PLACENTAL PATHOLOGY

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NORMAL HISTOLOGY REVIEW:

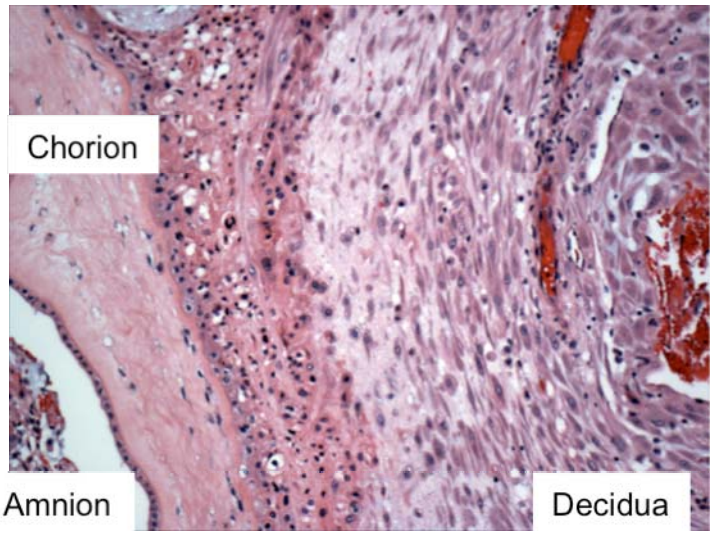
1) Membranes: The amnion lines the amniotic sac containing the fetus. The amnion consists of a single layer of cuboidal epithelium, its basement membrane, and a collagen layer. The fibrous chorion lies deep to the amnion and consists of a sparsely cellular collagen layer. The fibrous chorion rests on a layer of intermediate trophoblast cells (clear to eosinophilic cytoplasm). Amnion, chorion, and intermediate trophoblast cells are fetal in origin. Deep to the intermediate trophoblast layer is the decidual layer (maternal in origin). This layer contains decidualized maternal cells, vessels, fibrin, and often some degree of hemorrhage.

2) Umbilical cord: The outer surface of the umbilical cord is covered by a single layer of cuboidal amniotic epithelium. There is often focal squamous metaplasia. Normally, 3 vessels (2 arteries and 1 vein) are embedded in a myxoid paucicellular matrix (Wharton's jelly).

3) Placental disk: The chorionic plate (fetal surface) consists of a single layer of cuboidal amniotic epithelium overlying a paucicellular collagenous matrix (chorion) containing numerous large vessels of fetal origin. Separating the chorion from the intervillous space is a layer of fibrinoid (Langhans fibrinoid). Below this are the villi (large stem villi to small terminal villi) separated by clear or blood containing spaces (the intervillous space) where the maternal blood circulates around the fetal villi. The sizes of the villi vary depending on the gestational age. The villi consist of an outer layer of syncytiotrophoblast in immediate contact with the maternal blood in the intervillous space. Immediately deep to the syncytial trophoblast cells is a single, discontinuous layer of cytotrophoblast cells (larger, cuboidal cells). As gestation proceeds these become more difficult to identify. The cellular matrix consists of macrophages (Hofbauer cells) and fibroblasts with numerous vessels (capillaries in the terminal villi and larger muscular vessels in stem villi). As maturation proceeds the capillaries in the terminal villi appear to move closer to the syncytiotrophoblast layer, which increases diffusion efficiency. In addition, the terminal villi get smaller and more numerous, and syncytial knots increase (Tenny-Parker change). The resulting smaller and more highly branched terminal villi increase the effective surface area in contact with the intervillous space thereby increasing gas and nutrient exchange without a significant increase in placental mass. Calcifications also increase as term approaches.

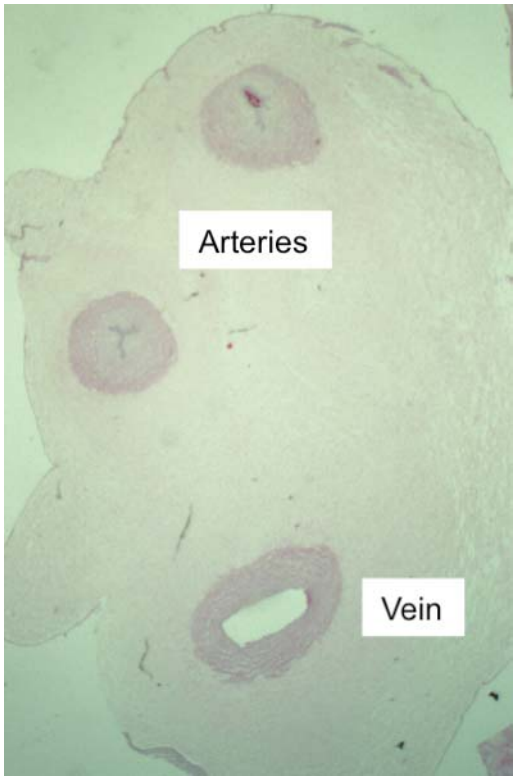
The basal plate (maternal surface) consists of intermediate trophoblast cells, decidualized maternal cells, at least two well defined fibrinoid layers (Rohr's (adjacent to intervillous space) and Nitabuch's (a deeper layer that separates intermediate trophoblast cells from decidua), and maternal vessels. The maternal vessels here should be assessed for fibrinoid necrosis and atherosis. Normally, these vessels are invaded by extravillous trophoblast cells, remodeled with elimination of surrounding smooth muscle layers and enlarging the lumens leading into the sinusoidal intervillous spaces. The invading trophoblast can resemble macrophages and the normal fibrinoid layers can surround these vessels mimicking fibrinoid necrosis.

Membranes

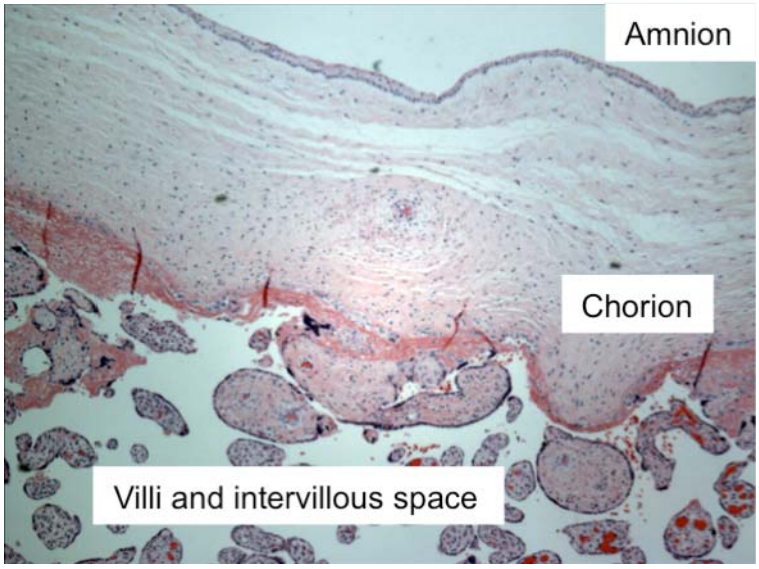


Intermediate Trophoblast

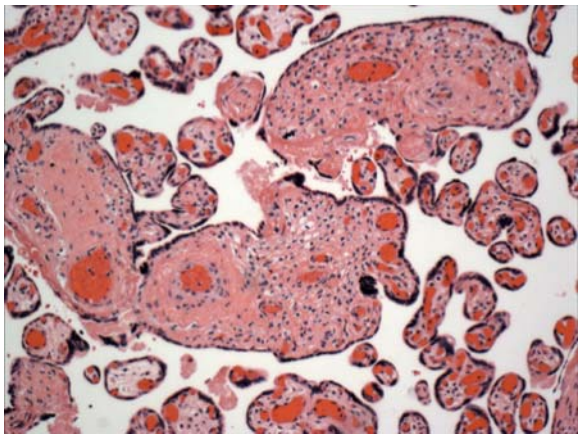
Umbilical Cord



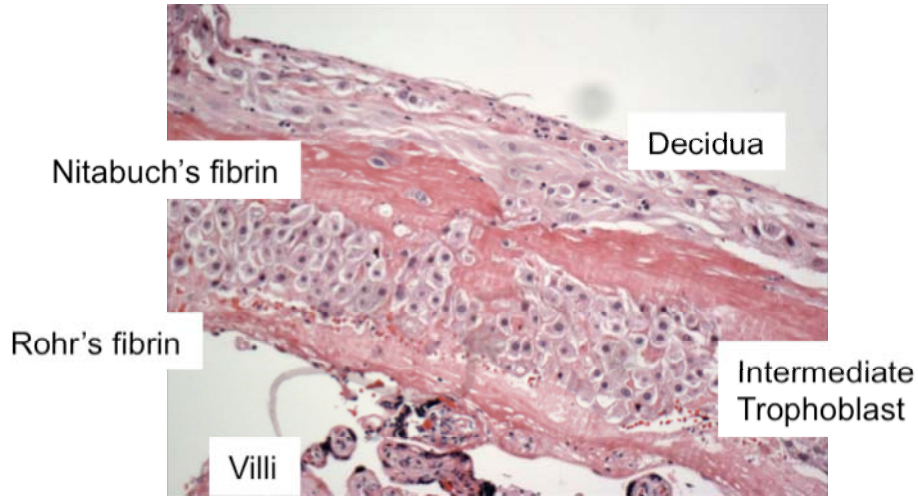
Chorionic Plate



Term Villi



Basal Plate



An Approach to the Histologic Examination of the Singleton Placenta

What to look for in each portion of the placenta:

Membranes

1) Acute inflammation (maternal inflammatory response)- Where is it (decidua, intermediate trophoblast layer, chorion, amnion)? How severe is it? Composition (PMNs, eosinophils)? Stage?
2) Maternal vessels - Look for thrombi, fibrinoid necrosis, atherosis (inflammation within the wall).
3) Amniotic epithelium - Is it intact, vacuolated, squamous metaplasia, amnion nodosum?
4) Pigment containing macrophages - Is it meconium (severity and depth of macrophage migration)? Is it hemosiderin (severity)?
5) Chronic inflammation - Where is it (chorion, intermediate trophoblast layer, or decidua)? How severe is it (mild, moderate, severe)? Composition (lymphocytes, plasma cells, macrophages)?
6) Laminar necrosis - Where is it (chorion or decidua)? How much?
7) Yolk sac remnant.
8) Fetus papyraceous.
9) Viral inclusions - very rare.

Umbilical cord

1) Number of vessels - Thrombi (occlusive, non-occlusive)? Dilatation (ectasia) or narrowing? Hemorrhage? Necrosis of smooth muscle wall of vessels (+/- meconium)?
2) Congenital remnants - Allantoic or vitelline (omphalomesenteric) duct remnants? Vitelline vessels?
3) Acute inflammation (fetal inflammatory response)- Where is it (inside vessel lumen, vessel wall (identify as artery or vein), periphery of cord (funisitis))? How severe is it (microabscesses)? Composition (PMNs, eosinophils, yeast)? Stage?
4) Chronic inflammation - Where is it (vessel walls, matrix)? How severe is it? Composition (lymphocytes, plasma cells, macrophages)?
5) Squamous metaplasia of amnion- Amount (focal vs. diffuse)? Severity?

Placental Parenchyma & Basal Plate

1) Villous maturation - Appropriate for gestational age (first, second or third trimester)? Hypermature (maternal vascular underperfusion). Appropriate. Immature – dysmature (diabetes, edema, syphilis).
2) Fetal vasculature changes - What vessels are affected (chorionic plate, stem)? Findings (fibrinoid necrosis, thrombi, karyorrhectic debris in villi, ectasia, endothelial cushions, avascular villi, hemorrhagic endovasculitis)? Extent (mild, moderate, severe)?
3) Decidual (maternal) vascular changes - What vessels are affected (basal plate)? Findings (thrombi, fibrinoid necrosis, atherosis, persistent muscularization of basal plate arterioles)? Extent? Associated decidual necrosis?
3) Villous edema - Extent (mild, moderate, severe)? *Caution in early gestation (first trimester) normal villi can appear edematous.
4) Villous ischemic changes - Where do the lesions occur (near chorionic or basal plate)? Findings: infarcts (age - early, recent +/- acute inflammation, old, extent as % of total

parenchyma), increased syncytial knots (focal or diffuse), increased calcification, reduction in size of terminal villi (distal villous hypoplasia)?

5) Intervillous space - Where do the lesions occur? Findings (intervillous thrombus, hemorrhage, acute inflammation, fibrin, chronic inflammation (macrophages))?

6) Acute inflammation - Where is it (villi, intervillous space, decidua, chorionic plate)? How severe is it? Composition?

7) Chronic inflammation - Where is it (intervillositis, villitis, deciduitis, chorionitis)? How severe is it? Composition (plasma cells)? Viral inclusions (TORCH)?

8) Decidual necrosis - Associated findings at basal plate/membranes (+/-inflammation or maternal vascular disease)? *Beware the normal fibrinoid layers!

9) Retroplacental hematoma - Where is it (marginal or central)? What is the age ((+/-hemosiderin) chronic or acute)? Does it disrupt villous spacing? Extent? Clinical significance (*this is critical - correlate with clinical history; DO NOT call an abruption without supporting clinical history)? Other associated findings? So-called “hidden abruption”? An acute abruption may show no gross or histologic changes at all.

10) Fibrin deposition - Where is it (intervillous space, basal plate, everywhere)? Extent (estimate % of intervillous space involved)?

11) Chorangiosis - Rule of 10s (10 areas with more than 10 villi with more than 10 blood vessels in cross-section in each terminal villous).

12) Nucleated fetal red blood cells - Extent of increase in number (mild, moderate, severe)? *Varies with gestational age (less with maturity). Look for other possible fetal intravascular disorders (leukemia, neuroblastoma).

13) Attached myometrial fibers – Is it accreta?

14) Weird stuff - Tumors, inborn errors in metabolism (is there increased vacuolization of the syncytiotrophoblast, Hofbauer cells?), chorangioma, etc.

IMPLANTATION AND EARLY PLACENTAL DEVELOPMENT

The fetal contribution to implantation involves primarily trophectoderm differentiation during blastogenesis. Following fertilization the zygote undergoes cell division producing smaller cells (blastomeres) while traveling through the Fallopian tube. The morula, still surrounded by the zona pellucida, is simply a ball of cells. The cells become tightly aligned – referred to as compaction. Compaction results in some internal cells which will eventually differentiate into the inner cell mass (destined to become the embryo), while the outer cells will differentiate into trophectoderm (eventually the trophoblast and placenta). The morula at about 12-16 cells enters the uterine cavity. In the morula a space develops between the inner cells and the outer cells resulting in the blastocyst. The zona pellucida disappears at this point (referred to as hatching). The blastocyst now rolls along the uterine cavity, eventually usually orienting the embryonic pole to face the endometrial lining. The embryo then attaches to the uterine wall (usually between days 5 and 6 post-fertilization).

