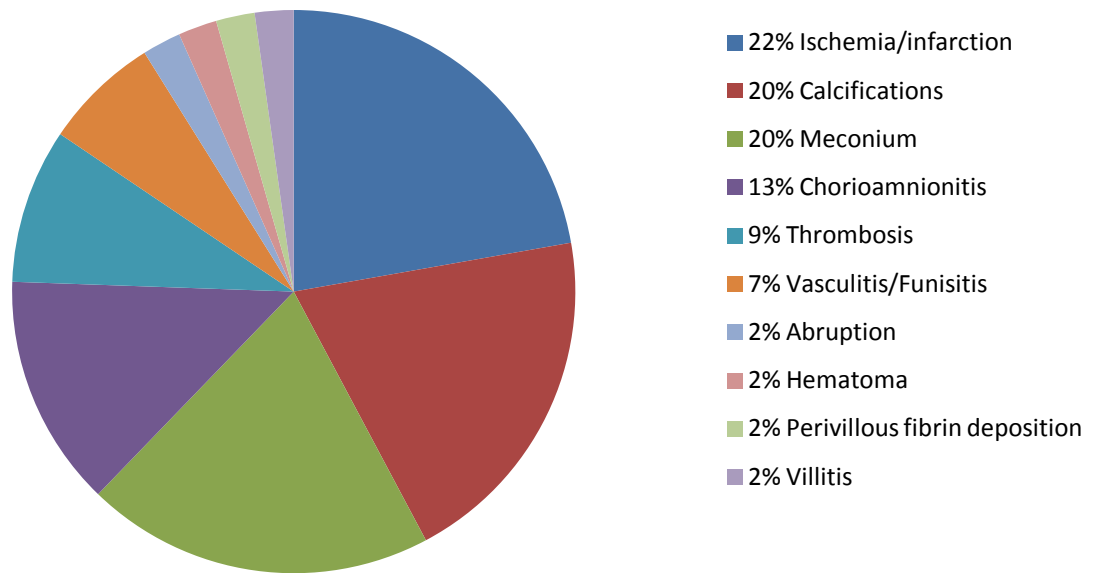


Figure 18. Frequency of placental features in infants in the non- HRFU program

5.7 Statistical Analysis

All histological findings that were included in the study were compared with a variety of clinical parameters using χ^2 . A sample 2 X 2 contingency table can be found in Appendix 2. Comparison of means and proportions were analyzed using the t-test.

5.7.1 HRFU and Non-HRFU Demographic Analysis

Data collected from the HRFU group whose infants had placentas submitted for examination (n=19) and the HRFU control group whose placentas were not submitted (n=20) (see Table 3) were compared using t-test. Their subsequent P-values are listed in Table 4. Furthermore, infants whose placentas

were submitted for examination were compared between those infants enrolled in the HRFU and those who were not (n=29). Their P-values are listed in Table 5.

Table 4. Comparison of means between HRFU group and HRFU controls

	P value
Gestational Age	0.0160
Maternal Age	0.8120
Caesarean delivery	0.2555
Diabetes	0.9569
Smoking	0.6759
Alcohol Abuse	0.9569
Birth weight (g)	0.1242
Apgar 1 min	0.6798
Apgar 5min	0.7626

Table 5. Comparison of means between HRFU group and non-HRFU group

	P value
Gestational Age	0.1291
Maternal Age	0.8295
Gravidity	0.7867
Caesarean delivery	0.0512
Prior term infants	0.8258
Obesity	0.6563
Diabetes	0.3217
Essential Hypertension	0.1476
Anemia	0.8189
Smoking	0.8189
Birth weight (g)	0.0814
Apgar score (1min)	0.2502
Apgar score (5min)	0.7461

5.7.2 Caesarean Section and Histological Outcomes

A total of nine histological findings and their corresponding mode of delivery were compared with the HRFU and non-HRFU groups. Their P-values are listed in Table 4.

