1 **Role of Platelets in Placentation**

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1

2   Abstract

3

4   In human placenta, embryo-derived trophoblasts aggressively invade maternal spiral

5   arteries and transform the arteries to low-resistant large-caliber vessels.   This process that

6   ensures adequate placental perfusion is called maternal vascular remodeling.   Histological

7   examination showed deposition of maternal platelets in the trophoblast aggregates formed in

8   the spiral arteries.   Several lines of evidence suggest that these platelets are activated.

9   Soluble factors released from the activated platelets, as whole, enhanced invasive capacity of

10   isolated trophoblasts in vitro.   These findings suggest importance of non-hemostatic platelet

11   function in maternal vascular remodeling.   In contrast, gene knockout studies suggest that

12   maternal platelet defects are compatible with successful pregnancy in mice.   Moreover,

13   pregnant women with severe platelet defects usually fulfill uneventful pregnancy.   Thus,

14   promotion of endovascular trophoblast infiltration by maternal platelets might not be the only

15   mechanism that regulates maternal vascular remodeling.

16   The maternal vascular remodeling is an essential component of human reproduction and

17   should be secured by several complementary mechanisms.   Future studies should aim to

18   elucidate other mechanisms that could regulate endovascular trophoblast infiltration.

19

1   Introduction

2

3   In human placenta, cytotrophoblasts show two distinct patterns of differentiation (Figure 1).

4   In floating villi, cytotrophoblasts differentiate into syncytiotrophoblast and form the syncytial

5   layer, where exchange of gas and nutrients takes place.   On the other hand, at

6   villus-anchoring sites, cytotrophoblasts differentiate into extravillous trophoblasts and form

7   the stratified structure called cell column.   After losing proliferative activity and acquiring

8   invasive activity in the cell column [1], extravillous trophoblasts begin to invade decidual tissue

9   (interstitial trophoblasts) or maternal blood vessels (endovascular trophoblasts).

10   Endovascular trophoblasts destroy the muscular linings and replace the endothelium of the

11   maternal spiral arteries, transforming them from small resistant vessels to flaccid large-caliber

12   vessels.   This process is called maternal vascular remodeling.   The maternal vascular

13   remodeling ensures adequate placental perfusion and contributes to establishment of

14   successful pregnancy [2].   In fact, endovascular trophoblast invasion is limited to superficial

15   decidua and the myometrial segments remain narrow in cases of preeclampsia and/or

16   intrauterine growth restriction [3].

17   Vascular infiltration by endovascular trophoblast is a unique phenomenon in primate

18   placenta [4].   Moreover, in most of non-human primates, vascular remodeling is restricted to

19   the decidua, which in human has to be considered as pathological.   Lack of proper

20   experimental model animal has hampered analysis of endovascular trophoblast invasion and

21   the mechanism is still largely unknown.

22

1   Platelet in human placentation

2

3   What directs extravillous trophoblasts towards the maternal spiral arteries?   It is intuitive

4   to consider that some factor(s) derived from the endothelium or blood constituents direct this

5   movement.   Relatively high oxygen tension in maternal arteries promotes trophoblast

6   differentiation toward invasive phenotype, which could be one of the mechanisms that

7   facilitate endovascular trophoblast invasion [5].   This hypothesis is derived from the findings

8   that isolated cytotrophoblasts cultured under hypoxic conditions (2% O_2) continued

9   proliferating, whereas those cultured in 20% O_2 stopped proliferating and differentiated to

10   invasive phenotype.   Another candidate that facilitates endovascular trophoblast invasion

11   could be maternal platelets [6].   Histological examination of human placental bed revealed that

12   maternal platelets are trapped by endovascular trophoblast aggregates that are formed inside

13   the lumen of the spiral arteries.   These platelets were attached to collagen deposited around

14   endovascular trophoblasts, suggesting that these platelets are activated.   Indeed, these

15   platelets expressed P-selectin.   P-selectin is one of activation markers of platelets that binds

16   to P-selectin glycoprotein ligand-1 (PSGL-1) on the surface of neutrophils, leading to

17   recruitment and activation of the neutrophils.   The activated neutrophils, in turn, release

18   platelet-activating factor (PAF) that promotes platelet aggregation [7].   In vitro, co-culturing

19   with platelets that were activated by collagen induced matrigel invasion of extravillous