The first true differentiation event in human development is the formation of the trophectoderm from the inner cell mass. This is regulated at a molecular level by a critical switch resulting in trophectoderm rather than inner cell mass and involves a decrease in Oct4 levels with an associated increase in Cdx2 (the relative ratio is critical). Oct4 is a major pluripotent gene necessary to maintain the pluripotent state of the developing embryo and eventually the epiblast. Cdx2 acts as the molecular switch resulting in trophectoderm differentiation. It has recently been shown that another transcription factor Tead4 acts upstream of Cdx2 and is also critical for trophectoderm differentiation. Other genes have also been implicated in regulating this early differentiation event including Sox2 (uncertain if upstream or downstream of Tead4), whereas Gata3 appears to be important downstream of Tead4 and in parallel with Cdx2. The exact details of this genetic cascade remains to be clarified.

Maternal contribution to implantation involves proper decidualization. In the normal menstrual cycle, following ovulation and fertilization, implantation will occur on the endometrium in the secretory phase, following estrogen priming and early decidualization induced by progesterone produced by the corpus luteum. The endometrium consists of a single layer of surface epithelium and associated underlying stroma. Under the influence of progesterone the stromal cells become decidualized and take on a different histologic appearance (larger with lipid and glycogen vacuoles) and altered function. Decidua impedes the movement of invasive trophoblast by forming a physical barrier and generating cytokines to promote trophoblast attachment (rather than invasion). Hence proper decidualization and continued production of progesterone is essential for implantation and maintenance of pregnancy. The early decidua also provides nutrients to the developing embryo as the placenta develops. The decidua aids in the development of immunologic tolerance so the maternal immune system does not “reject” the developing embryo. Initially the corpus luteum produces progesterone but as the placenta forms, it takes over the production of progesterone. By about week 6 or 7 post fertilization the placenta is the major source of progesterone production. Immediately below the endometrium/decidua is the myometrium (smooth muscle layers) of the uterus. Aberrant invasion of the placenta can result in a life threatening situation for the mother during delivery as the normal separation plane of the placenta following birth of the fetus is at the level of the decidua and a fetal fibrinoid layer. If decidua is absent the placenta may not completely separate and can result in excessive maternal hemorrhage

and/or retention of part of the placenta. Normal human blastocyst implantation occurs in the anterior or posterior wall of the body of the uterus. Implantation in the lower uterine segment over the cervical os is referred to as *placenta previa*. This results in the placenta blocking fetal exit from the uterus during parturition and can result in fetal hypoxia and death and significant maternal hemorrhage.

At a molecular level attachment of the blastocyst is mediated by L-selectin on the trophoblast surface interacting with the carbohydrate receptors on the uterine epithelium (this is a similar process to neutrophils attaching to endothelium prior to exiting as part of inflammation). Several other cell interaction systems are also important including integrins and laminin in facilitating attachment and fibronectin in regulating migration. These signaling systems also crosstalk with other intracellular signaling pathways to modulate trophoblast differentiation.

By day 7.5 to 8 the blastocyst is partially embedded in the endometrial stroma. The embryoblast is normally at the invasion site with differentiation of the underlying trophoblast into 1) cytotrophoblast (an inner layer of mononuclear cells that are rapidly proliferating) and 2) an outer multinucleated zone without cell membranes (a true syncytium) the syncytiotrophoblast. The cytotrophoblast contribute the cells to the syncytiotrophoblast by fusion. The syncytiotrophoblast are the lead point of invasion into the endometrial stroma. The inner cell mass differentiates into 2 layers 1) the hypoblast (destined to form the yolk sac) and 2) the epiblast destined to become the embryo proper. A small cavity appears within the epiblast which is destined to form the amniotic cavity. The epiblast cells adjacent to the cytotrophoblast are called amnioblasts and will give rise to the amnion (the cuboidal cell layer lining the amniotic sac in which the embryo/fetus will develop). The endometrial stroma (decidua) adjacent to the implantation site is highly vascular and edematous.

Day 9 represents the *lacunar stage* of development. The blastocyst is more deeply imbedded into the endometrium and the penetration defect is covered by a fibrin clot. Vacuoles begin to form within the invading syncytium and these fuse to form large lacunae (without blood at this stage).

Day 11-12 the blastocyst is completely embedded within the endometrial stroma with re-epithelialization of the uterine surface. The embryonic pole continues to be the site of syncytiotrophoblast invasion and lacunae formation. At the opposite (abembryonic) pole, the trophoblast consists almost entirely of cytotrophoblast. At the invasion front the syncytiotrophoblast begin to invade maternal vessels and sinusoids and erode endothelium resulting in the maternal blood filling the lacunae (these will become the intervillous spaces that will bathe the villi in the mature placenta). The syncytiotrophoblast are now in direct contact with maternal blood. This represents the establishment of the uteroplacental circulation. These spaces are fed by maternal spiral arteries in the uterus. A new population of cells arises at this time, referred to as extraembryonic mesoderm, from between the cytotrophoblast and the outer surface of the exocoelomic cavity (derived from yolk sac cells). These cells fill the space between the trophoblast and the amnion and are destined to become chorion. The chorion is mesenchymal tissue that will act as an interface between maternal decidua and embryonic amnion. Large spaces in the extraembryonic mesoderm form and become confluent to form the chorionic cavity, which completely surrounds the yolk sac and amniotic cavity, except for a connecting stalk (future umbilical cord) that leaves the embryo attached to the trophoblast. Decidualization of the entire endometrium continues.

By day 13 at the invasion front the cytotrophoblast proliferates and penetrates into the syncytiotrophoblast forming cell columns (surrounded by syncytiotrophoblast) which are referred to as primary villi. The chorionic cavity continues to form.

By the beginning of the third week the primary villi consist of a cytotrophoblast core covered by syncytiotrophoblast and extraembryonic mesoderm cells begin to invade the cytotrophoblast core growing towards the decidua and this is now referred to as a secondary villous. By the end of the third week these mesoderm derived cells will begin to differentiate into blood cells and small vessels (vasculogenesis – development of new blood vessels de novo without preexisting vessels to branch from). Remember angiogenesis represents new blood vessel formation from preexisting vessels, and angiogenesis will be important for expansion of the placental vascular network later once the initial vascular system is established. The villi are now referred to as tertiary or definitive villi. The capillaries in the villi will connect to the developing vessels in the chorionic plate and connecting stalk which will connect to the intraembryonic blood vessels, thereby connecting the fetal and placental circulations in preparation for the beginning of a continuous fetal circulation when the fetal heart begins to beat in the fourth week of development. The cytotrophoblast continue to invade the syncytiotrophoblast and eventually penetrate to the maternal endometrium and will form the outer cytotrophoblast shell which firmly attaches the chorionic sac to the endometrium. The cytotrophoblastic shell is in direct contact with the decidua. These will become the extravillous trophoblast at the basal plate. Remember one of the functions of the decidua is to help prevent further trophoblast invasion. Anchoring or stem villi are those that extend from the chorionic plate to the decidua basalis (basal plate) and terminal villi are those villi that arise as small branches from the stem villi.

By the second month many secondary and tertiary villi have formed from the stem villi. The surface of the villi in direct contact with the maternal blood of the intervillous space is covered by syncytiotrophoblast which rests on a layer of cytotrophoblast, which in turn covers a vascular mesodermal core. Erosion of the maternal spiral arteries (remodeling) to allow for emptying blood into the intervillous spaces occurs by endovascular invasion by specialized extravillous cytotrophoblast cells. The maternal spiral arteries are normally surrounded by smooth muscle and lined by endothelium. These vessels are normally (nonpregnant state) reactive to various cytokines especially under conditions of hypoxia/ischemia the vessels can constrict to divert blood away from the uterus. During pregnancy this would be disadvantageous as the fetus would be deprived of oxygen. Remodeling of the spiral arteries involves elimination of the outer smooth muscle layers and replacement of the endothelium by extravillous trophoblast cells that function as the endometrium during pregnancy. This effectively diminishes artery reactivity and helps maintain uterine and thereby fetal circulation even under conditions of maternal hypoxia/ischemia. During remodeling the extravillous trophoblast will partially obstruct (plug) these spiral arteries inducing a relative hypoxic/ischemic state, thought to be critical for early placental development (both villous and vascular). Within the terminal villi the layers separating the fetal and maternal circulations at this point are syncytiotrophoblast, cytotrophoblast, connective tissue, and fetal endothelium.

During placental development the villi at the embryonic pole continually expand and give rise to the chorion frondosum (bushy chorion), whereas on the abembryonic pole the villi degenerate and form the chorion laeve (smooth chorion). The decidua over the chorion frondosum is referred to as the decidua basalis (basal plate) consisting of large decidual cells with abundant lipid and glycogen and tightly connected to the chorion. The decidual layer over

the abembryonic pole is referred to as the decidua capsularis which becomes thin and degenerated. The chorion laeve will come in contact with the uterine wall (referred to as decidua parietalis) and fuse obliterating the uterine cavity. By the third month only the chorion frondosum and decidualis basalis will participate in gas and nutrient exchange and form the definitive placenta. Similarly the amnion overgrows the embryo to completely surround it and eventually fuses with the chorion, obliterating the chorionic cavity and forming the amniochorionic membranes, which are examined as part of our routine pathologic examination of the placenta. It is this membrane that ruptures during labor.

During development, the cytotrophoblast can differentiate into specialized functional cell types usually referred to as extravillous trophoblast, as well as generate syncytiotrophoblast. There are several distinct extravillous trophoblast subtypes predominantly based on differences in functions including endovascular trophoblast (invade and remodel maternal vessels), trophoblast giant cells which can invade into myometrium, and others. Histologically most of these extravillous trophoblast cells resemble cytotrophoblast.

The mature placenta consists of a chorionic plate adjacent to the fetus and the decidual or basal plate composed of both fetal and maternal tissues and attached to the uterus. By the 4^{th} to 5^{th} month several decidual septae form that project into the intervillous space and separate the maternal surface into 15-20 cotyledons. The placenta continues to enlarge throughout pregnancy covering about 15-30% of the internal surface of the uterus. Attached to the placenta is the umbilical cord, which usually consists of 3 vessels, although a 2 vessel cord does occur, and fetal membranes consisting of amnion fused to chorion with a small amount of attached maternal decidua.

In the third trimester the placental weight will increase from about 300 grams at 28-30 weeks gestation to about 500 grams at term (38-40 weeks gestation). During this same period the fetal weight will increase from 1000 grams at 28-29 weeks gestation to 2800 grams at term (39-40 weeks). How does the placenta provide adequate nutrition and gas exchange when the fetal mass is effectively outgrowing the placental mass? The answer lies in increased branching of secondary and tertiary villi into smaller terminal villi at term. This effectively increases the surface area of the villi in contact with the maternal blood, while the overall placental mass does not have to increase as much. In addition, at term within the terminal villi the blood vessels are essentially directly subjacent to the syncytiotrophoblast layer thereby resulting in a decreased diffusion distance compared to more immature larger villi. Therefore the 2 main mechanisms by which the term placenta optimizes gas and nutrient exchange is through smaller terminal villi with increased surface area and by decreasing the diffusion distance between the maternal and fetal circulations. If the fetus experiences chronic hypoxia/ischemia in utero prior to term the villi will become smaller sooner in an effort to increase surface area, however, the placenta then cannot usually continue to grow appropriately. These prematurely small terminal villi are referred to as "hypermature" villi, distal villous hypoplasia, or Tenney-Parker change. All are indicative of pathologic processes (see below). In addition, local ischemic/hypoxic change in the placenta results in clumping of the syncytiotrophoblast nuclei into "knots". This is also morphologic evidence of localized ischemia/hypoxia within the placenta.

PATHOLOGY OF THE PLACENTA:

I. GROSS PATHOLOGY

A. *Size and shape:* The size and shape of the placenta is critical and is the lead off line in our surgical pathology reports. The weight of the placenta and the three dimensions of its size are recorded in the gross description. The measured weight is compared to standard tables (Pinar H et al. Reference values for singleton and twin placental weights. Pediatr Pathol Lab Med 16:903, 1996) and reported as large, small or appropriate for gestational age depending on whether the weight is greater than the 10%, less than the 10% or within the expected range.

1. Shape: A normal placenta is round to ovoid although multiple irregular shapes are possible as a result of disease (large remote infarct), manner or site of implantation, or atrophy. Dumbbell shaped placentas can occur where there is still parenchyma attaching both lobes. Abnormally shaped placentas probably arise due to abnormalities in decidualization or vascularization of the uterus resulting in local atrophy and irregular growth (e.g. implantation over a leiomyoma).

In multilobed placentas there is true separation of the lobes by a segment of intervening membranes. A bilobed placenta has two lobes of approximately equal size (2-8% of placentas). If one lobe is smaller than the other then it is reported as an accessory or succenturiate lobe (5%). Umbilical cord insertion can be into either lobe (usually the dominant lobe) or velamentous. Membranous vessels are always present by definition. These vessels should be inspected carefully for possible thrombi, compression or rupture. Multilobed placentas are associated with vaginal bleeding, postpartum hemorrhage, placenta previa and retained placenta. The pathogenesis leading to each of these associations is not necessarily well understood.

2. Size: Large for gestational age placentas (>10 percentile) can occur in a number of clinical circumstances including diabetes, hydrops (from any cause), genetic background (normal variation), placental mesenchymal dysplasia, infections (classically syphilis), maternal obesity, unknown, others.

Small for gestational age placentas (<10 percentile) can occur as a result of maternal vascular underperfusion related to chronic hypertension or preeclampsia, from multiple infarcts, as a result of massive perivillous fibrin deposition, underlying maternal diseases (heart and kidney), associated with chronic villitis (either infectious or villitis of unknown etiology), in severe fetal thrombotic vasculopathy with extensive avascular villi, due to genetic background (normal variation including ethnicity), and frequently for unknown reasons.

Appropriate for gestational age placentas can still demonstrate severe pathology.

B. *Membranes & chorionic plate:* Normal membranes are clear and colorless. Acute chorioamnionitis can result in opaque membranes due to the presence of inflammation. The passage of meconium can also cause opacification and discoloration (brown to green). Chronic or large remote marginal of fetal plate hemorrhages can result in the accumulation of numerous hemosiderin laden macrophages in the membranes or at the chorionic plate and give a brown to tan discoloration to the membranes.

Normal membranes insert at the margin of the placenta. When the membranes insert closer to the central portion of the placenta than at the margin this is referred to as circummarginate insertion (reported in up to 25% of placentas). If there is folding of the membranes to form a ridge at the site of insertion this is referred to as circumvallate insertion

(occurring in 1-5% of placentas). Both are referred to as extrachorialis membrane insertion and this is relatively easy to assess by simply holding the membranes above the surface of the placenta and determining whether a portion of placental tissue protrudes past the insertion rim. We report the percentage of the circumference that is circummarginate (rarely complete) and the greatest amount of protruding placenta as X cm of extrachorialis placenta. Histology at the site of membrane insertion in these cases often reveals hemosiderin and increased fibrin. The pathogenesis leading to extrachorial placentation remains controversial, but a widely accepted model is that marginal hemorrhage undermines the membrane insertion site. Circummarginate and circumvallate membrane insertion seem to represent the same process but traditionally circummarginate insertion is not associated with adverse outcomes, whereas circumvallate insertion is associated with an increased risk of antenatal bleeding and premature delivery. Both can be associated with marginal hemorrhages, but the clinical significance of these hemorrhages is unknown.