Table 6. χ^2 results of placenta features and mode of delivery between HRFU and non-HRFU groups

Histological Outcome	P value
Meconium	0.3468
Ischemia/Infarction	0.7838
Chorioamnionitis	0.7358
Abruption	0.5436
Villitis	0.4762
Calcifications	0.8249
Vasculitis	0.7512
Thrombosis	0.5852
Umbilical Cord Vasculitis	0.7358

5.7.3 Apgar Scores and Histological Outcomes

One and five minute Apgar scores were recorded and grouped into two categories: Apgar scores 0-6 and 7-10. These scores were compared with the nine histological outcomes with the HRFU and non-HRFU groups; their subsequent P-values are listed in Table 5.

Table 7. χ^2 results of placenta features and neonatal indications of Apgar scores between HRFU and non-HRFU groups

Histological Outcome	Apgar 1 min (0-6)	Apgar 1 min (7-10)	Apgar 5min (0-6)	Apgar 5 min (7-10)
Meconium	0.9212	0.8795	0.8017	0.9697
Ischemia/Infarction	0.5288	0.1463	0.1714	0.3992
Chorioamnionitis	0.7321	0.4974	0.4825	0.9384
Abruption	0.4215	0.9610	0.9237	0.4007
Villitis	0.8070	0.7039	0.8351	0.8298
Calcifications	0.8663	0.3134	0.3283	0.7228
Vasculitis	0.4799	0.2466	0.2428	0.4208
Thrombosis	0.3329	0.1427	0.1466	0.2784
Umbilical Cord Vasculitis/Funisitis	0.7321	0.4974	0.4825	0.9384

5.7.4 Correlation of Gestational Age in non-HRFU and HRFU Groups and Total Number of Abnormalities

The total number of histological abnormalities found in each placenta was tallied and compared to the infants' gestational age. The Pearson correlation coefficient for those in the non-HRFU was calculated to be $r=0.2586$ with a P-value of 0.1756 (see Fig 19). While the HRFU group had a Pearson correlation coefficient of $r=0.2977$ with a P-value of 0.2158 (see Fig 20).

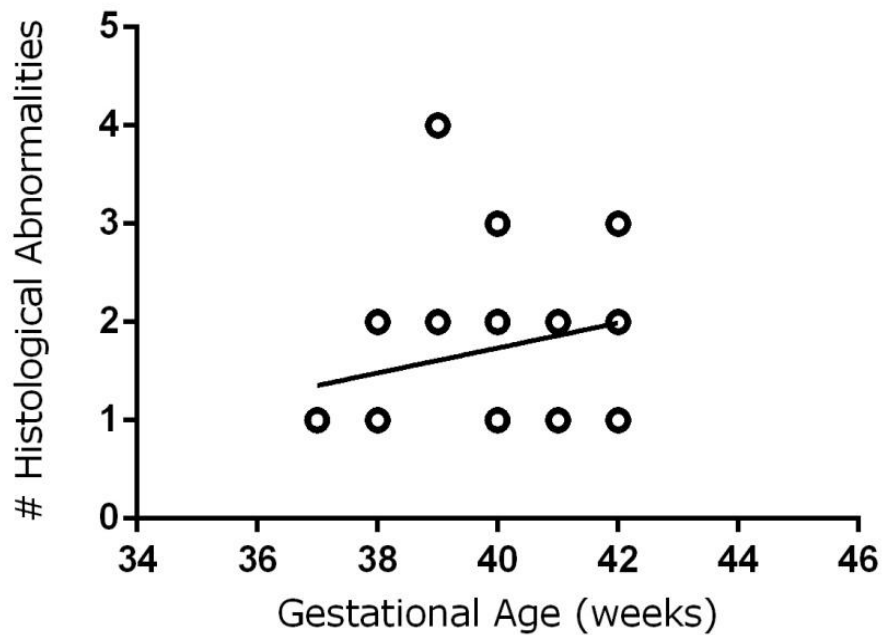


Figure 19. Correlation analysis of total number of abnormalities in each placenta with infants' gestational age in the non-HRFU group.

