

Congenital HCMV Infection, Impaired Placental Development & Rescue with Neutralizing Monoclonal Antibodies

L Pereira, T Tabata, M Petitt, M Zydek,
J Fang-Hoover & LM Kauvar*

Cell & Tissue Biology, U California San Francisco,
*Trellis Biosciences

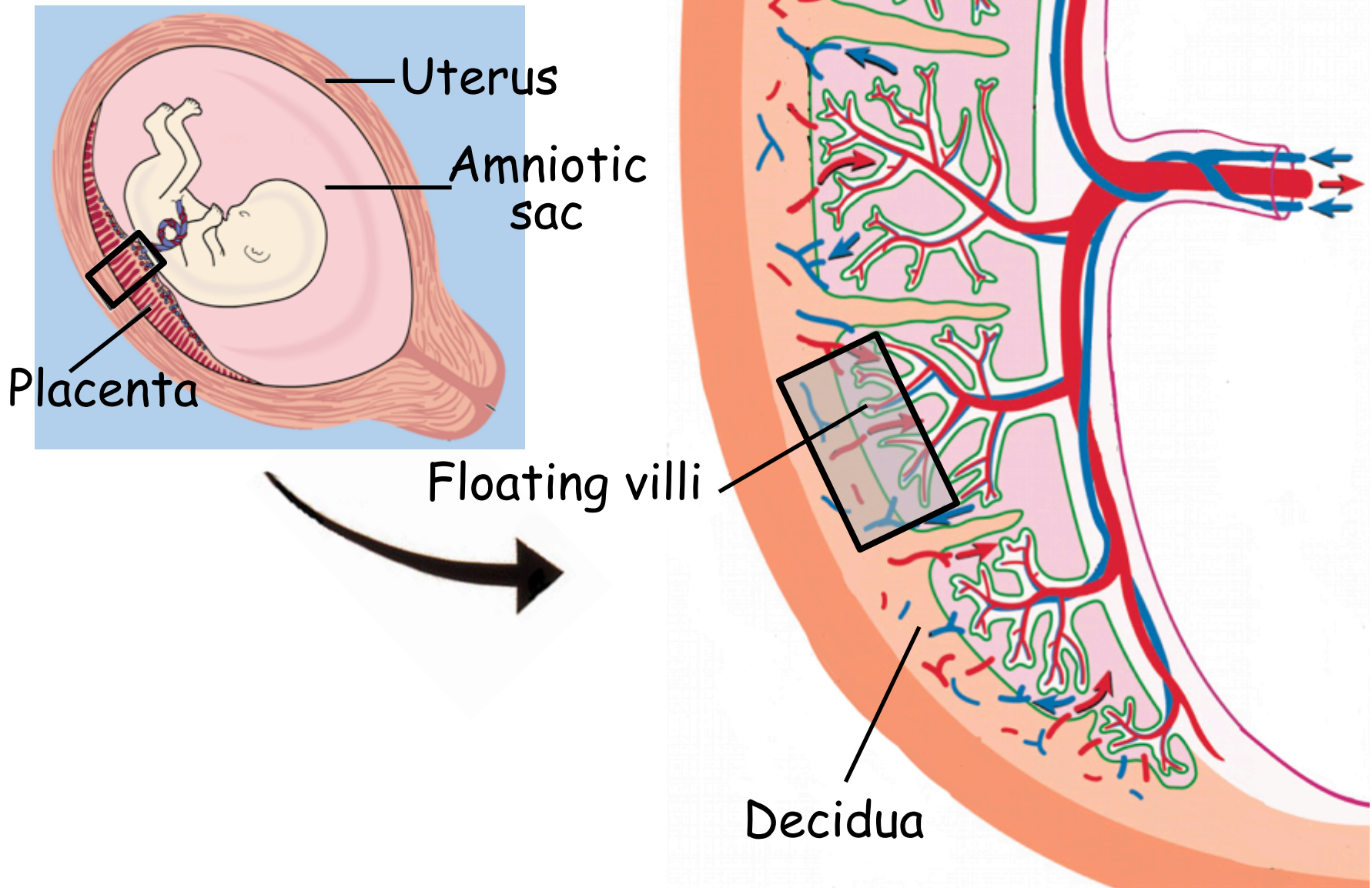
CMV Public Health & Policy, Salt Lake City, Utah Sept 26-27, 2014

Topics

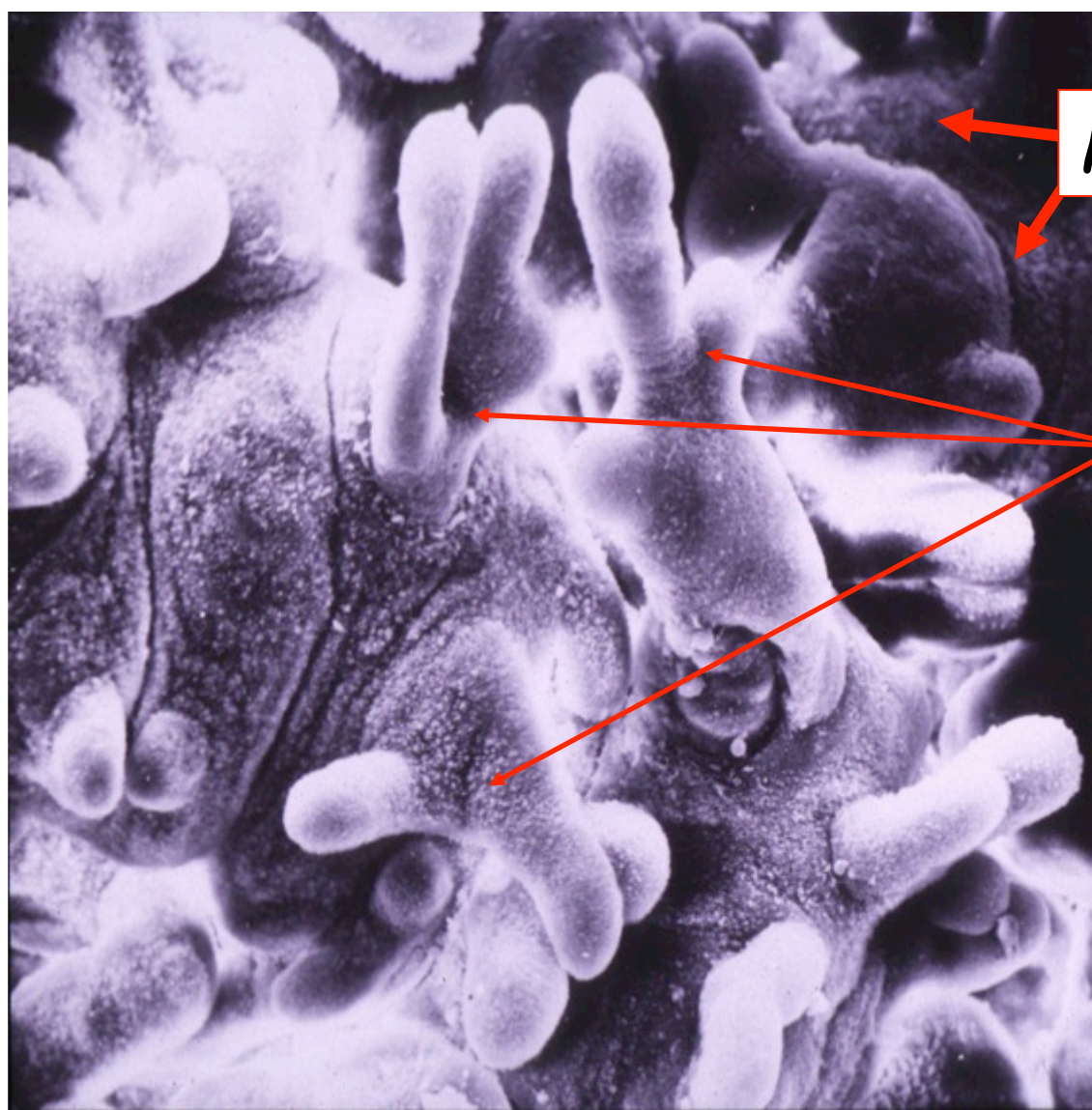
- Sites of HCMV infection in the placenta.
- Role of trophoblast progenitor cells.
- Antibodies reduce infection, rescue development.
- Viral replication in the fetal compartment.