We also indicate in our gross description the site of membrane rupture relative to the margin if it can be determined. Membrane sections should be taken to include the site of rupture.

Other membrane and chorionic plate findings include various cysts (amnionic epithelial inclusion cysts, subchorionic cysts and pseudocysts), yolk sac remnants, vernix caseosa (small collections of squamous cells and hair derived from the fetus and embedded beneath the amnion), and amnion nodosum (often associated with prolonged oligohydramnios). Subamnionic hemorrhages are common and usually occur intra- or post-partum. Subchorionic hemorrhages or fibrin thrombi are very common occurring in up to 60% of placentas. These usually appear as white plaques visible at the chorionic plate surface. Microscopically they are laminated fibrin thrombi and often contain hemosiderin. Subchorionic fibrin thrombi have been associated with preterm birth, abortion, vaginal bleeding, intrauterine growth restriction (IUGR), and fetal demise, however, most are associated with a normal pregnancy. They are more commonly found in placentas of mother's with severe heart disease or thrombophilias. A more extensive form of subchorionic hemorrhage is referred to as the Breus mole. The etiology leading to this large bosselated subchorionic hemorrhage is unknown.

C. *Basal plate & parenchyma:*

1. Hemorrhages (including abruption):

Background: A variety of hemorrhages can be identified grossly and are often described by their location. These include marginal or retroplacental hemorrhages (can be associated with abruption or occur peripartum), intervillous thrombi (usually pale laminated lesions that can occur anywhere in the placenta), and acute parenchymal hemorrhages (usually dark red and laminated but with more red blood cells). All retroplacental hemorrhages are not abruptions. A retroplacental hemorrhage is defined as hemorrhage at the site of placental separation from the uterus and may occur antepartum (these may be clinically silent or significant), peripartum, or following the delivery of the infant and as part of normal delivery of the placenta. Abruption is defined as a clinically significant antenatal detachment of the placenta usually with associated retroplacental hemorrhage. The result can be severe fetal hypoxic injury or death. Multiple causes for acute abruption include maternal vascular disease (hypertension, preeclampsia, thrombophilias, and autoimmune diseases), trauma, amniocentesis, uterine anomalies, placenta previa, folic acid deficiency, substance abuse including cocaine and nicotine, and multiparity. The most common causes being maternal vascular diseases and trauma from MVA. The frequency of abruption is estimated at about 1-4% of all deliveries. The

placental separation can be complete (rare) or partial. The clinical triad includes vaginal bleeding, pain, and rigid abdomen. Acute abruption is usually thought to involve disruption of maternal arteries.

Pathology: Acute retroplacental hemorrhages that occur less than an hour before delivery cannot be distinguished from normal postpartum blood clot. It takes several hours for an antenatal retroplacental hemorrhage to become firmly attached to the placenta grossly. The underlying placental parenchyma becomes compressed and focally necrotic +/- acute inflammation and with extended time the associated villi will infarct. The RBCs breakdown with hemosiderin accumulation (4-5 days) and increased perivillous fibrin deposition. Other findings suggestive of maternal vascular underperfusion should be sought. If the hemorrhage is large and has been present for an extended time it will indent the maternal surface. The correlation between pathologic and clinical abruption is poor. I rarely will call retroplacental hemorrhage an "abruption" straight out in the diagnostic line. If there is a good clinical history of suspected abruption and I find gross and histologic evidence of a retroplacental hemorrhage I will usually call it a "acute/subacute/remote retroplacental hemorrhage (measuring XXXcm in greatest dimension) consistent with the clinical history of possible abruption."

Clinical associations: Acute abruption is associated with numerous adverse outcomes including preterm delivery, IUGR, stillbirth, and hypoxic/ischemic brain injury. Chronic abruption is thought to involve venous disruption rather than arterial. Chronic abruption is associated with multiparity, smoking, oligohydramnios and deep uterine implantation. Chronic abruption is associated with other placental findings including remote marginal hemorrhage with hemosiderin, membranes or chorionic plate with hemosiderin laden macrophages, and circumvallate membrane insertion. Chronic abruption is associated with an increased risk of preterm delivery, cerebral palsy and other neurologic impairment in term infants.

2. Intervillous thrombi:

Background: Intervillous thrombi are common, occurring in up to 20% of term placentas. The origin of the blood has been found to be fetal in many cases. There is an association between fetomaternal hemorrhage and the presence of intervillous thrombi. The diagnosis of fetomaternal hemorrhage must be made by flow cytometry or a Kleihauer-Betke test on maternal blood as there are no specific gross or histologic findings in the placenta that are diagnostic. Intervillous thrombi can also result from thrombosis of maternal blood in the intervillous space. Maternal disease states such as thrombophilias or preeclampsia (diminished perfusion) are associated with increased likelihood of intervillous thrombi.

Pathology: Grossly these thrombi can be red if acute or pale tan to white if remote and are usually well demarcated parenchymal lesions. As they age the laminations become evident and these lesions can usually be accurately diagnosed grossly. Histology reveals expansion of the intervillous space by layers of fibrin and red blood cells. Villi do not routinely become "entrapped", but are more likely to be pushed away by the hemorrhage. If large, the adjacent villi can become infarcted and show evidence of ischemic change (distal villous hypoplasia and increased syncytial knots). These lesions should be sampled, examined histologically and reported.

Recent Reference:

Carles D, André G, Pelluard F, Martin O, Sauvestre F. Pathological Findings in Feto-maternal Hemorrhage. Pediatr Dev Pathol. 2014 Mar-Apr;17(2):102-6. doi: 10.2350/13-12-1419-OA.1. Epub 2014 Feb 27

3. Villous infarcts:

Background: Placental infarcts represent localized areas of ischemic necrosis of villi usually due to diminished maternal perfusion to the intervillous space. Small infarcts are common and can be seen in up to 25% of normal pregnancies and are commonly seen at the margin. They are found much more commonly in placentas from patients with hypertension or preeclampsia.
Pathology: Grossly acute infarcts tend to be red and change to white with time. They usually have well defined borders and lack the laminations seen in intervillous thrombi. Microscopically the earliest change is loss of the intervillous space with villous crowding. There is usually increased perivillous fibrin deposition in some of the remaining intervillous spaces and around the outside of the ischemic area. Acutely there can be a brisk acute inflammatory response to the infarcted necrotic tissue. With time the nuclear chromatin within cells of the affected villi clumps and eventually fades away leaving pink acellular villi (ghost villi) with increased surrounding fibrin. Remote infarcts can have increased calcification. The surrounding villi that survive often show evidence of hypermaturation (distal villous hypoplasia and increased syncytial knots). Organization and fibrotic scarring as occurs in myocardial and other organ infarcts do not occur in placental infarcts.
Clinical associations: It has been estimated that over 50% of the parenchyma must be infarcted to result in fetal demise, but I believe it is much more complicated than that and that any given percentage of infarction cannot directly predict the likelihood of fetal death, i.e. even a small amount of infarcted villi within a small placenta may be sufficient to result in death. Clinically infarcts are associated with maternal vascular underperfusion due to the disease states listed below. There is a strong correlation between the presence and amount of infarcted placenta and IUGR and a small placenta. The percentage of affected parenchyma should be estimated and reported.

4. Chorangioma:

Background: Chorangioma represents a discrete neoplasm of benign proliferating capillaries and stroma occurring within a villous and can be identified grossly as a bulging red to white mass. This is different from chorangiosis and chorangiomatosis which are not identifiable grossly (see later).
Pathology: Histologically chorangiomas are composed of proliferating fetal blood vessels and a variable amount of fibrous and cellular stroma.
Clinical associations: These are rare lesions but are seen more commonly in multiple gestations and neonates with congenital anomalies. The clinical consequences, which are often associated with the size of the lesion, include fetal hydrops, stillbirth, IUGR, fetal anemia and thrombocytopenia, fetal congestive heart failure, DIC, premature delivery, abruption, and preeclampsia.

5. Calcifications: Calcifications of the basal plate and parenchyma are more likely with increased gestational age and are commonly prominent in late and post-term placentas. Calcifications can occur with or without other placental pathology. In general I will comment on the presence of increased calcifications in a report only if there is extensive calcification in almost all fields on most slides. The clinical significance, if any, is not usually clear.

6. Intervillous fibrin: Increased basal plate and/or intervillous fibrin raises concern for possible massive perivillous fibrin deposition/maternal floor infarct (see below). Grossly visible intervillous fibrin appears as white to tan curvilinear strands running through the parenchyma. Increased basal plate fibrin appears as a rind of white firm material 1-3mm thick covering all or part of the maternal surface, and is best appreciated on cut section. Estimated pathologic amounts of intervillous fibrin range from 30-50% of the total intervillous space.

D. *Umbilical cord:*

1. Embryonic remnants: Different types of remnants that can be found in the umbilical cord include allantoic duct remnants (usually found between the 2 umbilical arteries), omphalomesenteric duct remnants (usually at periphery of cord), and vitelline vessels. We do not report these.

2. Umbilical cord insertion: Most commonly the umbilical cord inserts centrally or eccentrically (>90%). These insertions are not associated with pathology. Marginal insertion (< 1cm from the true disc margin) occurs in about 7% of placentas. The clinical significance remains debated, but marginal insertions have been reported to be associated with preterm labor, neonatal asphyxia, abortions and malformed infants. We do report these in the diagnostic line of our report. Velamentous cord insertion is when the cord inserts into the membranes rather than into the disc and occurs in about 1% of singleton placentas, but with a much higher likelihood in twins. The pathogenesis remains unknown. Velamentous cord insertion has important clinical consequences. The vessels within the membranes are prone to trauma, rupture, compression and thrombosis and velamentous cords can result in the histologic changes associated with fetal vascular obstruction (see later). The velamentous vessels can also present during delivery prior to the fetus (vasa previa) with adverse consequences including rupture of the vessel(s) with massive fetal hemorrhage. Velamentous cords are associated with low birth weight, low Apgar scores, abnormal fetal heart rate patterns, prematurity, cerebral palsy, death, early abortion, and congenital anomalies. We always report velamentous insertion in the diagnostic line. Furcate cord insertion is rare and is defined by the umbilical vessels splitting and leaving Wharton's jelly prior to reaching the chorionic plate surface. Most infants with furcated cord insertion are normal, however, there is a weak association with stillbirth, thrombosis of fetal vessels, IUGR, and hemorrhage. Velamentous and furcate cord insertion can be difficult to distinguish from each other grossly in some cases. Interpositional cord insertion is also a form of membrane insertion whereby the vessels remain covered by Wharton's jelly but are also inserted in the membranes. The cord can appear to be embedded in the chorionic plate surface. Interpositional cord insertion does not carry the risks associated with velamentous insertion.

3. Coiling: There are numerous papers written on umbilical cord coiling or "twist." A recent study demonstrated that particular patterns of coiling (rather than hypercoiling) are associated with much worse outcomes for the fetus. We determine the coiling index (number of twists (coils) per 10 cm of cord) for all placentas. A normal coiling index is between 1 to 3 coils per 10 cm of cord. Less than 1 coil per 10 cm is considered undercoiled (hypocoiled) and >3 coils/10 cm is considered hypercoiled (10-20% of placentas). Coiling of the umbilical cord is thought to be a result of fetal movement. Focal areas of hypercoiling and localized strictures can be identified. The significance of umbilical cord strictures in cases of prolonged intrauterine fetal demise remains controversial. Additional pathologic evidence of mechanical obstruction to fetal blood flow should be sought to invoke an umbilical cord stricture as the cause of IUFD. Both hypocoiling and hypercoiling are associated with an increased risk of IUGR, fetal distress, and perinatal death in older studies but more recent studies suggest hypercoiled umbilical cords are not associated with poorer clinical outcomes. We always report abnormal coiling in the diagnostic line of our reports.

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4. Cord length and diameter: Normal umbilical cord length at term is 60 +/- 13 cm. Cord length increases with gestational age and appropriate tables should be consulted for gestational age (Naeye RL. Umbilical cord length: clinical significance. J Pediatr 107:278, 1985). In addition, genetic factors and fetal movement have been proposed to determine the length of the umbilical cord. Excessively long cords occur in about 5% of placentas and short cords occur in about 1-2% of placentas. Care must be taken in reporting a short cord as seldom is the entire cord submitted to pathology for evaluation. Long UCs are associated with cord accidents and entanglements (including nuchal cord), cord prolapse, true knots, excessive coiling, constricture, and thrombi. The clinical consequences of excessively long cords have been studied and include fetal distress, neurologic impairment, IUGR, and IUFD. Abnormally short cords are associated with cord hemorrhage, abruption, failure of descent, prolonged second stage of labor, fetal distress, low Apgar scores and fetal developmental and congenital anomalies (abdominal wall defects and amniotic band disruption sequence). We always report an abnormally long cord length in the diagnostic line of our reports. The expected cord diameter at term is between 1.2 and 1.7 cm but there can be segmental variation. Significantly increased cord diameters are often secondary to edema. An edematous cord by itself is not associated with an increased risk of an adverse outcome. Abnormally thin cords are associated with IUGR. There is a potential risk that the diminished Wharton's jelly can predispose the vessels to injury and rupture. Thin cords can occur in the setting of oligohydramnios from a variety of causes including maternal vascular underperfusion.
5. Umbilical cord hemorrhage: Cord hemorrhages can be seen grossly and/or histologically. If acute, which most are, one must be careful in ascribing significance to the hemorrhage. Most are likely the result of cord traction during removal of the placenta and occur following delivery of the infant. There are cases where a cord hemorrhage may be significant, such as intrauterine fetal demise, and additional supporting pathologic evidence of a clinically significant UC hemorrhage must be sought.
6. Umbilical cord vessel number: The normal constellation of umbilical blood vessels is 2 arteries and 1 vein. In less than 1% of singleton pregnancies and in up to 9% of twin

pregnancies there may be a single umbilical artery (SUA) or two vessel umbilical cord. Occasionally a small remnant of the other artery can be identified. The umbilical arteries can fuse away from the insertion site so one section may have three vessels and another only two. This fusion is called Hyrtl's anastomosis. Although most infants with a single umbilical artery have a normal outcome there is an associated increased risk for IUGR, antepartum hemorrhage, polyhydramnios and oligohydramnios. Mothers with diabetes are more likely to have infants with SUA than nondiabetics. In autopsy studies fetuses with a SUA have a high likelihood of other congenital anomalies, especially involving the kidneys. Interestingly, it is usually (about 75%) the left umbilical artery that is absent. The etiology is not well understood but in many cases it is thought to represent atrophy of one artery due to a diminished downstream area of villous perfusion. Accessory blood vessels in the umbilical cord are rare and can represent an aberrant right umbilical vein or more likely persistence of one or more vitelline vessel.

7. Knots: True umbilical cord knots are rare (<1%) and are classified as tight (clinically relevant) and loose (probably not important). Tight knots can result in umbilical vein compression with stasis and congestion of the vein. If long standing an umbilical vein thrombus can develop. The knot should be untied and sections should be taken from both sides of a knot to look for congestion and thrombosis. Tight knots are often seen in association with long cords, monoamnionic twinning, and multigravidas. Clinically tight knots increase the risk for intrauterine and intrapartum demise (up to 10% mortality) and the risk for a poor neurologic outcome. We report all true knots in the diagnostic line as either tight or loose. False knots are due to redundancy in fetal vessels in the cord and have no clinical significance.