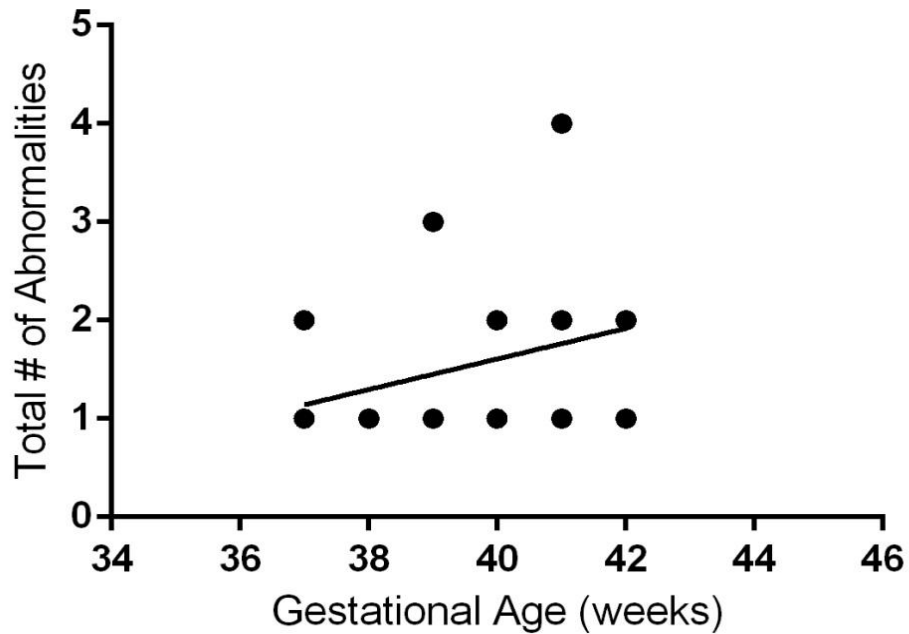


Figure 20. Correlation analysis of total number of abnormalities in each placenta with infants' gestational age in the HRFU group.

5.7.5 Correlation of Infant Weight in the non-HRFU and HRFU groups and Total Number of Abnormalities

The Pearson correlation coefficient for those in the non-HRFU group was calculated as $r = -0.1737$ with a P-value of 0.5359 (see Fig 20). While in the HRFU group $r = -0.1908$ with a P-value of 0.4341 (see Fig 21).

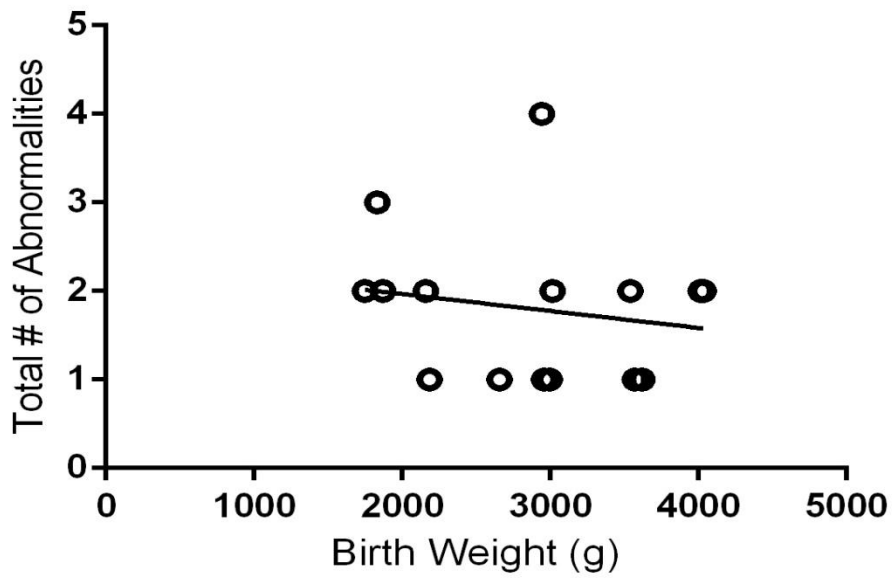


Figure 21. Correlation analysis of total number of abnormalities in each placenta and infant birth weight in the non-HRFU group.

