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## NORMAL AND ABNORMAL TRANSFORMATION OF THE SPIRAL ARTERIES DURING PREGNANCY

**Jimmy Espinoza<sup>1,2</sup>, Roberto Romero<sup>1,3</sup>, Yeon Mee Kim<sup>1,4</sup>, Juan Pedro Kusanovic<sup>1</sup>, Sonia Hassan<sup>1,2</sup>, Offer Erez<sup>1</sup>, Francesca Gotsch<sup>1</sup>, Nandor Gabor Than<sup>1</sup>, Zoltán Papp<sup>5</sup>, Chong Jai Kim<sup>1,4</sup>**

<sup>1</sup>Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, Maryland, and Detroit, Michigan USA

<sup>2</sup>Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, USA

<sup>3</sup>Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan, USA

<sup>4</sup>Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan, USA

<sup>5</sup>First Department of Obstetrics and Gynecology, Semmelweis University, Budapest Hungary

### Abstract

This article reviews the anatomy and physiology of the uterine circulation, with emphasis on the remodeling of spiral arteries during normal pregnancy, and the timing and anatomical pathways of trophoblast invasion of the spiral arteries. We review the definitions of the placental bed and basal plate of the placenta, their relevance to the study of the physiologic transformation of the spiral arteries, as well as the methods to obtain and examine placental bed biopsy specimens. We also examine the role of the extravillous trophoblast in normal and abnormal pregnancies, and the criteria used to diagnose failure of physiologic transformation of the spiral arteries. Finally, we comment on the use of uterine artery Doppler velocimetry as a surrogate marker of chronic uteroplacental ischemia.

### Keywords

atherosis; immunohistochemistry; impedance to blood flow; integrin; physiologic transformation of the spiral arteries; placental bed

### 1. Anatomy and physiology of the uterine circulation:

The blood supply of the uterus is provided by the uterine and ovarian arteries [102]. After entering the myometrium, the uterine arteries give rise to the “arcuate arteries,” which branch into the “radial arteries” [102]. The radial arteries divide into the “basal arteries,”

which supply the basal portion of the endometrium (critical for endometrial regeneration after menstruation) and the “spiral arteries,” which continue toward the endometrial surface [95]. The term “spiral arteries” reflects the coiled appearance of these vessels, whose role is to supply blood to the upper functional layer of the endometrium, thought to be shed during menstruation [95]. The spiral arteries branch into a subepithelial capillary plexus [95]. Markee reported that each spiral artery supplies approximately 4–9 mm<sup>2</sup> of endometrial surface [71]. Blood from the intervillous space is drained through the utero-placental veins, whose openings are on the floor of the intervillous space. The venous endothelium lines part of the floor of the intervillous space floor [62]. The uteroplacental veins undergo less dramatic changes than the arteries. Trophoblast may or may not invade the venous wall, even though free-floating endoluminal trophoblast have been found in these vessels [36].

### **Endometrial bleeding during menstruation and hemostasis:**

The endometrium has evolved to meet the needs of menstruation, implantation, placentation, as well as post-partum hemostasis. The specific mechanisms responsible for endometrial bleeding (during menstruation) and hemostasis (after menstruation) are relevant to the study of pregnancy, since uterine bleeding during pregnancy (i.e., hemostatic failure) is a risk factor for adverse pregnancy outcome [75, 101].

The current understanding of the vascular changes that occur during menstruation is based upon classic physiologic studies conducted by Markee, who examined the behavior of the endometrium from rhesus monkeys implanted into the anterior chamber of the eye of non-human primates [71]. The explants underwent a reduction in size of 25–75%, 2–6 days before the onset of bleeding. The spiral arteries became more coiled, and blood flow either slowed down or came to a complete stop. Red blood cells were reported not to move for 60–90 seconds [71]. Menstrual bleeding was considered to occur when vessel vasodilated after a period of intense vasoconstriction. Five mechanisms were proposed to account for the bleeding: 1) blood could pass through a damaged arteriole and form a hematoma, which would then rupture into the uterine cavity; 2) blood would pass directly from a ruptured arteriole into the uterine cavity; 3) red blood cells would extravasate by crossing the wall of the arterioles; 4) venous bleeding, which may occur either through the damaged venous wall or drainage following extravasation from arterioles; and 5) secondary bleeding may happen following violent movement. Markee proposed that 75% of bleeding derived from arterioles, and 25% from the venules. Cessation of bleeding is thought to be due to vasoconstriction [71].

The fact that menstrual fluid generally does not clot [94] should not be taken as an indication that coagulation is not important for uterine hemostasis. For instance, patients with Von Willebrand disease have excessive menstrual blood loss [29]. During normal menstruation, platelets and fibrin plugs are formed within the lumen of the spiral vessels [23]. Consumption of platelets and fibrinogen is thought to result in decreased concentrations of platelets and fibrinogen in menstrual fluid [28]. Lockwood et al. proposed that tissue factor and plasminogen activator inhibitor-1 (PAI-1) are implicated in both decidual hemostasis during implantation and bleeding during menstruation [67]. Tissue factor would induce coagulation and prevent hemorrhage during implantation, while PAI-1 would prevent

extracellular matrix degradation and fibrinolysis. At the end of the menstrual cycle, progesterone withdrawal would reduce tissue factor and PAI-1 production and facilitate menstruation [67].

## 2. Changes in the uterine circulation during pregnancy:

The spiral arteries in the placental bed are normally transformed into large dilated vessels undergoing dramatic structural changes in their vessel wall. The spiral arteries are also known as the utero-placental arteries [16]. The terms “physiologic changes” or “physiologic transformation” of the spiral arteries were first introduced by Brosens, Robertson and Dixon in 1967 to emphasize that these changes were part of normal pregnancy. The key findings of physiologic transformation are: 1) dilatation of the lumen; 2) trophoblast invasion of the vessel wall, which includes both the media and endothelium; and 3) replacement of the muscular and elastic tissue of the arterial wall by a thick layer of fibrinoid material that generally stains positive on Periodic Acid Schiff (PAS) staining [16] (Figure 1). Brosens et al. proposed that these structural changes, particularly the destruction of muscle in the media, would lead to loss in vasomotor control [16]. Collectively, these changes are thought to maximize the delivery of maternal blood to the intervillous space by making the arterial lumen wider as well as reducing the responsiveness of these vessels to vasoconstrictor agents. Invasion of the utero-placental veins has been implicated as a mechanism responsible for the lateral placental growth [25]. Much less is known, however, about changes in the morphology of the utero-placental veins [36].