8. Thrombi:

Background: Umbilical cord thrombi occur in about 1:1300 deliveries. Umbilical cord vein thrombosis is more common, whereas arterial thrombosis is more likely to result in fetal death.

Pathology: Thrombi within the umbilical cord can result in focal areas of vascular swelling and/or discoloration. These areas should be examined closely and if a thrombus is suspected, histologically examined. Microscopic features of a true thrombus need to be identified including dilation of the caliber of the involved vessel, a fibrin thrombus (multiple layers of fibrin and blood) within the lumen, attachment to the wall of the vessel, and/or organization. Depending upon how acute the thrombus may be, identification as a true thrombus can be difficult. Fibrin thrombi do not occur post-mortem.

Clinical associations: Umbilical cord vascular thrombi are associated with cord abnormalities (velamentous insertion, true knot, inflammation, entanglement (nuchal or shoulder), hypercoiling, amnionic bands, diabetes, thrombophilias (controversial) and others). The clinical consequences of umbilical cord thrombosis can be grave including death, IUGR, and neurologic injury including neonatal stroke and/or thrombosis possibly from embolic events.

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II. AMNIONIC FLUID INFECTION (AMNIONIC INFECTION SYNDROME)

Background: Infections during pregnancy that affect the placenta can occur via 2 main routes: ascending (usually bacterial) infections from the vagina/cervix and hematogenous spread from the maternal circulation (more commonly viral and parasitic – TORCH, but bacterial infections can also occur – Listeria, Fusobacterium nucleatum (*from the oral cavity)). Less commonly, infections involving the placenta and/or fetus can be acquired by direct inoculation from a diagnostic procedure such as amniocentesis or chorionic villous sampling or from direct extension of infections from adjacent organs including the endometrium (maternal chronic deciduitis) or appendix. This section focuses on the pathologic findings associated with an ascending bacterial infection resulting in amnionic fluid infection that is often referred to as acute chorioamnionitis. The presence of the infectious agent induces an acute inflammatory response, usually first inciting maternal neutrophils and later fetal neutrophils. Early studies implicated the extent and severity of the inflammation in being associated with poor fetal outcomes such as preterm premature rupture of membranes, anoxic brain injury including cerebral palsy, thrombosis, and fetal sepsis. In an effort to better classify the inflammation associated with ascending bacterial infections a systematic pathologic classification scheme was developed about 10 years ago (**Redline RW et al. Amniotic infection syndrome: Nosology and reproducibility of placental reaction patterns. Pedatr Devel Pathol 6:435-448, 2003). At Northwestern we use a slightly modified version of this scheme for reporting acute amnionic fluid infection.

Pathology: Acute chorioamnionitis can be divided into two components, the maternal inflammatory response and the fetal inflammatory response. Each of these is then assigned a stage (based on the location and extent of inflammation) and grade (based on severity). We only report stage. The stage of the maternal inflammatory response in the membranes and chorionic plate are referred to as stage 1) acute marginating choriodeciduitis (sub-chorionitis) if the neutrophils are restricted to the subchorionic fibrin and decidual-chorionic interface (6-12h of infection); stage 2) acute chorioamnionitis if the neutrophils have entered the chorion and/or amnion (12-36h of infection); and stage 3) necrotizing chorioamnionitis if there is clear evidence of amnion necrosis (not just separation of the amnion from the chorion) (>36 h of infection). These stages of maternal inflammatory response have been roughly estimated to be associated with the duration of infection (see reference 3 for discussion). The timing can be affected by multiple factors so they should be considered rough estimates. The grading of the maternal inflammatory response is based on the number of neutrophils within a cluster with less than 10-20 neutrophils per cluster being grade 1 and anything more than 10-20 being grade 2. We do not routinely grade the maternal inflammatory response. The stage of the fetal inflammatory response in the umbilical cord and chorionic plate vessels are referred to as stage 1) chorionic plate vasculitis or umbilical cord phlebitis (only the vein is involved); stage 2) if one or more of the umbilical arteries is involved by the inflammation and referred to as umbilical arteritis. If all vessels of the umbilical cord are involved this is still stage 2 but we

refer to this as panvasculitis; stage 3) acute inflammation encircling the vessels with associated vessel wall or Wharton's jelly necrosis (this is relatively rare). Grading of the fetal inflammation involves the extent of the inflammation and the presence of confluent collections of neutrophils (grade 2) and if less than this, it is assigned grade 1. By this definition a stage 3 fetal inflammatory response is also a grade 2. Again we only report the fetal inflammatory response stage. Studies have shown that only stage 2 or 3 fetal inflammatory responses are associated with poorer fetal outcomes. We always comment on the presence of associated intravascular thrombi as this has been reported to be associated with an increased risk for neurologic impairment. If the acute inflammation extends out into Wharton's jelly but does not reach stage 3 levels we refer to the vessels involved and state there is also "funisitis."
Peripheral funisitis: Candida infections frequently result in microabscesses at the outer periphery of the umbilical cord +/- fetal vasculitis, so we always survey the entire cord under the microscope at low power. If peripheral microabscesses are seen we always perform a PAS or GMS stain. If there are only peripheral microabscesses and no vasculitis we use the terminology "peripheral funisitis with microabscesses". If yeast/fungus is identified within the umbilical cord (candida funisitis) of a live neonate we consider this a "critical value" and call the clinician. While the risk of the neonate becoming ill is relatively low (<10%) those that do show signs of sepsis within the first week of life are at increased risk for poor outcomes because they are usually treated as bacterial infections and not as yeast sepsis.
Subacute chorioamnionitis represents mixed acute and chronic inflammation involving the chorionic plate and/or membranes and is thought to represent a more chronic infection perhaps by an organism with low pathogenicity.
Acute villitis and intervillositis represents acute inflammation (neutrophils) involving the villi and adjacent intervillous space, usually with direct injury to the involved villi. There may be microabscesses. This should raise suspicion for Listeria monocytogenes infection. However, other organisms (E. coli, group B streptococcus (GBS), and others) can less commonly result in a similar pattern. In addition, acute villitis or acute intervillositis can be seen in severe maternal septicemia.
The pathology report should include a description of both the maternal and fetal inflammatory responses (you not have to use the specific stage/grade) involving both the membranes and at the chorionic plate.
Clinical associations: The clinical features that define acute chorioamnionitis include fever >37.5 C, uterine tenderness, abdominal pain, foul smelling vaginal discharge, maternal and fetal tachycardia, and an elevated maternal white blood cell count. The pathologic findings of acute chorioamnionitis with the associated fetal vasculitis represent the maternal and fetal reactions, respectively, to an ascending maternal bacterial infection. Chorioamnionitis in a preterm pregnancy can result in preterm premature rupture of membranes and/or premature labor. It is estimated that 50-60% of all preterm placentas have evidence of amnionic fluid infection. In most cases there is a general consensus that the infection precedes and probably causes the rupture of membranes. However, prolonged rupture of membranes for any reason (prior infection, incompetent cervix, hemorrhage, others) predisposes the mother to a secondary ascending infection. Bacterial vaginosis is a known risk factor for infection as well. These premature neonates often develop complications associated with prematurity including possible fetal/neonatal sepsis. In near-term and term fetuses, chorioamnionitis is rarely the definitive cause of fetal death unless there is also evidence of fetal pneumonia, sepsis or thromboemboli. In a term stillbirth with no other cause of death and with only a stage 1 or 2

maternal inflammatory response in the placenta and no direct evidence of fetal infection (histology and cultures are negative) is usually listed as an unknown cause of death. However, from a medical-legal perspective one cannot totally exclude the possibility of overwhelming sepsis related to a cytokine storm. Chorioamnionitis at or near term is common (5-15%) and is usually an incidental finding. The presence of a fetal inflammatory response, especially if one or more arteries are involved by inflammation, indicates a higher likelihood of fetal exposure to the inciting agent and possible fetal complications. In addition, studies have indicated that the amount of the fetal inflammation is associated with higher likelihood of cerebral palsy and poor neurologic outcomes. The older term for the fetal inflammatory response was funisitis, but should now be referred to as fetal vasculitis if 2 or more vessels are involved or phlebitis (vein) or arteritis (artery) if a single vessel is involved. The term funisitis is now used to refer to neutrophils in Wharton's jelly. While there is little doubt that the specific causative organism leading to the ascending infection is important for neonatologists in order to treat suspected neonatal sepsis, routine identification of the causative organism from the placental tissue is difficult for the pathologist. Gram stains can be helpful in selected cases (GBS, fusobacteria, or listeria), but placentas are frequently contaminated by vaginal and fecal flora so interpretation of a causative organism should be made with care. We do not routinely culture placentas and rarely perform gram stains. In rare cases of stillbirth overwhelming bacterial infection can result in IUFD without a significant inflammatory response. Post-mortem cultures and histology can be extremely helpful in these cases.

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III. CHRONIC VILLITIS

Background: Chronic villitis is defined as chronic inflammation (lymphohistiocytic with or without plasma cells) involving the chorionic villi. Chronic villitis can be separated into 2 broad categories based upon etiology, although the histologic findings seldom distinguish between them. Chronic villitis can be due to infectious agents or villitis of unknown etiology (VUE).

Chronic villitis from the combined causes is relatively common, being found in about 10-15% of all placentas. Infectious causes for chronic villitis are due to the hematogenously spread transplacental agents which include the TORCH infections. Common infectious agents leading to chronic villitis include CMV and syphilis and others less commonly encountered include Toxoplasma gondii, rubella, herpes, varicella zoster, hepatitis B and C, poxviruses, enteroviruses, parvovirus B19, HIV, spirochetes, parasites, and others. Chronic intervillositis without villitis is more commonly seen due to P falciparum (malaria) and B burgdorferi (lyme disease). The details of each of these infectious agents and the associated placental pathology are well described in Manual of Pathology of the Human Placenta 2nd ed. RN Baergen Springer Science 2011. In general, infectious causes for chronic villitis are reported to represent only 5% of all chronic villitis cases, however, our experience is that it likely even less (more likely <1%). The inflammatory infiltrate in infectious causes and non-infectious causes are similar (lymphohistiocytic), although it is reported that the presence of plasma cells, fibrosis, and mineralization of the involved villi should raise greater concern for infectious etiologies. Our practice is to look carefully for viral inclusions by H&E (CMV, parvovirus, herpes) or toxoplasmosis cysts and order a CMV immunohistochemical stain if the amount of inflammation is severe (see below) or we are concerned based on the H&E findings. If the inflammation is predominantly granulomatous-like (this could still be VUE) or there are necrotizing granulomas we will order stains for acid fast bacteria (concern is for TB or leprosy – both very rare). Villitis of unknown etiology (VUE) which accounts for more than 95% of chronic villitis is essentially defined by the lack of a clear cause. It is now thought that most of these cases represent alloimmune mediated villous injury. The majority of the lymphocytes and histiocytes involved in the villous destruction have been shown to be of maternal origin although within the villi fetal macrophages participate in the inflammatory process and it is thought that there is a maternal inflammatory response (CD3 positive maternal T-cells) to some unknown fetal antigen with a resulting graft-versus-host like reaction (see reference 11). This would explain the high recurrence rate. This is also supported by the higher incidence of chronic villitis associated with donor oocyte IVF compared to non-donor IVF (see reference 8).
Pathology: Chronic villitis due to VUE usually shows nonuniform (multifocal) involvement of the placenta. The chronic inflammatory infiltrate of lymphocytes, histiocytes (including histiocytic giant cells) and plasma cells has been classified into low and high grade villitis based upon the number of involved villi (see **Redline RW Villitis of unknown etiology: noninfectious chronic villitis in the placenta Hum Pathol 381439-1446, 2007). Less than 10 villi per cluster involved by inflammation is classified as low grade and if there are more than 10 villi per focus this is classified as high grade. Each of these is then further staged based upon the extent of placental involvement. Low grade chronic villitis can be focal (only one slide involved) or multifocal (more than one slide involved). High grade villitis can be staged as diffuse if >5% of all placental villi are involved by inflammation and called patchy if <5% are involved. There are multiple patterns of involvement of the placenta by chronic villitis (see above paper for details). In chronic villitis involving the parenchyma, the inflammation is thought to result in fetal vascular destruction in terminal villi and can result in avascular villi which are a common associated finding. The presence of avascular villi should raise concern for one of two entities (chronic villitis or fetal vascular obstruction (fetal thrombotic vasculopathy)) and prompt a search for additional evidence for these. In severe forms of chronic villitis obliterative fetal vasculopathy can be seen. These areas show villous vascular wall injury and these vessels may contain thrombi. Concern should be raised for possible

syphilis which results in a villous endarteritis that can appear similar, but usually has more plasma cells. Chronic villitis is often associated with placental findings including chronic deciduitis with plasma cells, chronic chorionitis, increased perivillous fibrin, occasionally chronic intervillositis, and more recently eosinophilic/T-cell vasculitis.

Chronic chorioamnionitis has recently been described as the most common placental lesion seen in late preterm birth and correlated with a rise in incidence of VUE in this population. The significance of this observation remains to be determined.

Clinical association: Diffuse high grade chronic villitis is associated with a high recurrence rate (10-25%) and poor outcomes including IUGR, premature delivery, adverse neurologic outcomes, and stillbirth. A recent meta-analysis, however, failed to confirm a link between VUE and stillbirth. Low grade or basal chronic villitis are usually not associated with adverse outcomes. Basal villitis is defined by the restriction of the chronic inflammation to villi near the basal plate. Basal villitis can be seen in association with leiomyomas, uterine malformations, chronic endometritis and abnormal placental implantation (retained placenta, placenta accreta). A poorly understood association exists between fetal alloimmune thrombocytopenia and VUE (Althaus J et al. Chronic villitis in untreated neonatal alloimmune thrombocytopenia: an etiology for severe early intrauterine growth restriction and the effect of intravenous immunoglobulin therapy. Am J Obstet Gynecol 2005; 193:1100-1104). We have seen 3 cases of neonatal alloimmune thrombocytopenia associated with severe diffuse chronic villitis in the last 2 years and it is important for the neonatologists to recognize this association to provide proper treatment for the thrombocytopenia.