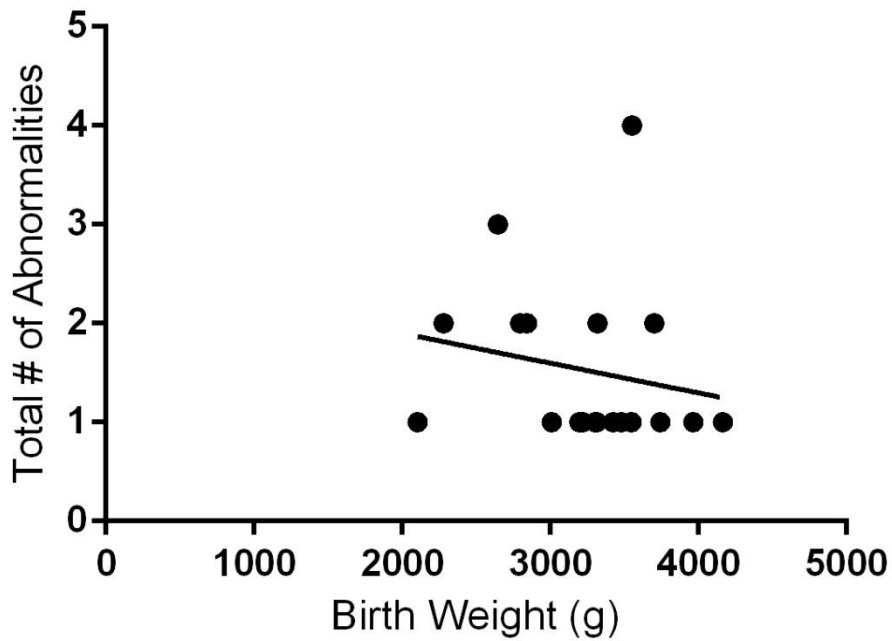


Figure 22. Correlation analysis of total number of abnormalities in each placenta and infant birth weight in the HRFU group.

5.7.6 Neurological Outcome in HRFU infants and HRFU Age-Matched Controls

Three infants in the HRFU that had placentas submitted had moderate to severe HIE and one infant in the control group had moderate HIE. 2/3 infants in the HRFU group that had HIE also had meconium, while meconium was absent in the control group (P=0.4386). No infants in the HRFU program that had placentas submitted for examination and their age-matched controls (n=20) suffered from neonatal stroke, meningitis, or hydrocephalus. Two infants in the HRFU group suffered from seizures and one was diagnosed with bilateral ventriculomegaly. The control group had one infant with grade 3 bilateral interventricular hemorrhage (IVH), while one infant each had grade 1 left-sided IVH and grade 2 left sided IVH.

6. DISCUSSION

In this study, we report maternal, neonatal and placental characteristics of 48 neonates who had placentas submitted for pathologic examination. The main objective of this study was to examine the nature and extent of pathologically significant placentas with various clinical outcomes in infants who were either referred to the HRFU program and those who were not.

6.1 Population Demographics

The mean gestational age of the HRFU and non-HRFU group is 39.35 and 40.17 weeks, respectively. Infants in the control group had a mean gestational age of 38.05 weeks that was statistically significant compared to infants in the HRFU that had placental submission. These findings were expected and can be explained by the restriction of the inclusion criteria to term infants. The lower gestational age in the control group without placental submission may be an indication that age may play a part in placental submission. With the exception of two unknown cases, all infants presented vertex in the HRFU group, one case of breech presentation was reported in the non-HRFU group, and three in the control group. Furthermore, 55% of cases in both the non-HRFU group and HRFU controls underwent caesarean section. Although, not a direct clinical indication for placental submission (see Appendix 3), Booth et al, report that surgical delivery was a major driving force for submission of placentas and placentas were more often submitted for children delivered by caesarean section

than for children delivered vaginally. However, even though the higher rate of placental submission among caesarean deliveries may reflect the common practice of submitting surgical specimens for pathologic examination regardless of secondary indications or elective caesarean delivery, our control group indicates that this practice is left entirely to the discretion of the clinician.

With the exception of diabetes mellitus and smoking, all maternal indications including obesity, essential hypertension and history of substance abuse were higher in the non-HRFU group than those in the HRFU group. Of note, 3 out of 29 mothers in the non-HRFU group (10%) reported having essential hypertension compared to zero in the HRFU and control group. However, two mothers in our control group reported PIH. These findings are slightly lower than those reported by Beaudet et al, who found 15.5% of mothers to have had hypertension in a cohort of infants admitted to a neonatal intensive care unit. Conversely, Chabrier et al report pre-existing maternal hypertension at 2% in a cohort of infants with arterial ischemic stroke, thus, the present study's findings fall within the current literature. Interestingly, smoking was highest among mothers in the control group without placental submission. One would deem the submission of placentas in this group appropriate; however, studies examining maternal cigarette smoking with certain placental abnormalities including abruption, placenta previa and uterine bleeding appear to have no common etiology with respect to cigarette smoking (Ananth et al, 1996).