## 3. Timing and anatomical pathways of trophoblast invasion of the spiral arteries:

Robertson et al. [86] and Pijnenborg et al. [82] proposed that trophoblast invasion occurs in two stages: 1) the transformation of the decidual segment of the spiral arteries by a “wave” of endovascular trophoblast migration in the first trimester; and 2) the transformation of the myometrial segments of the spiral arteries in the second trimester [82, 86]. In 1972, Brosens and colleagues [17] reported that in patients with preeclampsia and SGA, the physiologic transformation of spiral arteries is limited to the decidual segment of the uteroplacental arteries, and Robertson proposed that this process is due to inhibition of the second “wave” of endovascular trophoblast migration that occurs from about 16 weeks onwards [51]. However, Lyall et al. have concluded that morphological evidence of this proposal is unclear and that the molecular mechanisms to control such a process would be very complex [68]. Moreover, recent studies have suggested that trophoblast invasion of the spiral arteries is a continuous process [68, 69].

The anatomic pathways taken by the endovascular trophoblast (either the extravasation or intravasation model) have also been a matter of controversy [48]. The extravasation model, which is based on animal studies, proposes that trophoblast cells gain access to the uteroplacental arterial lumen via the point of entrance to the intervillous space, or close to it [48]. Thereafter, the cells migrate along the arterial lumen retrograde to the blood flow by adhering to and replacing the endothelium, forming intraluminal trophoblastic plugs. Some of these cells were thought to leave the arterial lumen and invade the media as well as

adventitia (Figure 2A) [8, 48, 82, 83]. In contrast, the intravasation model is based on studies in human tissues and proposes that endovascular trophoblast represents differentiated interstitial trophoblast (Figure 2B) [27, 34, 48]. According to this model, the trophoblast invades the arterial wall from the surrounding junctional zone and migrates inside the arterial lumen [27, 34, 48]. More recently, a combination of both hypotheses was proposed by Kam et al. [47].

#### **4. Number and location of spiral arteries supplying the placental bed:**

A comprehensive assessment of the vascular supply to the placental bed can only be determined by examination of cesarean section hysterectomy specimens. Indeed, Ivo Brosens studied 15 cesarean section hysterectomy specimens, including 3 cases in which the placenta had been left in situ. He estimated that there were 120 arterial openings in the placental bed, with each artery containing only one opening into the intervillous space [15]. However, the estimates have ranged widely in studies reported during the past century [36]. The number of arteries and openings in the placental bed has been the subject of disagreement. Some investigators report more openings than arteries, while others propose that the number of openings equals that of utero-placental arteries. Moreover, other investigators have emphasized the difficulty in distinguishing arterial from venous openings [36]. The density of the spiral artery opening at term varies from 0.5 to 1 per centimeter square [36]. Brosens et al. reported that spiral artery openings are topographically adjacent to the placental septae, and 96% of spiral arteries from normal pregnancies have findings of physiologic transformation. Spiral arteries in the center of the placenta are more likely to have physiologic transformation than those located on the periphery [16].

#### **5. Definition of placental bed and basal plate**

The term “placental bed” refers to part of the uterus (decidua and myometrium) lying underneath the placental insertion site [36]. The maternal-fetal junction comprises the basal plate of the placenta, which is composed of the tissues normally detached with the placenta and the placental bed, which is what is left in the uterus (Figure 3) [36]. The term “placental bed” should not be confused with the “basal plate of the placenta,” which is the floor of the intervillous space. Its surface is visible with the naked eye on the maternal surface of the placenta, and it is composed of the part of the maternal-fetal junction adhering to the delivered placenta [7].

#### **6. Methodologies to obtain placental bed biopsy specimens:**

A comprehensive and accurate study of the placental bed requires access to the post-partum uterus, knowledge of the site of placental implantation, and systematic sectioning of the specimen. Cesarean section hysterectomy specimens were used several decades ago to gain understanding about the microscopic morphology of the placental bed. However, these operations, once performed as a method of sterilization, are no longer in use, as the morbidity associated with the procedure is higher than that of other sterilization techniques (e.g., tubal ligation). Thus, uteri for these types of studies become available generally in the instance of post-partum hemorrhage refractory to medical and surgical maneuvers (e.g.,

uterine artery ligation), or when hysterectomy is clinically indicated (such as early cervical or ovarian cancer diagnosed during pregnancy or after maternal death). The underlying pathology, which was the indication for hysterectomy, introduces potential biases in these studies. For example, some patients with intractable post-partum hemorrhage to conventional treatment may have placenta accreta, and to consider them representative of a physiologic state may not be appropriate.

Dixon and Robertson introduced in 1958 a new technique for obtaining tissue in order to study the placental bed at the time of cesarean section. They called this technique a “placental bed biopsy” [31]. The authors were clear in understanding that the biopsy specimen had to include not only the decidua but also the myometrium [31], since the pathology observed in preeclampsia consisted of failure of physiologic transformation of the myometrial segment of the spiral arteries. This technique has been used extensively to study the placental bed in patients with complications of pregnancy [37, 87]. Robertson et al. published a review of the experience of placental bed biopsies from three European centers and concluded that the technique was safe, and contributed to the understanding of the biology of this interface [87]. An important limitation of placental bed biopsies is the potential for sampling problems. The number of vessels present in one biopsy is limited and the biopsy may not be representative of the lesions present in the entire uterus. Obtaining a placental bed biopsy can be carried out either at the time of cesarean section or by using a transvaginal method under ultrasound guidance.

