Placenta accreta and placenta increta: an approach to pathogenesis based on the trophoblastic differentiation pathway

PLACENTA ACCRETA AND PLACENTA INCRETA - AN APPROACH TO PATHOGENESIS BASED ON THE TROPHOBLASTIC DIFFERENTIATION PATHWAY

Stewart F. Cramer, M.D. and Debra S. Heller, M.D.

Department of Pathology, Rochester General Hospital, University of Rochester School of Medicine; and Department of Pathology, Rutgers-New Jersey Medical School, Newark, New Jersey.

Supported by a grant from the Rochester General Hospital Foundation and the Genesee Hospital Foundation. We thank Sue Chase, Leslie Trifiro, and Julie Warner, M.D. for their assistance.

Corresponding Author: Debra S. Heller, M.D., Pathology and Laboratory Medicine; UH E/158, New Jersey Medical School, Rutgers, The State University of New Jersey, 185 S. Orange Ave, Newark, NJ 07103. Email: hellerds@njms.rutgers.edu. Phone 973-972-0751, Fax 973-972-5724.

Running title: PLACENTA ACCRETA AND PLACENTA INCRETA
KEY WORDS: Decidua, trophoblast, placenta accreta

Abstract

Morbid adherence remains a puzzling disease, characterized by both decidual and trophoblastic abnormalities. The purpose of this paper is to suggest that progress may come from trying to apply stem cell theory. We propose that normal and abnormal placentation may best be viewed in terms of trophoblastic stem cells, and the mutually exclusive branches of the trophoblastic differentiation pathway. The latter include villous trophoblast, two nonvillous trophoblast (NVT) branches at the implantation site – interstitial and endovascular, and a positional variation in chorionic NVT of the fetal membranes. Based on hysterectomies for morbid adherence seen over 30 years at a community hospital; analyzed with routine keratin stains, with actin and trichrome stains as indicated, and attempts at sonographic-pathologic correlation; we present selected observations. In true accreta, we suggest the site of morbid adherence is to dilated basal plate vessels in the decidua, infiltrated by endovascular NVT; with scant interstitial NVT, and normal myometrium. Excess blood flow into the placenta was due to excessively deep keratin-positive endovascular NVT that spread, independently of intersititial NVT, in an angiocentric fashion, in both accreta and increta. Retroplacental abnormalities on sonograms were due to myometrial destruction by interstitial NVT in increta, sometimes requiring actin stains for detection; and to an admixture of markedly dilated endometrial glands and vessels in true accreta, best appreciated with keratin stains. Morbidly adherent fetal membranes are described. Possible explanations for partial accreta, and variable myometrial invasion are suggested. The role of C-section scars in incretas is addressed.

Introduction

Morbid adherence remains an unsolved puzzle. Standard teaching has been that chorionic villi adhere to normal myometrium in placenta accreta; while villi invade myometrium in placenta increta; but this has been questioned by experts (1-3). Although increased morbid adherence after Cesarean section has established that loss of the decidual barrier promotes life-threatening myometrial invasion; many continue to doubt that decidual deficiency can fully explain all morbid adherence (4-9). Although a limited degree of myometrial infiltration can be considered normal (1,9,10), diagnostic criteria are ill-defined, so gross myometrial thinning can be the key to diagnosis (1). Sonographic diagnosis can save lives, partly by promoting Cesarean hysterectomy; but more work is needed on sonographic-pathologic correlations (6,11,12). There is an increasing trend towards immunohistochemical and molecular approaches to improve understanding of morbid adherence, but there has been little comparison of the protease-antiprotease balance in normal vs. abnormal trophoblast (2,6,13-18)

The purpose of this article is to suggest that progress may come from viewing the difference between normal placentation and morbid adherence in terms of stem cell theory; based on the concept of a trophoblastic differentiation pathway. Despite universal belief in stem cell theory, many pathologists are not accustomed to interpreting slides of hysterectomies for morbid adherence using this approach. We will start by explaining our preferred concepts and terminology in regard to the trophoblastic differentiation pathway.

Trophoblastic Stem Cells

The fundamental principle of stem cell theory is that "Normal stem cells arise during development, are present in adult organs as tissue-determined stem cells, and are little changed, if at all, from their embryonic counterparts" (19-21). In placentas, the starting point is the trophoblastic shell; which can be seen both in early products of conception specimens, and in early ectopic tubal pregnancies (Figure 1a). We believe that these are the trophoblastic stem cells. These stem cells proliferate tremendously in order to generate both villous and nonvillous trophoblast, which have low proliferative rates; even in exaggerated placental sites (1,10,22).

Although this has been referred to as the cytotrophoblastic shell (9); this terminology is incompatible with stem cell theory. This is because villous cytotrophoblast is a more restricted cell type than stem cells in the trophoblastic shell, which are pluripotential (Figure 1a) and highly proliferative. Figure 1a shows that the trophoblastic shell gives rise to villous trophoblast (VT) in a way that is mutually exclusive with its generation of nonvillous trophoblast (NVT); which includes both interstitial NVT and endovascular NVT (23).

The trophoblastic shell withers away, so as to integrate the VT differentiation pathway, and the bidirectional NVT differentiation pathway on the other side of the early placenta, into a fully developed placenta. Note that in Figure 1a, both VT and interstitial and endovascular NVT are already present while the trophoblastic shell still dominates. We suggest that the anchoring villi thought by some to play a role in morbid adherence (6,11,24) may represent the final stage in this process of withering away. Ki67 stains support the interpretation that the trophoblastic

columns at the tips of anchoring villi are the last conspicuous remnants of trophoblastic stem cells (9,10). It is fundamental to our analysis of normal placentation vs. morbid adherence that stem cell theory suggests that villous trophoblast does not give rise to nonvillous trophoblast. Others have implied that extravillous trophoblast between villi and myometrium may be derived from adherent or invading villous trophoblast.