20   trophoblasts isolated from early human placental tissue.   After 48-hour culture, most of the

21   isolated extravillous trophoblasts exhibit elongated spindle-shaped morphology mimicking

22   interstitial trophoblasts, whereas they are transformed to round-shaped morphology

23   mimicking endovascular trophoblasts after 48-hour co-culture with platelets (Figure 2).   The

24   round-shaped cells share similar molecular property with endovascular trophoblasts in vivo,

1   such as up-regulation of integrin α1 and CCR1 [8] [9].   These changes do not require direct

2   contact of platelets and isolated extravillous trophoblasts, suggesting that some soluble

3   factor(s) derived from the activated maternal platelets direct trophoblast invasion towards

4   maternal arteries and induce differentiation towards endovascular phenotype.

5   Then, what are the platelet-derived soluble factors that enhance trophoblast invasion?

6   Besides regulating hemostasis, platelets contain a number of bioactive peptides, including

7   growth factors (EGF, VEGF, PDGF-B, TGF-β1, IGF-1, HGF, bFGF), cytokines (soluble

8   CD40 ligand), chemokines (RANTES, βTG, PF4), and bioactive phospholipids (S1P).

9   These mediators are stored in platelet granules and released upon stimulation [10].   The effect

10   of platelet-conditioned medium to promote invasion of the isolated extravillous trophoblasts

11   was completely abrogated by heat treatment, but not by charcoal stripping [9].   This suggests

12   that some growth factors, cytokines, or chemokines, but not bioactive phospholipids

13   contribute to the invasion-promoting effect of platelets.   Immunocytochemistry revealed that

14   isolated extravillous trophoblasts express a chemokine receptor, CCR1.   Matrigel invasion

15   assay revealed that RANTES, one of CCR1 ligands, enhances invasion of isolated

16   extravillous trophoblasts, indicating that CCR1 expressed on isolated extravillous trophoblasts

17   is a functional receptor.   Interestingly, immunohistochemisry of the placental bed showed

18   that CCR1 is predominantly expressed from the cell column through endovascular

19   trophoblasts.   These findings suggest that circulating maternal platelets are trapped by

20   endovascular trophoblast aggregates and activated to release CCR1 ligands, which in turn

21   attracts CCR1-positive extravillous trophoblasts into maternal spiral arteries to encourage the

22   vascular remodeling (Figure 3).

23   In this theory, activation of platelets in the spiral arteries requires the preexistence of

24   endovascular trophoblast aggregates.   In this respect, platelet is not a primary initiator of

1   extravillous trophoblast invasion into the spiral arteries; rather it might provide a positive

2   feedback mechanism that facilitates endovascular trophoblast infiltration.

3

1   Platelet in mouse placentation

2

3   Gene knockout has generated several mice lineages that have quantitative or qualitative

4   platelet defect.   The transcription factor NF-E2 is essential for megakaryopoiesis and

5   NF-E2-deficient mice have severe quantitative platelet defect [11] [12].   NF-E2-null murine

6   embryos are able to complete intrauterine life, although they show significant growth

7   restriction at birth.   The growth restriction is associated with abnormal vascularization of the

8   labyrinthine layer in NF-E2-null placenta.   Since the number of blood vessels in the

9   labyrinthine layer is not reduced, abnormal vascularization is considered to be due to failure

10   in the maturation of preexisting vessels.   These indicate that embryonic platelets are required

11   for normal placentation [13].

12   In mice lacking α-subunit of the heterotrimeric guanine nucleotide binding protein Gq

13   (Gαq), platelet count is normal but these platelets cannot be activated in vitro with

14   physiological agonists including thrombin, ADP, and collagen [14].   In contrast to NF-E2 null

15   mice, vascularization of Gαq-null placenta is not affected, suggesting that hemostatic platelet

16   function is not required for normal placentation.   In other words, non-hemostatic function of

17   platelets, which is only revealed in mice with quantitative platelet defect, is essential for the

18   maturation of the fetus-derived vessels in the placenta [13].

19   Both quantitatively and qualitatively platelet-deficient mice are able to complete

20   intrauterine development and some of them even survive to reproductive age.   These adult

21   female mice allow analysis of possible effects of maternal platelet defect on placentation.   In

22   contrast to NF-E2 null embryos, the litter size of NF-E2 null mothers is not reduced as long as

23   embryo has normal platelet count.   Detailed analysis of the placental histology showed that

24   massive placental hemorrhage occurs in approximately half of the placentas of NF-E2 null

1   mothers most probably due to maternal bleeding diathesis [15].   Thus, maternal platelet is not

2   required for normal murine placentation.