#### 6.1. Transabdominal placental bed biopsies:

The transabdominal approach is used at the time of cesarean section. It is of the utmost importance that the placental location be determined by ultrasound examination immediately before the procedure and then recorded. Robertson et al. recommended that the surgeon identifies the true center of the placenta, which may not be underneath the umbilical cord insertion [87]. One approach is to deliver the uterus during the cesarean section after delivery of the fetus, identify the placental center, and place the digit of one of the operators on the serosal surface of the uterus. The placenta is then delivered and the biopsy taken from this place. Robertson et al. recommended taking biopsies of 1.5 cm in diameter [87]. The specimen needs to contain myometrium; this is easily achieved, as only a few millimeters of depth (5mm) would include this tissue [87]. When the placenta is implanted in the fundus, biopsies are difficult to obtain. In this case, after uterine contraction, reaching the proper site for the biopsy may not be possible

The instruments used for the biopsy could be a curved scissors and a scalpel. It is recommended that non-toothed forceps be employed in order to avoid crushing the specimen [87]. Another alternative is to use dermatologic punch biopsies, as they provide an easy method to standardize the procedure. Robertson et al. indicated that an experienced individual using this technique can sample the true placental bed in 70% of cases [87]. Hemostasis may sometimes be required, and this can be accomplished by placing absorbable sutures.

## 6.2. Transvaginal approach:

The first transvaginal approach, reported in 1981, consisted of introducing forceps through the cervix after delivery of the placenta to obtain placental bed biopsies [37]. This procedure was subsequently modified by Michel et al., who performed ultrasound examinations to determine the location of the placenta, The authors used cervical biopsy forceps under ultrasound guidance to sample the placental bed in women who were diagnosed with a blighted ovum (or anembryonic pregnancies) and underwent first trimester termination of pregnancy [72]. Khong et al. were successful in retrieving placental bed biopsies using curettage under ultrasound guidance in patients with spontaneous abortions [52]. Robson et al. modified this technique and reported that placental bed biopsies (containing a spiral artery in 50% of cases) could be obtained using a transvaginal approach. The forceps they employed were 310 mm long, with a 5mm-wide moveable jaw with a cutting adage. The goal was to take four biopsy specimens from each case. This procedure was attempted in first trimester terminations of pregnancy (n=201), missed abortions (n=104), cesarean sections at term (n=95), and after a vaginal delivery (n=13) [88]. Overall, in 67% (281/417) of the cases, the placental bed was sampled, and in 55% at least one spiral artery was present. The authors did not encounter any significant complications attributable to the procedure [88].

## 7. Immunohistochemical evaluation of a placental bed biopsy:

The four key questions when examining a biopsy are: 1) Does the specimen come from the placental bed? 2) Does it contain more than one myometrial spiral artery? 3) Is there a physiologic transformation of the myometrial segment of the spiral arteries? and 4) Is there atherosclerosis? Placental bed biopsies are generally fixed in formaline and then stained with cytokeratin 7 and PAS to answer these questions.

### 7.1. Does the specimen come from the placental bed?

Evidence that the biopsy derives from the placental bed (rather than the non-placental bed or decidua parietalis) requires identification of interstitial trophoblast. Interstitial trophoblast may be difficult to distinguish from maternal cells in the decidua and basal plate [83]. Immunohistochemistry is therefore used to detect trophoblast. The most widely used antibody to accomplish this procedure is anti-human cytokeratin 7. This type of antibody recognizes epithelial cells (trophoblast has epithelial characteristics). In placental bed biopsy material, this antibody would identify both trophoblast cells and glandular epithelium of the endometrium. We have used anti-cytokeratin 7 (DAKO, OV-TL 12/30), which reacts with the 54kDa cytokeratin intermediate filament protein [26]. Cytokeratins are types of “intermediate filament proteins” belonging to a supergene family that includes tubulin polymers and actin microfilaments, and are part of the cytoskeleton and karyoskeleton of eukaryotic cells [40]. The term “intermediate” is used because their diameter (7–22 nm) is between that of actin (5–7 nm) and tubulin (22–25 nm) [40]. The anti-cytokeratin 7-specific antibody OV-TL 12/30 has been reported to be the only one that binds all trophoblast populations (and uterine glands) without any reactivity to mesenchymal stromal cells [11]. Thus, anti-cytokeratin 7 is considered to be the most useful antibody to identify trophoblast



cells [56]. While the choice of marker to purify trophoblast *in vitro* is the subject of debate, there is general acceptance of the value of anti-cytokeratin 7 for histologic studies [11, 56].

## 7.2. Does the specimen contain one myometrial spiral artery?

Spiral arteries in the myometrium have a luminal diameter of approximately 200  $\mu\text{m}$  (Boyd and Hamilton, 1970, cited in Frank et al. [36]). However, as the arteries travel through the decidua to the intervillous space, they widen and can attain a diameter of 500–1000  $\mu\text{m}$  [91, 92]. Close to the ostia or the opening of the artery in the intervillous space, the diameter may reach 2000  $\mu\text{m}$  [36]. Arteries with a lumen of <120  $\mu\text{m}$  within the myometrium generally represent “basal” or straight arteries, and should not be confused with spiral arteries. Several decades ago, some authors proposed that the spiral arteries have a sphincteric mechanism close to the ostia to regulate the entry of blood into the intervillous space (Spanner 1935, Debiasi 1963, cited in Frank et al. [36]). Subsequent investigations [36] have disproved this concept. Biopsy specimens that have segments of a spiral artery within the decidua, but not the myometrium, are of limited value for the diagnosis of failure of physiologic transformation.

## 7.3. Is there physiologic transformation of the spiral arteries?

The characteristics of physiologic transformation include: 1) a dilated myometrial segment with a luminal diameter of greater than 120  $\mu\text{m}$ ; 2) infiltration of the vessel wall by trophoblast; 3) destruction of the media (i.e., smooth muscle and elastic tissue); and 4) fibrinoid deposition in the media, recognized as PAS positive material [16]. Therefore, physiologic transformation of the spiral arteries can be documented by staining the placental bed biopsy specimens with PAS and an anti-cytokeratin antibody (Figure 1).

## 7.4. Identification of fibrinoid:

The term “fibrinoid” has been used to refer to an acellular, homogeneous material, which binds to acid stains such as eosin and PAS. Two types of fibrinoid material have been identified: fibrin-type and matrix-type [49]. These subtypes cannot be distinguished with hematoxylin and eosin stain. However, matrix-type fibrinoid contains trophoblast cells, while fibrin-type does not [49]. With trichrome staining, fibrin-type fibrinoid stains red to orange, whereas the matrix-type has a blue to light-green appearance. Lang has also called attention to the usefulness of a modified paraldehyde fuchsin stain in differentiating two types of fibrinoids [61]. Fibrin-type stains green/orange, while matrix-type stains blue, violet, or green [49]. The type of fibrinoid found in the vessel wall is fibrin-type (Figure 1). Thought to be derived from the deposition of fibrin, the fibrin-type is therefore generated by activation of the coagulation cascade [49]. This observation is based upon immunohistochemistry studies demonstrating that the material stains with antibodies against fibrin, which do not cross-react with fibrinogen [42].