The Trophoblastic Differentiation Pathway

Despite some similarities in cytology and immunostains (2,6), VT and the 2 types of implantation site NVT are not only in mutually exclusive locations (Figure 1a); they are also arranged differently, to mediate different functions, suggesting different patterns of gene activation and protein expression. VT manifests orderly progression from cytotrophoblast to syncytiotrophoblast, so as to regulate transplacental diffusion; which requires intact villous basement membranes.

In striking contrast, it is essential for NVT to destroy basement membranes. Interstitial NVT anchors the placenta to the uterus, largely by attachment to laminin in basement membranes (15). Fully differentiated decidual cells comprise a massive laminin factory (25). To achieve stable anchoring, interstitial NVT uses proteases to break down basement membranes (15-17,26,27), so it can invade deeper. Decidua not only provides a deep anchor (25), but is also a barrier to pathologic myometrial invasion, promoting survival of pregnant women (5,8,10). Although limited myometrial infiltration by interstitial NVT is considered normal (1,9,10); it is not considered normal for interstitial NVT to destroy myometrium.

In order to promote fetal growth, a 2nd branch of the NVT differentiation pathway is induced when NVT encounters arterioles (Figure 1a, lower left, arrow). This implies that pluripotential trophoblastic cells may be present amongst the population of interstitial NVT. To our knowledge, no immunohistochemical marker yet exists to help identify pluripotential NVT cells in the early placenta; or to identify residual trophoblastic stem cells in the fully developed placenta. The difficulty of identifying residual stem cells in developed tissues is well recognized in other differentiation pathways (20).

Endovascular NVT binds to vascular basement membranes, invades these vessels, transiently plugs vascular lumens; and transiently replaces the endothelial lining. In striking contrast to interstitial NVT, which does not produce fibrinoid in the myometrium; normal endovascular NVT uses proteases to convert smooth muscle in spiral arterioles to fibrinoid, so as to prevent vasospasm that might compromise the fetus. Although destruction of smooth muscle in deeper myometrial arteries can be incomplete (23), endovascular NVT permits physiologically transformed vessels to massively increase blood flow into the intervillous space.

In comparison to interstitial NVT, endovascular NVT not only has a mutually exclusive location (Figure 1a), it also has:

a) different target tissues (vascular smooth muscle and endothelium vs. decidua and myometrium),

b) different functions (vascular remodeling to amplify and redirect blood flow vs. stable anchoring that nonetheless facilitates placental separation); and

c) different normal outcomes (smooth muscle destruction and replacement with

fibrinoid, combined with transient replacement of endothelium vs. failure to destroy decidua or produce fibrinoid in the myometrium).

We suggest that this is strong evidence that endovascular and interstitial NVT are mutually exclusive branches of the NVT differentiation pathway. Our observations will demonstrate further evidence of mutual exclusivity in morbid adherence.

Lastly, in the fetal membranes, there appears to be a positional variation in the NVT differentiation pathway. It appears that endovascular NVT does not occur; and that protease activity of chorionic NVT is downregulated, so there is no myometrial infiltration. Sections of normal fetal membranes do not have adherent myometrium. However, chorionic NVT in early products of conception can have keratin-positive dendrites that connote their infiltrative nature (Figure 1b).

Although the term intermediate trophoblast is useful in diagnosis of trophoblastic neoplasia (13,22), we suggest that it is inadequate to fully represent the separate branches of the NVT differentiation pathway. Similarly, despite the utility of the term extravillous trophoblast (EVT) (2,11), this term merely connotes a difference in location. EVT does not distinguish between stem cells in the trophoblastic shell, cells in the 2 branches of the bidirectional NVT pathway at the implantation site, and chorionic NVT in fetal membranes. Although much work remains to be done, we demonstrate that viewing morbidly adherent placentas in terms of stem cell theory suggests new ideas that merit further study.

MATERIALS AND METHODS

This is neither a review article; a systematic prospective study; or even a comprehensive retrospective review with “normal controls” (9,10). Its limited

ambition is merely to present evidence in support of the idea that viewing morbid adherence in terms of stem cell theory suggests new ideas that merit future study.

This report presents selected illustrations, collected from a few dozen cases of morbid adherence examined over a 30 year period at a university-affiliated community hospital (about 2/year); with routine use of keratin stains (MAK6 or AE1/AE3); actin stains (MSA or SMA) and connective tissue stains (trichrome, Elastic van Gieson, Kreyberg) (28) as needed; and attempts at sonographic-pathologic correlation. They include cases diagnosed on sonograms, leading to Cesarean hysterectomy, to avoid massive blood loss (5,7,8); typical cases with blood loss following attempted placental delivery (24); increta s involving Cesarean scar pregnancies ranging from 1st trimester to term (5,7,8); and cases of uterine inversion (1,29). Pertinent observations on delivered placentas are included (2,9).

RESULTS

Selected Observations on Morbid Adherence

In a term Cesarean hysterectomy, the placenta was attached from the lower uterus to the upper corpus (Figure 2a); although the attachment was discontinuous. This Cesarean hysterectomy was done because a sonogram predicted that placental delivery might lead to massive hemorrhage (6,11). Because this placenta never invaded the myometrium, it qualifies as a true accreta (1,9,10,24). All other cases diagnosed as accreta in this series had placental delivery, with hemorrhage leading to postpartum hysterectomy. This case may be unique in the literature, since other pathologic studies reported no term Cesarean hysterectomies for true accreta (9,10). Fetal membranes were normal in this case.

Despite sonograms indistinguishable from this unique true accreta , other term Cesarean hysterectomies to prevent massive bleeding showed incretas of the inner third. These were also adherent from the lower uterus to the upper corpus, but the attachment was continuous (Figure 2b). Degree of myometrial thinning was quite variable. In these cases, fetal membranes could be so diffusely adherent that the uterus could be lifted by a clamp on the morbidly adherent fetal membranes (MAFM)(Figure 2c).