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IV. MATERNAL VASCULAR UNDERPERFUSION

Background: Maternal vascular underperfusion of the intervillous space (also known clinically as uteroplacental insufficiency or placental malperfusion) occurs from a variety of causes. Systemic maternal diseases such as severe heart disease, diabetes mellitus, maternal hypertension (chronic or pregnancy induced hypertension and preeclampsia/eclampsia), thrombophilias (factor V Leiden mutation, deficiency of antithrombin III, protein C or S deficiency, hyperhomocysteinemia, prothrombin mutation), autoimmune disorders (particularly systemic lupus erythematosus), antiphospholipid antibody syndrome, chronic renal failure, and abnormalities of the maternal arteries supplying the uterus can result in diminished blood flow to the uteroplacental vascular bed. Despite years of research the exact pathophysiologic events mediating diminished placental perfusion in preeclampsia remains poorly understood. The currently widely accepted model is that failure of proper extravillous trophoblast invasion and remodeling of maternal spiral arteries results in a chronically underperfused placental bed with resulting decreased fetoplacental growth (small placenta and IUGR). The persistence of the smooth muscle cells around basal plate decidual vessels is proposed to result in persistent vasoreactivity to circulating vasoactive mediators and resulting in diminished lumen size and decreased blood flow into the placental intervillous vascular bed. This chronic ischemia can result in the release of additional vasoactive mediators (from fetus and/or placenta) and result in maternal hypertension and preeclampsia. A second event is proposed to be necessary following improper remodelling of basal plate arteries prior to the development of preeclampsia. Preeclampsia is defined as maternal hypertension associated with pregnancy after 20 weeks gestation and proteinuria. Preeclampsia is relatively common (2-7% of primigravidas), however, 75% of these are only mild cases. Risk factors include primigravida, young age, multiple gestation, history of chronic hypertension, previous preeclampsia, diabetes and thrombophilia. There are paternal risk factors as well. Most recently maternal obesity especially (morbid obesity with a BMI >40) is associated with an increased risk of preeclampsia. The pathogenesis leading to obesity related adverse effects in pregnancy including preeclampsia remains unclear but the number of obese pregnant women is increasing. The pathologic placental findings associated with obesity have recently been reported in a large cohort of women. Most of the findings are non-specific and include a higher rate of maternal vascular lesions, maternal origin villous lesions, fetal neutrophilic infiltration, and meconium affecting the fetal membranes (see reference 6).

Pathology: Probably the most significant gross pathologic finding associated with chronic maternal vascular underperfusion is small placental size (<10%). The histologic features of maternal vascular underperfusion are shared by all the various etiologies that lead to underperfusion such that it is impossible to distinguish maternal vascular underperfusion due to preeclampsia from that due to antiphospholipid antibody syndrome, etc. We divide the histologic findings that define maternal vascular underperfusion into “vascular lesions” and “villous changes.” The vascular lesions include fibrinoid necrosis (acute atherosis) of maternal (decidual) vessels found in the membranes or at the basal plate. The maternal vessels become surrounded by dense pink material (fibrinoid) and are often associated with acute or chronic inflammation. If clear evidence of foamy macrophages is seen we use the term acute atherosis. This process is, at best patchy, with only scattered vessels affected. The number of

affected vessels is not necessarily associated with the severity of the clinical presentation. This vascular injury is thought to result from vasospasm and diffuse endothelial injury although the molecular details are still being explored. These injured vessels can also contain fibrin thrombi. In thrombophilic states the thrombi may precede the vascular injury. Often associated with thrombosed decidual vessels are areas of decidual necrosis that can have acute inflammation. Another vascular lesion associated with maternal vascular underperfusion includes persistent muscularization of basal plate arteries. Any basal plate artery with smooth muscle cells is abnormal, as these should all be remodeled in the first and early second trimester. Finally hypertrophy of the smooth muscle cells in the membrane decidual arterioles is abnormal and reflective of maternal vascular underperfusion. The criteria for mural hypertrophy of membrane arterioles is that the thickness of the smooth muscle layers must be >50% (some studies used 30%) of the total diameter of the vessel or the vascular lumen is <50% (70% in other studies) of the total diameter (both sides of smooth muscle layers and the lumen together = total diameter). The villous changes associated with maternal vascular underperfusion include villous infarcts. Villous infarcts are quantitated as best as possible and we report the largest size and the total amount (%) of parenchyma affected. We also try to estimate the relative age of the infarcts which is a less than precise process. We report evidence of villous hypermaturation due to chronic underperfusion including increased syncytial knots and distal villous hypoplasia (abnormally small size of the terminal villi for that gestational age). The underperfusion of the intervillous space can also result in local areas with increased perivillous fibrin and villous agglutination. These latter 2 findings are nonspecific, however. The greatest problem with all of these criteria is determining how much of each criteria is needed to assign the diagnosis of "Findings consistent with maternal vascular underperfusion?" All of the above histologic findings can be found in varying amounts in many placentas that are small, large or normal size. Finally, there is the increased risk of abruption seen in patients with preeclampsia. We report the retroplacental hemorrhages separately and discuss any possible association with maternal vascular underperfusion in a note.

Clinical associations: Maternal complications associated with preeclampsia include abruption, DIC, acute renal failure, pulmonary edema, liver failure, stroke and death. Preeclampsia is associated with maternal HELLP syndrome (hemolysis, elevated liver enzymes, low platelets). Fetal complications of preeclampsia include preterm delivery, IUGR, abruption, hypoxic ischemic neurologic injury, and death. Mother's with severe preeclampsia or eclampsia are delivered as soon as possible as removal of the placenta usually ameliorates the symptoms. However, a premature neonate will face the usual potential for numerous complications associated with prematurity. The placental underperfusion can also result in a thin umbilical cord, which is predisposed to injury, and underperfusion can lead to oligohydramnios with potential adverse effects on normal lung development.

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V. FETAL VASCULAR OBSTRUCTION

Background: The pathogenesis leading to thrombus formation within the fetal vasculature is dictated by Virchow's triad, whereby thrombi can form as a result of endothelial damage, hypercoagulable states, or turbulent flow with stasis. A number of etiologies can cause one of these processes to be initiated and thereby result in thrombi within the fetal circulation. Possible etiologies include acute chorioamnionitis (this precludes the diagnosis of fetal thrombotic vasculopathy as fetal vascular thrombi can occur as part of an ascending maternal infection or fetal sepsis – a hypercoagulable state), umbilical cord obstruction/accident (can result in local endothelial injury and more significantly stasis of flow), fetal and/or maternal thrombophilia (this is a weak and controversial association), possibly toxic causes (meconium myonecrosis), polycythemia, fetal heart failure, chronic villitis with obliterative vasculitis and others. Overlap of many of the features of involutional changes associated with prolonged intrauterine fetal demise (avascular villi, hemorrhagic endovasculitis, recanalization of stem vessels) can make diagnosis of premortem fetal vascular obstruction difficult in stillborns. The distinction is best made by global involutional changes compared to histologic evidence of regional fetal vascular obstruction (more likely to be pre-mortem). Multiple risk factors may be present at one time and contribute to the final placental pathology. The most severe form of fetal vascular obstruction is defined by histologic changes referred to as "fetal thrombotic vasculopathy". The less severe forms may be best described as "findings consistent with fetal vascular obstruction". The severity does not necessarily relate to the etiology. Terminology and specific histologic criteria have been established to help categorize this type of placental pathology. What appears to be lacking, as with other forms of placental pathology, are good quantitative criteria for each histologic finding to better assign a specific cause.

Pathology: The placenta can be small or normal size. If the obstruction to fetal blood flow is long standing and severe there will be many avascular villi and the placenta is often small for gestational age. The histologic criteria for these lesions are well described (see references listed below). As with maternal vascular underperfusion we divide the histologic findings into vascular lesions and villous changes. The vascular lesions that define obstruction to fetal blood flow include thrombi involving chorionic plate or stem vessels as well as thrombi within umbilical cord vessels. These thrombi can be acute and difficult to definitively diagnose or organizing with attachment to the vascular wall (long-standing thrombi may be calcified). The acute thrombi are often difficult to identify grossly, whereas more remote thrombi are often white and more firm/solid. Vascular ectasia (dilation) is listed as a minimal histologic criterion for diagnosing a possible cord accident in stillbirths with abnormal umbilical cords (Parast and colleagues, see reference below). Mural intimal fibrin thrombi (also known as endothelial cushions) can occur in chorionic and villous stem vessels. These represent non-occlusive intramural fibrin thrombi usually characterized by residual hard-pink fibrin and fibroblast proliferation bulging from the wall into the lumen of a vessel. Long standing cushions can be calcified. Another vascular lesion frequently seen in placentas with fetal vascular occlusion are

recanalized chorionic and stem vessel lumens. If associated with extravasated and fragmented red blood cells these recanalized vessels have been referred to as "hemorrhagic endovasculitis". Recanalized vessels have been the focus of many heated discussions with regards to their true relevance and can occur as a part of involutional changes associated with intrauterine fetal demise. These are therefore not considered a specific criterion in fetal vascular obstruction. Villous changes include villous stromal-vascular karyorrhexis, which represents an early stage of apoptosis and degeneration of endothelial cells and stromal fibroblasts in response to vascular occlusion upstream. This is usually seen in focal areas associated with obstruction and more diffusely as an involutional change associated with IUFD. The end result of long standing fetal vascular occlusion is avascular villi. Avascular villi are usually seen in small to large localized collections representing the region with diminished fetal blood flow. They appear as collagenized pink villi without intravillous vessels. Unlike an infarct the syncytiotrophoblast is spared and remains basophilic. In addition, viable stromal cells can also usually be found. The villi are not necrotic but simply avascular. Remember the differential diagnosis of collections of avascular villi includes chronic villitis. The extent of each of the villous changes should be noted. Fetal thrombotic vasculopathy (FTV) is the terminology used to represent the most severe forms of fetal vascular obstruction and is defined by the presence of thrombi (the number is not defined) and significant numbers of avascular villi (>15 avascular villi per slide with at least 3 slides examined (45 total avascular villi)). There remains debate as to the significance of this particular number of affected villi. In general, FTV is characterized by more extreme vascular and villous changes than the less severe forms and pathologists should develop a threshold for specifying FTV using the above criteria as a guideline. In the study by Parast et al., the authors defined the minimal histologic criteria for designating a cord accident as the likely cause of death in stillborns with umbilical cord abnormalities. They defined the minimal histologic criteria to be vascular ectasia and thrombi with either avascular villi or stromal-vascular karyorrhexis in a regional and not diffuse pattern (the exact number of affected villi were not designated). There could not be abruption, infarction or chronic villitis.

Clinical associations: Obstruction to fetal vascular blood flow can result in IUGR, fetal monitoring abnormalities, neurologic injury, hepatic failure, limb infarction, renal vein thrombosis, infarcts in other organs including the GI tract, and intrauterine fetal demise. In our cohort of near-term and term stillbirths, fetal thrombotic vasculopathy represents a frequent cause of death (over 20% of cases). Fetal vascular thrombi in the setting of severe acute chorioamnionitis in very low birth weight infants is a major risk for neurologic impairment. Fetal thrombotic vasculopathy is highly associated with maternal thrombophilias, at least in one small study (see reference 6). In a relatively large study (see reference 7) placentas with fetal thrombotic vasculopathy have been shown to be associated with a 9-fold increase risk of stillbirth, a 2-fold increase risk of IUGR, and a 6-fold increased risk of fetal cardiac abnormalities.

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VI. MISCELLANEOUS PLACENTAL LESIONS

A. MASSIVE PERIVILLOUS FIBRIN DEPOSITION (and maternal floor infarct):

Background: In massive perivillous fibrin deposition (MPVFD) (considered in the spectrum with maternal floor infarction (MFI)) there is markedly increased perivillous fibrin and extracellular matrix fibrinoid surrounding distal villi in the lower 2/3 of the placenta usually including the basal plate. Regionally increased perivillous fibrin can occur in the setting of maternal vascular underperfusion but rarely affects a large percentage of the placenta and definitive villous infarcts can be identified histologically with MVU. MPVFD is a rare disorder affecting 0.03 to 0.5% of deliveries. The etiology for MPVFD remains unknown but it can recur in future pregnancies (12-78% reported). There is an association of this disorder in patients with autoimmune diseases, preeclampsia/maternal hypertension, thrombophilias, long-chain 3-hydroxyacyl-CoA (LCHAD) deficiency or mutations, and in patients with an imbalance in angiogenic and anti-angiogenic factors. More recently MPVFD has also been associated with an increased risk of renal tubular dysgenesis in a small number of cases. Prenatal ultrasound usually shows IUGR, oligohydramnios and a dense hyperechoic placenta in cases of MPVFD.

Pathology: Placentas affected by MPVFD are usually small for gestational age and often tongues of firm fibrinoid material can be seen grossly, often best after fixation. There are two histologic patterns of presentation one being a rind-like basal plate-associated deposition of fibrin which was originally called maternal floor infarction. The second presentation is diffuse lacy strands of fibrin marbling the lower 2/3 of the parenchyma. These strands can extend to the chorionic plate. Histologically there is extensive perivillous fibrin surrounding but not displacing distal villi with large numbers of associated intermediate trophoblast cells. The entrapped villi can become infarcted due to diminished perfusion. Localized lesions should not be confused with this more diffuse process. Perivillous fibrin occupying >30-50% of the intervillous space has been proposed to be potentially lethal to the fetus. Care must be taken with stillbirths as increased perivillous fibrin can occur as part of involutional changes. A semiquantitative scoring system has been proposed by Katzman and Genest for making the diagnosis of MPVFD:

1. Classic pattern: basal villous involvement by fibrinoid along the entire maternal floor (MFI) and of >3mm thickness on at least one slide.
2. Borderline MPVFD: 25-50% of villi encased by fibrinoid in a transmural distribution on at least one slide.
3. Transmural MPVFD: >50% of villi encased by fibrinoid material with transmural extension on at least one slide.

Scattered acute and/or chronic inflammation can be present including intervillositis or villitis. However, the predominant pattern should be massive perivillous fibrin deposition for a

diagnosis of MPVFD. The differential diagnosis includes normal perivillous fibrin deposition, chorionic villous ischemia/infarction, fetal thrombotic vasculopathy, diffuse chronic villitis (VUE), and massive histiocytic chronic intervillositis.

Clinical associations: This is a serious disease that can recur and is associated with recurrent pregnancy loss (both spontaneous abortions and stillbirths (13-50%)), severe IUGR (24-100%), preterm delivery (26-60%), and neurologic impairment. It often begins in late second trimester or early third trimester. If suspected clinically early delivery is recommended. Therapy using anticoagulants remains controversial. While the exact cause for this disorder remains unknown, several possibilities have been proposed (see reference 9 for discussion).

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B. CHORANGIOSIS AND CHORANGIOMATOSIS

Background: Chorangiosis is a diffuse increase in the number of villous capillaries in terminal villi, whereas chorangiomatosis is a focal to multifocal lesion characterized by increased villous capillaries involving secondary and tertiary stem villi (often seen in preterm placentas). Chorangiosis occurs in the setting of chronic prenatal hypoxia and is seen in large placentas from diabetics, associated with other maternal chronic disease (severe anemia and preeclampsia), and in placentas delivered at high altitude. Chorangiosis is seen in placentas of smokers and in mothers with chronic hypoxia due to heart disease. It is proposed to take weeks to develop chorangiosis. Localized chorangiomatosis is associated with preeclampsia, late preterm birth, and multiple births. Diffuse multifocal chorangiomatosis is associated with

advanced maternal age, multigravidity, IUGR, prematurity, preeclampsia, an enlarged placenta and congenital anomalies. Both of these lesions (chorangiosis and chorangiomatosis) are relatively uncommon.