Physiological transformation of the spiral arteries is not an “all or none” phenomenon, as it may vary among spiral arteries in the same patient as well as within the same artery. In some of them, even the decidual segment is not transformed [51, 60], while in others the myometrial segments may be only partially transformed [51]. Thus, a more objective assessment of the degree of physiologic transformation of the spiral arteries may be achieved

by referring to the proportion of the artery that is transformed in the placental bed biopsy specimens [53, 54]. Physiologic transformation is more likely at the center of the placental bed than on the periphery [15, 68, 87]. Therefore, the determination of the true center of the placenta, by ultrasound or during the cesarean section before the biopsy, may reduce sampling bias [87].

## 8. Is there atherosclerosis?

“Acute atherosclerosis” of the spiral arteries was first described in 1945 by Herting in patients with preeclampsia [41]. The typical findings are: 1) fibrinoid necrosis of the vessel wall; 2) accumulation of lipid-laden macrophages in the vessel wall; and 3) a mononuclear perivascular infiltrate [85] (Figure 4). Sexton et al. coined the term “atherosclerosis” [90] and reported that it could be observed in patients with chronic glomerulonephritis with superimposed preeclampsia. However, Zeek and Assali subsequently proposed that the lesion was confined to preeclampsia [104]. Acute atherosclerosis may be seen in the myometrial or decidual segments of the spiral arteries, and it is believed to occur only in vessels that have failed to undergo physiologic transformation (i.e., trophoblast invasion of the arterial wall). The lesion resembles those observed in atherosclerosis [100], and has been reported in other complications of pregnancy (such as growth restriction [50, 93], intrauterine fetal demise [52], diabetes mellitus [6, 57, 58], systemic lupus erythematosus (SLE), discoid lupus erythematosus [2, 59], and antiphospholipid antibody syndrome [64, 76, 97]). The frequency of this lesion in the “great obstetrical syndromes” was recently described by Kim et al [55].

## 9. The role of extravillous trophoblast invasion

### 9.1. Does decidual spiral artery remodeling begin before or after trophoblast invasion?

Several authors have proposed that maternal vessels may undergo changes before interstitial trophoblast invasion [14] (Harris and Ramsey 1966, Boyd and Hamilton 1970, as cited in Craven et al [24]). Craven et al. provided evidence that vasodilatation and some degree of vascular remodeling (expression of VCAM-1 by endothelial cells) could be detected in very early intrauterine pregnancies, in the endometrium of patients with ectopic gestations, as well as in the decidua parietalis of two patients who underwent hysterectomy at 5 and 11 weeks of gestation. The control group consisted of endometrial samples from patients in the secretory phase of the menstrual cycle. The authors suggested that the arterial changes observed in early pregnancy may be maternal responses associated with early endometrial decidualization [24].

Following these initial changes, further structural modifications have been described, such as reduction of the smooth muscle in the vascular media and deposition of fibrinoid material before infiltration of the media by trophoblast [48]. The trophoblast invasion of the arterial wall induces further dilation of the spiral arteries [48].

### 9.2. Invasion of extravillous trophoblast:

According to Kaufmann and Huppertz, the invasion of the extravillous trophoblast requires: 1) a source of proliferating cells; 2) expression of extracellular matrix receptors, which will facilitate adhesion of invasive cells to the extracellular matrix of the uterus; 3) the presence



of invasive cells, which produce extracellular matrix; 4) matrix degrading enzymes to form channels in the uterine matrix for migration; 5) cellular motion; and 6) arrest of invasion.

**9.2.1. Proliferation:** The stem cell for extravillous trophoblast invasion is considered to be attached to the basement membrane of anchoring villi. These cells stain positive for proliferation markers such as H thymidine, Ki67 (MIB-1), and antibodies against proliferating cell nuclear antigens (PCNA) and, thus, have a proliferative phenotype [9, 18] (Muhlhauser, 1993, Konsake, 1994, in Frank et al [36]). Upon departure from this location, cells abandon the cell cycle and acquire an invasive or migratory phenotype [36]. However, in pathologic conditions such as choriocarcinoma [103], ectopic pregnancy (Ketschanska, 1998, Kemp 1999 in Frank et al. [36]), and placenta accreta/percreta [36], invasive trophoblast retains its proliferative capacity. While this is easy to understand in the case of a neoplasia, the dissociation in ectopic pregnancy and placenta accreta suggests that factors intrinsic to the decidua play a role in altering the proliferative/migratory switch [36].

**9.2.2. Integrin switch:** Cells interact with extracellular matrix components through a set of transmembrane proteins linking the cytoskeleton of the cell to the extracellular matrix components such as fibronectin, laminin, and collagen [4]. Integrins are a family of proteins that bind to their ligands with lower affinity ( $K_a$   $10^6$ - $10^9$  liter/mol) and are present in higher concentrations (10- to 100-fold) than receptors for hormones and other soluble signaling molecules [4]. These properties allow a cell to explore its environment without becoming irreversibly attached to it [4]. Integrins are important as they are the main molecules allowing cells to bind and respond to extracellular matrix. Integrins are formed by two non-covalently associated transmembrane glycoprotein subunits called alpha and beta. The matrix binding domain projects more than 20nm from the plasma membrane [4]. The intracellular end of the dimmers binds to the actin cytoskeleton (via proteins such as talin and alpha actinin) [12]. The functional importance of integrin bindings in trophoblast migration has been demonstrated through in *vitro* experiments in which blocking antibodies against integrins results in loss of trophoblast cell adhesion to extracellular matrix [45].

The following integrins have been detected in extravillous trophoblast [12]:

1.  $\alpha_3\beta_1$ , which is the receptor for laminin;
2.  $\alpha_6\beta_4$ , which binds to laminin and through it to collagen IV;
3.  $\alpha_5\beta_1$ , a fibronectin receptor;
4.  $\alpha_1\beta_1$ , a receptor for laminin and collagens I and IV;
5.  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ , which are the receptors for vitronectin, as well as fibronectin and osteopontin.