In contrast, term Cesarean hysterectomy for Cesarean scar pregnancy (CSP) could be attached only in the lower uterus, qualifying as partial increta (9,10,24), while penetrating deep into the outer third (Figure 2d). In contrast to a report of 10 Cesarean scar pregnancies (5), percreta was not universal in our experience with Cesarean scar pregnancies. A possible histologic explanation for this was noted (see below). Fetal membranes could be grossly normal in Cesarean scar pregnancies.

In uterine inversion with placental delivery, there was no grossly evident increta of the dome implantation site, but there could be morbidly adherent retained membranes in the mid-corpus, so tightly bound that the uterus could be lifted off the dissecting table with a clamp on the retained membranes (Figure 2e).

Pathology of True Placenta Accreta in a Cesarean Hysterectomy

The site of morbid adherence was to hugely dilated basal plate vessels in the decidua. This explained the discontinuous morbid adherence, and validated the sonographic warning that placental delivery might lead to hemorrhage. Furthermore, vascular wall smooth muscle might well have been seen on the maternal surface of the delivered placenta (30). These basal plate vessels had

markedly irregular walls; shown on the actin stain to vary from thick to thin to moth-eaten to almost totally destroyed (Figure 3a). There was more preservation of smooth muscle than in normally transformed spiral arterioles. Keratin stain showed conspicuous endovascular NVT in these basal plate vessels, with markedly dilated subjacent endometrial glands (Figure 3b). Both actin (Figure 3c) and keratin stains showed intravascular villi in these vessels. When similar basal plate vessels with intravascular villi and irregular smooth muscle walls were seen in other delivered placentas, this was reported to clinicians as raising the question of occult accreta.

Although the endometrium above these abnormal vessels looked like normal implantation site, the lower endometrium lacked conspicuous decidualized stromal cells and interstitial NVT. Instead, there was a spongy zone (Figure 3d), correlating with the retroplacental abnormality seen on ultrasound. Keratin stain showed that some spaces were markedly dilated endometrial glands, while others were markedly dilated vessels (Figure 3e). These anomalies distinguished this true accreta from implantation sites in hysterectomies for uterine atony.

Routine keratin stain showed that the dilated vessels received excess blood from deep vessels involved by endovascular NVT; and actin stain showed these vessels had plenty of muscle in their walls. No vessels had the replacement of smooth muscle by fibrinoid that is considered “physiological” (9). Keratin stain with a Kreyberg counterstain showed irregularity of the endomyometrial junction; which had markedly dilated endometrial glands; and essentially normal myometrium (Figure 3f). Even if one might describe this true accreta as partly deficient in decidual cells, it did not predispose to myometrial infiltration by interstitial NVT.

Selected Observations on Sonographic-Pathologic Correlation of Chaotic Intraplacental Blood Flow (Venous Lakes).

Sonograms of true accreta or increta show abnormal intraplacental blood flow, sometimes referred to as chaotic, or as "venous lakes" (6,11, 31). The pathologic correlate appears to be excessively deep endovascular NVT in larger vessels, best seen in most cases with a keratin stain (Figure 4a). In both true accreta and inner third increta, endovascular NVT went deeper than interstitial NVT, and deeper than seen in normal implantation. These larger deeper vessels were demonstrated in other accreta cases with a trichrome stain (Figure 4b), showing endovascular NVT in hyalinized vessels lacking intact muscle. These larger deeper vessels may cause excessive blood flow into the placenta.

In our limited experience, routine keratin stains do not always demonstrate endovascular NVT in association with morbid adherence. Endovascular NVT was not seen in dome implantation associated with uterine inversion; in sections of morbidly adherent membranes, or in either 1st trimester or some term incretas associated with Cesarean scar pregnancy. In a term deep increta invading the CS scar, there was insufficient myometrium deep to the scar to detect endovascular NVT.. In a 1st trimester Cesarean scar pregnancy with increta (Figure 4c), detected on a sonogram, the keratin stain was negative for endovascular NVT (Figure 4d). The trophoblast shell had not yet withered away entirely, with anchoring villi appearing on the surface (Figure 4d). Dilated vessels were seen in the C-section scar (Figure 4c), and deep to both the scar and implantation site were large parametrial arteries; providing an alternative explanation for increased blood flow.

Selected Observations on the Retroplacental Zone of Placenta Increta

In 30 years of practice at a university-affiliated community hospital, retroplacental myometrial pathology has always been present in placenta increta; although it was sometimes necessary to do actin stains to demonstrate this. In a term increta of the inner third, diagnosed on ultrasound, the retroplacental zone showed conspicuous degeneration, edema, and mild chronic inflammation of inner myometrium (Figure 5a). Myofibers infiltrated by interstitial NVT were small, ragged, and irregular, as compared to normal gestational hypertrophy of myometrial cells. In this and other similar incretas (Figure 4b), intravascular chorionic villi were seen in the retroplacental myometrium. The actin stain in Figure 4b also demonstrates small, degenerated myofibers in the basal plate of the placenta. Inflamed degenerated retroplacental myometrium was seen even in a 1st trimester laparoscopic hysterectomy after sonographic diagnosis of placenta creta associated with a Cesarean scar pregnancy at 6 weeks; best appreciated with an actin stain (Figure 4c). At the other end of the spectrum was a huge retroplacental hypocellular zone in a term Cesarean scar pregnancy with deep increta; which had only a few scattered NVT and shrunken myofibers in myometrium adjacent to the CS scar (Figure 4d).