Pathology: Chorangiosis usually involves only the distal villi and the increased capillaries are lined by endothelium. The rule of tens apply for the diagnosis: there needs to be 10 or more villous capillary cross sections seen in 10 or more villi in clusters in several (10) fields using a 10x objective in 3 different noninfarcted areas of the placenta (diffuse involvement). Often there will be 15-20 capillary cross-sections in some capillaries. The most difficult differential diagnosis is when there is marked vascular congestion in an otherwise normal term placenta. The distinction can be nearly impossible at times. Some pathologists use the terms focal chorangiosis or borderline chorangiosis in equivocal cases where the process does not seem to be truely diffuse. Chorangiomatosis has 2 patterns. Localized (divided into focal or segmental) chorangiomatosis involves a focal or single localized area of adjacent stem villi with increased vessels and cellularity (segmental chorangiomatosis involves greater than 5 villi whereas focal chorangiomatosis involves five or less villi). It has been proposed that localized chorangiomatosis and chorangiomas are related lesions. Multifocal chorangiomatosis is a distinct lesion. Multifocal chorangiomatosis is characterized by a network of small anastomosing capillaries at the margins of placental stem and immature intermediate villi in multiple independent areas of the placenta. While chorangiosis involves primarily capillary proliferation, chorangiomatosis can also involve surrounding perivascular cells, increased stromal cells and increased stromal collagen (similar to chorangiomas).

Clinical associations: Chorangiosis is associated with perinatal morbidity (cerebral palsy) and mortality and is probably a marker for chronic hypoxia. Chorangiomatosis has similar associations with hypoxic states and is increased in patients with Beckwith-Wiedemann syndrome and with other congenital anomalies.

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C. CIRCULATING NUCLEATED FETAL RED BLOOD CELLS

Background: An increased number of circulating nucleated fetal red blood cells is indicative of fetal hypoxic/ischemic stress. The finding is nonspecific for etiology but is extremely helpful in an effort to determine whether there was fetal hypoxic stress in utero prior to delivery. In the third trimester there should be essentially no circulating nucleated fetal red blood cells. The presence of these cells indicates premature release of red cell precursors from the bone marrow and/or if chronic, indicates increased fetal erythropoiesis. Increased circulating fetal red blood cells can be seen in association with several of the placental pathologies already discussed (maternal vascular underperfusion, massive perivillous fibrin deposition, severe fetal

thrombotic vasculopathy) but in addition nucleated fetal red blood cells are commonly seen in fetuses with anemia for any reason (Rh or ABO incompatibility, fetomaternal hemorrhage, many others).

Pathology: Nucleated fetal red blood cells can be difficult to distinguish from fetal lymphocytes especially if the placental tissue is poorly preserved or not adequately fixed. Once identified as nucleated red blood cells within the fetal circulation an effort should be made to quantitate the numbers as mild, moderate, or severe. We use the method of examining the distal villi at 40X and counting the number of nucleated RBCs. A mild increase is defined by 2-3 unequivocal nucleated fetal red blood cells in one or more terminal villi in 3 or more 40X fields. A moderate increase is greater than 3 nucleated red blood cells in one or more terminal villi in 3 or more 40X fields. A marked increase is defined as clusters of > 3 nucleated red blood cells and immature erythroblasts in most 40X fields. Cases of severe anemia are more likely to have a marked increase in the number of circulating nucleated fetal red blood cells. Others use a critical cutoff value for increased circulating nucleated red blood cells as >10/10HPF (see reference 8).

Clinical associations: Increased nucleated fetal red blood cells in the circulation indicate fetal hypoxia, but this does not identify the etiology. The time interval necessary to see increased circulating nucleated fetal red blood cells after the hypoxic event remains controversial although most individuals believe it takes several hours (2-24 hours). Dr. Redline has indicated that a marked increase in nucleated fetal red blood cells in an otherwise normal fetus probably takes 6-12 hours to develop. One cause for fetal hypoxia that can occur acutely in term fetuses and result in increased nucleated fetal red blood cells is a significant fetomaternal hemorrhage. This can only be definitively diagnosed by flow cytometry or the Kleihauer-Betke test run on maternal blood. The half-life of fetal blood cells in the maternal circulation is about 100 days (less if there is blood group incompatibility) so this test can be ordered several days following delivery. Several nonspecific placental findings can be supportive of the diagnosis of fetomaternal hemorrhage including increased circulating nucleated fetal red blood cells, placental edema, and intervillous thrombi. Intervillous thrombi represent sites of fetomaternal hemorrhage, but they are very common and most do not reflect a large fetomaternal hemorrhage. Multiple or large intervillous thrombi increase the probability of a significant fetomaternal hemorrhage. The result of a large fetomaternal hemorrhage is severe fetal anemia with associated congestive heart failure, CNS injury, hydrops, and/or death.

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D. MECONIUM

Background: Hypoxic fetal stress in utero in the late third trimester can result in the passage of meconium into the amniotic fluid. The trigger in many cases is thought to be transient umbilical cord obstruction which is common in late pregnancy. Meconium represents the fetal stool and is composed of bile acid, phospholipases and other components. Meconium can have detrimental effects on umbilical and chorionic plate blood vessels, particularly resulting in smooth muscle coagulative necrosis. Multiple factors can alter the toxic effects of meconium including the amount passed, the duration of exposure and the volume of the amniotic fluid. Meconium passage and the presence of chorioamnionitis often coexist and can likely synergize to lead to vascular injury. Meconium passage at or after term is common (affecting 15-20% of all placentas). Prior to 32-34 weeks gestation meconium passage is seldom seen.

Pathology: Grossly meconium passage can result in deep green discoloration of the chorionic plate and membranes or to just mild discoloration/opacification of the membranes. Meconium passage can result in toxic effects on the amnion resulting in cytoplasmic vacuolization, reactive changes (change from cuboidal to tall columnar morphology), edema, or even amniocyte necrosis. The meconium is picked up by tissue macrophages and appears as red-yellow-tan pigment within vacuolated macrophages in the amnion, chorion, extravillous trophoblast layer or decidua, dependent upon the duration of exposure. The differential diagnosis of this brown tinged pigment includes hemosiderin, which can be assayed by iron stains. Determining the duration of meconium passage in utero is an inexact science with studies showing disparate results. Estimates from one study indicate that it takes about 1 hour for meconium to begin to accumulate in the amnion. Spread through the superficial chorion likely takes 3 or more hours and for the pigmented macrophages to reach the deeper layers of the chorionic plate and/or invade Wharton's jelly (with resulting green umbilical cord) may take as long as 12-16 hours. A small more recent study demonstrated no correlation between the duration of meconium exposure in utero to meconium uptake by macrophages, when the exposure was less than 8.5 hours. Care should be taken in trying to estimate the duration of intrauterine meconium exposure based only on histologic findings of the amount and depth of meconium laden macrophages. One of the known detrimental effects of prolonged meconium exposure is focal necrosis of the smooth muscle cells surrounding the umbilical cord vessels, referred to as myonecrosis. This is characterized by rounding up of the smooth muscle cells with cytoplasmic hypereosinophilia and pyknosis of the nuclei with eventual loss of nuclear basophilia of the smooth muscle cells surrounding one or more of the umbilical vessels (usually one of the arteries). Meconium induced myonecrosis of umbilical cord vessels is a relatively rare lesion (about 1% of meconium stained placentas).

Clinical associations: Meconium passage is thought to be indicative of some degree of hypoxic/ischemic stress but it is nonspecific. Meconium is most commonly seen in fetuses without any other signs of fetal distress. Many fetuses that have undergone serious

intrauterine stress do not pass meconium. However, meconium associated umbilical vessel myonecrosis has been associated with adverse CNS outcomes. Meconium passage also predisposes the affected neonate to possible meconium aspiration syndrome, however, there are no predictive findings within the placenta to identify the subgroup of neonates that will develop this complication.

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E. CHRONIC DECIDUITIS WITH PLASMA CELLS

Pathology: The decidua at the basal plate or associated with the membranes can contain increased numbers of lymphocytes, macrophages, and plasma cells. The presence of lymphocytes and macrophages are usually considered non-pathologic. However, the presence of plasma cells is always abnormal. How many plasma cells (1, 2, etc.) are needed to consider them pathologic, remains to be determined. Some pathologists consider even one plasma cell pathologic.

Clinical associations: While increased chronic inflammation in the decidua is non-specific (lymphocytes and macrophages are common), the presence of plasma cells should raise concern for a possible chronic maternal infection (possible chronic endometritis). This is not a 100% association (closer to about 30%). Chronic deciduitis with plasma cells often, but not always, accompanies chronic villitis. Increased lymphocytes surrounding decidual vessels in the membranes and basal plate are frequently seen in association with MVU. Focal lymphoplasmacytic deciduitis is proposed to take at least weeks to develop, or if associated with maternal chronic endometritis may be present from the beginning of pregnancy. Chronic lymphoplasmacytic deciduitis has been associated with recurrent pregnancy loss and preterm delivery.

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F. PLACENTAL EDEMA

Background: Patchy villous edema can be seen in association with many of the placental pathologies already discussed including chorioamnionitis, maternal vascular underperfusion, cytogenetic abnormalities, and others as well as in placentas with no other pathology. Severe villous edema known as a hydropic placenta is less common.

Pathology: Microscopically the villous stroma is edematous (clear white spaces) and villi appear enlarged. There can be syncytiotrophoblast cell necrosis, villous capillary lumens may be reduced, and Hofbauer cell number may be increased. Increased circulating nucleated fetal red blood cells may be present. Usually villous edema is easily assessed and it is important to determine the extent of involvement (mild, moderate, severe). Some very rare inborn errors of metabolism can mimic edema. However, in many storage diseases the inclusions also occur in an intracellular manner, often affecting the trophoblast cells.

Clinical associations: The etiology leading to edema is important to consider but the differential diagnosis is extremely broad including chorioamnionitis, genetic disorders, fetomaternal hemorrhage, congenital heart disease, other infections (parvovirus), and many more. It can be helpful to establish whether there is also fetal/neonatal edema. The finding of villous edema is usually nonspecific and in the absence of placental evidence of chorioamnionitis the etiology is often not identified from placental examination alone.

Selected References:

1. In: Atlas of Nontumor Pathology – Placental Pathology. Kraus FT et al. eds. American Registry of Pathology, Washington DC, 2004.
2. In: Manual of Pathology of the Human Placenta, 2nd ed. Baergen RN. Springer, New York, 2011.
3. In: Pathology of the Placenta – Major Problems in Pathology, 3rd ed. Fox H and Sebire NJ. Saunders-Elsevier, China, 2007.

G. ABERRENT IMPLANTATION (placenta accreta, increta and percreta):

Background: Placenta accreta represents abnormal implantation characterized by failure of normal decidua to form with resulting insertion of chorionic tissue directly into the myometrium. Placenta accreta occurs when chorionic tissue is directly attached to myometrium without intervening decidua. Placenta increta represents deeper invasion of the myometrium by villi, and placenta percreta is when the placental villi have penetrated the uterine wall (serosal surface). The true defect here is deficiency of decidua and not the direct contact of villi with myometrium. Normally the presence of decidua seems to prevent deep invasion of the myometrium by the extravillous trophoblast. Focal placenta accreta is probably much more common than previously reported with the newer definition of myometrium in direct contact with chorionic tissue (fibrinoid layers or extravillous trophoblast) without intervening decidua. The incidence of accreta is rising and this is probably due, at least in part, to the newer definition and the increasing incidence of cesarean sections. The risk factors for the development of accreta are prior cesarean section (resulting in scar tissue deficient in endometrium for decidualization after pregnancy is initiated), prior endometrial curettage, and placenta previa (insertion over the cervical os). Other risk factors include submucosal leiomyomas and uterine anomalies.

Pathology: Normal separation of the placenta from the uterus occurs just peripheral to Nitabuch's fibrinoid layer along the decidua. In the absence of decidua the separation plane is incomplete and the placenta may bleed or be partially retained. A missing cotyledon due to

focal placental accreta should be reported if accurately assessed grossly. Histologically it is the absence of decidua that defines the pathology. As stated above, chorionic elements in direct contact with myometrium without intervening decidua is the definition of placenta accreta and this can be very focal, multifocal or diffuse. Difficulty can arise in misdiagnosing extravillous trophoblast cells as decidua. Over diagnosis can occur if deeply penetrating placental-site giant cells are interpreted as accreta. Placental-site giant cells normally invade the myometrium. It is common to find myometrial fibers attached to the basal plate with intervening decidua and although this is not associated with an adverse outcome, it is abnormal and we report it (a new study on this topic will be presented at the SPP Fall interim meeting in England 2014).

Clinical association: Placenta accreta is associated with an increased risk of retained placenta, pre- or post-partum hemorrhage, and uterine rupture in the setting of increta or percreta. Older studies report life threatening hemorrhage with maternal and/or fetal death in 9-10% of cases of placenta accreta. These most likely represented near complete accreta and not the focal accreta seen more commonly now. The presence of focal accreta is predictive of a high recurrence of accreta in subsequent pregnancies. The treatment of increta and percreta is cesarean delivery followed by hysterectomy.

Selected References:

1. In: Atlas of Nontumor Pathology – Placental Pathology. Kraus FT et al. eds. American Registry of Pathology, Washington DC, 2004.
2. In: Manual of Pathology of the Human Placenta, 2nd ed. Baergen RN. Springer, New York, 2011.
3. In: Pathology of the Placenta – Major Problems in Pathology, 3rd ed. Fox H and Sebire NJ. Saunders-Elsevier, China, 2007.
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H. CHRONIC (HISTIOCYTIC) INTERVILLOSITIS (massive chronic intervillositis)

Background: Chronic histiocytic intervillositis is defined histologically as a diffuse infiltration of the intervillous space by a monomorphic population of CD68 positive maternal histiocytes accompanied by increased perivillous fibrinoid and extravillous trophoblast. A small number of intervillous T-cells may also be present. The villi are not involved by the histiocytes (this is not chronic villitis). This lesion occurs more commonly in spontaneous abortions (about 10 per 1000) especially in the first trimester and is rare in later pregnancy (<1 in 1000). The incidence is reported to be much higher in women with recurrent spontaneous abortions (80 per 1000). The recurrence rate is high (67-100% in selected studies). The differential diagnosis includes placental malaria, which is exceedingly rare in the US. Other infectious etiologies should also be considered, especially if any inflammation involves villi. The etiology remains unknown, but it is proposed to be related to an underlying immunologic dysfunction, as the number of Treg lymphocytes have been reported to be elevated in both the basal plate and intervillous space.

Pathology: The placentas involved by chronic histiocytic intervillositis are usually small for gestational age. The histologic findings as described above define this entity. If chronic villitis or a polymorphous intervillous inflammatory infiltrate is present then this entity is excluded. Malaria should be considered with the clinical history reviewed and malaria organisms should be sought (only present in 40% of infected placentas) as well as the black malaria pigment (only present about 35% of the time). A note should probably be added that malaria cannot be

excluded based on the absence of diagnostic findings. There is a histologic grading system for chronic histiocytic intervillositis proposed by Rota et al.:
Grade 1: Inflammatory infiltrate (histiocytes) and fibrin occupy less than 10% of the intervillous space.
Grade 2: 10-50% of the intervillous space is occupied by inflammatory cells and fibrin.
Grade 3: greater than 50% of the intervillous space is affected.
The severity of some outcomes has correlated with the histologic grade.