The extravillous trophoblast in normal pregnancy undergoes a gradual switch from the proliferative to the invasive phenotype. This phenotypic change is also associated with a switch in integrin expression [36]. The extravillous trophoblast with proliferative phenotype, located on or close to the basal lamina of the anchoring villi, expresses predominantly  $\alpha_6\beta_4$  and, in part,  $\alpha_3\beta_1$  [36]. The integrin  $\alpha_6\beta_4$  is a laminin receptor that functions to anchor epithelial cells to the basal membrane [19]. This function may be aided by the long

intracytoplasmatic tail of the subunit  $\beta_4$  [19]. Cells that express this integrin type also express proliferative markers, exhibit polar secretion of extracellular matrix or absence of visible matrix secretion, and do not have an invasive behavior [36]. In contrast, invasive trophoblast in the decidua expresses interstitial integrins (such as  $\alpha_5\beta_1$ ,  $\alpha_1\beta_1$ ,  $\alpha_v\beta_3$ , and  $\alpha_v\beta_5$ ), which bind to fibronectin, show apolar secretion of the extracellular matrix, and upregulate certain metalloproteinases (e.g., MMP-2, MMP-11) [36].

Inadequate trophoblast migration and shallow trophoblast invasion of the decidua and spiral arteries, as seen in preeclampsia, have been associated with failure to down-regulate the  $\beta_4$  integrin subunit and upregulate the  $\alpha_1$  integrin [105]. This suggests that changes in the  $\alpha_6\beta_4$  and  $\alpha_1\beta_1$  integrins may be important in the trophoblast migration and invasion during pregnancy [19]. These findings, however, have not been confirmed by other studies [30].

### 9.2.3. The role of matrix degrading enzymes in trophoblast

**invasion:** Trophoblast invasion requires the degradation of extracellular matrix in the decidua and spiral arteries and the proximal third of the myometrium [38]. Proteolytic enzymes involved in the extracellular matrix degradation include serine proteases of the plasmin system [43, 74] and matrix metalloproteinases (MMPs) [43, 98]. Immunohistochemical studies demonstrated that MMP-1 [43, 65], MMP-2 [10, 43], MMP-3 [43, 98], MMP-9 [43, 65], and its inhibitors TIMP-1 [43, 44] and TIMP-2 [43, 44] are present in the extravillous trophoblast.

**9.2.4. Control of invasion:** Huppertz et al. have proposed that trophoblast invasion can be controlled by several mechanisms, including: 1) a change from a proliferative to an invasive phenotype. Unlike neoplastic cells, which proliferate and invade tissue, invasive trophoblast is not proliferative except under special circumstances (e.g., ectopic pregnancy and placenta accreta); 2) programmed cell death or apoptosis; 3) endoreduplication; and 4) syncytial fusion (personal communication). After delivery, trophoblast cells in the uterine wall undergo apoptosis generally within weeks. However, some trophoblast cells have been identified in hysterectomy specimens several years after pregnancy (personal communication). It is uncertain whether some trophoblast cells may persist for months or even years.

## 10. Failure of the physiologic transformation of the spiral arteries

### 10.1. Is interstitial trophoblast invasion “shallow” in preeclampsia?

Kadyrov et al. [46] reported a study in which the depth of trophoblast invasion was examined in hysterectomy specimens from patients with normal pregnancies, early-onset preeclampsia, and anemia. The depth of invasion was determined by identifying the endometrial-myometrial junction by actin staining (smooth muscle). Compared to normal pregnancies, trophoblast invasion was shallower in patients with preeclampsia and deeper in patients with anemia. In contrast, apoptosis was decreased in both anemia and preeclampsia [46]. Redline et al. proposed the diverging view that preeclampsia is associated with an excess in proliferative immature intermediate trophoblast [84].

## 10.2. Failure of physiologic transformation of the spiral arteries and pregnancy complications

Brosens and Robertson, using specimens from placental bed biopsies and cesarean hysterectomies, described that while physiological changes of the spiral arteries extended both to the decidual segment and 1/3 of the myometrial segment in normal pregnancy, the key feature of “failure of physiologic transformation of the spiral arteries” in preeclampsia was the lack of invasion of the trophoblasts into the spiral arteries of the myometrial segment [17] (Figure 5). Subsequent studies using specimens from cesarean hysterectomy from patients with preeclampsia and intrauterine growth restriction demonstrated that physiological transformation of the spiral arteries is not an “all or none” phenomenon and varies among spiral arteries in the same patient. In some spiral arteries from these patients, even the decidual segment was not transformed [51]. More recently, failure of physiologic transformation of the spiral arteries has also been reported in spontaneous abortion [52], placental abruption [32], preterm labor and intact membranes [53], as well as preterm PROM [54].

## 11. The relationship between Doppler of the uterine artery and “failure of physiologic transformation”

Failure of physiologic transformation of the spiral arteries in placental bed biopsies has been associated with high impedance to blood flow in the uterine arteries, as measured by Doppler velocimetry in patients with preeclampsia [1, 66, 79, 99], intrauterine growth restriction [1, 66, 70, 79], preterm labor with intact membranes, and preterm PROM [33].

Pulsed Doppler ultrasound has been utilized since its introduction by Campbell et al. [21] for the non-invasive assessment of impedance to blood flow in the uterine arteries [3, 5, 13, 22, 39, 63, 80, 81]. High impedance to blood flow in the uterine arteries between 20 and 24 weeks, defined as bilateral uterine artery notches and a pulsatility index or resistance index above the 95<sup>th</sup> percentile [3, 13, 39], has been associated with high risks of developing early-onset preeclampsia, delivering an SGA neonate, and perinatal death [3, 39, 63, 80]. Indeed, 80% of patients developing preeclampsia before 34 weeks [3, 39] (as opposed to only 27% of those who develop the disease after 34 weeks [3]) have a high impedance to blood flow in the uterine arteries between 22 and 24 weeks.