The CS scar itself was infiltrated by interstitial NVT in a zone of massive globular elastosis (Figure 4e)(28). The pale staining zone of elastosis in the trichrome stain was confirmed with dark black staining on the Elastic van Gieson stain, as in our previous work (28). Keratin stain confirmed that the cells in the elastosis were interstitial NVT. We have seen massive globular elastosis in unstable

C-section scars that ruptured, and it is common both in postablation scars and the outer wall scars that were formerly diagnosed as fibrosis uteri (28). This elastosis may possibly have resisted protease digestion, allaying progression to percreta (5). Globular elastosis was also seen in the 1st trimester C-section scar in Figure 3c.

Selected Observations on Uterine Inversion

Examination of the implantation site in the dome - in a case with a corrected term uterine inversion and no significant bleeding - showed absent decidua, despite the lack of a known predisposing factor (Figure 6a). Although subjacent muscle fibers were shrunken and degenerated, consistent with inner third increta (Figure 6b), the interstitial NVT was indistinguishable from normal myometrial infiltration (1), and endovascular NVT was not observed.

This and other delivered placentas had myofibers on the maternal surface, and there were also small irregular spindle cells, and isolated NVT cells, in basal plate fibrinoid. Actin stain showed degenerated muscle both on the maternal surface and higher up in the basal plate (Figure 6c). Report of this finding led to a postpartum ultrasound, which demonstrated retained cornual placenta, but no sonographic diagnosis of accreta (31). This led to a delayed postpartum hysterectomy, for fear of cornual perforation.

During hysterectomy for retained cornual placenta 5 days later, the cervix unexpectedly fell apart in the surgeon's hands, with massive blood loss. No percreta of cervix was seen by the surgeon, but only a mm separated the placenta from the outside of the cervix. On pathologic examination, there was deep increta in both the cornu and the cervix. There were many involuted and obliterated placental site

303 blood vessels in the myometrium; and there were also dilated thrombosed
304 subinvoluted vessels (1).

305 In another corrected term inversion, with continued bleeding after placental
306 delivery, requiring immediate postpartum hysterectomy; there was hemorrhagic
307 necrosis of superficial myometrium, consistent with early venous infarction, due to
308 grossly obvious myometrial venous thrombosis; secondary to the inversion. Lines of
309 Zahn were seen in the thrombi. Deeper dome myometrium was markedly
310 edematous (29). This case had morbidly adherent retained fetal membranes, but no
311 gross increta. Despite microscopic implantation in endocervix as well as dome, there
312 was no increta in the cervix.

313 **Selected Observations on Pathology of Morbidly Adherent Fetal Membranes**

314 Sections of morbidly adherent fetal membranes (MAFM) included both
315 decidua and myometrium. In a term inner third increta with diffusely attached
316 MAFM, there was an undulating chorion, with variable distance between chorion
317 and myometrium, and a heterogeneous decidual matrix (Figure 7a). Unlike sections
318 of normal membranes, fibrinoid was focally prominent in the decidua (Figure 7b).
319 The keratin stain showed alternating zones of infiltrative and noninfiltrative
320 chorionic NVT (Figure 7c). An infiltrative pattern on the left in Figure 7c
321 corresponds to an area on the left with prominent fibrinoid in Figure 7a. The lower
322 decidua had dilated endometrial glands, which are not seen in normal membranes.
323 When dilated endometrial glands have been seen in fetal membranes from a
324 delivered placenta, basal plate myofibers were looked for and found on the maternal

surface of the placenta; suggesting that this can be a sign of clinically occult morbid adherence.

In a case of uterine inversion with no gross increta, but with retained MAFM after placental delivery, the keratin stain showed less undulation of the chorion, diffusely infiltrative chorion, and more dilated endometrial glands (Figure 7d), as compared to Figure 7c. At high magnification, a raggedly irregular infiltrative pattern was reminiscent of protease-mediated stromal invasion in cancers (32)(Figure 7e). Actin stain in this case showed focal muscle destruction under MAFM (Figure 7f). Infiltrative interstitial NVT was seen at this site; but in no case have we found endovascular NVT in a section of MAFM.

DISCUSSION

The Role of Villous Trophoblast in Morbid Adherence

Standard teaching about morbid adherence is that chorionic villi adhere to or invade the myometrium, but some experts have taken tentative steps away from this dogma (1-3,9,11). Kraus, Redline, Gersell et al noted in the AFIP Fascicle that: "In placenta accreta, it is a common misperception that well vascularized villi must directly abut smooth muscle"(3). Stanek and Drummond used actin and keratin stains to demonstrate that "between the myometrium and chorionic villi there are only the extravillous trophoblasts and Rohr fibrinoid, but no decidua" (2). An article on ultrasound and MRI by Benirschke et al illustrated accreta with the comment: "Note that the extravillous trophoblast is adherent directly to myometrium…"(11), and this was also observed by Khong and Robertson (9). An open break with

prevailing dogma in the former paper was avoided by the statement that defective decidua allows "anchoring villi to penetrate myometrium" (9,11,15). However, current evidence suggests to us that the trophoblastic columns at the tips of anchoring villi (9,10) are residual trophoblastic stem cells, remnants of the withering trophoblastic shell.

We assert that 30 years of experience with routine keratin stains in hysterectomies for morbid adherence always demonstrates nonvillous trophoblast at the invading front. Based on our view that NVT derives from pluripotential and highly proliferative trophoblastic stem cells, as a mutually exclusive branch of the trophoblastic differentiation pathway; we suggest that it is time to reclassify morbid adherence as a disease of nonvillous trophoblast (NVT).