6.2 Histological Features

Forty-four percent of placental abnormalities in the HRFU group and 26% in the non-HRFU group were single abnormalities ($P=0.1214$). Infants in the non-HRFU group had a greater number of placentas with multiple abnormalities than those in the HRFU group. The reason for these differences may be due to the initial reasons for placental submission. Placentas in the HRFU group may have been likely submitted for fetal indications while non-HRFU placentas submitted for grossly remarkable abnormalities. Ventolini et al (2011) found the prevalence of placental findings in their study to be up to 42% with 35% of placentas with gross abnormalities.

Approximately two-thirds of all placental abnormalities identified in both groups included meconium staining, calcifications, and ischemic changes. Only 10 of the 48 cases (20%) had pathologic findings other than ischemic changes, meconium staining, calcifications, chorioamnionitis, hematoma, and perivillous fibrin deposition. The HRFU group each reported one case of chorionitis and chronic deciduitis, while four cases of thrombosis, three cases of vasculitis/funisitis and one case of abruption were reported in the non-HRFU. These pathologic findings between the two groups however, were not statistically significant (see Table 6). Nonetheless, the present findings are consistent with several epidemiological studies. For example, Beebe and Cowan reported that in term placentas ischemic changes and meconium were dominant features whereas chorioamnionitis was less frequent at 17%. Furthermore, Katz and

Bowes reviewed clinical publications related to meconium and estimated from studies of amniotic fluid to occur in 7-22% of live births.

The number of infants whose one minute Apgar score was ≤ 6 , was 13 and eight in the non-HRFU and HRFU group, respectively. Conversely, 18 infants in the non-HRFU group and 13 in the HRFU group had five minute Apgar score ≥ 7 . When one and five minute Apgar scores were compared to the total number of each pathologic finding within the HRFU and non-HRFU group using χ^2 , no statistically significant results were reported (see Table 7). However, placental features varied considerably depending on Apgar scores. Findings associated with low Apgar scores such as ischemic changes, calcifications and thrombosis were most common in placentas from the non-HRFU group. Whereas findings associated with high Apgar scores like meconium staining and villitis were seen more commonly in placentas from the HRFU group. It is possible that the lack of significant results between the two groups may reflect biases introduced because of the reasons for referral to pathology. Furthermore, there was a marked improvement between one and five minute Apgar scores among the non-HRFU infants compared to those infants in the HRFU group. Clinically, prolonged low Apgar scores in the HRFU can be expected as difficult birth or suspected birth anoxia may have been the reason for admission to the intensive care unit. In addition, low Apgar score has been shown to be associated with poor cognitive function and neurologic impairment (Ehrenstein, 2009). In a study review conducted by Ehrenstein et al (2009) showed that Apgar score < 7 at five minutes is associated with increased risks of neurologic disability that tends to persist for

years postnatally. As a result, these infants would be expected to be enrolled in the HRFU, and their placentas submitted secondary to fetal indications.

Several neonatal indications in this study show a weak-moderate relationship associated with the number of pathological features per placenta. In particular, the number of placental abnormalities increased in infants with a greater gestational age. This relationship was noted in both the HRFU and non-HRFU groups. Conversely, the number of placental features increased in those infants with a lower birth weight in both groups.

Neurological outcomes including neonatal stroke, HIE and abnormal CNS MRI were examined amongst infants in the HRFU group. The study found that there was an increase in the number of HIE cases in the group with placental submission versus the age-matched controls. In addition, two out of three infants (66%) with HIE that had placental submission also showed evidence of meconium changes ($p=0.4386$). These findings correspond to the criteria of indications for placental submission based on the unusual appearance of the placenta and membranes. It is expected of those infants with meconium stained placentas to be submitted for histological examination. Furthermore, these findings in the HRFU group are slightly higher than previous studies, but those in the control group fall within the current literature. Previous studies show that 50% of infants that developed HIE were born through meconium stained amniotic fluid (Ojah et al, 2006).