### **Bilateral diastolic notches in the uterine arteries represent very high impedance to blood flow.**

Patients with bilateral notches in the Doppler waveform of the uterine arteries at 23–24 weeks are at increased risk for adverse pregnancy outcome (preeclampsia, SGA, and perinatal death) [20]. Studies in which gelfoam has been used to embolize the uterine circulation in pregnant animals showed that the development of uterine artery notches are associated with high impedance to blood flow [77, 78]. Indeed, progressive embolization of the spiral arteries in pregnant animals produces a diastolic notch only when the uterine blood flow has been reduced to approximately one third, and the vascular resistance has been increased three to four times compared to its normal values [77, 78].

Longitudinal studies in uncomplicated pregnancies indicate that diastolic notches in uterine arteries disappear at 24 weeks of gestation [35, 89]. Therefore, persistence of uterine artery notches in the third trimester may indicate that high impedance to flow and the consequent uteroplacental ischemia are chronic in nature. The pathophysiology of the uterine artery diastolic notch is not completely understood. However, two theoretical computer models have been used to explain the physiopathology of uterine artery diastolic notches. Mo et al. proposed that the notch is the result of a wave reflection due to a high placental bed resistance [73]. In contrast, Talbert et al. put forward the idea that the uterine artery diastolic notch is due to increased arterial wall compliance [96]. During labor, there is a high impedance to blood flow in the uterine arteries. However, labor is not generally associated with uterine artery notches [96].