Pathogenesis of True Placenta Accreta

The pathologic analysis of true accreta in a term Caesarian hysterectomy in this report may be unique in the literature (9,10,24). Postpartum hysterectomies after placental delivery (9,10) may be suboptimal for demonstrating the dilated basal plate vessels, spongy zone, and essentially normal myometrium with little or no interstitial NVT seen in this case. We suggest that limited understanding of the pathogenesis of true accreta may often lead to classification of early increatas as true accretas. More study of true accretas in Cesarean hysterectomies is needed, but several points bear emphasis:

1) Morbid adherence of this true accreta occurred in the endometrium. The risk of massive hemorrhage, and discontinuous attachment on gross exam,

reflected morbid adherence to strikingly dilated basal plate vessels with keratin-positive endovascular NVT and degenerated vascular smooth muscle. Deficient endovascular NVT effects in accreta have been seen by others (9,10). True accreta is among the diseases of endovascular NVT (23,33).

2) As per standard teaching (1), this true accreta had normal myometrium; despite morbid adherence, and sonographic recognition of both an abnormal retroplacental zone and blood lakes in the placenta (6,11). Interstitial NVT appeared less than normal, with little or no myometrial infiltration.
3) The retroplacental zone seen on ultrasound in true accreta was composed of admixed markedly dilated endometrial glands and vessels. Dilated endometrial glands are characteristic of implantation sites (23,34,35). This may reflect NVT-induced secretion of glycogen-rich fluid to nourish the early embryo. In true accreta, endometrial glands are markedly dilated. More study of this subject is needed.Intravascular chorionic villi in the basal plate are currently considered normal (1). However, they were observed in this true accreta in vessels with abnormal smooth muscle architecture; in superficial myometrium of inner third increta; and in deep myometrium of percreta. Further study of intravascular villi in the basal plate, in relation to normal vs. abnormal vascular smooth muscle, appears to be warranted.

Role of Endovascular NVT in the Pathogenesis of Sonographic Blood Lakes

Chaotic intraplacental blood flow, also known as blood lakes or venous lakes, is characteristic of both true accreta and increta on ultrasound (6,11,31). These blood

lakes are inside the placenta. Transformation of endometrial and inner third myometrial arteries by endovascular NVT is the generally accepted mechanism of adaptation to support normal intraplacental blood flow; although deeper vessels can be involved to a lesser degree, with some residual intact smooth muscle (23). Thirty years of experience with keratin stains in hysterectomies for morbid adherence suggests that larger deeper vessels are transformed by endovascular NVT, leading to the excessive intraplacental blood flow seen on ultrasound. This has been seen by others in increta s (9,10), but it appears that routine keratin stains are needed to detect deep endovascular NVT in true accreta. Dilated vessels in the basal plate may reflect resistance and backup of the intervillous space to increased blood flow.

The increased blood flow due to deep vessel involvement by endovascular NVT may possibly explain intravascular villi in the basal plate and myometrium, if villi are "carried along" in the increased venous drainage from the intervillous space. Their presence in inner myometrium in early increta and deep myometrium in percreta may not reflect significantly greater displacement from intravascular villi in the basal plate, if myometrium is driven up towards the placenta by myometrial tone (see below). Alternatively, intravascular villi may be developmental anomalies that occur as the trophoblastic shell withers away. It is our subjective impression that intravascular villi are seen more in term placentas than earlier placentas, suggesting that they are an acquired anomaly; but this needs more study.

Discordance between depth of endovascular NVT and interstitial NVT is common

in incretas, and has been seen by others (9,10); and significantly greater penetration than normal depth of endovascular NVT has been measured (10). We suggest that endovascular NVT is a mutually exclusive branch of the NVT that can spread deeper (more obvious with routine keratin stains) because it spreads along blood vessels in an angiocentric fashion. A similar mechanism of spread has been observed during prenatal nevogenesis, where the favored descriptive term was angiotropic spread (22,36). Further work is needed to explore if there are different proteases and/or protease inhibitors in endovascular NVT. It is known that proteases unique to the placenta appeared during evolution (26), and that proteases and their inhibitors are very heterogeneous (18,27).

The present report suggests that the endovascular NVTDP may not be induced in all morbid adherence. In 1st trimester Cesarean scar pregnancy, increased blood flow through the scar was suggested by vascular ectasia, possibly deriving from large parametrial arteries that normally occur in this region. This may be an alternative explanation for blood lakes in the placenta in first trimester Cesarean scar pregnancies diagnosed as creta on ultrasound.

Pathogenesis of Sonographic Retroplacental Zones in Placenta Increta

Despite literature since 2000 on occult placenta accreta and “early placenta accreta”; most recent attention has focused on increta or percreta (2,6,7,12,13). Occult accreta was defined as basal plate myofibers in association with deficient decidua in delivered placentas (2). In marked contrast, “early placenta accreta” was defined from a clinical perspective as a 2nd trimester placenta with ”no myometrium

437 between the placenta and the serosa or bladder"; so from a histologic perspective,
438 these were actually cases of increta or percreta (7).

439 We assert that 30 years of experience with hysterectomies done for increta
440 always shows retroplacental myometrial degeneration, edema, and mild chronic
441 inflammation, explaining abnormal retroplacental zones on sonograms. Myometritis
442 and hyaline degeneration have been observed by others (9), but shriveled myofibers
443 are more easily recognized by actin and trichrome stains; as illustrated in this
444 paper; so these may be underdiagnosed on routine H&E stains. Shriveled myofibers
445 were also seen in the basal plate in increta s. They are also commonly seen in
446 postablation scars and C-section scars (28). This finding may help diagnose increta s,
447 both in cases without sonograms (e.g., inversion cases) and in first trimester
448 increta s; where actin stains may be necessary (2).

449 Enhanced placental protease activity in IS NVT (15,16) is the likely cause of
450 myometrial degeneration; which could be due to a deficiency of protease inhibitors
451 and/or other decidual factors (14,17,18). This idea is supported by comparison to
452 protease-mediated lung diseases:

453 1. When bacteria secrete proteases to generate nutrients (37), bacterial
454 proliferation can lead to massive tissue destruction in lung abscesses. However,
455 proliferation of interstitial NVT in morbid adherence is minimal (< 1mm, and <twice
456 normal) (2,10,13); and to date there is no evidence to associate exaggerated
457 placental sites with morbid adherence (1,13,22).