Clinical associations: Chronic histiocytic intervillositis is associated with recurrent spontaneous abortion, IUGR, and intrauterine fetal demise. Overall perinatal mortality rate has been reported to be as high as 77%. The death rate is lower in the third trimester (18%). A recent review of treatments for this disorder failed to show any significant improvement in outcome with treatment and there was a small trend (not statistically significant) that treatment resulted in a lower incidence of live birth than no treatment.

Selected References:

1. In: Atlas of Nontumor Pathology – Placental Pathology. Kraus FT et al. eds. American Registry of Pathology, Washington DC, 2004.
2. In: Manual of Pathology of the Human Placenta, 2nd ed. Baergen RN. Springer, New York, 2011.
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6. Rota C et al. Perinatal prognosis of pregnancies complicated by placental chronic intervillitis. J Gynecol Obstet Biol Reprod 35:711-719, 2006.
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I. EOSINOPHILIC/T-CELL VASCULITIS

Background: Eosinophilic/T-cell vasculitis (ETV) is an unusual and rare (0.2 to 0.6% of placentas) entity first described in 2002. ETV consists of a fetal derived chronic inflammatory infiltrate composed of eosinophils and CD3 positive T-lymphocytes that focally involves a single chorionic artery or vein. Additional sampling of involved placentas has failed to reveal additional involved vessels. Neutrophils are not a component of the inflammation. The vast majority of cases involve term placentas and the remainder of cases are 34 weeks gestation or older. Most cases have a maternal or fetal abnormality, likely leading to the placental

examination, however, a consistent pattern of abnormalities has not been observed. The etiology remains unknown. Recurrences in subsequent pregnancies have not been observed.

Pathology: The pathologic findings are as described above. There is no obvious gross lesion and usually only a single vessel is involved by inflammation composed of eosinophils and T-lymphocytes. In about 10-15% of cases there can be an associated intraluminal thrombus. The fetal inflammatory response associated with amnionic fluid infection can involve numerous eosinophils, but there are usually also neutrophils and minimal lymphocytes. The most recent study of 51 placentas with ETV showed a statistically significant association of ETV with villitis of unknown etiology (43% of ETV cases versus 13% of control cases had chronic villitis), although most of the cases were low grade in both groups. In addition, ETV was associated with fetal vascular obstructive disease in 23% of cases compared to 11% in controls. Other placental lesions failed to show statistically significant differences between placentas with ETV and controls.

Clinical associations: The clinical significance, if any, has not yet been observed. Thus far, ETV is not associated with known adverse outcomes although only a few studies have been performed and long term followup is lacking. Clearly the presence of associated intraluminal thrombi is a risk factor for fetal thrombosis.

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1. Fraser RB & Wright JR. Eosinophilic/T-cell chorionic vasculitis. Pediatr Devel Pathol 5:350-355, 2002.
2. Jaiman S & Johansen T. Eosinophilic/T-cell chorionic vasculitis and intrauterine fetal demise at 34 weeks: case report and review of the literature. Pediatr Devel Pathol 13:393-396, 2010.
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VII. APPROXIMATE TIMING OF VARIOUS PLACENTAL PATHOLOGIES:

Chronic placental lesions (proposed to take greater than 1 week prior to delivery to develop) -

1. Findings consistent with maternal vascular underperfusion (small placenta, infarcts, distal villous hypoplasia, etc.).
2. Findings consistent with fetal vascular obstruction (multiple foci of avascular villi).
3. Chronic villitis (VUE) +/- obliterative fetal vasculopathy.
4. Increased perivillous fibrinoid material (surrounding > 10% of villi).
5. Chronic abruption including evidence of chorionic hemosiderosis.

Subacute placental lesions (proposed to develop over a period of at least 6-12h and less than 1 week prior to delivery) -

1. Acute chorioamnionitis with a substantial fetal vasculitis (stage 1 or 2).
2. Findings consistent with prolonged meconium exposure (numerous meconium laden macrophages in chorionic plate +/- meconium associated vascular necrosis).
3. Organizing fetal vascular thrombi.
4. Retroplacental hemorrhage with associated villous infarct (subacute abruption).
5. FTV with villous stromal vascular karyorrhexis.
6. Chronic intermittent UC compression.
7. Fetomaternal hemorrhage (some).

Acute onset placental lesions (proposed to develop less than 6 to 12 h before birth) -

1. Maternal acute chorioamnionitis without a fetal inflammatory response.

2. Membranes with meconium laden macrophages.
3. Significant retroplacental hemorrhage without tissue injury (acute abruption).
4. Complete sudden UC occlusion.
5. Lacerated large fetal vessel.
6. Fetomaternal hemorrhage (most).

VIII. PLACENTAL LESIONS ASSOCIATED WITH NEUROLOGIC DYSFUNCTION AND OTHER ADVERSE OUTCOMES

Neurologic impairment in term infants including cerebral palsy has been shown to be associated with subacute to chronic placental lesions including:
Avascular villi.
Chronic villitis with obliterative fetal vasculopathy.
Increased perivillous fibrinoid deposition.
Acute chorioamnionitis with a stage 2 fetal inflammatory response.
Fetal thrombi.
Subacute abruption.
Meconium associated vascular necrosis.

Placental lesions as predictors of CP or poor neurocognitive test scores at 8 y.o. in extremely low birth weight premature infants was studied by Redline and colleagues:
MVU lesions were associated with CP.
Villous edema was associated with a single low neurocognitive test score.
In patients with histologic acute chorioamnionitis, only those with a severe fetal inflammatory response showed a lower score.

In a series of medicolegal cases studied by Redline the following placental lesions were associated with neurologic dysfunction compared to controls (not a perfect set of controls):
Findings of fetal thrombotic vasculopathy.
Chronic villitis with obliterative vasculopathy.
Acute chorioamnionitis with a fetal inflammatory response.
Meconium myonecrosis.
In a follow-up paper 63% of these cases were associated with evidence of umbilical cord obstruction. He then classified the cases into 5 groups based on clinicopathologic criteria.

The following adverse pregnancy outcomes have been shown to be associated with the listed placental pathologies:
1. Preterm birth - acute chorioamnionitis, chronic deciduitis with plasma cells, MVU, acute and chronic abruption.
2. IUGR - MVU (more common in preterm births), fetal vascular obstruction (including FTV), high grade chronic villitis (more common at term), increased perivillous fibrinoid, chronic abruption.
3. Hypoxic/ischemic injury (including long term neurologic dysfunction) - fetal vascular obstruction (FTV), chronic villitis with obliterative fetal vasculopathy, acute chorioamnionitis with an intense fetal inflammatory response, prolonged meconium exposure with myonecrosis, pathologic lesions of the umbilical cord, less common intrapartum fetal/maternal hemorrhages.
4. Maternal recurrent pregnancy loss - chronic deciduitis with plasma cells, MVU, VUE, MPVFD, chronic histiocytic intervillositis.

Previously known associations of increased circulating nucleated fetal red blood cells include maternal diabetes, smoking, IUGR, and anemia. In a study by Redline, the presence

of nucleated fetal red blood cells was determined to be associated with subacute to chronic placental lesions (implies >6-12h for them to appear at a significant level (>10/10HPF in this study).

Placental pathologies associated with increased circulating nucleated fetal red blood cells included avascular villi and chronic villitis. Interestingly, clinical indicators of birth asphyxia including acidosis were not significantly associated with increased nucleated fetal red blood cells if no placental lesion was present.

Dr. Redline recently wrote an excellent review on this topic (see reference 7). Here is brief summary of his approach (for details see his review).

Neurodisability affects 2-3/1000 livebirths. Of these 50-60% involve term to near-term gestations (>34 weeks). VLBW infants have a higher incidence.

Sentinel events/birth asphyxia. Severe placental perfusion defects that result in asphyxia are referred to as sentinel events. The obstetric syndrome associated with a sentinel event is called birth asphyxia and is defined clinically by a low cord pH and elevated base excess with resulting neonatal encephalopathy (NE). The 4 major categories of sentinel events are 1) abruptio placenta or uterine rupture, 2) umbilical cord occlusion, 3) fetal hemorrhage, 4) maternal hypotension (this has no placental findings). If clearly documented each of these can result in NE.

Classification of other placental findings associated with CNS injury:

I. Fetal Vasculopathy:

A) FTV is associated with NE, CP and developmental delay in term infants and neuronal injury in stillbirths. A few studies have implicated FTV in preterm CNS injury and perinatal stroke.

B) Chronic villitis with obliterative fetal vasculopathy is a strong risk factor for CP and NE. High grade chronic villitis is associated with neonatal seizures and perinatal stroke.

II. Prolonged Partial Asphyxia/Chronic Intermittent Hypoxia:

A) Chronic partial/intermittent UC compression due to -

1. Abnormal insertion (membranous, furcate).
2. Decreased Wharton's jelly or hypercoiling.
3. Entanglement due to nuchal or body coiling or tight UC knot.

These UC findings have been associated with neuronal damage in stillbirths and with both CP and NE in liveborn infants. UC abnormalities are reported in 34-43% of all patients with neurodisability. These abnormalities include decreased UC diameter (<8mm), hypercoiled UC (>5 coils/10 cm), abnormally long cords (>70 cm at term), dilated umbilical vein relative to artery (>4:1 ratio), intimal mural fibrin thrombus (cushion), or downstream effects (small clusters of avascular villi (<10 villi per focus)).

B) Subacute and/or chronic abruption -

Abruptions can be categorized into 4 types including acute abruption (abruptio placenta) which is listed as a sentinel event or an acute marginal abruption which are rarely associated with CNS injury. A subacute abruption (centrally located) due to rupture of a basal plate artery with associated histologic evidence of early tissue injury can result in intermittent hypoxia and is related to CNS injury in stillbirths (not well studied in livebirths but thought to be associated with CNS injury). Chronic abruption can be marginal or central and is thought to represent venous bleeding. Chronic abruption can also result in intermittent hypoxia and is a clear risk factor for CP at term.

III. Uteroplacental Insufficiency/Decreased Placental Reserve -

A) Maternal malperfusion (accelerated maturation or maternal vascular underperfusion (MVU). Villous infarcts correlate with CNS injury in stillbirths and are a risk factor for CP and NE at term. Severe MVU is a risk factor for CP, NE and developmental delay in VLBW infants, whereas mild MVU may be protective for CNS injury in this same group.
B) Distal villous immaturity (DVI) (delayed villous maturation). This represents a placental maturation defect with retarded villous development thought to represent pathologic persistent villous growth rather than differentiation (maturation) and is usually diagnosed after 37 weeks gestation. The true gestational age of the fetus must be known to avoid overdiagnosing this entity. The histologic features include excess villous stroma, more centrally placed villous capillaries and clear persistence of excessive numbers of cytotrophoblast cells. This is more common in diabetic mothers. The association of DVI with CNS injury is not yet proven. However, there is a high prevalence of DVI in stillbirths. Also 2 small studies have shown an association between DVI and other markers for adverse outcomes.
C) Perivillous fibrinoid deposition -
Maternal floor infarcts are associated with and increased rate of late neurodevelopmental abnormality at all gestational ages. Increased perivillous fibrin deposition weakly correlates with CP and neurodevelopmental disorders in term infants.

Finally this review discusses 3 placental findings that act as biomarkers for potential poor outcome:
1) The fetal inflammatory response to acute amnionic fluid infection is related to poor outcome. In particular, studies have demonstrated that grade 2 (confluent PMNs) fetal inflammatory response is associated with neurologic impairment in preterm and term infants. Older studies correlated "funisitis" (not uniformly defined) with CP, intraventricular hemorrhage, white matter damage, and developmental delay. Interestingly, the maternal inflammatory response is <u>not</u> related to CNS outcomes.
2) Meconium release as previously discussed is thought to represent a fetal stress response to hypoxia. Meconium is common with up to 30% of all deliveries at 41 weeks demonstrating meconium passage. Prolonged meconium exposure (>6-12h) has been shown to be a low risk factor for CNS injury. The problem is that the exact timing of meconium passage is seldom attainable. Meconium myonecrosis as noted above is a highly significant risk factor for CP, severe NE and other neurodisability.
3) Increased circulating nucleated fetal red blood cells are a risk factor for CNS injury in term infants above a level of 10/10 HPF.

This review closes with a discussion of an algorithm to use to relate placental findings to adverse neurologic outcome. The idea is to identify a possible causative factor from the placental pathology to account for any neurologic injury. The first step is to determine whether a clear cut sentinel event occurred. If not then assess whether there is significant fetal vasculopathy to account for the adverse outcome. If not assess for findings within the other classes as possible definitive causes or as a combination of causes. If not then no definitive placental causation is determined.

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IX. PATTERNS OF PLACENTAL FINDINGS IN HYPOXIA

The following discussion is primarily based on placental studies from J Stanek, whereby he defines hypoxic patterns of placental injury. His method of classifying the patterns of chronic hypoxic placental injury into preuterine (related to pregnancy at high altitude, maternal anemia or multiple pregnancies), uterine (related to preeclampsia at term and late onset IUGR), and postuterine (related to antepartum fetal death, fetal thrombotic vasculopathy, and early onset IUGR) has not been widely accepted, but certainly provides an interesting way of viewing these constellations of placental findings.

He defines several histologic features of placental hypoxia and their associated criteria in a recent review (see reference 3). These include villous infarcts, membrane laminar necrosis, villous maturation, villous vascularity, syncytial knots, villous cytotrophoblasts, villous extracellular matrix, vasculosyncytial membranes, villous agglutination, fetal thrombotic vasculopathy, placental site giant cells, excessive extravillous trophoblasts, Hofbauer cells, increased perivillous fibrinoid, and microscopic chorionic pseudocysts. The timing and development of both villous infarcts and membrane laminar necrosis are discussed in this review (Stanek, 2013). Fetal hypoxia is assessed in this classification scheme by meconium and the number of circulating nucleated fetal red blood cells. Meconium by itself has shown only a weak correlation with fetal hypoxia and stress. The timing of meconium accumulation shows a great deal of variability with 2-3 h minimum for meconium laden macrophages to appear in the superficial chorion of the chorionic plate, 6-12h for deep penetration into the membranes, and anywhere from 12-16 to 48h for meconium induced myonecrosis of the umbilical cord vasculature (the only meconium lesion clearly associated with a worse fetal outcome (see above)). This timing for meconium exposure remains controversial.

I will briefly discuss the histology of 2 lesions implicated in placental hypoxia: 1) *membrane laminar necrosis* and 2) *placental site giant cells*. Membrane laminar necrosis is defined as a band of decidual, trophoblastic or mixed coagulative necrosis occupying greater than 10% of the membranes. Neutrophils can be associated with this lesion. Membrane laminar necrosis is a controversial placental finding in terms of its significance and etiology. Membrane laminar necrosis has been associated with fetal growth restriction, maternal hypertensive disorders including preeclampsia and conditions associated with in utero hypoxia. However, these findings have not been reproducible in all studies to date. The estimated incidence of membrane laminar necrosis is between 6.6 to 18% of placentas. There is no

doubt that membrane laminar necrosis occurs, but the etiology leading to the findings and its actual significance is uncertain. I do report membrane laminar necrosis if it exceeds 30% of the membrane roll and there is no associated acute inflammation, but I consider it a nonspecific finding possibly associated with hypoxia. The second placental lesion is the presence of increased placental site giant cells consists of the finding of 3 or more trophoblastic cells with 3 or more nuclei in the decidua basalis. This finding was originally described as associated with other features of MVU in preeclampsia. This has since been validated but also it is proposed that the presence of these multinucleated trophoblastic giant cells correlate with IUGR, small placental weight and other features of placental hypoxia (infarcts, hypertrophic decidual arteriopathy, atherosis, and membrane laminar necrosis, as well as with some umbilical cord abnormalities). The number of multinucleated trophoblastic giant cells normally found in the decidua basalis decrease with age. Multinucleated trophoblastic giant cells are normally found deep in the myometrium and their function there is poorly understood. Recently decreased numbers of these cells has been reported in retained placentas. While the increased presence of these multinucleated cells in the basal plate is one of the defining features described by Redline and colleagues for MVU, we do not routinely report them unless extremely prominent. However, their presence would certainly support the other features that define MVU.