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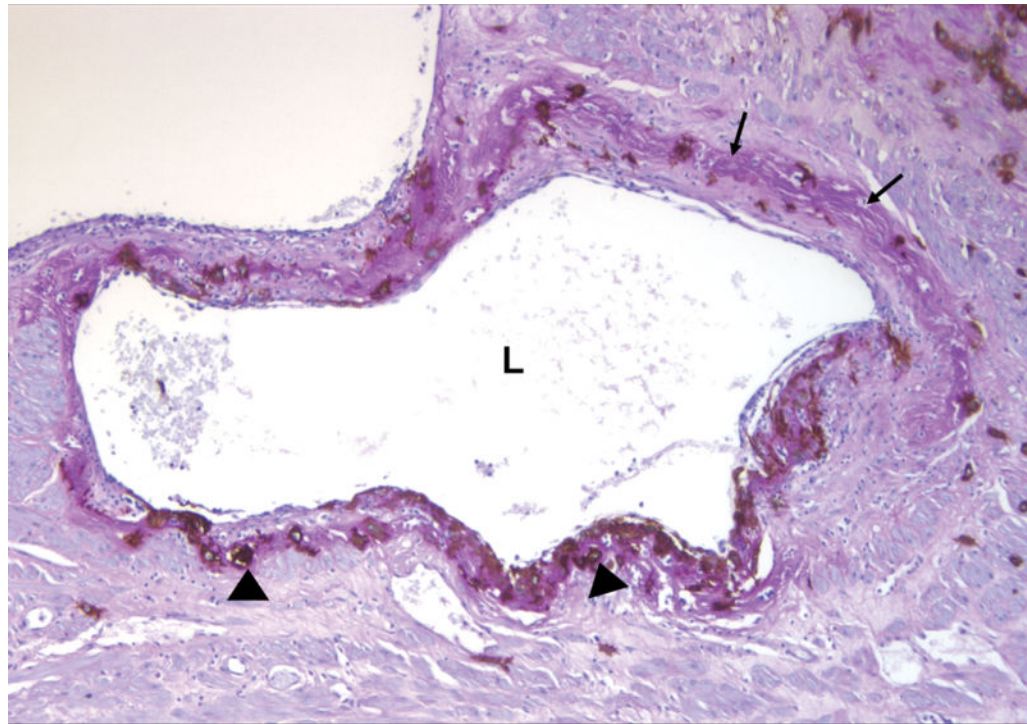
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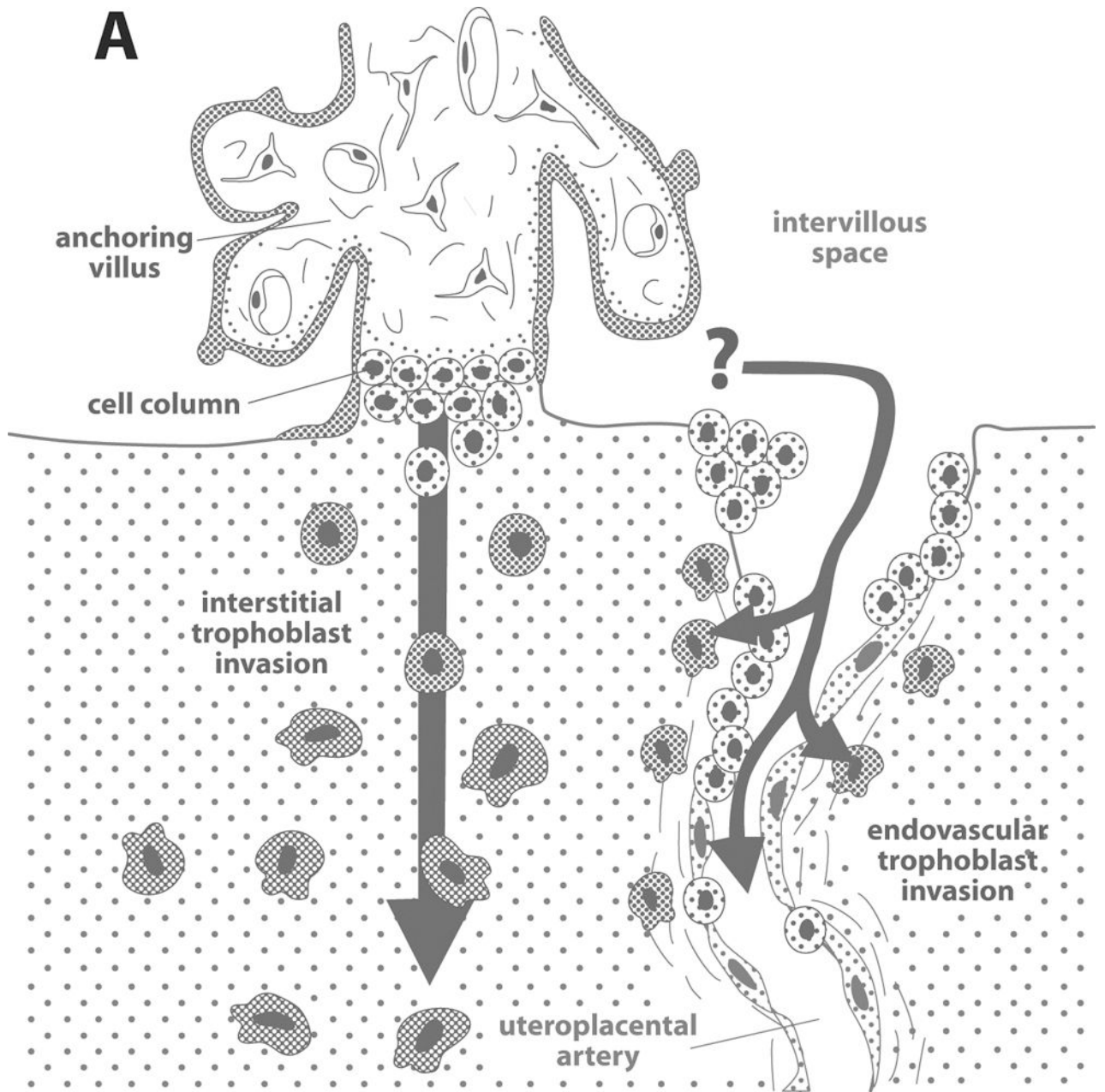
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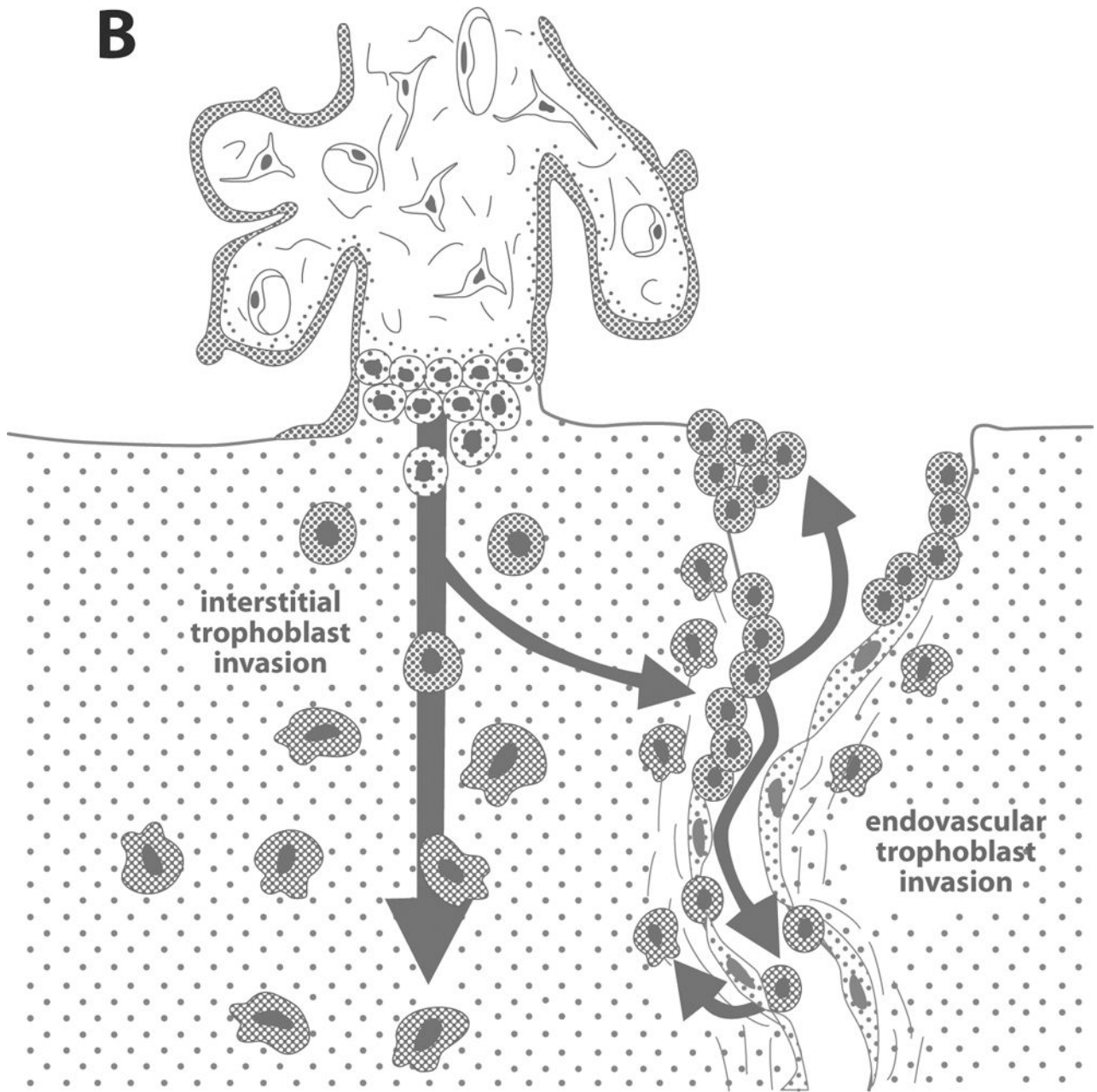


**Figure 1. Microscopic demonstration of physiologic transformation of myometrial segment of the spiral artery in normal pregnancy at term.** Placental bed biopsy specimen was immunostained for cytokeratin-7 to locate trophoblasts (arrowheads) infiltrating the arterial wall, and additional Periodic Acid Schiff (PAS) staining was performed to identify fibrinoid (arrows). Spiral arterial lumen is dilated, and fibrinoid replaces the media and intima. L: spiral arterial lumen (Cytokeratin 7-PAS staining, X100).