2. Neutrophil serine proteases can overwhelm antiproteases in cystic fibrosis, leading to potentially fatal chronic lung infections (38). There is no evidence for excess neutrophil serine proteases in morbid adherence, although further work on macrophage proteases merits consideration.
3. Serine proteases in alveolar macrophages lead to premature emphysema in young smokers with alpha-1-antitrypsin deficiency (A1ATD)(18). This mechanism remains unexplored in morbid adherence, despite the fact that smoking is a risk factor for accreta (39).

The wide variety of alleles with highly variable phenotypes in protease inhibitor deficiency (18) might help to explain some of the wide variation in depth of penetration by interstitial NVT in increta (9,10,24). This wide variation accounted for skepticism of the decidual barrier concept in the past (9); since it was not clear why every increta did not go all the way through the wall. Species variations in the interstitial NVT protease-antiprotease balance may also explain why thinner decidua in other species is not associated with deep myometrial invasion (9).

Also unexplained to date is the pathogenesis of partial vs. complete increta. Previous studies have confirmed our observation that previa creta often is not restricted to the C-section site of decidual deficiency (9,10,25). Protease inhibitor deficiency as a cofactor may explain involvement of the upper uterus in these cases.

It appears unlikely that increased interstitial NVT proliferation or invasion explains deeper incretas (10). Interstitial NVT usually does not “look” more invasive

in increta, as compared to "normal" infiltration (1). Indeed, the most ragged, irregular NVT we have encountered, resembling stromal invasion in cancers (32), has been infiltrative chorionic NVT in morbidly adherent fetal membranes. There is, in fact, reason to question that increta moves deeper in the wall as the myometrium beneath it disappears. The reverse is more likely, in our view.

It is often said, perhaps nonchalantly, that increta "eats" its way through the wall; but we suggest that this analogy to eating may be the literal truth. We point out that during true eating, swallowing occurs, with partly digested material moving away from the site of "ingestion". In morbid adherence due to increta, this implies that partly digested muscle fibers may end up in the basal plate, between the maternal surface and the villi; as observed in this study. This implies that some clinically occult cases with basal plate myofibers may actually be occult increta.

Rather than the interstitial NVT moving down, we suggest that the myometrium may be moving up. This may be driven by myometrial tone, with the greatest pressure exerted by the larger, more parallel myofibers of the outer third (40-43). Since myometrium is a complex and heterogeneous muscular tissue (42) that can contract in various directions, uneven myometrial tone may at least partly explain the variable degree of myometrial destruction in increta.

Similar considerations may explain why normal placental separation can occur despite myometrial infiltration by interstitial NVT. We suggest that "normal myometrial infiltration" may leave the involved myometrium sufficiently strong and firm to resist shear stress, as the placenta is cleaved from the uterus (10). In contrast, degenerated myometrium in "occult cretas" may be too weak to resist

shear stress, so that some myofibers come out with the delivered placenta; leading to suspicion of placenta creta on placental examination (2).

Pathogenesis of Uterine Inversion

Morbid adherence in the dome, causing uterine inversion, has received little attention (29). Although percreta of the dome was seen when the dome was the site of Cesarean section incision (24), we and others have not seen deep increta and percreta in the dome (29). Inversion cases were not diagnosed by sonograms, but microscopic retroplacental myometrial degeneration was seen; consistent with early incretas. The degree of myometrial NVT infiltration was within the spectrum of what is considered "normal" in other locations (1). We will suggest that this can be explained in terms of basic dome biology.

The dome, cornus, and tubes derive from fusion of the upper Mullerian ducts (43). Although cornus do not decidualize, decidua is seen in Fallopian tube segments resected at the time of delivery. However, this decidua is not generally observed in ectopic tubal pregnancies; supporting the suggestion that decidual deficiency can – to some extent – be the result of placental invasion and destruction (9,14). The lack of decidua at the dome implantation site in uterine inversion raises the question of inherent decidual deficiency. This needs study.

Although deficient dome decidualization might promote myometrial infiltration at implantation sites, it is suggested that the dome may have special defenses to pathologic myometrial invasion (deep increta and percreta). This is based on the observation that dome myometrium is both unique and essential to

526 successful labor and delivery. Dome myometrium has a unique set of muscle
527 bundles that contract so as to pull the cornus towards each other (28), which may
528 help the dome provide downward pressure during uterine contraction. Perhaps the
529 protease-antiprotease balance (15-18,27) is modified in the dome, to preserve and
530 protect this vital function. More study is needed.

531 Two other aspects of dome biology merit further investigation. First is the
532 observation that during development, hypoxia in utero may cause large fibrous
533 scars in the dome (43). Such scars might negate effective dome contraction, leading
534 to myometrial exhaustion. A search for dome scars in cases of uterine atony (29)
535 should be on the to-do list of obstetric research.

536 Secondly, the shear stress that allows placental separation from decidua (10)
537 needs further analysis in uterine inversion with dome implantation. When the dome
538 functions properly, it provides the downward force necessary to promote placental
539 separation from myometrium, despite "normal" myometrial infiltration by
540 interstitial NVT in the corpus. However, when implantation is in the dome,
541 insufficient shear stress may be generated to disrupt what would otherwise be
542 considered "normal" myometrial infiltration; so that attempted placental delivery
543 results in uterine inversion.

544 Lastly, we note that uterine inversion due to dome implantation can coexist
545 with endocervical implantation. This can range from incidental microscopic
546 involvement to advanced increta with impending percreta. This wide phenotypic
547 variation might be partly explained by a wide range of protease inhibitor

548 deficiencies (18); with the fetus (placenta) providing protease variations, and the
549 mother providing protease inhibitor variations. This needs more study.