Placenta infection: The Early Steps

Uterine-placental interface



Human placenta floating villi

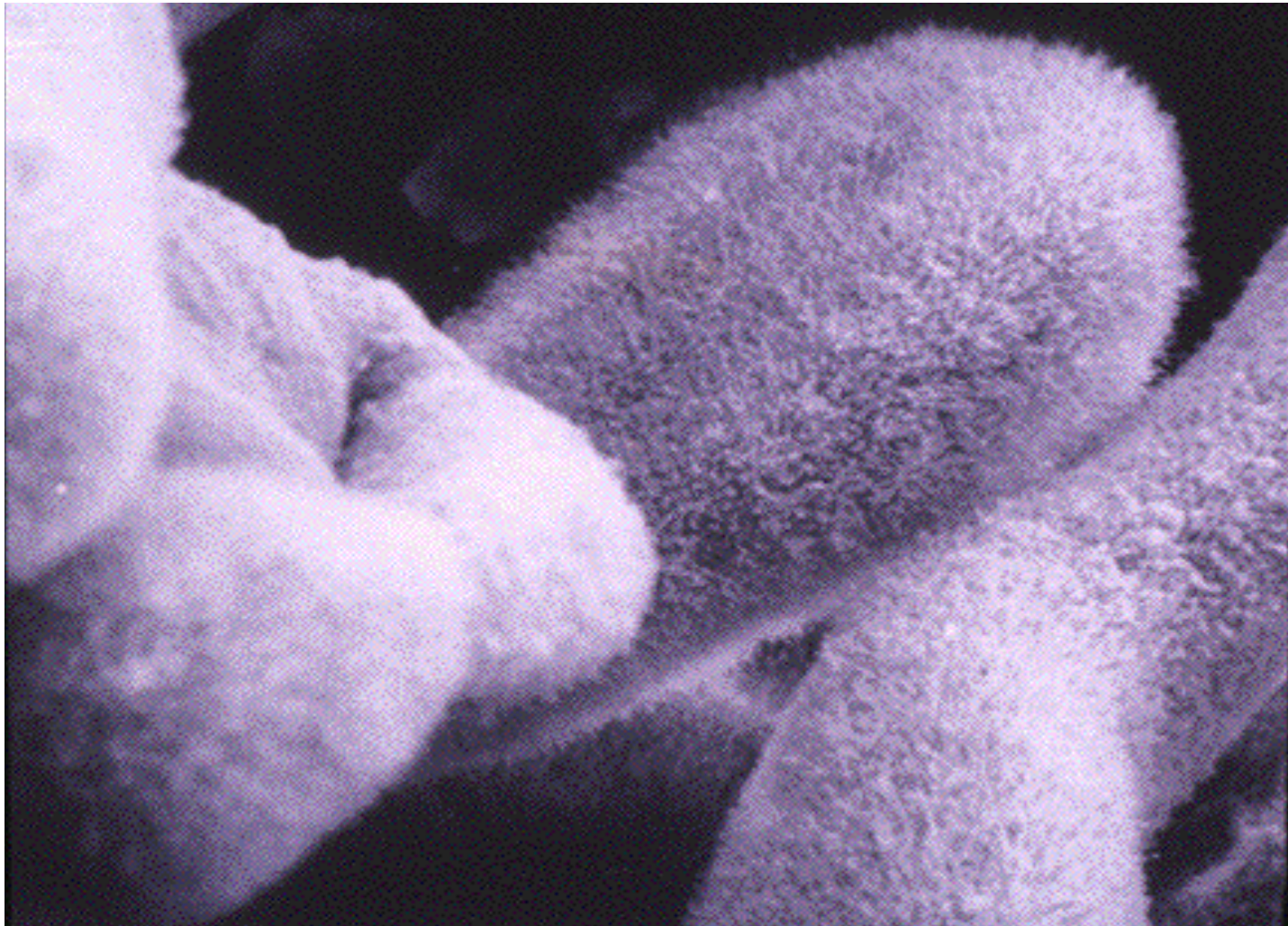


Maternal blood space

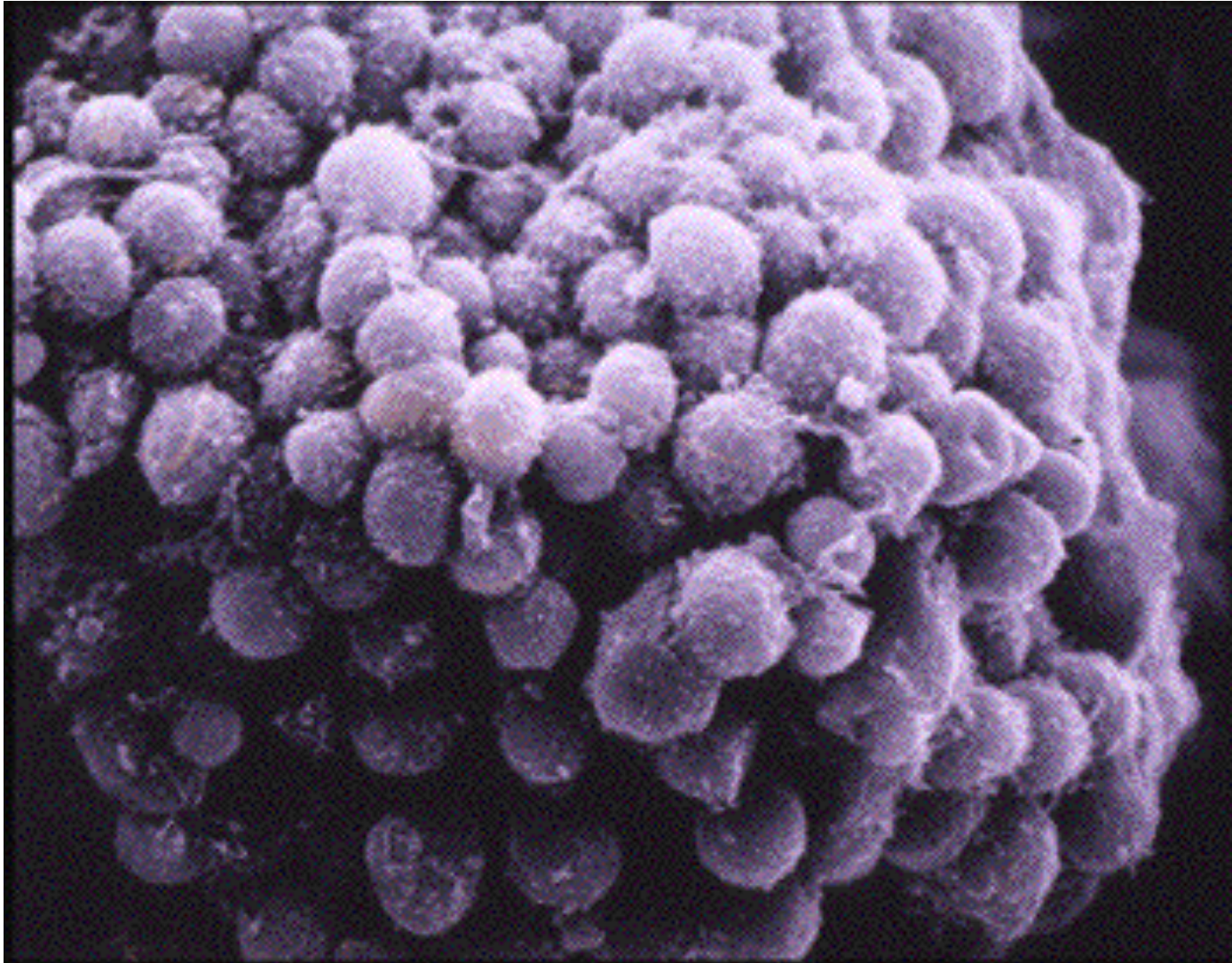
Branching increases transport of oxygen, nutrients & IgG for passive immunity

Susan Fisher

Floating villi have branched microvilli
increase surface perfusion



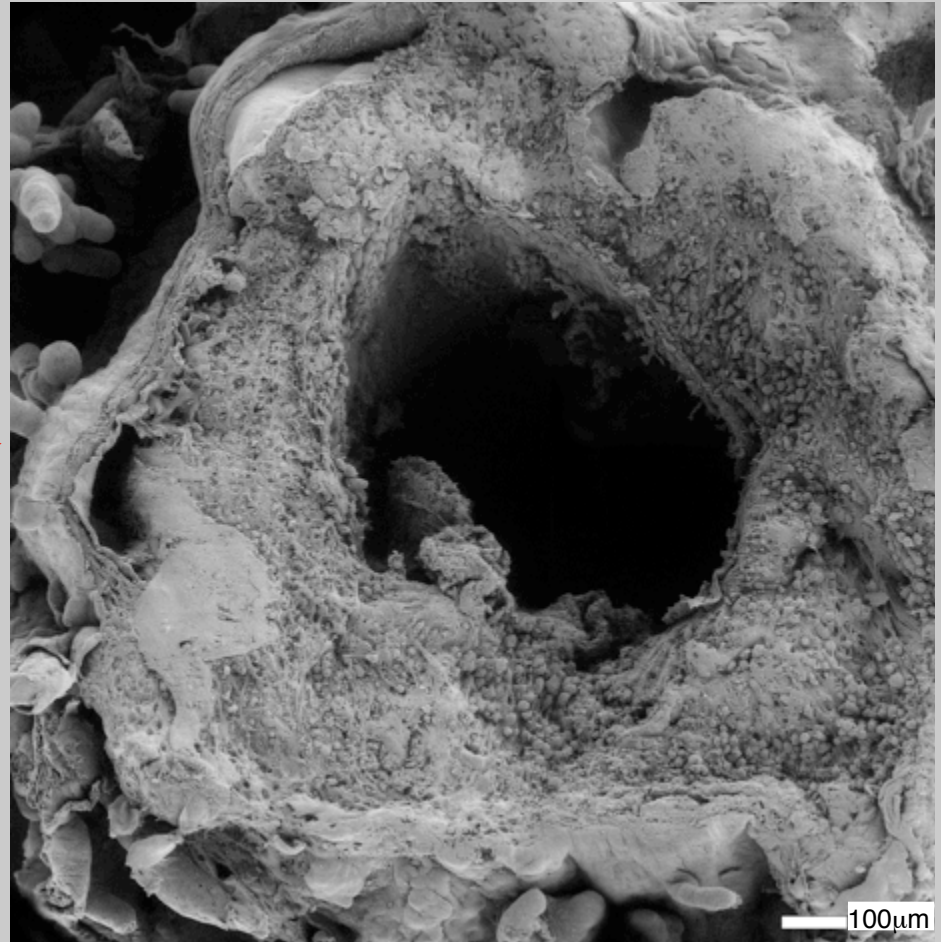
Underlying villus cytotrophoblasts



CTBs invade & remodel uterine spiral arteries

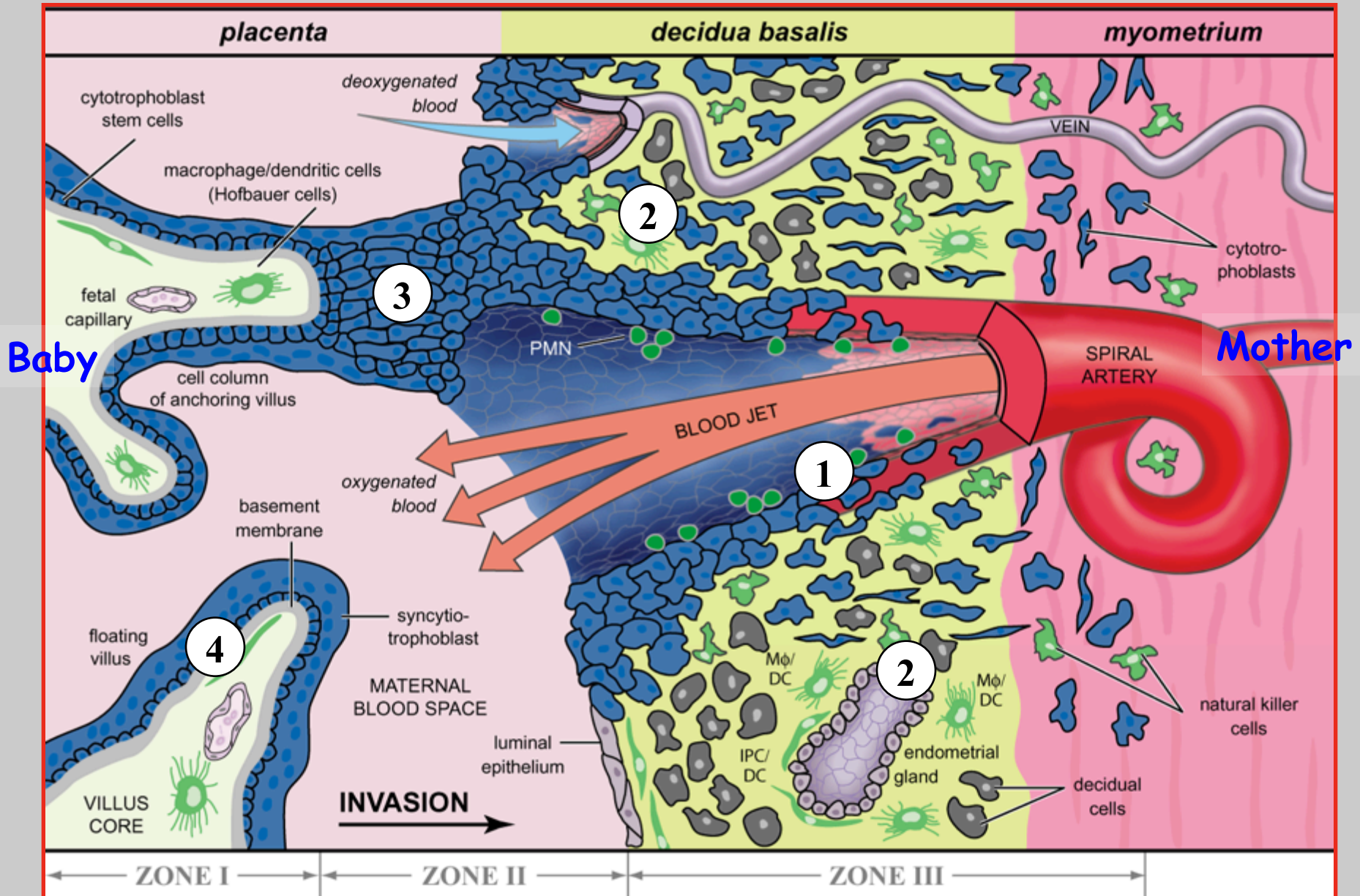


Increase lumen size 1000-fold



Kristy Red-Horse & Susan Fisher

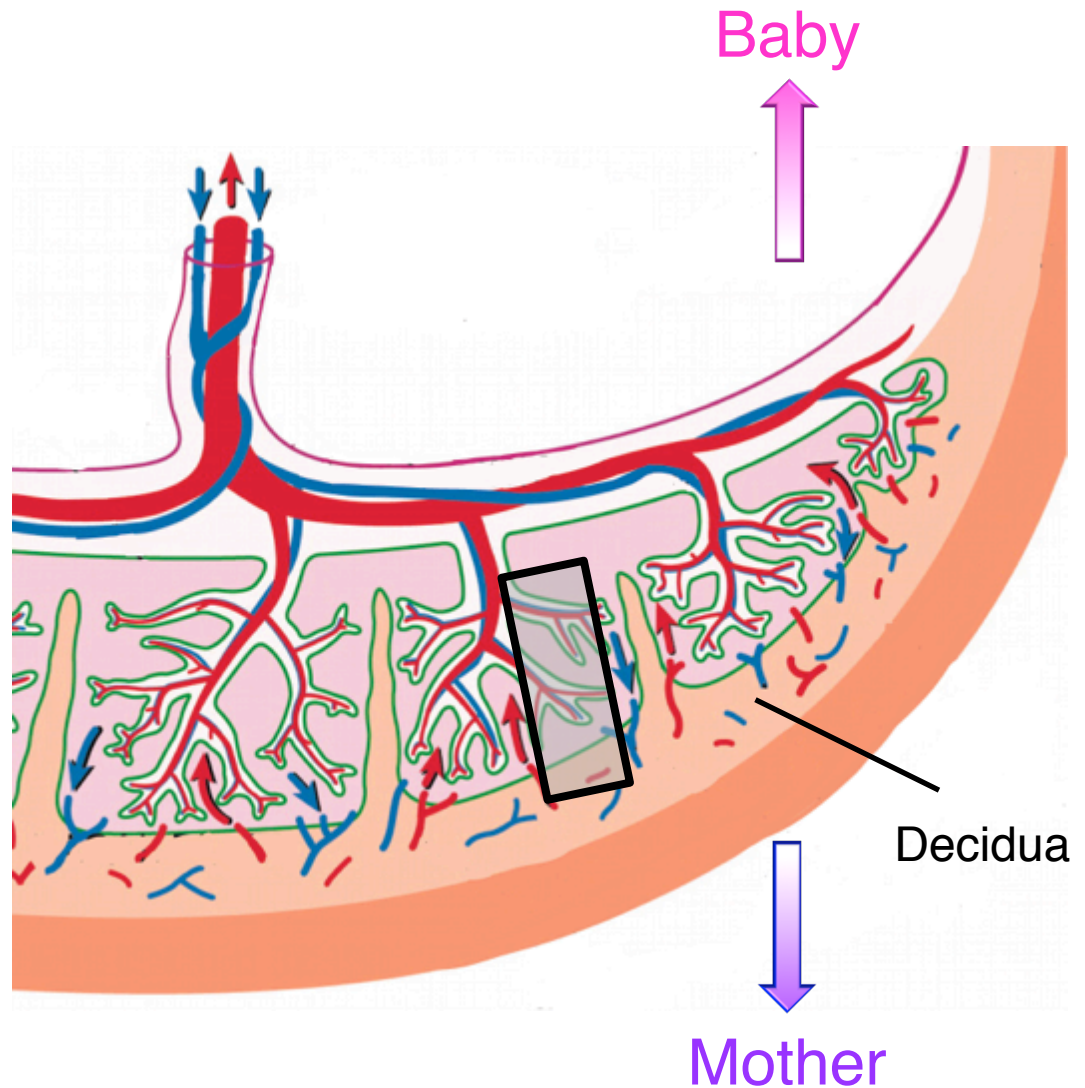
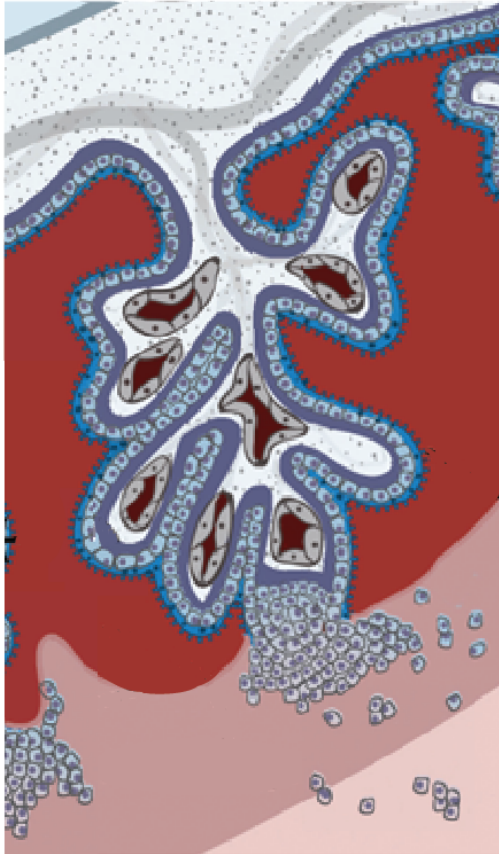
HCMV infects uterine-placental interface