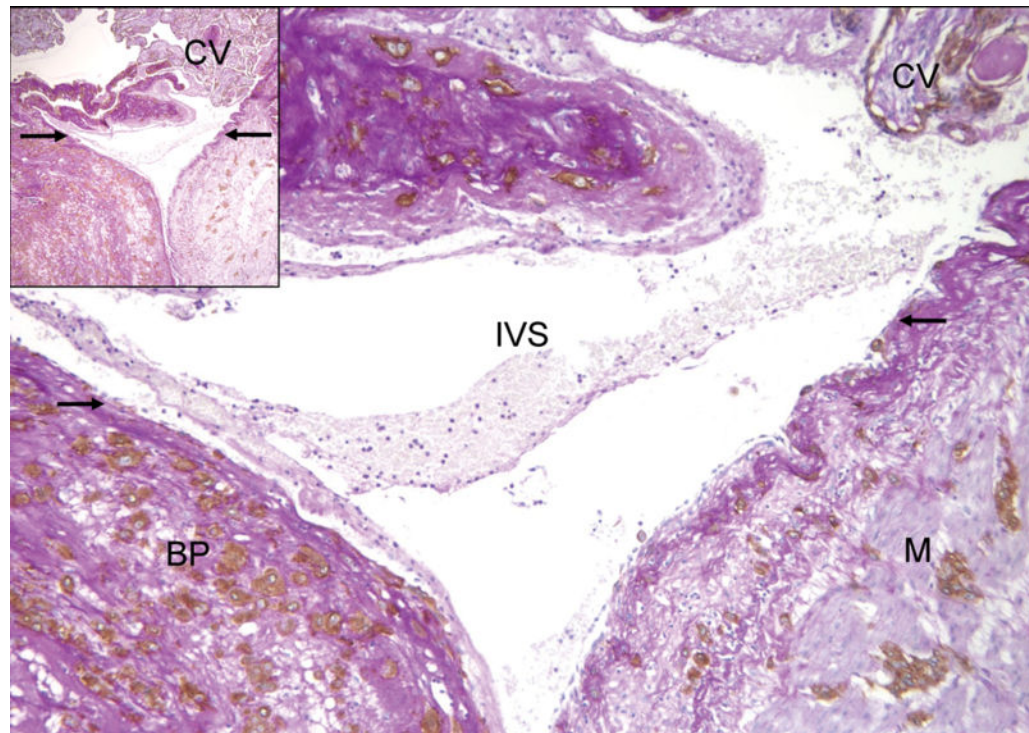
## Endovascular trophoblast invasion by extravasation



## Endovascular trophoblast invasion by intravasation

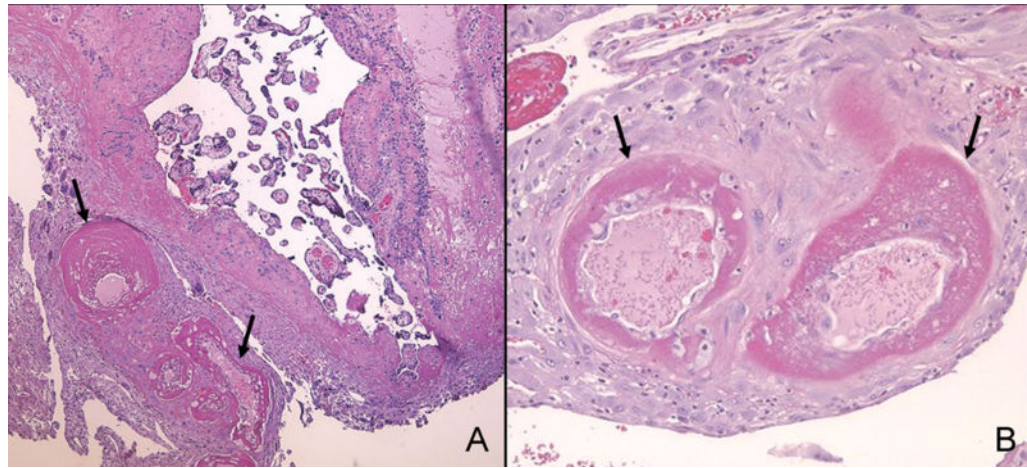
**Figure 2. Intravasation (Figure 2A) and extravasation (Figure 2B) routes trophoblast invasion.** Reproduced with permission from Kaufmann P et al. *Biology of Reproduction* 2003; 69: 1–7.





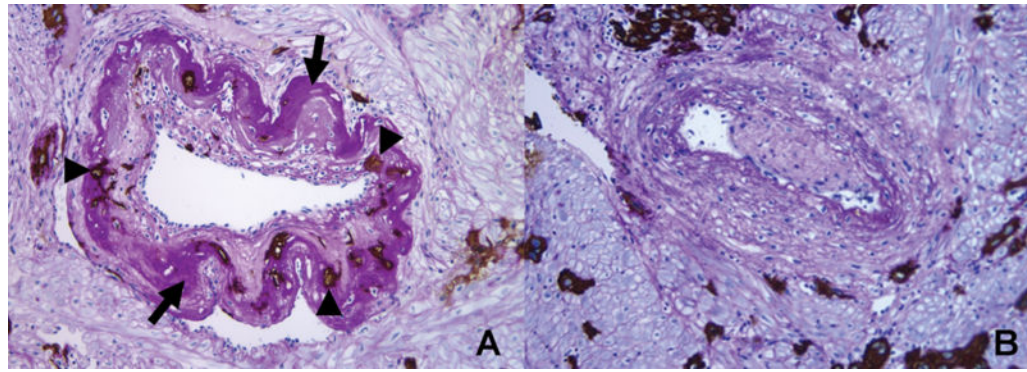
**Figure 3. Microscopic feature of fetomaternal junction (basal plate and placental bed) showing the distal end of the spiral artery (arrows) emptying into the intervillous space (IVS).**

Inset shows a longitudinally running spiral arterial segment at a lower magnification. BP: basal plate, CV: chorionic villi, M: myometrium (Cytokeratin 7-PAS, X40 and X100).



**Figure 4. Atherosclerosis of spiral arteries and decidual arterioles in the basal plate (A) and decidua of the fetal membrane (B), respectively.**

Arrows indicate vessels affected by atherosclerosis. The hallmark of atherosclerosis is fibrinoid necrosis of the wall (arrows) and the presence of lipid-laden macrophages. (H&E, A: X40, B: X100)



**Figure 5. Comparison of transformed and non-transformed spiral arteries in the myometrium.** Contours of the two arteries are in stark contrast. **(A)** A transformed spiral artery is characterized by the presence of intramural trophoblasts (arrowheads) and fibrinoid degeneration (arrows) of the wall. **(B)** Absence of intramural trophoblasts, fibrinoid degeneration, and intact arterial contour mark spiral artery with failure of physiologic transformation (Cytokeratin 7-PAS, X200).