550 **Pathogenesis of Morbidly Adherent Fetal Membranes (MAFM)**

551 The pathogenesis of MAFM is previously unexplored. Some might question
552 whether diffusely adherent fetal membranes may reflect failure of labor to induce
553 membrane separation in Cesarean hysterectomy; but we disagree because MAFM
554 can be so tightly adherent that a pregnant uterus could be lifted off the dissecting
555 table with a clamp on the membranes. We doubt that diffusely adherent fetal
556 membranes in a Cesarean hysterectomy reflect failure of labor, since retained membranes
557 in a postpartum hysterectomy looked more infiltrative and more deeply adherent. We
558 note that a previous case of increta was illustrated as showing adherent
559 myometrium to fetal membranes (9), but that retrospective study had no details
560 about gross pathology. We have seen 17 cases of MAFM, although they have not
561 been systematically analysed other than to note that MAFM is usually associated
562 with morbid placental adherence in the upper corpus, dome, and/or cornu.

563 Microscopic study of MAFM suggests that morbid adherence can be limited
564 to the upper decidua, or can have superficial myometrial infiltration with subjacent
565 myometrial degeneration detected on actin stains. The lower decidua can have
566 dilated endometrial glands, which is more striking in association with retained
567 membranes. Keratin stains demonstrate a more infiltrative phenotype in retained
568 membranes, as compared to diffusely adherent MAFM in Cesarean hysterectomies.
569 There is heterogeneity of the decidual matrix, with more prominent fibrinoid at

sites of more infiltrative chorionic NVT. More study is needed, but in our experience, dilated endometrial glands and decidual fibrinoid in sections of fetal membranes may merit the use of actin stains to evaluate for basal plate myofibers and/or subchorionic myometrial degeneration.

Proteases in Morbid Adherence

It is generally accepted that placental proteases mediate both anchoring of the placenta by interstitial NVT and destruction of vascular wall smooth muscle by endovascular NVT. We suggest that further study of placental proteases may lead to better understanding both of the trophoblastic differentiation pathway, and of morbid adherence. There is no inherent reason to expect that trophoblastic stem cells in the trophoblastic shell have proteases that can break down basement membranes, unless they are blocked by antiproteases (16,17, 27). While it is already established that interstitial NVT has such proteases (15,16), it would seem that such proteases must be either absent or blocked by antiproteases in VT, since regulation of transplacental diffusion requires intact basement membranes.

It will go a long way to confirming our hypothesis that the trophoblastic columns at the tips of anchoring villi are remnants of trophoblastic stem cells if they are found to have the same protease-antiprotease phenotype as the trophoblastic shell. It is already known that Ki67 stains distinguish VT and NVT from trophoblastic columns in anchoring villi (10).

Endovascular NVT proteases that destroy smooth muscle in spiral arterioles may differ from interstitial NVT proteases which destroy basement membranes. Endovascular NVT proteases appear to be abnormal in true accreta (9,10). The same

may be true in increta (9,10). This may reflect variation in the protease-antiprotease balance in endovascular NVT in morbid adherence. Paradoxically, endovascular NVT goes deeper in both true accreta and increta (9,10). Depth of angiocentric spread may be independent from degree of vascular smooth muscle destruction; since depth of angiocentric spread is greater than normal (10), but replacement of vascular smooth muscle by fibrinoid is less than normal.

Although infiltration of inner myometrium by interstitial NVT is considered normal; destruction of myometrium by the proteases of interstitial NVT is evidence of placenta increta; including clinically occult incretas. We suggest that variation in the protease-antiprotease balance (16-18,27) may distinguish the interstitial NVT of increta from normal interstitial NVT.

Infiltrative chorionic NVT has a different morphology than noninfiltrative chorionic NVT. Variation in the protease-antiprotease balance would be an expected correlation with this finding. Since chorionic NVT does not normally infiltrate myometrium; and since morbid adherence of fetal membranes can occur in the decidua, without myometrial destruction; we suggest that protease-antiprotease variations in chorionic NVT may differ from those in other locations.

Uterine Scars and Morbid Adherence

It has recently been suggested that increta and percreta may gain access to the deep uterine wall via uterine scars (10). This was based in part on analysis of the data showing only a limited amount of proliferation and invasion by interstitial NVT; an analysis with which we agree. The authors only observed 3 uterine scars in their series of 38 cases, but 11 additional cases were located at the site of the prior

C-section incision. Although a previous series with 18 hysterectomies (9) reported no detectable uterine scars , the possibility of limited sampling of C-section scars applies to both series (9,10). However, ACOG Practice Bulletin 115 emphasizes that not all CS scars are an obstacle to vaginal delivery (44), so the mere history of prior C-section does not constitute evidence of the kinds of altered wound healing seen in our previous work (28). Based on our experience with morbid adherence, and our histopathologic analysis of uterine scars that led to hysterectomy for pain or bleeding (28); we enthusiastically endorse the recommendation (10) for further histologic evaluation of the relationship of uterine scars to morbid adherence.

We note that it is not uncommon for cases of previa with suspected accreta on sonograms to lack pathologic confirmation of accreta (11, 39, 45). We have suggested that in such cases, myofiber disarray and globular elastosis consistent with abnormal wound healing in the isthmic scar may preclude the contraction required to achieve isthmic hemostasis (28). Thus, we recommend that the research on C-section scars be extended to include cases of previa where the accreta suspected on ultrasound cannot be confirmed on pathologic examination (28,39,45).