3

4

1   Platelet and human pathological pregnancies

2

3   Primary hemostasis begins immediately after endothelial disruption.   The exposed

4   subendothelial collagen recruits circulating von-Willebrand factor, which binds to platelet

5   surface glycoprotein Ib-IX-V complex and thus mediates the contact between collagen and

6   platelets.   Collagen-activated platelets form pseudopods that stretch out to cover the injured

7   surface and express receptors for fibrinogen that mediates primary platelet aggregation.

8   Next, bioactive substances such as thromboxane A2 are released from the activated platelets

9   (platelet degranulation) and further activate surrounding platelets.   These activated platelets

10   express glycoprotein IIb-IIIa complex that can bind to von-Willebrand factor.   Secondary

11   platelet aggregation is mediated by interaction between von-Willebrand factor and

12   glycoprotein IIb-IIIa complex, leading to primary thrombus formation.   In the secondary

13   hemostasis, coagulation cascade efficiently progresses on the phospholipid exposed on the

14   surface of the aggregated platelets to solidify the primary thrombus (secondary thrombus

15   formation).

16   Low-dose aspirin has been used worldwide to prevent placenta-mediated obstetric

17   complications such as recurrent pregnancy loss, intrauterine growth restriction, and

18   preeclampsia [16].   Aspirin inhibits platelet cyclooxygenase to suppress synthesis of

19   thromboxane A2 that induces secondary platelet aggregation and systemic vasoconstriction.

20   Thus, theoretically, aspirin can inhibit thrombus formation in the placenta to help maintain

21   placental blood flow as well as ameliorate hypertension in preeclampsia.   In fact, recent

22   meta-analysis established moderate but consistent preventative effect of low-dose aspirin

23   therapy on preeclampsia [17].   Since aspirin does not interfere with platelet adhesion to

24   collagen or subsequent platelet degranulation, platelet release of bioactive soluble factors

1   other than thromboxane A2 should be maintained during low-dose aspirin therapy.

2   Therefore, in terms of maternal vascular remodeling, aspirin does no harm to endovascular

3   trophoblast invasion enhanced by platelet-derived soluble factors.

4   Secondary thrombus formation generally ensues after platelet activation. Given that

5   maternal platelets trapped by the endovascular trophoblasts are activated, it seems peculiar

6   that no fibrin deposition is detected among the endovascular trophoblast aggregates.

7   Although the actual mechanism for this discrepancy remains to be elucidated, abundant

8   thrombomodulin [18] and tissue- and urokinase-type plasminogen activators [19][20][21] expressed by

9   trophoblasts could inhibit the coagulation cascade and/or facilitate rapid degradation of the

10   fibrin at the surface of the endovascular trophoblasts. In pathological pregnancies such as

11   preeclampsia, it is fascinating to consider that inhibitory mechanism against the secondary

12   thrombus formation around the endovascular trophoblasts is defective, thus leading to

13   excessive placental thrombosis. In this situation, aspirin could be particularly beneficial,

14   because it can inhibit platelet secondary aggregation that triggers the initiation of the

15   coagulation cascade without affecting endovascular trophoblast invasion.

16   Bernard-Soulier syndrome is characterized by deficiency of platelet glycoprotein Ib-IX-V

17   complex that mediates contact of platelets with collagen [22]. In this pathological condition,

18   collagen-activation of platelets is severely impaired and most of the patients exhibit

19   spontaneous bruising, epistaxis, and bleeding after minor trauma. In pregnancy, entrapment

20   of maternal platelets by collagen-deposited endovascular trophoblasts and the subsequent

21   activation should be restricted, resulting in insufficient maternal vascular remodeling.

22   However, pregnant women with Bernard-Soulier syndrome as well as those with congenital

23   thrombocytopenia generally complete uneventful pregnancy despite an increased risk of

24   postpartum hemorrhage [23-25]. This suggests that maternal platelets are not an essential

1   component of human placentation process including maternal vascular remodeling.

2   Although in vitro data suggest importance of maternal platelets in endovascular trophoblast

3   infiltration, their actual role in vivo still needs to be defined.