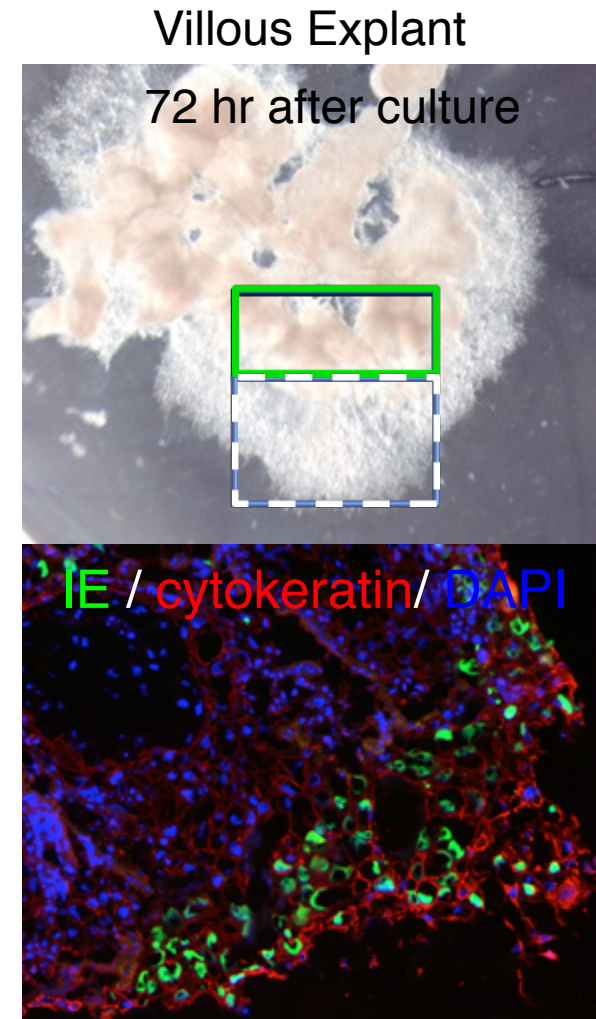
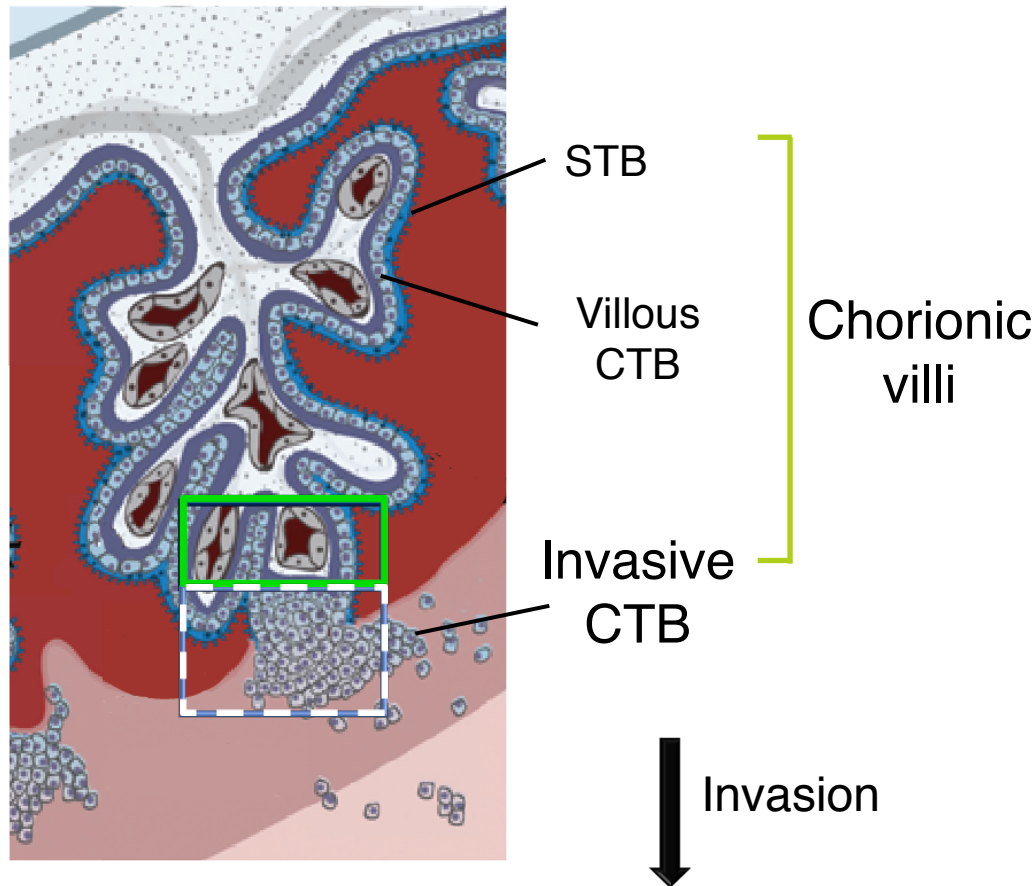
HCMV infection interferes with maintenance
& differentiation of human trophoblast
progenitor cells of the placenta

Takako Tabata, Matthew Petitt, Martin Zydek, *et al* submitted

Uterine-placental interface



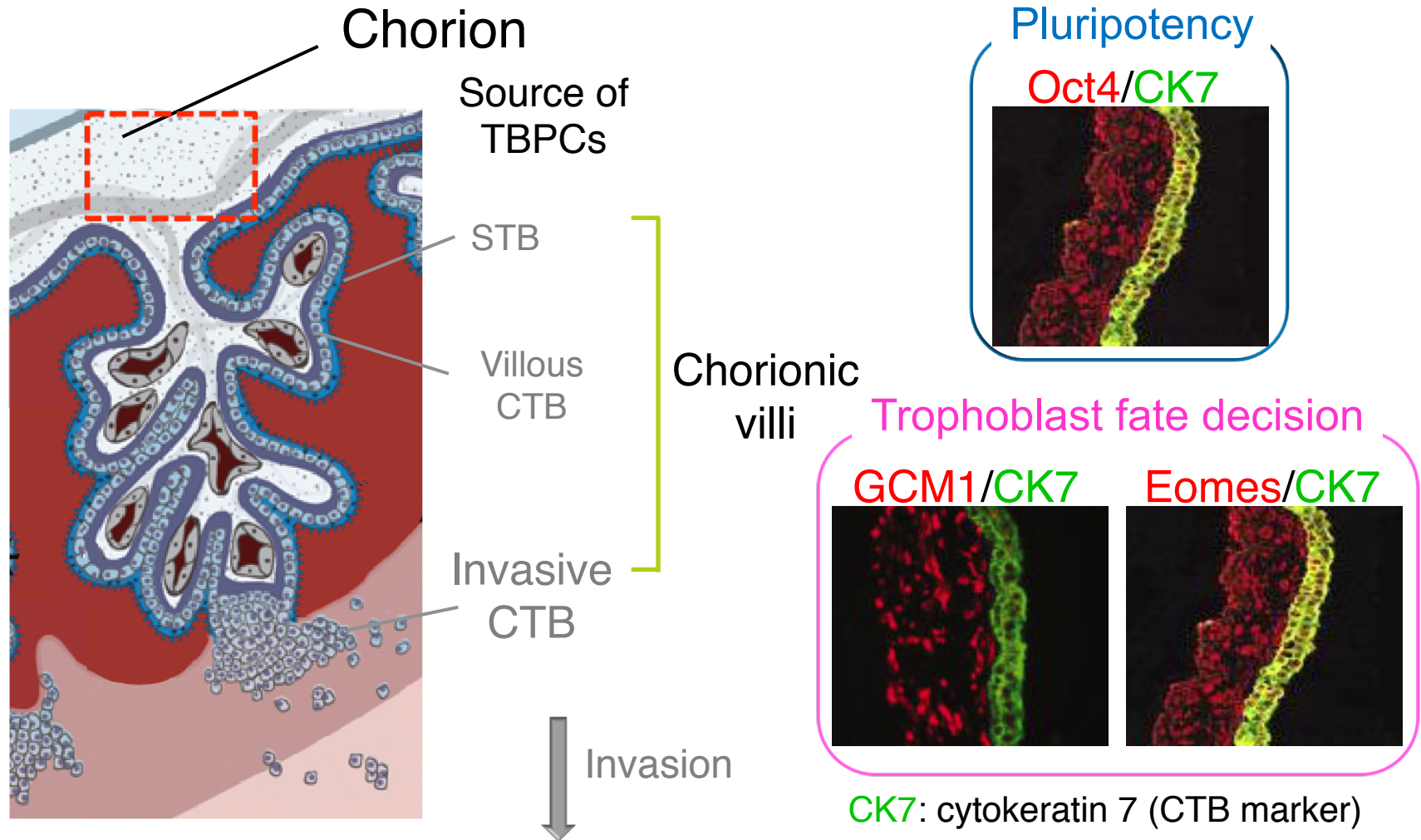
HCMV replicates in villus CTBs, differentiating/invading CTBs & anchoring villous explants



STB: syncytiotrophoblast, CTB: cytotrophoblast

Tabata et al (2012) Am J Pathol

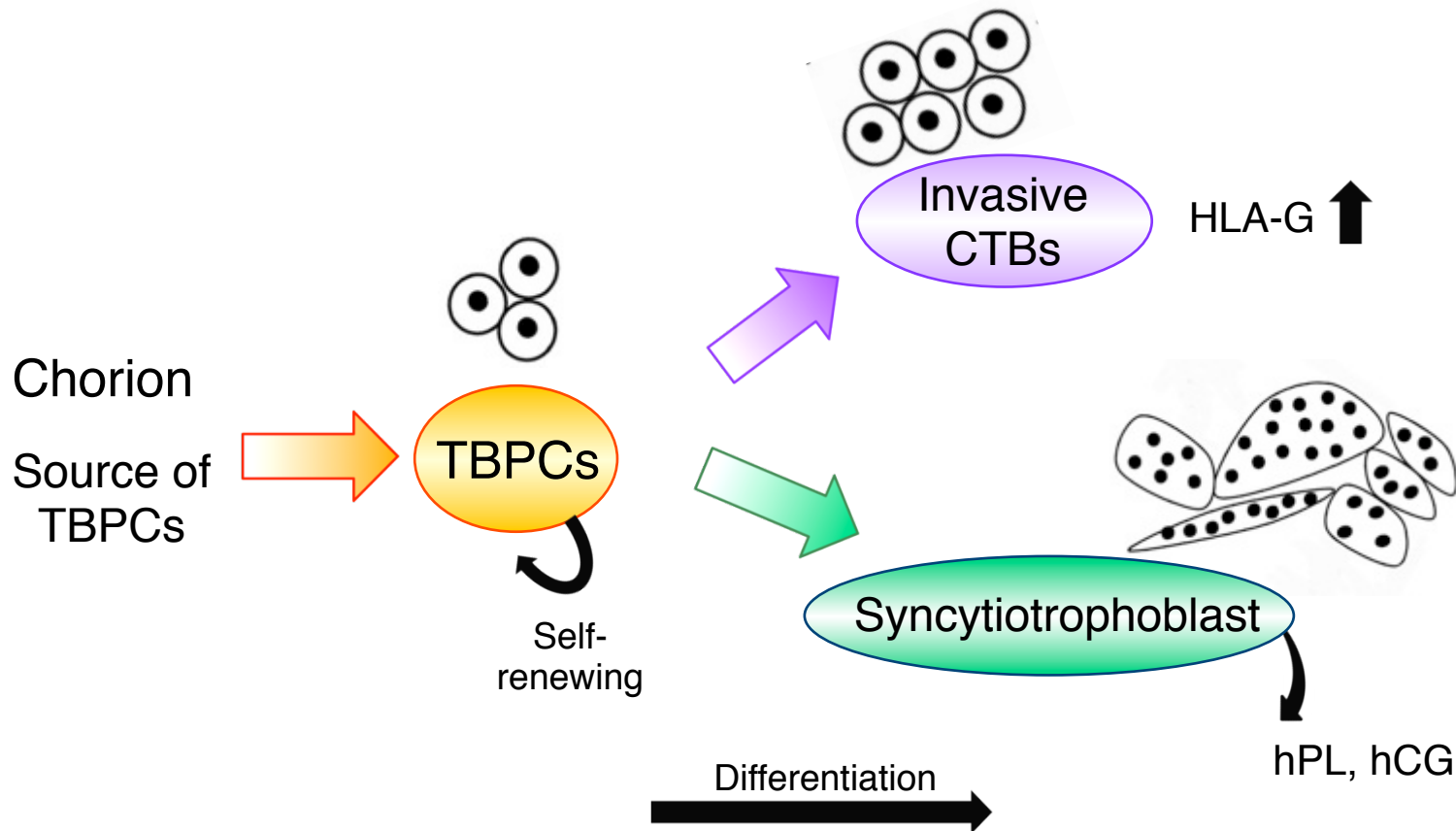
Chorion is a source of self-renewing human trophoblast progenitor cells (TBPCs)