We do not have data on the frequency of increta in C-section scars in our material, but our experience suggests that some uterine scars may retard progression of myometrial destruction. We suggest that unstable scars with globular elastosis (28, 46) may be obstacles to protease-mediated destruction; since some proteases are less active than others against elastic tissue (18). These scars may be C-section scars of the isthmus; but may also be the elastotic outer wall scars of the corpus previously labeled as fibrosis uteri, or the newly described pattern in

639 the corpus of inner myometrial elastosis, found in hysterectomies done for pelvic
640 pain (28, 46). We have suggested an alternative mechanism for access to deeper
641 myometrium; namely that myometrial tone may push myometrium up into a zone of
642 enhanced protease-mediated myometrial destruction by interstitial NVT in increta;
643 possibly due to a maternal deficiency of antiprotease activity against the fetally
644 programmed placental proteases.

645 **Conclusion**

646 In the Introduction, we presented evidence that the VT differentiation
647 pathway is mutually exclusive with the NVT differentiation pathway in normal
648 placentas; and that the interstitial and endovascular branches of the NVT pathway
649 are also mutually exclusive. Our selected observations on morbid adherence further
650 suggest mutual exclusivity in the branches of the trophoblastic differentiation
651 pathway. Stem cell theory and the concept of a trophoblastic differentiation
652 pathway may help to better understand morbid adherence in true accreta, increta,
653 morbidly adherent fetal membranes, and clinically occult cretas that are risk factors
654 affecting future patient management.

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LEGENDS FOR ILLUSTRATIONS

Figure 1 – Trophoblastic differentiation Pathway

Figure 1a – Keratin stain of tubal pregnancy. The primitive trophoblastic shell gives rise to villous trophoblast and nonvillous trophoblast (NVT) on opposite sides of the shell. Interstitial NVT predominates, but endovascular NVT can be seen plugging the

lumen of vessels in the lower left. A similar pattern has been seen in intrauterine products of conception.

Figure 1b – Keratin stain, products of conception. Interstitial NVT in myometrium has dispersed ”starfish cells” with keratin-positive dendrites that connote their infiltrative nature.

Figure 2 – Morbid Adherence

Figure 2a – Morbidly adherent true placenta accreta extends to upper corpus, in a discontinuous fashion. Freshly sliced.

Figure 2b – Morbidly adherent placenta increta irregularly invades and thins inner third of wall, from lower uterus to upper corpus, with continuous attachment.

Figure 2c - Morbidly adherent fetal membranes (MAFM) allow uterus to be lifted off dissection table with a clamp attached to MAFM.

Figure 2d– Placenta of Cesarean scar pregnancy at term was attached only in lower uterus.

Figure 2e– Uterine inversion case had retained MAFM after placental delivery, so uterus could be lifted with clamp on retained membranes.

Figure 3 True Accreta

Figure 3a– Actin stain shows highly abnormal smooth muscle in huge basal plate vessels.

Figure 3b– Keratin stain of abnormal basal plate vessels showed endovascular NVT.

Figure 3c– Actin stain shows intravascular villi in abnormal basal plate vessels.

Figure 3d – True accreta in situ has spongy retroplacental zone in lower endometrium. Kreyberg stain.

Figure 3e - Keratin stain with alcian blue counterstain shows dilated vessels and dilated endometrial glands in spongy zone.

Figure 3f – Keratin stain with Kreyberg counterstain shows irregular endomyometrial junction, with essentially normal myometrium.

Figure 4 – Blood Lakes

Figure 4a – Term inner third increta has angiocentric endovascular NVT going deeper than interstitial NVT, involving larger deeper vessels than usual. Keratin stain.

Figure 4b – Deep large dilated vessel with endovascular NVT was clearly apparent on the trichrome stain, in a case classified as accreta after placental delivery led to hemorrhage. Clearcut myometrial thinning at lower uterine implantation site was not obvious (1), and myometrial infiltration by interstitial NVT appeared "normal" (1).

Figure 4c -Vascular ectasia in C-section scar next to 1st trimester inner third increta. Trichrome stain.

Figure 4d – This 1st trimester increta had no endovascular NVT on keratin stain.

Figure 5 – Retroplacental Zone in Placenta Increta

Figure 5a – Retroplacental myometrial pathology of term inner third increta, correlating with sonogram.

Figure 5b - Intravascular chorionic villi were often seen in retroplacental degenerated myometrium in increta. Actin stain.

Figure 5c – 1st trimester Cesarean scar pregnancy had placenta increta of inner third, with retroplacental inflamed degenerated myometrium, correlating with sonogram. Actin stain

Figure 5d – Extremely hypocellular retroplacental myometrium was adjacent to C-section scar in term increta of lower uterus, with only a few NVT and a few shriveled myofibers.

Figure 5e - Trichrome stain of term deep increta (same case as Figure 5d) shows shriveled myofibers, normal myofibers (upper right), and infiltration of interstitial NVT into massive globular elastosis of CS scar.

Figure 6 - Uterine Inversion

Figure 6a – Implant site in dome lacks decidua, with shriveled muscle in retroplacental zone.

Figure 6b - Interstitial NVT infiltrates shriveled retroplacental myometrium of dome. The NVT was indistinguishable from that seen in "normal" myometrial infiltration (1).

Figure 6c – Actin stain of delivered placenta in this case of uterine inversion was positive for myofibers both on the maternal surface, and higher up in the basal plate.

Figure 7 Morbidly Adherent Fetal Membranes

Figure 7a - Undulating chorion and heterogeneous decidua of diffusely attached MAFM.

Figure 7b – Fibrinoid in decidua under MAFM.

Figure 7c – Keratin stain of diffusely attached MAFM shows alternating zones of infiltrative vs. noninfiltrative chorionic NVT. Deep decidua has dilated endometrial glands.

Figure 7d – Keratin stain of retained MAFM in postpartum hysterectomy after uterine inversion shows less undulation, more infiltration, more dilation of endometrial glands.

Figure 7e – Raggedly infiltrative chorionic NVT in retained MAFM "looks invasive" on keratin stain.

Figure 7f – Actin stain shows retrochorionic myometrial degeneration under retained membranes.