4

1   Conclusion and future direction

2

3   In human placenta, maternal platelets are deposited in trophoblast clusters that have

4   invaded the uterine spiral arteries.   These platelets are likely to be activated and release

5   various soluble factors, which as whole enhance invasive capacity of extravillous trophoblasts

6   in vitro.   Thus, maternal platelets might be a candidate that attracts extravillous trophoblasts

7   into the spiral arteries and encourages maternal vascular remodeling.   As demonstrated in

8   gene-knockout mice with quantitative or qualitative platelet defects, however, maternal

9   platelets are not required for murine placentation.   The fact that pregnant women with severe

10   platelet defects are compatible with uneventful pregnancy suggests that maternal platelets are

11   not an essential component of human placentation.

12   The maternal vascular remodeling that ensures adequate placental perfusion is an essential

13   component of human reproduction and should be secured by several complementary

14   mechanisms.   In this respect, promotion of endovascular trophoblast infiltration by maternal

15   platelets could be one of the mechanisms that regulate the vascular remodeling, although the

16   vascular remodeling might occur in the absence of maternal platelets.

17   Precise characterization of endovascular trophoblasts, which is a crucial step for

18   clarification of the mechanism(s) of maternal vascular remodeling, has been hampered by the

19   extreme difficulty in their isolation from human placenta.   As mentioned above, co-culture

20   with platelets could induce differentiation of isolated extravillous trophoblasts towards

21   endovascular phenotype in vitro.   These endovascular-like trophoblasts are potentially useful

22   to characterize endovascular trophoblasts, leading to identification of novel mechanisms of

23   maternal vascular remodeling.

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1   References

2

3   [1]   Damsky CH, Fitzgerald ML, Fisher SJ. Distribution patterns of extracellular matrix

4   components and adhesion receptors are intricately modulated during first trimester

5   cytotrophoblast differentiation along the invasive pathway, in vivo. *J Clin Invest*. 1992; **89**(1):

6   210-22.

7   [2]   Ramsey EM. The story of the spiral arteries. *J Reprod Med*. 1981; **26**(8): 393-9.

8   [3]   Khong TY, De WF, Robertson WB, Brosens I. Inadequate maternal vascular

9   response to placentation in pregnancies complicated by pre-eclampsia and by

10   small-for-gestational age infants. *Br J Obstet Gynaecol*. 1986; **93**(10): 1049-59.

11   [4]   Frank HG, Kaufmann P. Nonvillous parts and trophoblast invasion. In: Benirschke

12   K, Kaufmann P, (eds) *Pathology of Human Placenta*. New York: Springer 2000; 171-272.

13   [5]   Genbacev O, Zhou Y, Ludlow JW, Fisher SJ. Regulation of human placental

14   development by oxygen tension. *Science*. 1997; **277**(5332): 1669-72.

15   [6]   Sato Y, Fujiwara H, Zeng BX, Higuchi T, Yoshioka S, Fujii S. Platelet-derived

16   soluble factors induce human extravillous trophoblast migration and differentiation: platelets

17   are a possible regulator of trophoblast infiltration into maternal spiral arteries. *Blood*. 2005;

18   **106**(2): 428-35.

19   [7]   Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions:

20   linking hemostasis and inflammation. *Blood Rev*. 2007; **21**(2): 99-111.

21   [8]   Zhou Y, Genbacev O, Damsky CH, Fisher SJ. Oxygen regulates human

22   cytotrophoblast differentiation and invasion: implications for endovascular invasion in normal

23   pregnancy and in pre-eclampsia. *J Reprod Immunol*. 1998; **39**(1-2): 197-213.

[9]   Sato Y, Higuchi T, Yoshioka S, Tatsumi K, Fujiwara H, Fujii S. Trophoblasts

acquire a chemokine receptor, CCR1, as they differentiate towards invasive phenotype.

Development. 2003; **130**(22): 5519-32.

[10]   Boehlen F, Clemetson KJ. Platelet chemokines and their receptors: what is their

relevance to platelet storage and transfusion practice? *Transfus Med*. 2001; **11**(6): 403-17.

[11]   Shivdasani RA, Rosenblatt MF, Zucker-Franklin D*, et al.* Transcription factor

NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF

in megakaryocyte development. *Cell*. 1995; **81**(5): 695-704.