STB: syncytiotrophoblast, CTB: cytotrophoblast

Genbacev et al (2011) Stem Cell

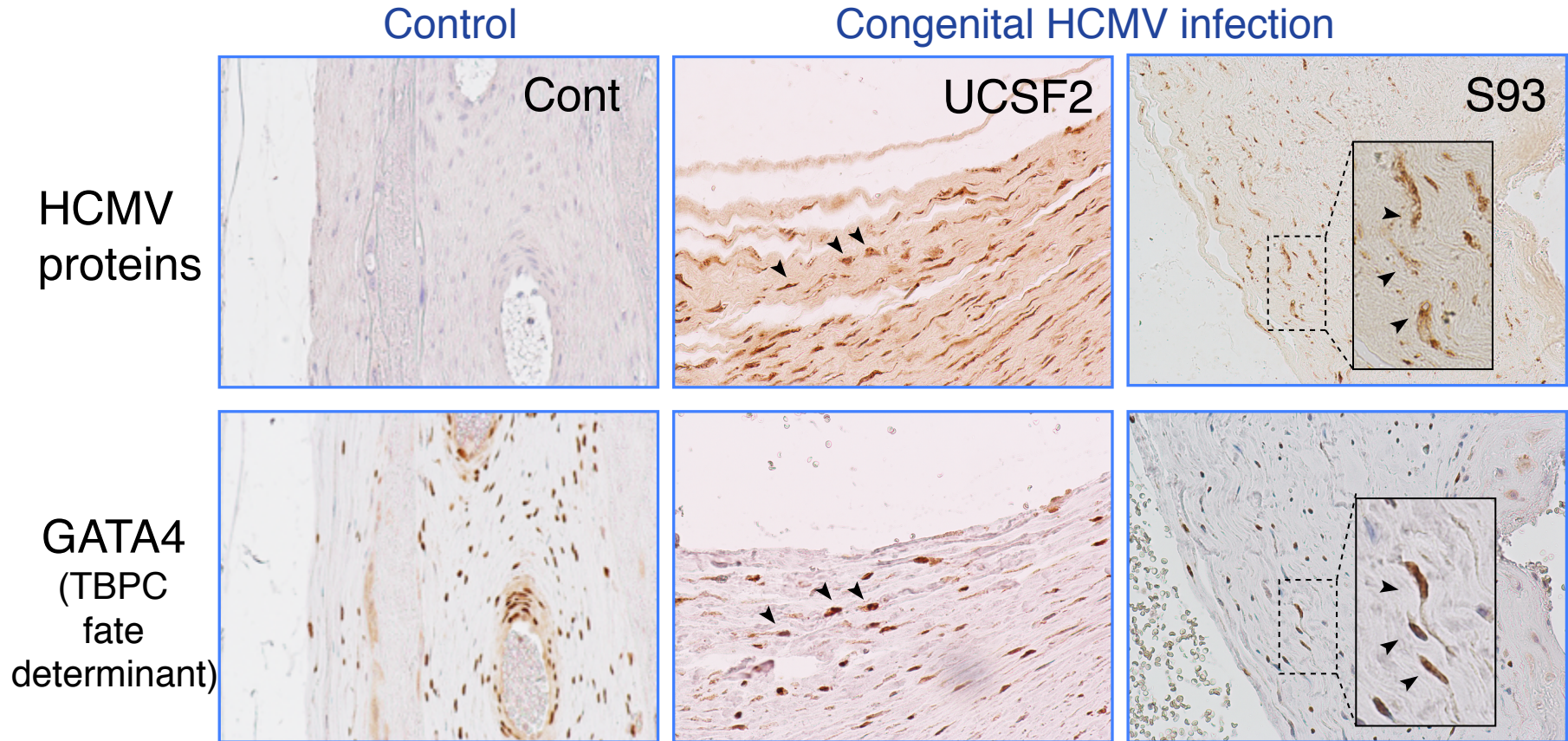
TBPCs differentiate into invasive CTBs & fuse to form STBs that cover chorionic villi



hPL : human placental lactogen
hCG : human chorionic gonadotropin

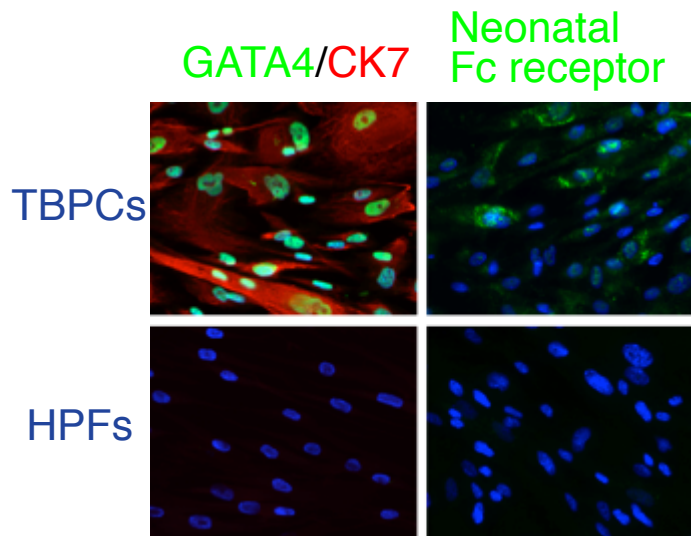
Genbacev et al (2011) Stem Cell

HCMV replicates in TBPCs of the chorion from symptomatic congenital infection

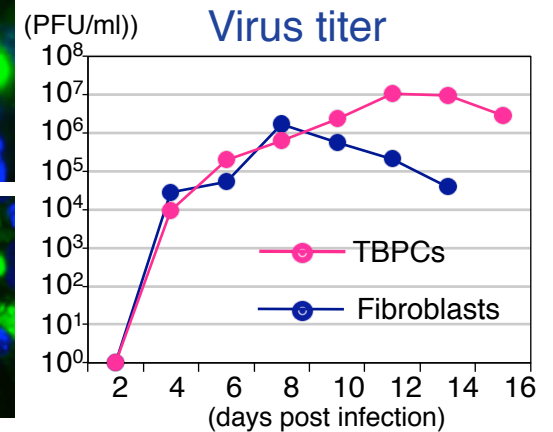
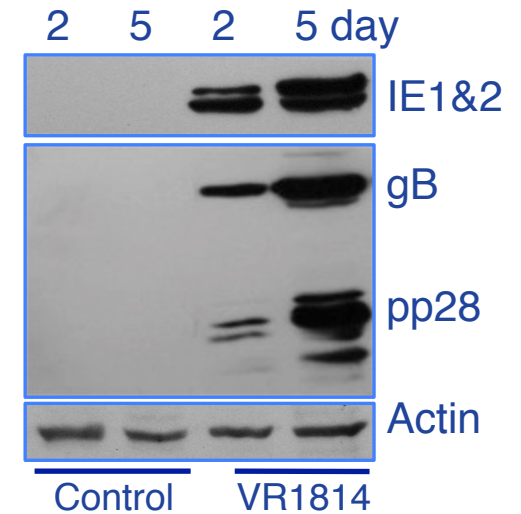
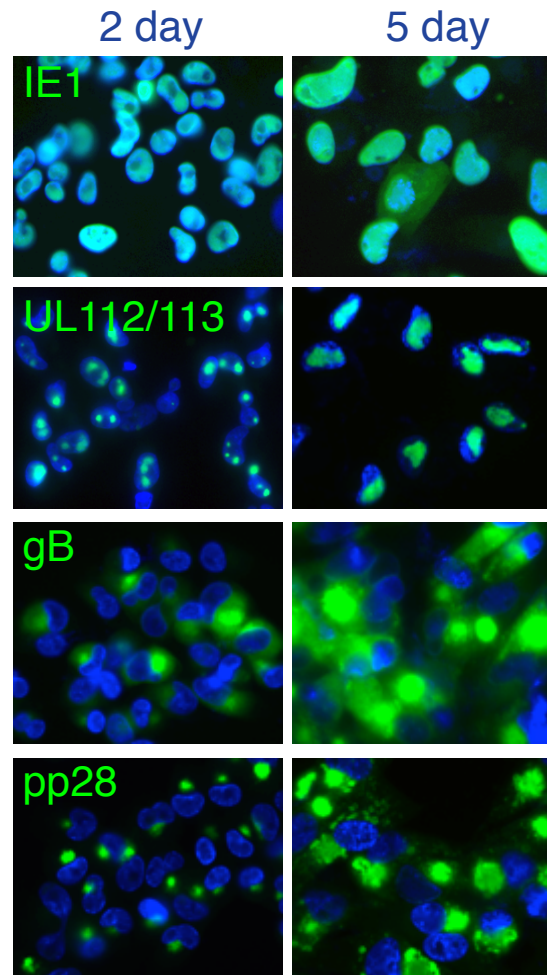
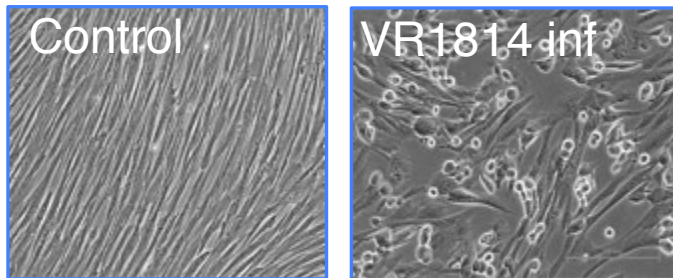


Studies in continuously self-renewing TBPCs yield insights into mechanisms whereby HCMV impairs early placental development.

HCMV replicates in TBPCs

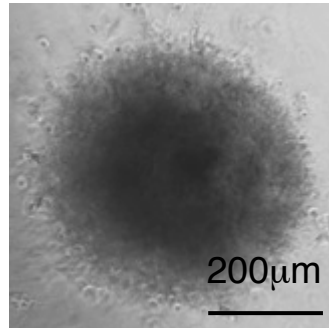


HPFs: placental fibroblasts

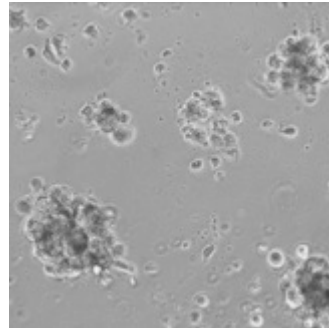


HCMV infection impairs TBPC functions: differentiation/sphere formation

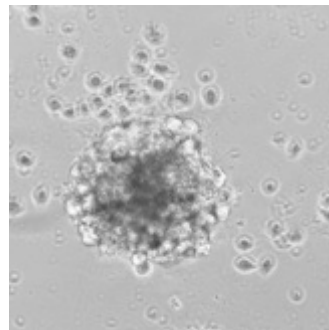
Uninfected control



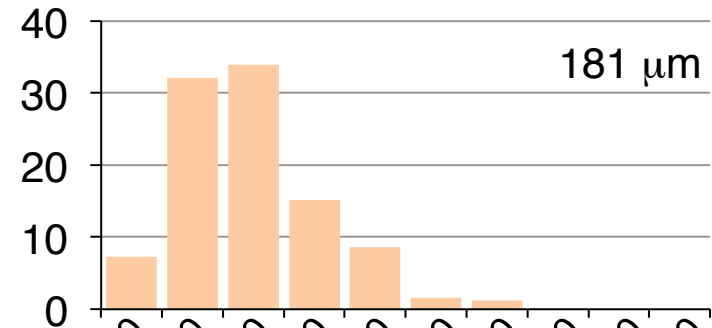
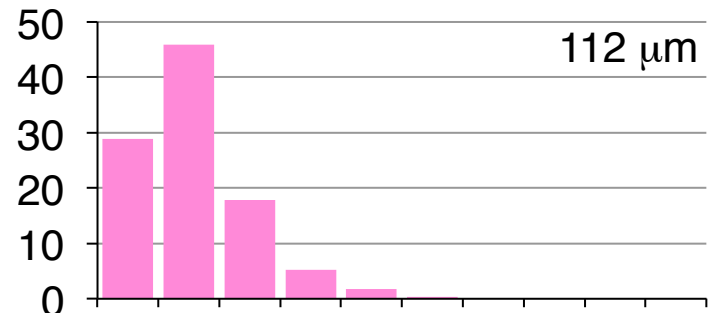
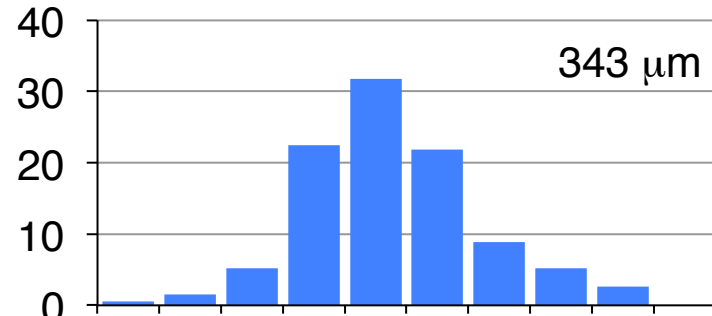
VR1814 inf