7. CONCLUSION

This study found that 61% of placentas in the HRFU group and 62% of placentas in the non-HRFU group had placental features of ischemic changes, calcifications and meconium staining. The findings that were reported suggest similar rates of placental abnormalities between the two groups. Although the data are not statistically significant between the two groups, the findings are consistent with previous publications. However, the sample size for this study is small. The study found evidence of meconium with those infants in the HRFU group with HIE. No neonatal stroke was identified within the HRFU group. The analyses indicate placental abnormalities in the absence of any immediate clinically detectable abnormality. This study found no difference in placental pathology between non-HRFU infants and infants enrolled in the HRFU Program. This raises questions and warrants further study on the efficacy for placental submission as a predictive measure for neonatal outcomes.

8. STUDY LIMITATIONS

Several limitations including a small sample size made it difficult to adequately interpret statistical analyses. This in part, was due to (1) a number of cases with incomplete data – neonatal and maternal information was often scarce in the non-HRFU group and HRFU controls were missing data that is normally found on the placental pathology reports; (2) the relatively low enrollment in the HRFU; (3) the limited number of placentas submitted for examination; (4) inadequate placental submission within the HRFU age-matched

controls; and (5) the relatively stringent inclusion criteria. The latter, however, was necessary to limit the number of confounding variables within the study.

In addition, due to the retrospective nature of the study, the study author could not control inter-observer variability amongst pathologists and pathologists' assistants. In the future, synoptic reporting of placenta cases may aid in this matter.

9. APPENDIX

9.1 Sample calculation between total number of term placentas sent for examination and total term infants in HRFU

Calculation: $z = \frac{a-b}{\sqrt{a+b}}$

$$= \frac{174-132}{\sqrt{17.49}}$$

$$Z=2.40$$

P=0.9920 P>0.01

9.2 Analyze a 2x2 contingency table

	Meconium C-section Total		
HRFU	8	6	14
non-HRFU	9	16	25
Total	17	22	39


Chi-square with Yates correction

Chi squared equals 0.885 with 1 degrees of freedom.

The two-tailed P value equals 0.3468

The association between rows (groups) and columns (outcomes) is considered to be not statistically significant.

9.3 Placental examination request form

THIS SPACE FOR LAB USE ONLY PLACE LIS LABEL HERE	 DIAGNOSTIC SERVICES OF MANITOBA SERVICES DE DIAGNOSTIC DU MANITOBA PATHOLOGY SERVICES REQUEST FOR PLACENTAL EXAMINATION	THIS SPACE FOR LAB USE ONLY PLACE AP LABEL HERE
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NAME OF PHYSICIAN ORDERING TEST: <small>(LAST) (FIRST)</small> Copy of report to: <small>Address</small> <small>Fax/Phone</small> REFERRING INSTITUTION NAME AND ADDRESS OR CODE (FOR EXTERNAL LOCATIONS): CONTACT TELEPHONE PAGER PHYSICIAN'S SIGNATURE	LOCATION/WARD: PATIENT NAME: <small>(LAST) (FIRST)</small> DATE OF BIRTH: GENDER: <input type="checkbox"/> M <input type="checkbox"/> F <small>DD/MM/YYYY</small> FACILITY HEALTH RECORD NO.: PERSONAL HEALTH ID NO. (PHIN): <small>(PROV OR INST)</small> PHYSICIAN (PRINT): <small>(LAST) (FIRST)</small> COLLECTION DATE and TIME:
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***** Specimens may not be examined without the appropriate Demographics and Clinical Information *****