[12]   Levin J, Peng JP, Baker GR*, et al.* Pathophysiology of thrombocytopenia and

anemia in mice lacking transcription factor NF-E2. *Blood*. 1999; **94**(9): 3037-47.

[13]   Palumbo JS, Zogg M, Talmage KE, Degen JL, Weiler H, Isermann BH. Role of

fibrinogen- and platelet-mediated hemostasis in mouse embryogenesis and reproduction. *J

Thromb Haemost*. 2004; **2**(8): 1368-79.

[14]   Offermanns S, Toombs CF, Hu YH, Simon MI. Defective platelet activation in G

alpha(q)-deficient mice. *Nature*. 1997; **389**(6647): 183-6.

[15]   Isermann B, Nawroth PP. The role of platelets during reproduction. *Pathophysiol

Haemost Thromb*. 2006; **35**(1-2): 23-7.

[16]   James AH, Brancazio LR, Price T. Aspirin and reproductive outcomes. *Obstet

Gynecol Surv*. 2008; **63**(1): 49-57.

[17]   Askie LM, Duley L, Henderson-Smart DJ, Stewart LA, Group PC. Antiplatelet

agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. *Lancet*.

2007; **369**(9575): 1791-8.

[18]   Isermann B, Sood R, Pawlinski R*, et al.* The thrombomodulin-protein C system is

essential for the maintenance of pregnancy. *Nat Med*. 2003; **9**(3): 331-7.

1   [19]   Strickland S, Richards WG. Invasion of the trophoblasts. *Cell*. 1992; **71**(3): 355-7.

2   [20]   Teesalu T, Blasi F, Talarico D. Expression and function of the urokinase type

3   plasminogen activator during mouse hemochorial placental development. *Dev Dyn*. 1998;

4   **213**(1): 27-38.

5   [21]   Hu ZY, Liu YX, Liu K, *et al.* Expression of tissue type and urokinase type

6   plasminogen activators as well as plasminogen activator inhibitor type-1 and type-2 in human

7   and rhesus monkey placenta. *J Anat*. 1999: 183-95.

8   [22]   Nurden AT, Nurden P. Inherited defects of platelet function. *Rev Clin Exp Hematol*.

9   2001; **5**(4): 314-34; quiz following 431.

10   [23]   Khalil A, Seoud M, Tannous R, Usta I, Shamseddine A. Bernard-Soulier syndrome

11   in pregnancy: case report and review of the literature. *Clin Lab Haematol*. 1998; **20**(2): 125-8.

12   [24]   Turner RJ, Spencer RA, Miyazawa K. Successful cesarean section in a gravida with

13   the thrombocytopenia with absent radius syndrome. A case report. *J Reprod Med*. 1986; **31**(4):

14   260-2.

15   [25]   Peitsidis P, Datta T, Pafilis I, Otomewo O, Tuddenham EG, Kadir RA. Bernard

16   Soulier syndrome in pregnancy: a systematic review. *Haemophilia*. 2010.

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1   Figure legend

2

3   Figure 1   Schematic representation of early human placenta.

4   In floating villus, cytotrophoblasts (light gray cells) differentiate into multinucleated

5   syncytiotrophoblast (dark gray cells) and form the syncytial layer, where exchange of gas and

6   nutrients takes place. At villus-anchoring sites, cytotrophoblasts differentiate into extravillous

7   trophoblasts (spotted cells) and form the stratified structure called cell column.   Extravillous

8   trophoblasts acquire invasive activity in the cell column and begin to invade the decidual

9   tissue (interstitial trophoblasts) or the spiral arteries (endovascular trophoblasts).

10

11   Figure 2   Platelet-derived soluble factors induce endovascular differentiation of isolated

12   human extravillous trophoblasts.

13   Isolated human extravillous trophoblats were cultured for 48 hours in the absence (A,

14   control) or in the presence of human platelets (B, platelet co-culture) that were plated in the

15   collagen type I-coated upper chamber.   Note that most of extravillous trophoblasts exhibit

16   round-shaped morphology (arrows) mimicking endovascular trophoblasts after platelet

17   co-culture (B).

18

19   Figure 3   An illustration showing possible chemokine gradient produced by platelets in

20   spiral arteries and its estimated effects on endovascular trophoblast infiltration.