VR1814 inf
+ ganciclovir

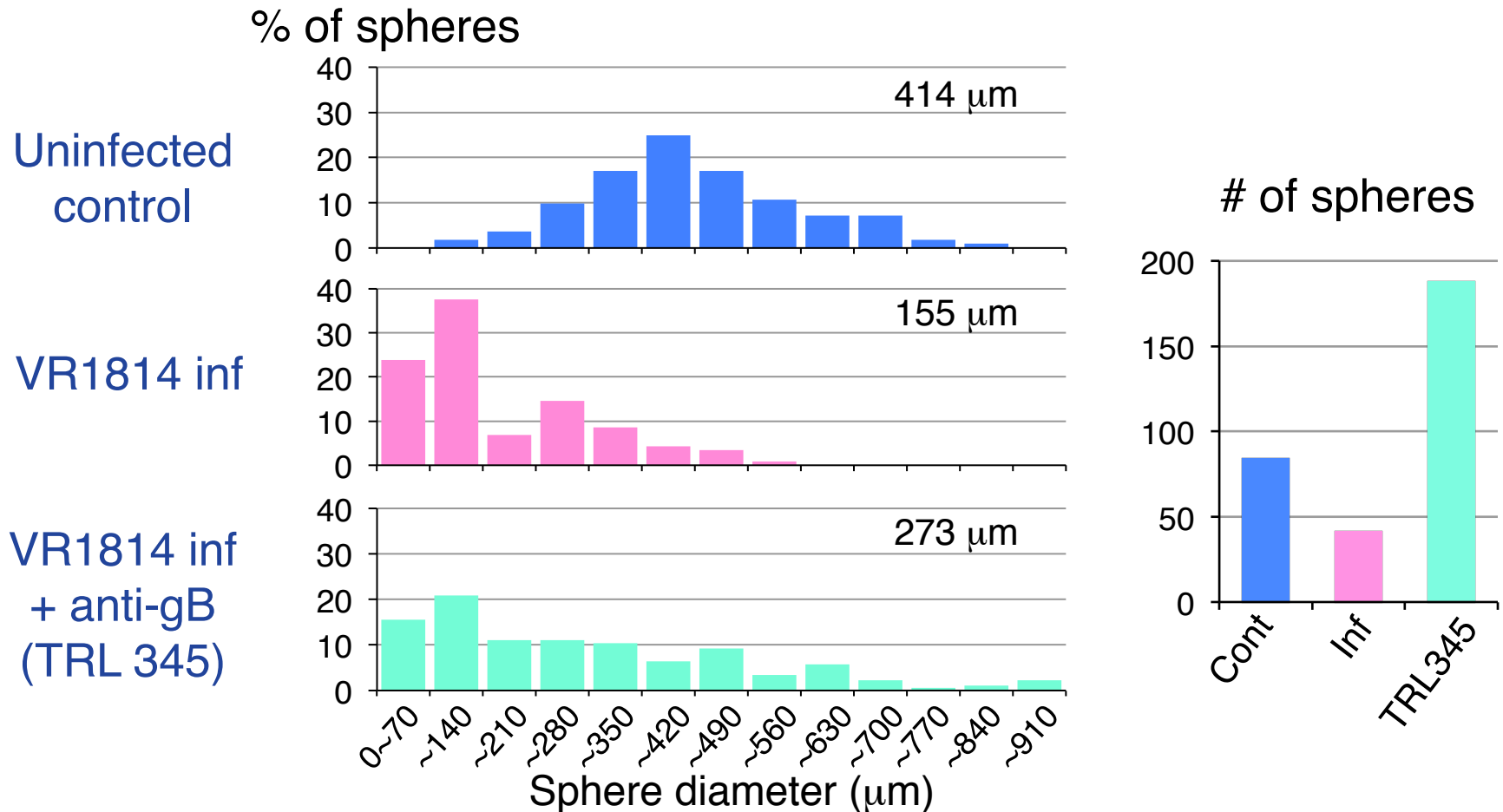


% of spheres



Sphere diameter (μm)

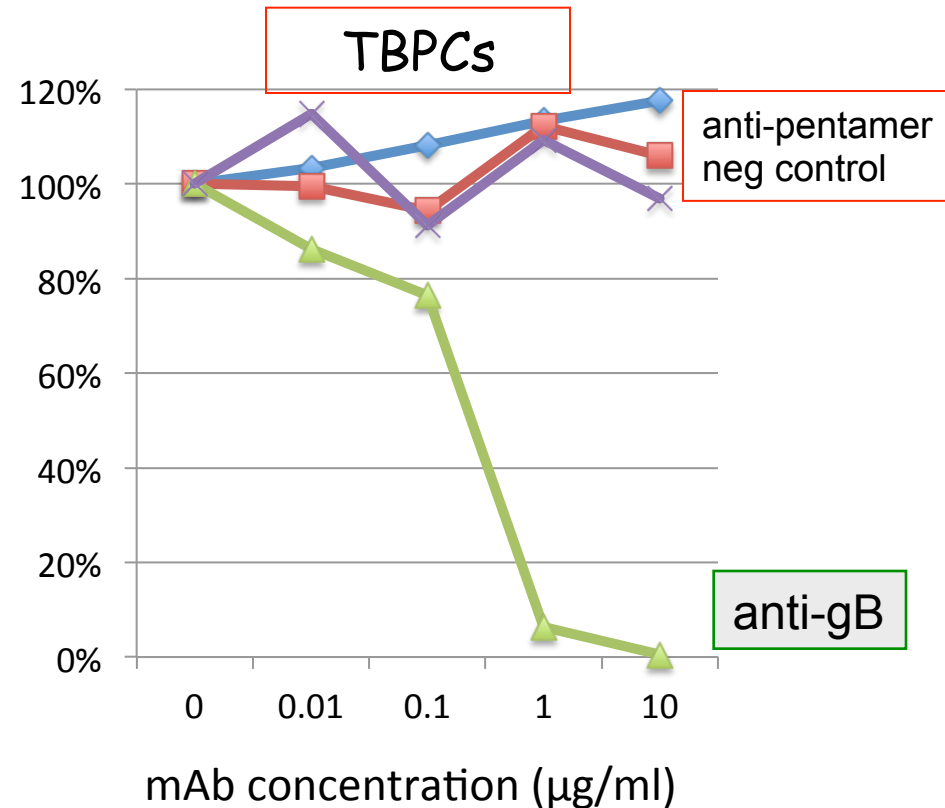
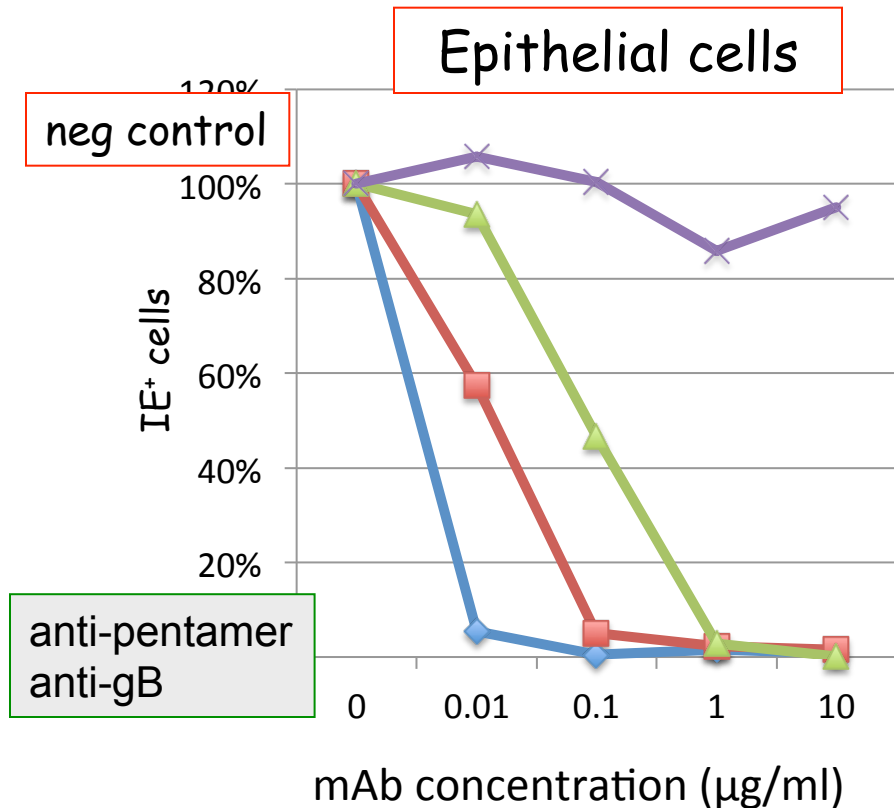
Anti-HCMV antibody added after infection partially restores size & number of spheres



Explanation for compensatory development of villi with HIG treatment for congenital infection

HCMV neutralizing monoclonal antibodies (Mabs) rescue TBPCs & anchoring villi from infection