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X. TEMPLATES USED AT NORTHWESTERN

Placental Gross Description

Received (fresh/fixed) labeled with the patient's name and "_____" is a (ovoid/irregular shaped/bilobed) placenta with attached membranes and umbilical cord. The membranes are (intact/ruptured) and the site of rupture is (# cm from the edge). Membranes are inserted (at the margin/circummarginate (% of circumference involved and greatest amount (in cm) of extrachorialis). The membranes are (clear/opaque/green/other). The umbilical cord is inserted # cm from the margin and insert (normally/velamentously/furcated). The umbilical

cord measures #cm in length and averages # cm in diameter. The external surface of the umbilical cord is (unremarkable/with amnion nodosum/white lesions) and there are # twists per 10 cm segment. The cut surface shows # vessels. Trimmed weight of the placenta is # grams and the disc measures
__X__X__ cm. The fetal surface is (color/lesions). The maternal surface is (color/lesions/intact). Cut surface shows (beefy red parenchyma/lesions). Representative sections are submitted as follows:

1	membrane rolls (2-3 rolls per cassette)
2	umbilical cord (2-3 pieces per cassette)
3	maternal surface biopsies (3 biopsies in one cassette)
4	full thickness parenchyma
5	full thickness parenchyma
additional sections	lesions/abnormalities

General Templates

PLWT **PLACENTA, DELIVERY:**
- PLACENTAL WEIGHT, # GRAMS, SMALL/LARGE/APPROPRIATE FOR GESTATIONAL AGE (EXPECTED AT (GESTATIONAL AGE) # GRAMS).

BPMYO BASAL PLATE WITH ATTACHED MYOMETRIAL FIBERS.

CDPC CHRONIC DECIDUITIS WITH PLASMA CELLS.

MEMHEM MEMBRANES WITH REMOTE PARIETAL DECIDUAL HEMORRHAGE WITH HEMOSIDERIN.

MEC (MILD/MODERATE/NUMEROUS) MECONIUM-LADEN MACROPHAGES IN (AMNION/CHORION/DECIDUA/AT CHORIONIC PLATE).

TERMGEST VILLOUS MATURATION CONSISTENT WITH TERM GESTATION (OR APPROPRIATE FOR GESTATIONAL AGE).

MEMB MEMBRANES AND UMBILICAL CORD WITHOUT SIGNIFICANT ABNORMALITY.

Amnionic Fluid Infection

- FINDINGS CONSISTENT WITH ACUTE AMNIONIC FLUID INFECTION:
  - MATERNAL INFLAMMATORY RESPONSE, STAGE _____.
    - MEMBRANES WITH
      Pick one:
      ACUTE MARGINATING CHORIODECIDUITIS.

ACUTE CHORIOAMNIONITIS.
ACUTE NECROTIZING CHORIOAMNIONITIS.
- CHORIONIC PLATE WITH
Pick one:
ACUTE SUBCHORIONITIS
ACUTE SUBCHORIONITIS AND CHORIONITIS.
ACUTE SUBCHORIONITIS, CHORIONITIS, AND AMNIONITIS.

- FETAL INFLAMMATORY RESPONSE, STAGE _____.
Pick all that apply:
  - ACUTE CHORIONIC VASCULITIS.
  - ACUTE UMBILICAL PHLEBITIS.
  - ACUTE UMBILICAL ARTERITIS.
  - ACUTE UMBILICAL PANVASCULITIS WITH FUNISITIS.

Maternal Vascular Underperfusion

- FINDINGS CONSISTENT WITH MATERNAL VASCULAR UNDERPERFUSION.
  - VASCULAR LESIONS:
    - ACUTE ATHEROSIS/FIBRINOID NECROSIS OF BASAL PLATE OR MEMBRANE ARTERIOLES.
    - PERSISTENT MUSCULARIZATION OF BASAL PLATE ARTERIES.
    - MURAL HYPERTROPHY OF MEMBRANE ARTERIOLES.
  - VILLOUS CHANGES:
    - VILLOUS INFARCTS, ACUTE/SUBACUTE/REMOTE, __CM, MULTIPLE OCCUPYING __% OF PARENCHYMA.
    - INCREASED SYNCYTIAL KNOTS.
    - DISTAL VILLOUS HYPOPLASIA.
    - VILLOUS AGGLUTINATION.
    - INCREASED PERIVILLOUS FIBRIN.

Chronic fetal vascular obstruction (fetal thrombotic vasculopathy)

- FINDINGS CONSISTENT WITH CHRONIC FETAL VASCULAR OBSTRUCTION (INCLUDES FETAL THROMBOTIC VASCULOPATHY).
  - VASCULAR LESIONS:
    - THROMBI INVOLVING CHORIONIC PLATE VESSELS/STEM VESSELS.
    - ACUTE/SUBACUTE/REMOTE MURAL INTIMAL FIBRIN THROMBI (ENDOTHELIAL CUSHIONS), CHORIONIC PLATE VESSELS/STEM VESSELS.
    - RECANALIZED LUMENS, CHORIONIC PLATE/STEM VESSELS.
  - VILLOUS CHANGES:
    - VILLOUS STROMAL-VASCULAR KARYORRHEXIS.
      - EXCLUSIVELY SMALL FOCI.

        - VARIABLY SIZED FOCI.
    - AVASCULAR VILLI.
        - EXCLUSIVELY SMALL FOCI.
        - VARIABLY SIZED FOCI.
        - GREATER THAN 45 AVASCULAR VILLI (3 FOCI OF MORE THAN 15 VILLI EACH).

Chronic Villitis

- FOCAL/MULTIFOCAL LOW GRADE CHRONIC VILLITIS OR PATCHY/DIFFUSE HIGH GRADE CHRONIC VILLITIS.
    - NO VIRAL INCLUSIONS SEEN.
    - IMMUNOHISTOCHEMICAL STAIN FOR CYTOMEGALOVIRUS (CMV) IS NEGATIVE. (OPTIONAL)
    - ____ AVASCULAR VILLI.
    - CHRONIC DECIDUITIS WITH PLASMA CELLS.
    - OBLITERATIVE ARTERITIS IN VESSELS OF TERMINAL/STEM VILLI.
    - SCATTERED GRANULOMATOUS INFLAMMATION SEEN.
    - FOCAL CHRONIC CHORIONITIS.
    - ASSOCIATED PATCHY CHRONIC INTERVILLOSITIS.
    - SEE NOTE.

Note: Chronic villitis, although inflammatory, is associated with infection in only a small number of cases. In these instances, the most common infections are T. gondii, CMV, or syphilis. However, most of the time the inflammatory process is not associated with infection and is termed “villitis of unknown etiology.” High grade patterns of chronic villitis are associated with an increased risk of fetal growth restriction or fetal demise, and may recur in future pregnancies.

Abruption Note:
In case of clinical suspicion of abruption without retroplacental or marginal hemorrhage the following NOTE can be added.

Note: While there is no gross or histologic evidence of abruption, it should be noted that acute abruption may show no pathologic findings on placental examination as the clot forms acutely without time to attach to the maternal surface, indent, or infarct the placental parenchyma.

I do not routinely use the term “ABRUPTION” in the diagnostic line, as this is really a clinical term. I describe the hemorrhage in the diagnostic line and often add a NOTE.

ACUTE/SUBACUTE/REMOTE RETROPLACENTAL/MARGINAL/PARENCHYMAL HEMORRHAGE (____ CM IN GREATEST DIMENSIONS).

Note: The described hemorrhage is consistent with the clinical history of abruption.
OR The significance of the described hemorrhage is unknown. Clinical correlation may be helpful.

CHROMOSOMAL MICROARRAY VERSUS KARYOTYPE IN OBSTETRICS AND PERINATAL PATHOLOGY (LECTURE 3)

The original gold-standard for assessing for cytogenetic abnormalities in prenatal, stillbirth, and post-natal patients has been conventional karyotyping. Fluorescent in situ hybridization (FISH) has found limited use in supplementing karyotype as it can only detect prespecified targets and is not a cost-effective whole genome screening method. The emerging new technology of chromosomal microarray (CMA) has been shown to have numerous advantages over karyotype and is rapidly replacing the former gold-standard. Karyotyping requires culture of viable fetal cells which occurs in about 84% of prenatal amniocentesis or chorionic villous sampling and often with less than 50% success rates in stillbirths. In addition, karyotyping has a turn-around time of up to 3 weeks. Conventional G-banding karyotyping in general can detect gene defects (deletions or amplifications) of 3 to 10 Mb, whereas CMA can detect microdeletions or microamplifications of 50-200 kb, thereby resulting in higher diagnostic yield. CMA does not require cell culture as small amounts of DNA, even from formalin fixed paraffin embedded tissue samples, can be used and therefore CMA has a faster turn around time (about 1 week). In 2009 the American College of Obstetricians and Gynecologists (AGOG) recommended that evaluation of stillbirths include a perinatal autopsy, placental examination, and karyotype. In 2013 ACOG modified it's recommendations for the use of karyotype (see end of these notes).

Microarray Technology (a simplified view):

CMA can be performed on uncultured amniocytes, chorionic villous cells, fetal cells, or cell free fetal DNA for prenatal diagnosis or from formalin fixed or fresh frozen tissue from stillbirths including macerated fetuses. Some CMA platforms can use highly degraded DNA even down to 40bp (molecular inversion probe array method).

CMA can identify major chromosomal aneuploidy and submicroscopic amplifications and deletions not detected by karyotyping. Deleted or duplicated fragments of DNA detected by CMA are known as copy number variants (CNV). CNV associated with human disease have been estimated at 15%. CNV are more likely detected in individuals with grossly visible structural abnormalities but are also detected in 1-2% of normal individuals. Not all CNV are associated with known disorders and these are referred to as variants of unknown significance (VOUS). CNV can be detected using comparative genomic hybridization (CGH) arrays. The other CMA method is the use of SNP arrays. A SNP (single nucleotide polymorphism) is a single nucleotide variation in the genome. There are limitations to both methods (see below), but neither CNV detected by CGH nor SNPs identified by CMA are associated with increased maternal age, whereas chromosomal defects detected by karyotype are associated with maternal age. CGH arrays detect localized areas of chromosomes with gains or losses including whole chromosome alterations such as trisomies. However, CGH arrays do not detect triploidy or balanced translocations. SNP arrays involve hybridizing fetal DNA to arrays with large numbers of known specific DNA sequences and CNVs are detected by alterations in signal intensity. SNP arrays can demonstrate triploidy, consanguinity, and uniparental disomy. Arrays usually screen the whole genome, but targeted arrays are also available for use in identifying CNV highly associated with clinical findings, such as 22q11 deletion in certain forms of congenital heart disease. Targeted arrays also reduce the identification of VOUS.

Advantages of CMA versus conventional G-banding karyotype:

1. Higher diagnostic yield due to smaller changes detected by CMA.
Prenatal results: A 2013 review (reference 7) demonstrated clinically significant CNVs by CMA in 2.4% of prenatal cases with normal karyotype and in 6.5% of prenatal cases with a normal karyotype and an abnormal ultrasound. A 2014 review (reference 5) demonstrated that 3.1 to 7.9% of fetuses with one structurally abnormal organ by ultrasound had a clinically significant CNV that explained the phenotype (all were karyotypically normal).
Stillbirth results: In the study by Reddy et al. (reference 4) 8.3% of all stillbirths showed a significant CNV whereas only 5.8% had a karyotype abnormality. In stillbirths with congenital anomalies 29.9% showed a CNV by CMA and only 19.4% had an abnormal karyotype. These differences were highly significant in this study. CMA was successful 87.4% of the time versus 70.5% success rate for karyotype in stillbirths.
2. Faster turnaround time for CMA.
Disadvantages of CMA versus G-banding karyotype:
1. CMA does not detect balanced structural rearrangements.
There is no CNV in a completely balanced translocation. Completely balanced translocations are rare and do not usually result in pathology.
2. CMA does not detect triploidy if the platform is CGH.
While a 69,XXX fetus can be missed a 69,XXY fetus would be detected by CGH.
3. CMA has variable ability to detect low level mosaicism.
SNP arrays are more sensitive for the detection of mosaicism.
4. CMA detects variants of unknown significance.
VOUS can result in parental anxiety with regards in how to proceed with an affected pregnancy. VOUS in CMA mandates both pre-CMA and post-CMA genetic counseling thereby raising the overall cost. VOUS is estimated to occur in 1 to 2% of cases. The number of VOUS should drop as CMA is performed more frequently and more data on clinically significant CNVs and benign CNVs are acquired. The other considerations for the need for patient counseling are discussed in detail in references 1 and 2.
5. CMA is more expensive than karyotype and the costs of counseling have not been assessed. (see reference 8).
6. CMA demonstrates variable clinical sensitivity.
Some clinically defined disorders are clearly associated with deletions or duplications whereas other disorders may present with a similar phenotype due to deletion or point mutations (the latter would not be detected by CMA).
Indications for CMA
Post-natal: CMA has been shown useful in detecting CNVs in patients with altered cognitive abilities, congenital anomalies (including congenital heart disease) and in patients with autism spectrum disorder.
Pre-natal: See ACOG bulletin opinion (reference 2) and list below.
Stillbirth (>20 weeks): CMA has been shown to significantly increase the identification of clinically relevant CNVs in stillbirths, especially if congenital abnormalities are identified at autopsy.
ACOG and Society for Maternal-Fetal Medicine Recommendations:
1. CMA should replace karyotype for patients with a fetus with 1 or more anomalies identified by US and undergoing invasive prenatal diagnosis.
2. Either CMA or karyotype can be used after undergoing invasive prenatal diagnosis if the fetus is structurally normal by US.

3. Maternal age is not related to CMA so patients of all ages should be offered this test.
4. IUFD or stillbirth CMA is recommended due to higher yield and higher success rate of the assay.
5. Data of the usefulness of CMA in first and early second trimester loss is limited and does not recommend the use of CMA at this time.
6. Comprehensive pretest and post-test genetic counseling must be offered. *CMA should not be ordered without informed consent.* (This impacts the use of CMA at autopsy, without clinician involvement. The autopsy pathologist can supply the tissue but the clinician must order the test.). Discussion of VOUS, nonpaternity, consanguinity and identification of adult onset diseases are all issues that need to be discussed prior to ordering CMA.

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