21   In the spiral artery undergoing vascular remodeling, platelets are deposited among the

22   endovascular trophoblasts. These platelets are likely to have been activated by extracellular

23   matrix secreted from endovascular trophoblasts and to have released various soluble factors

24   including CCR1 ligands, forming local chemokine gradient.   The chemokine gradient directs

1   CCR1-positive extavillous trophoblasts into the spiral artery, providing a positive feedback

2   cascade for trophoblastic arterial infiltration.   Note that CCR1 is expressed from the cell

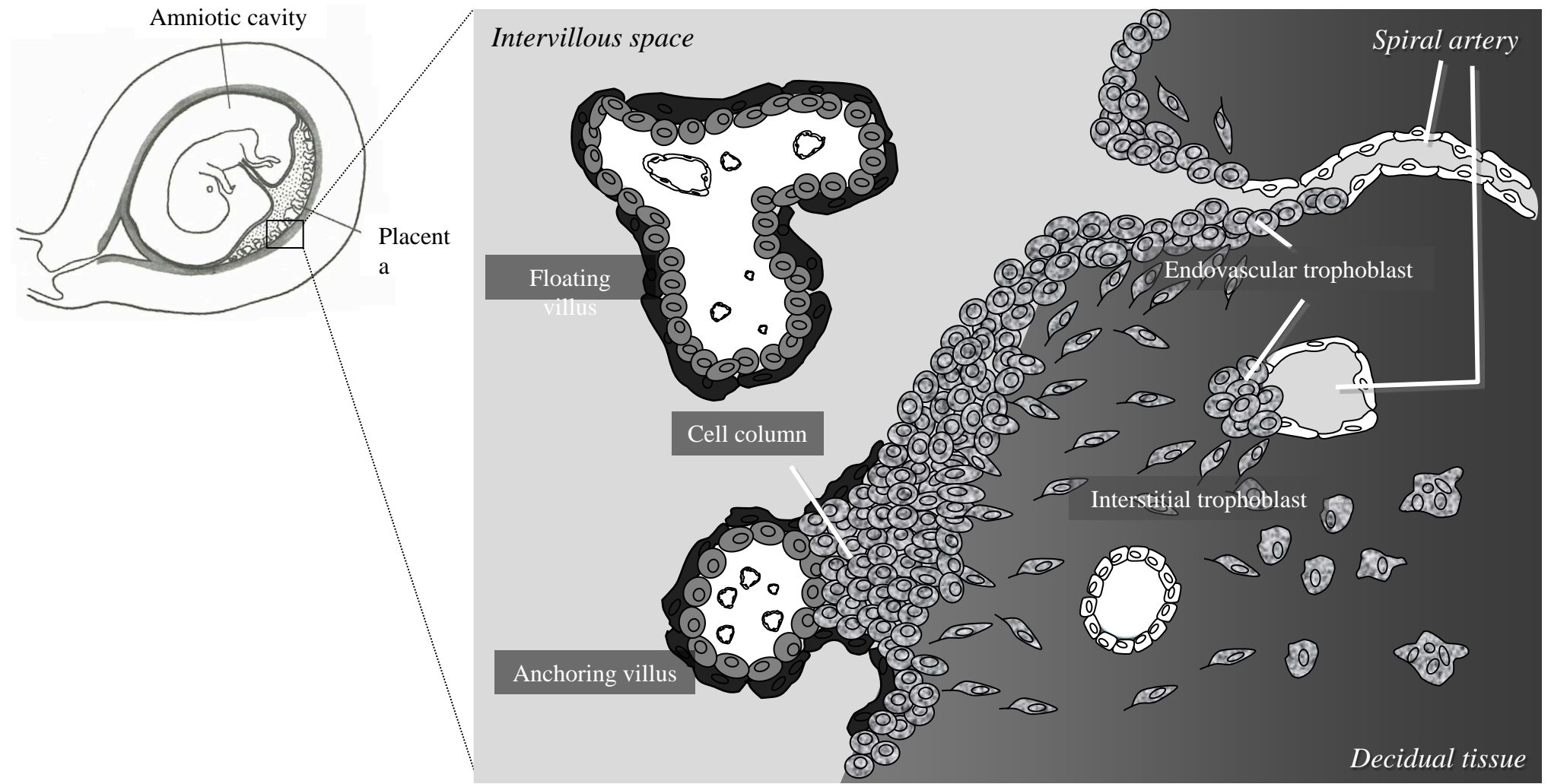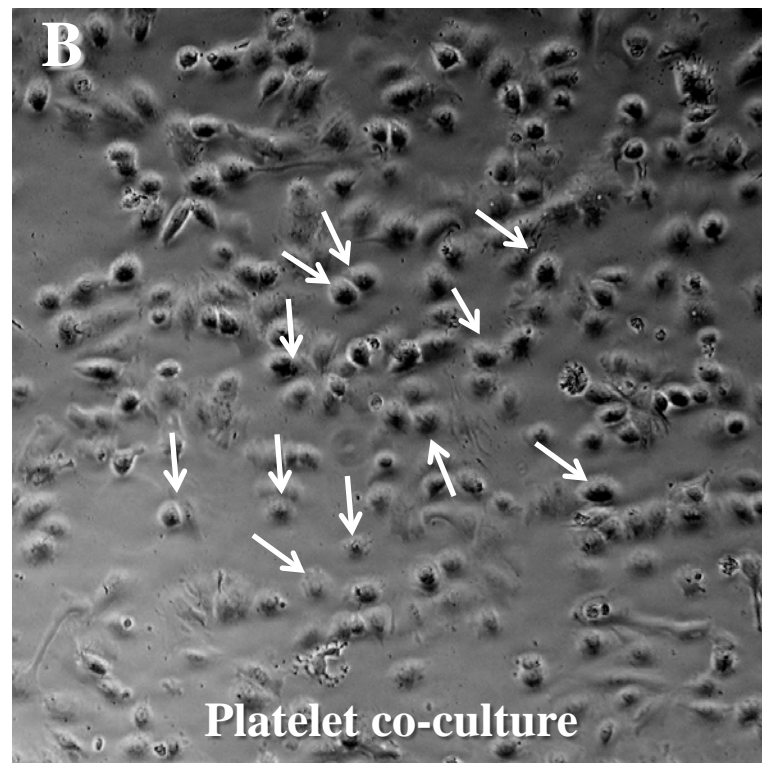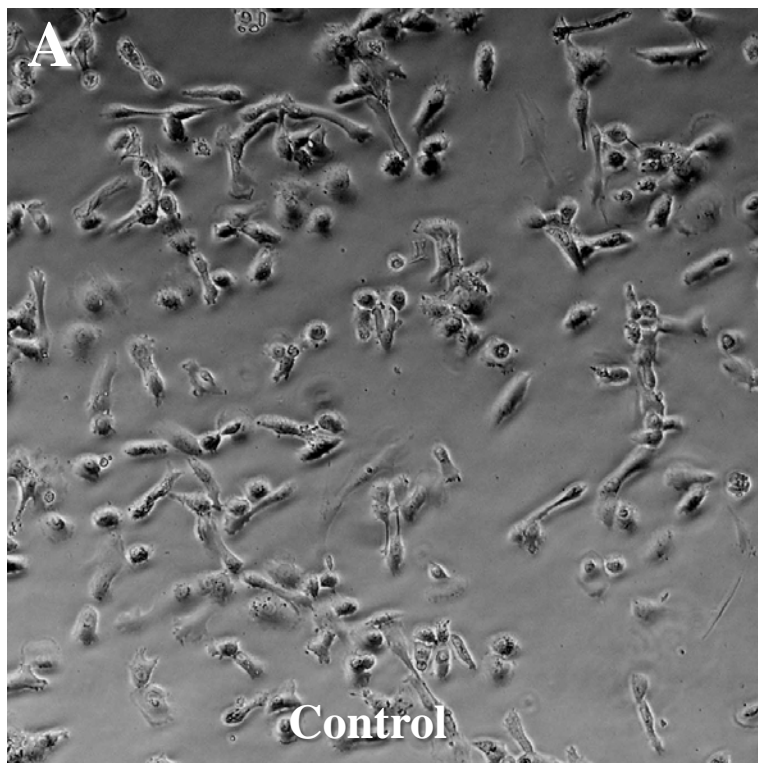3   column through endovascular trophoblasts.

Figure 1

Amniotic cavity

Placenta

Intervillous space

Spiral artery

Floating villus

Endovascular trophoblast

Cell column

Interstitial trophoblast

Anchoring villus

Decidual tissue

Figure 2

Figure 3

Spiral artery under vascular remodeling

CCR1-positive trophoblasts

Chemokine gradient

Endothelial cell

Platelet