Anti-HCMV Mabs reduce infection

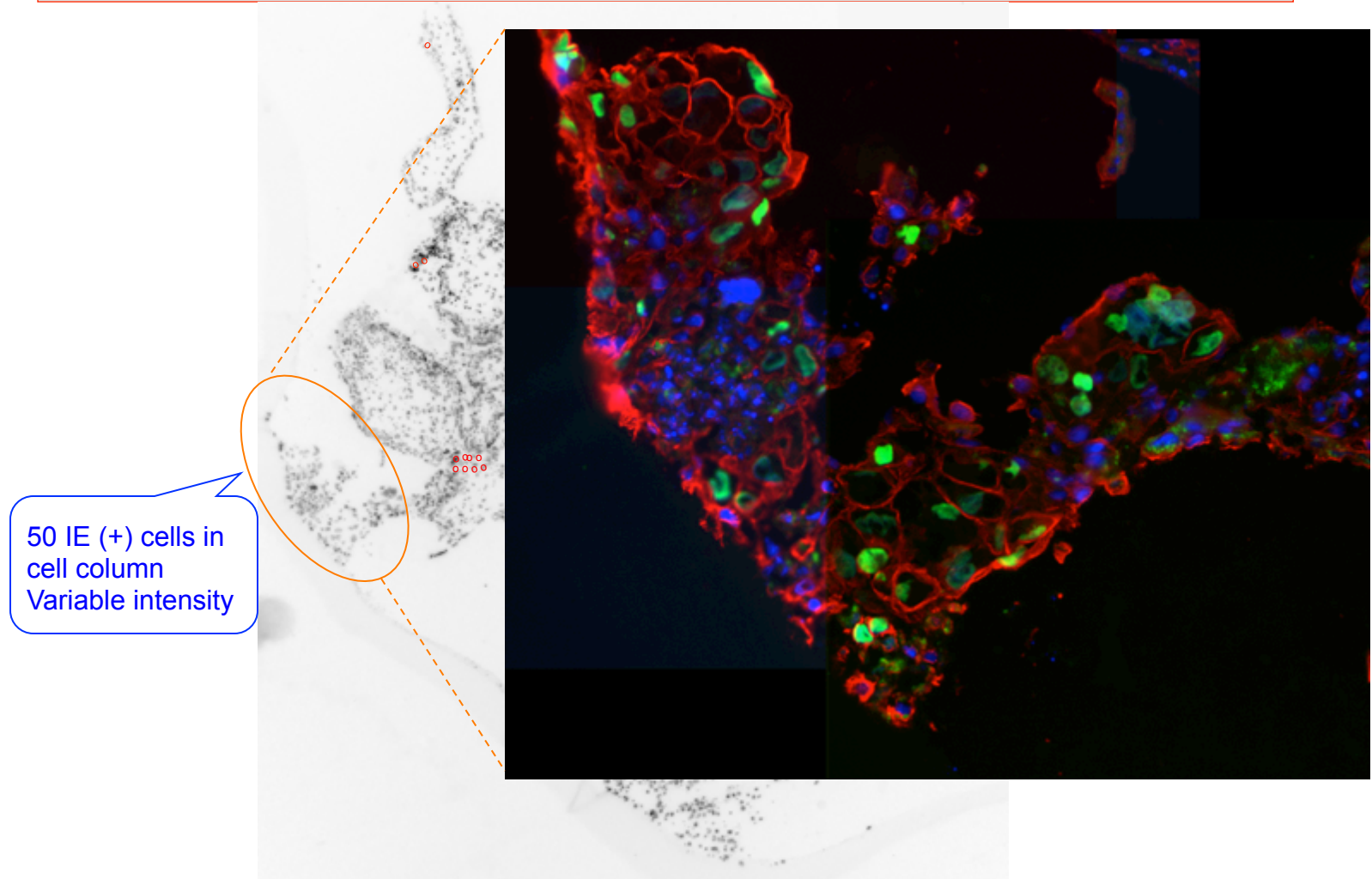


Mabs TRL310 & TRL345 protect
epithelial & endothelial cells

TRL345 protects TBPCs

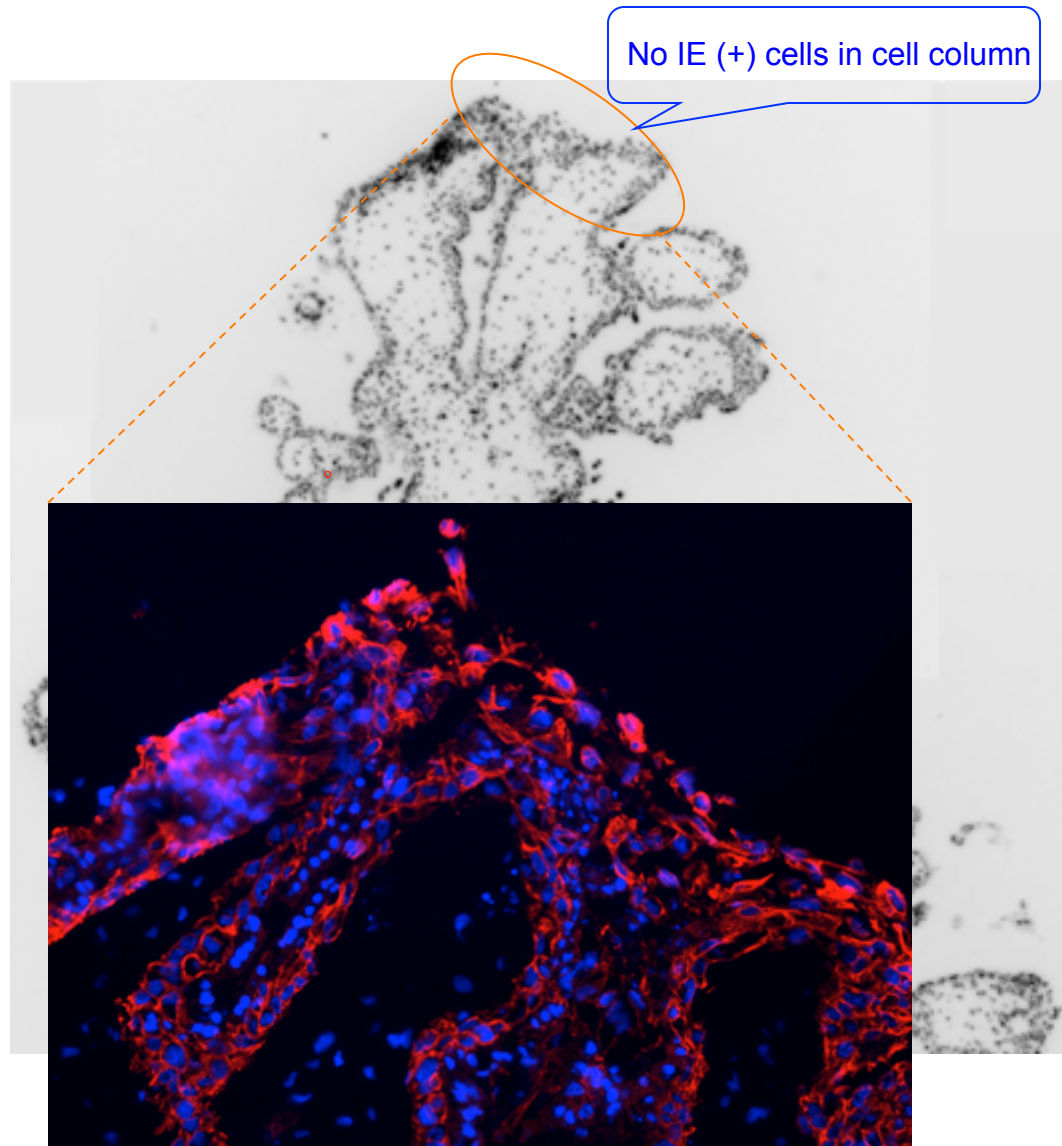
Anti-gB: TRL345
Anti-pentamer: TRL310

HCMV-infected villus explants



CTBs express HCMV proteins & cytokeratin

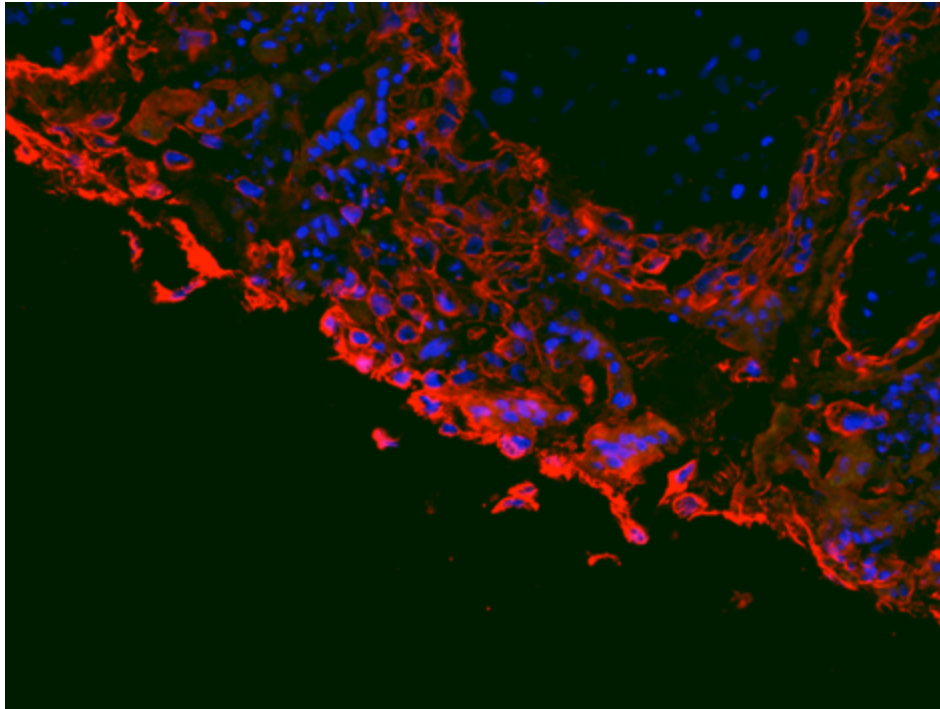
HIG rescues villus explants from infection



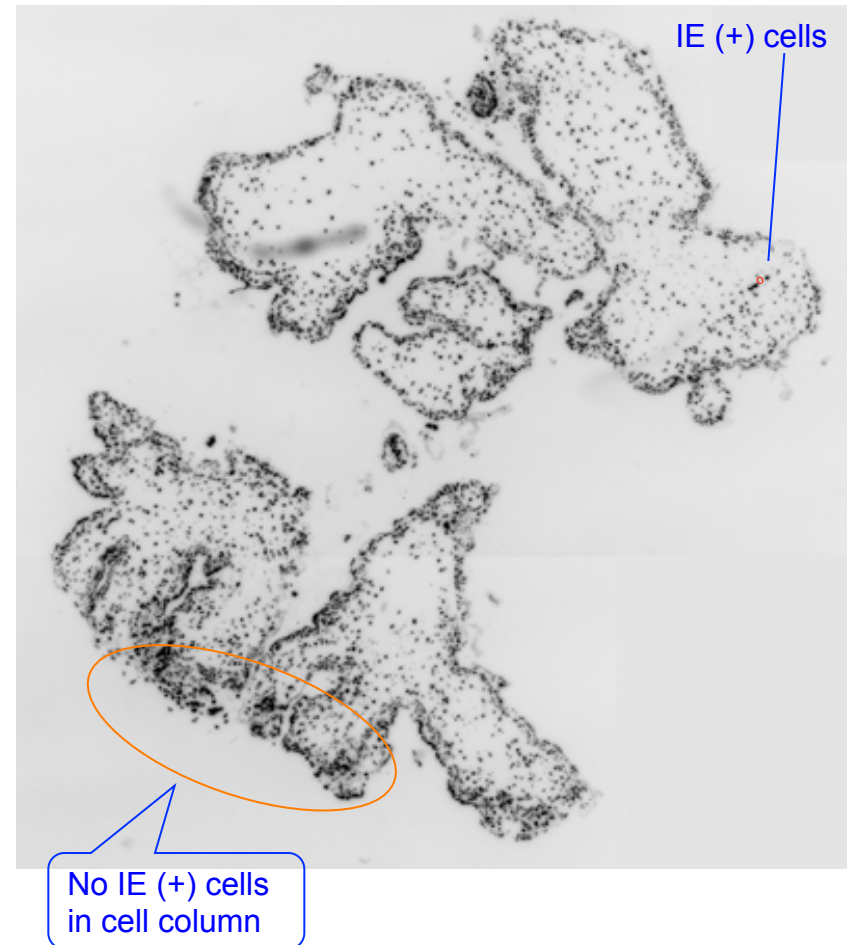
HIG (100 $\mu\text{g/ml}$)

anti-HCMV Mabs rescue explants from infection

anti-HCMV gB Mab (10 $\mu\text{g/ml}$)



anti-HCMV pentamer Mab
(0.05 $\mu\text{g/ml}$)



Neutralizing Mabs more potent than HIG

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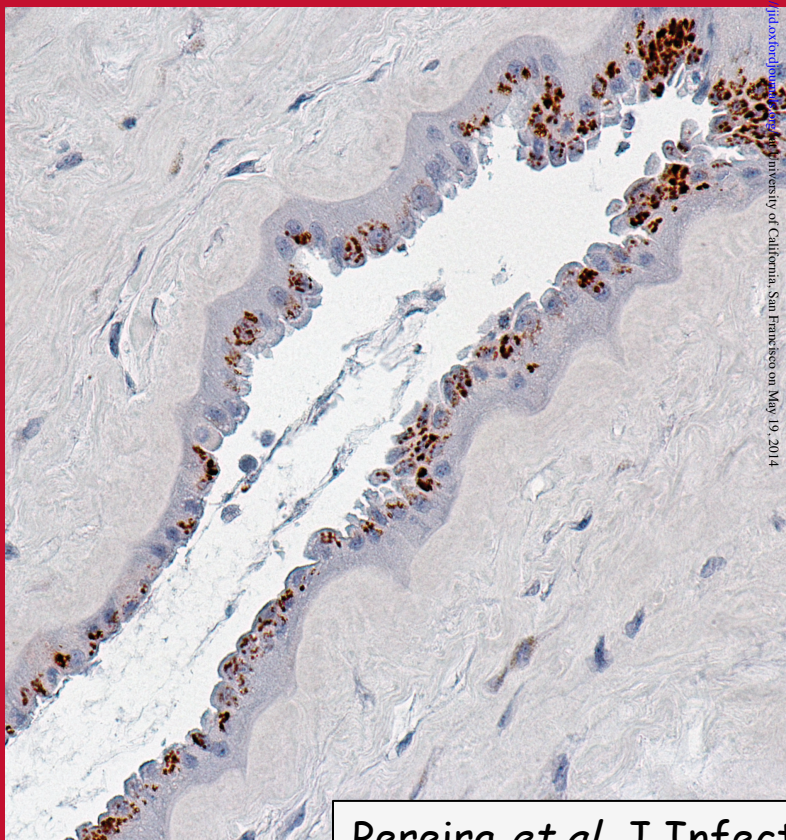


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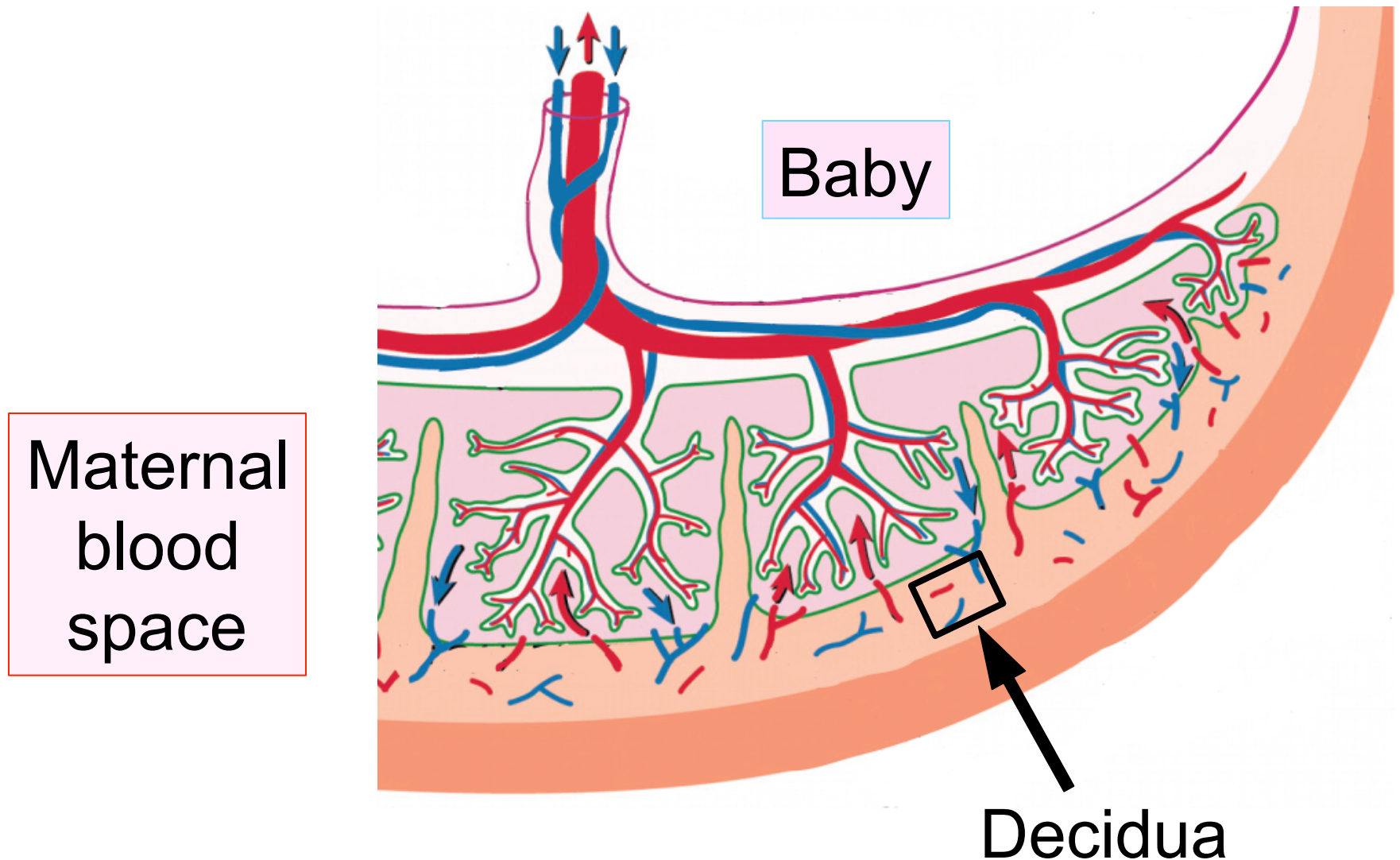
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Pereira *et al*, J Infect Dis 209:1537, 2014