MATERNAL	PRENATAL HISTORY	Absent	Present
1. Age	7. Pre-eclampsia	<input type="checkbox"/>	<input type="checkbox"/>
2. Last Menstrual Period	8. Body Mass Index greater than 30	<input type="checkbox"/>	<input type="checkbox"/>
3. Expected Date of Delivery	9. Essential Hypertension	<input type="checkbox"/>	<input type="checkbox"/>
4. Gravida (number of pregnancies).....	10. Gestational Hypertension	<input type="checkbox"/>	<input type="checkbox"/>
5. Number of:	11. Diabetes Mellitus	<input type="checkbox"/>	<input type="checkbox"/>
a) Prior Full-term	12. Anaemia	<input type="checkbox"/>	<input type="checkbox"/>
b) Prior Premature	13. Alcohol/Drug Abuse	<input type="checkbox"/>	<input type="checkbox"/>
c) Prior Spontaneous Abortions	14. Seizure Disorder	<input type="checkbox"/>	<input type="checkbox"/>
d) Prior Stillborn	15. Herpes, Genital	<input type="checkbox"/>	<input type="checkbox"/>
e) Living Children	16. Viral Syndrome	<input type="checkbox"/>	<input type="checkbox"/>
f) Other (e.g. Ectopic)	17. Haemorrhage	<input type="checkbox"/>	<input type="checkbox"/>
6. Abnormalities of Previous Pregnancies:	17. Other (describe):	<input type="checkbox"/>	<input type="checkbox"/>
.....	18. Medications During Pregnancy:		
.....		

INFANT 19. <input type="checkbox"/> Liveborn <input type="checkbox"/> Stillborn <input type="checkbox"/> Single Gestation <input type="checkbox"/> Multiple Gestation 20. <input type="checkbox"/> Vaginal <input type="checkbox"/> Cesarean 21. Malformations: <input type="checkbox"/> Absent <input type="checkbox"/> Present 22. Birth: Date <small>DD/MM/YYYY</small> Time <small>HH:MM</small> 23. Spontaneous Rupture of Membranes/Artificial Rupture of Membranes: Date <small>DD/MM/YYYY</small> Time <small>HH:MM</small> 24. Birth Weight: _____ grams 25. Gender: <input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Indeterminate 26. Presentation: <input type="checkbox"/> Vertex <input type="checkbox"/> Breech <input type="checkbox"/> Occiput Posterior <input type="checkbox"/> Other Condition at Birth 27. APGAR: _____ 1 minute _____ 5 minute 28. Fetal Distress 29. Cord Prolapse 30. Cord Entanglement	<div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;"> Consent for Release of Remains of Perinatal Loss <input type="checkbox"/> Copy Attached </div> <div style="border: 1px solid black; padding: 5px;"> PLACENTA <input type="checkbox"/> Return to Family </div> <table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 60%;"></th> <th style="width: 20%; text-align: center;">Absent</th> <th style="width: 20%; text-align: center;">Present</th> </tr> </thead> <tbody> <tr> <td>31. Meconium Staining</td> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;"><input type="checkbox"/></td> </tr> <tr> <td>32. Oligohydramnios</td> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;"><input type="checkbox"/></td> </tr> <tr> <td>33. Polyhydramnios (describe):</td> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;"><input type="checkbox"/></td> </tr> </tbody> </table>		Absent	Present	31. Meconium Staining	<input type="checkbox"/>	<input type="checkbox"/>	32. Oligohydramnios	<input type="checkbox"/>	<input type="checkbox"/>	33. Polyhydramnios (describe):	<input type="checkbox"/>	<input type="checkbox"/>
	Absent	Present											
31. Meconium Staining	<input type="checkbox"/>	<input type="checkbox"/>											
32. Oligohydramnios	<input type="checkbox"/>	<input type="checkbox"/>											
33. Polyhydramnios (describe):	<input type="checkbox"/>	<input type="checkbox"/>											

Indications for Examination of Placenta

- A. Stillbirth
- B. Live-born babies admitted to the neonatal intensive care unit

Additional Clinical Data

- A. **Fetal/Neonatal Factors**
 - Sepsis or other serious illness in the neonate
 - Prematurity
 - Chromosomal aneuploidy
 - Growth disturbances (Intra-uterine Growth Retardation/Light for Gestational Age)
- B. **Maternal Factors**
 - Prolonged rupture of membranes or prolonged labour
 - Peripartum haemorrhage
 - Abruptio placenta
 - Spontaneous abortion
 - Radiation, drug or toxin exposure
 - Recurrent obstetrical catastrophe
 - Very low or high serum alpha fetoprotein levels
- C. **Placental Factors**
 - Morbid adherence
 - Tumours
 - Single umbilical artery
 - Unusual appearance at delivery (e.g. marked circumvallate membrane insertion, etc.)
 - Cord accidents (prolapse, true knots, etc.)
 - Other: _____

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