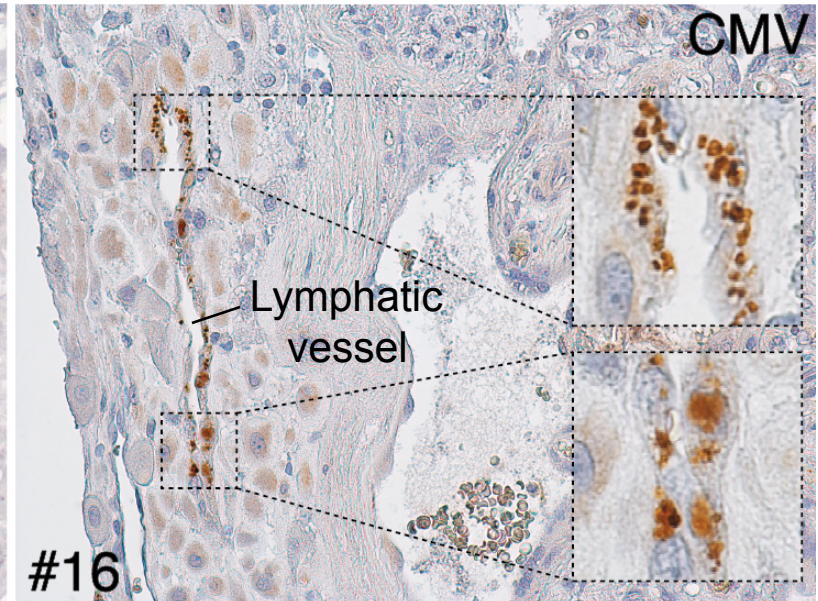
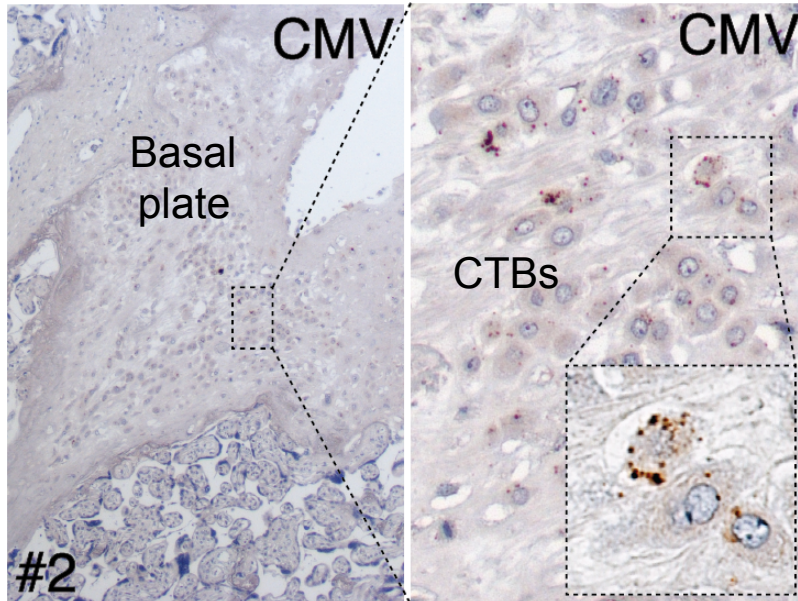
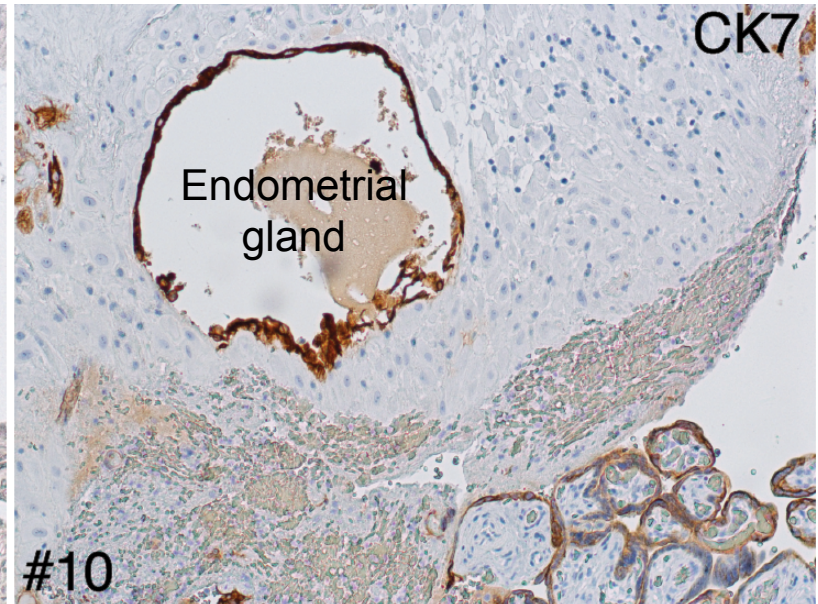
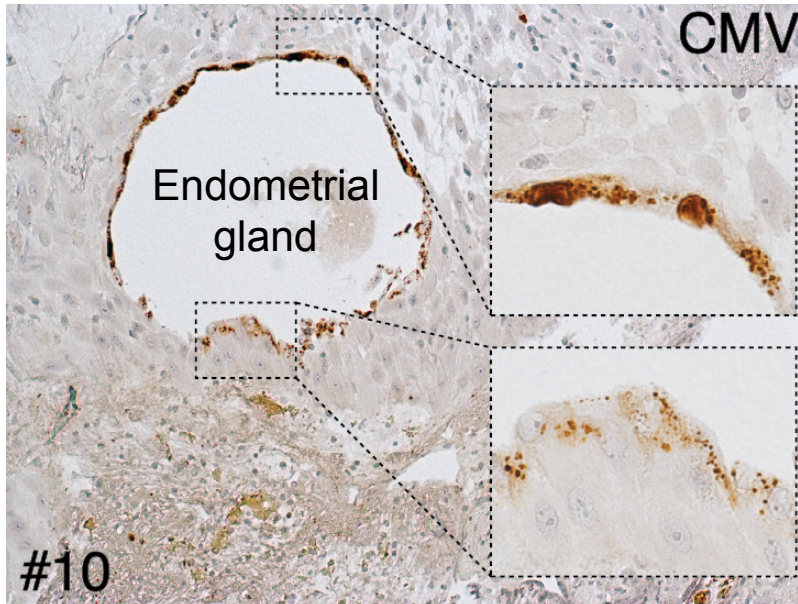
Congenital HCMV infection: 5/7 IUGR cases

IUGR		CMV Avidity	Neut. Titer		Immunoblot Profile	CMV DNA	cmvIL-10	CMV Infection
			HPF	HUVEC				
2	M	63.4	1:128	1:512	p150, CM2 , p65, gB1, gB2	1/5	+	Recurrent
	B	73.5	1:64	1:512				
3	M	64.3	1:32	1:1024	IE1 , p150, CM2 , p65, gB1	1/5	+	Recurrent
	B	67.1	1:32	1:1024				
18	M	63.4	NT	1:512	IE1 , p150, CM2 , p65, gB1	—	—	Recurrent
	B	73.5	NT	1:512				
16	M	18.5	1:16	1:512	IE1 , p150, CM2 , p65, gB1	5/5	+	Primary
	B	11.1	1:32	1:512				
12	M	36.0	NT	—	IE1 , CM2 , p65	—	—	Primary + PE
	B	33.5	NT	—				

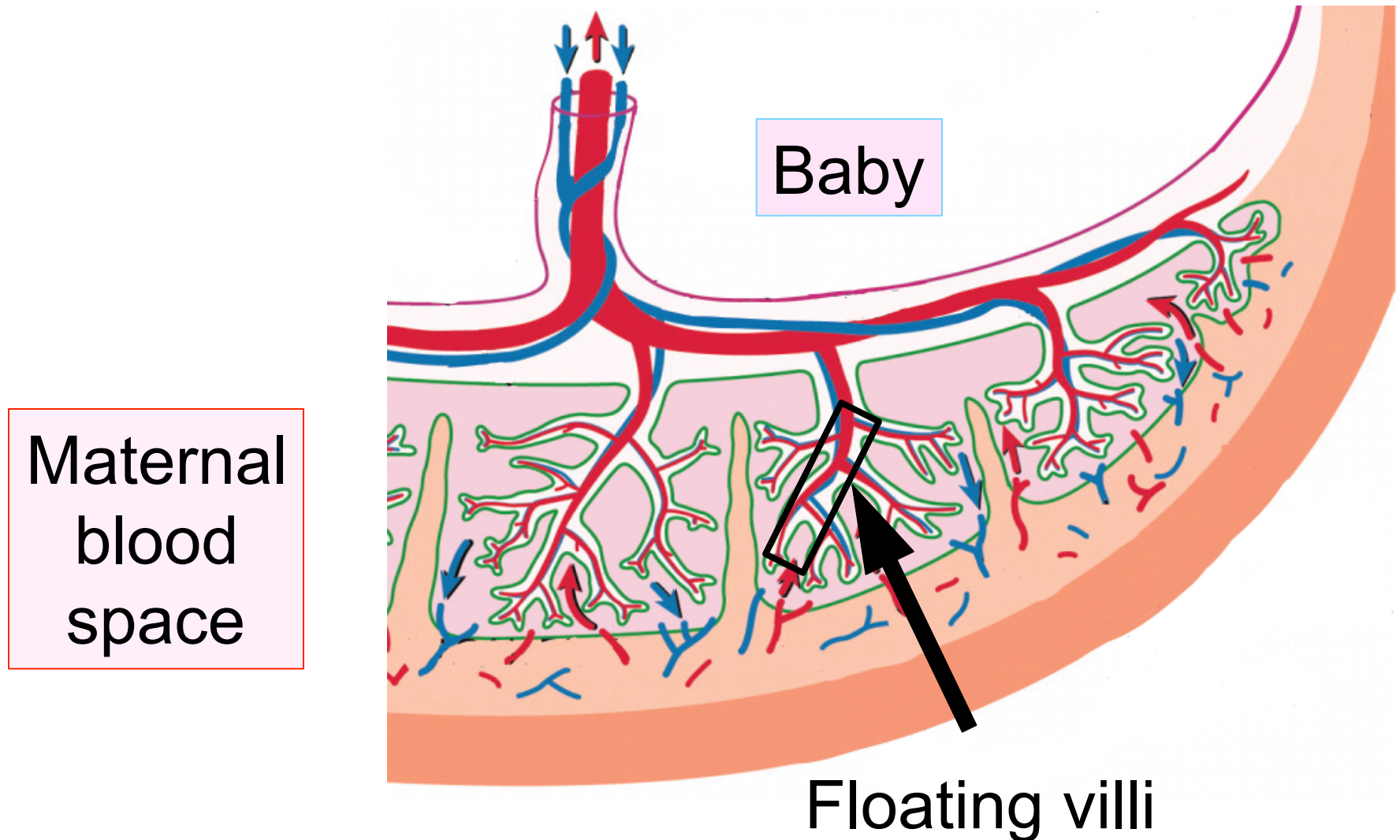
HCMV infects sites in uterine wall



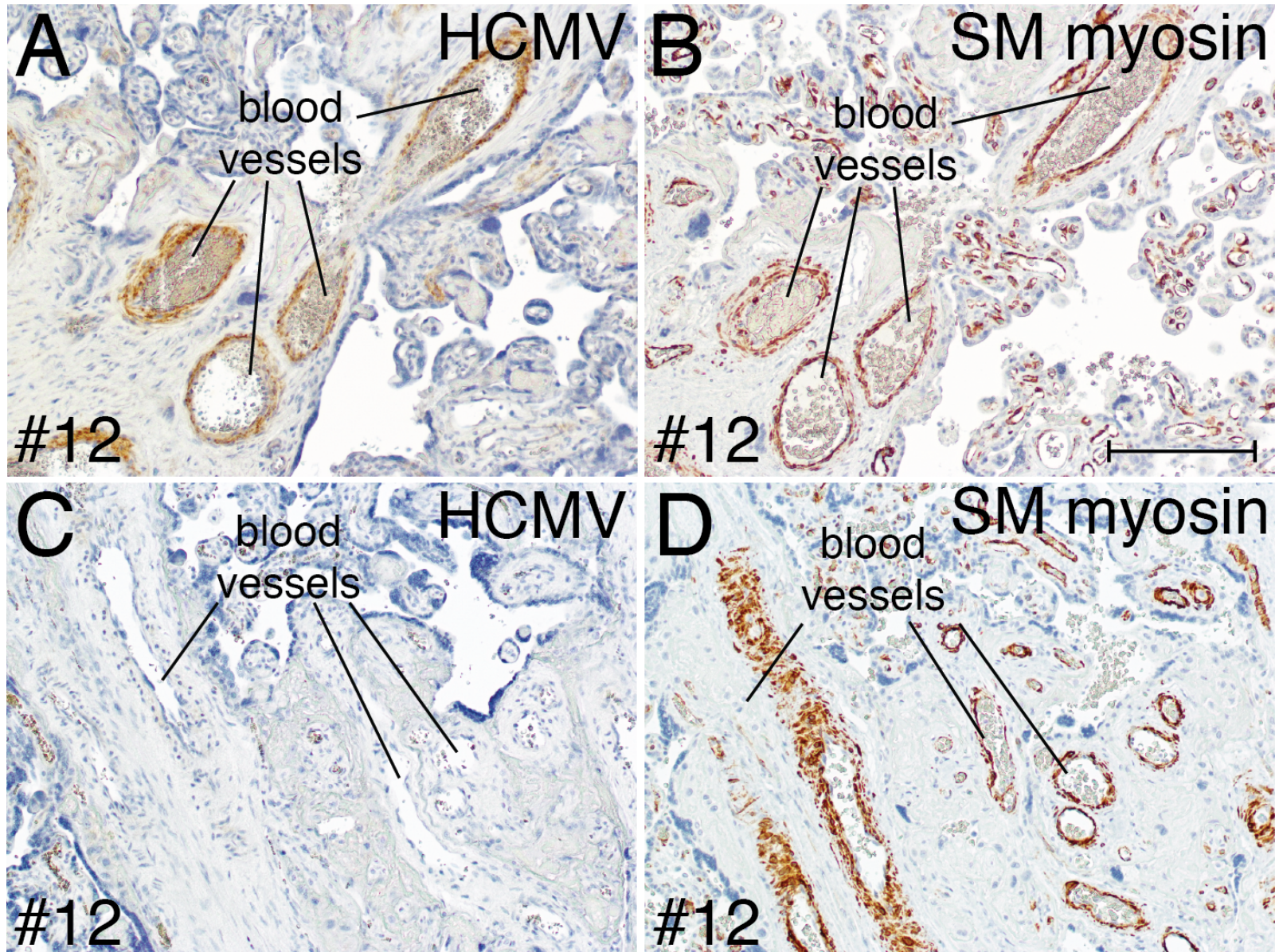
HCMV infects CTBs, epithelium, endothelium



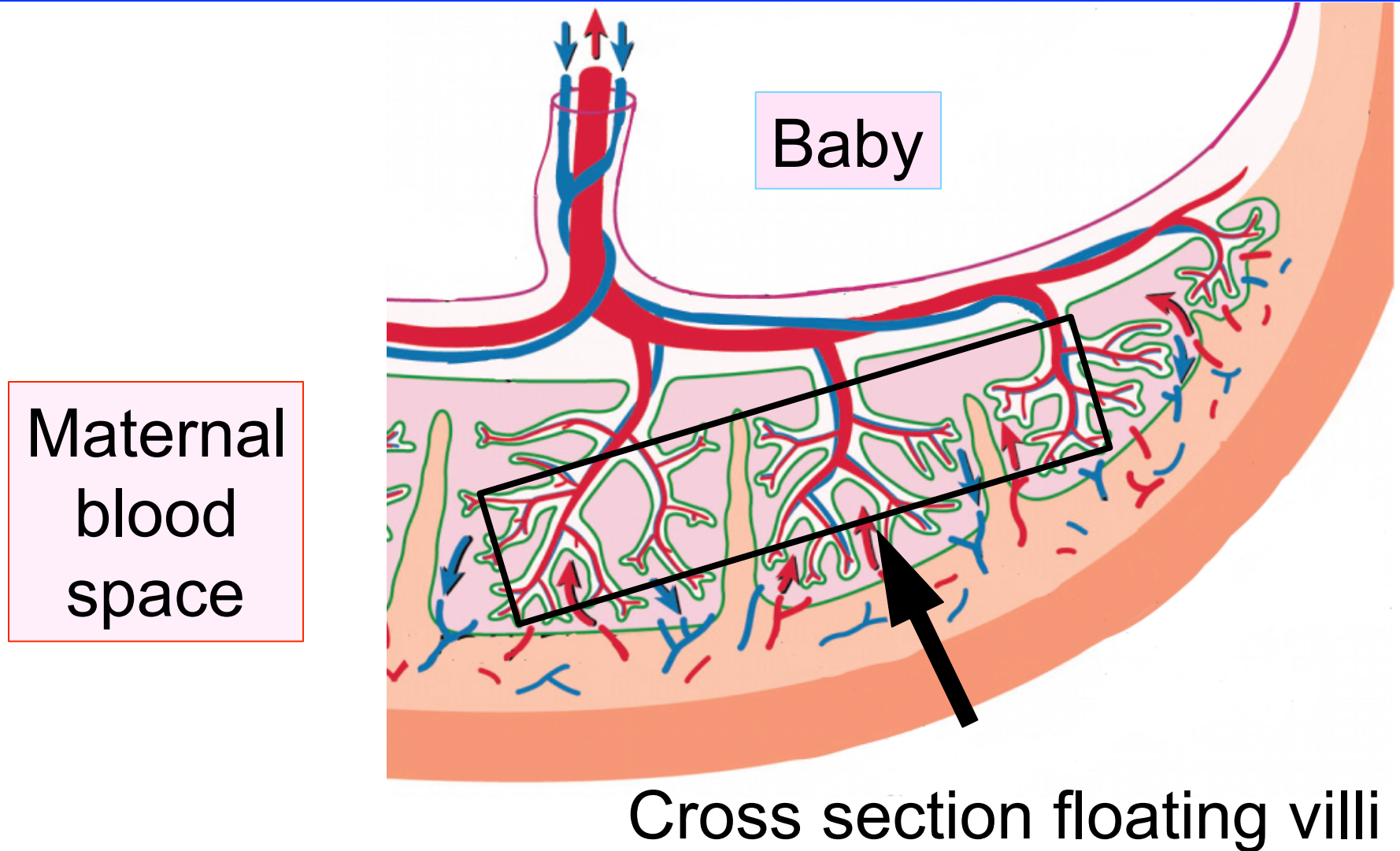
HCMV infects villous blood vessels



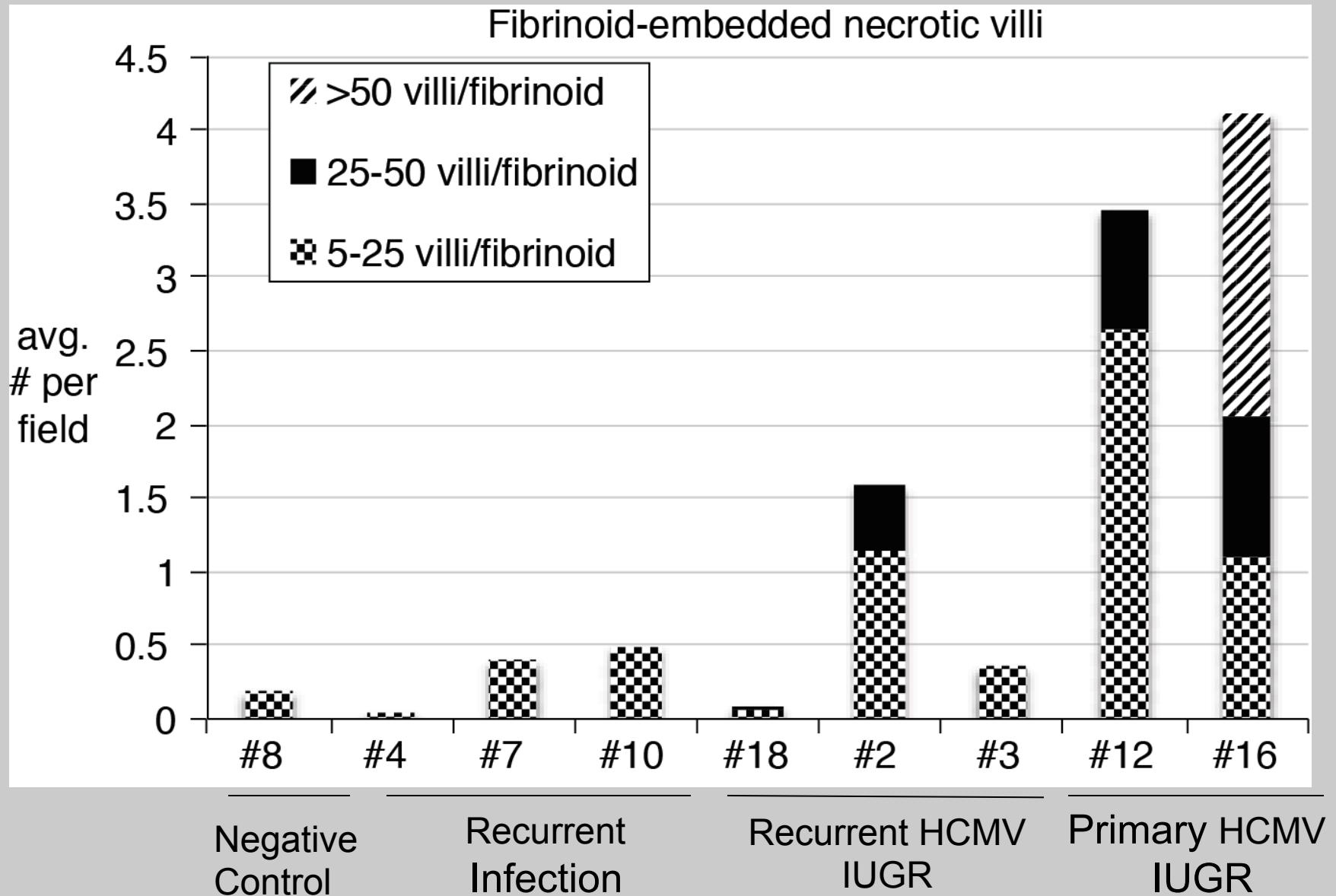
Infected blood vessels in stem villi



HCMV infection, hypoxia & inflammation progressively injure the placenta

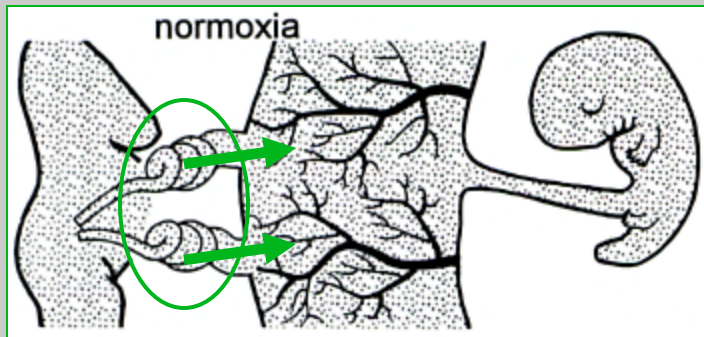


Primary infection increases villous fibrosis

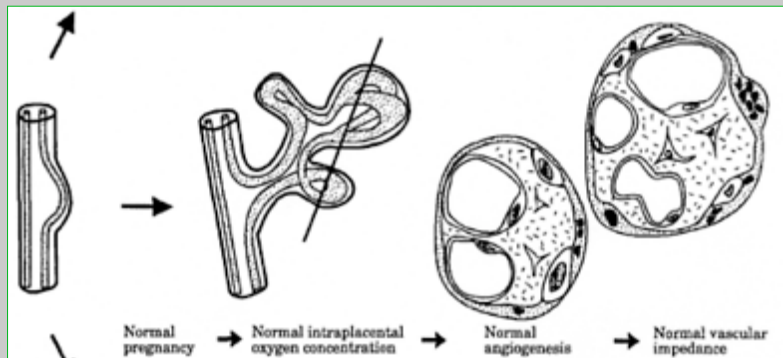


Healthy Placenta

CTBs remodel uterine blood vessels increase blood perfusion

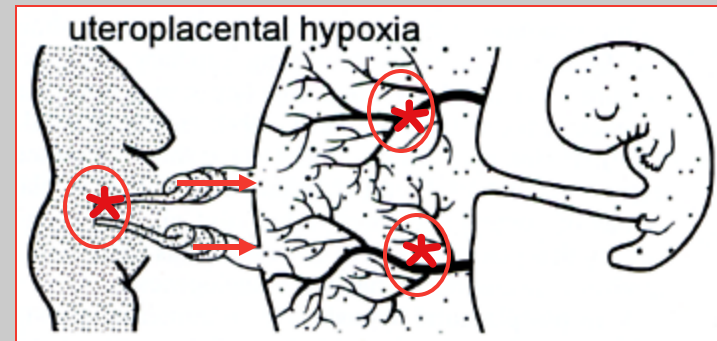


Dilated blood vessels increase oxygen diffusion in terminal villi

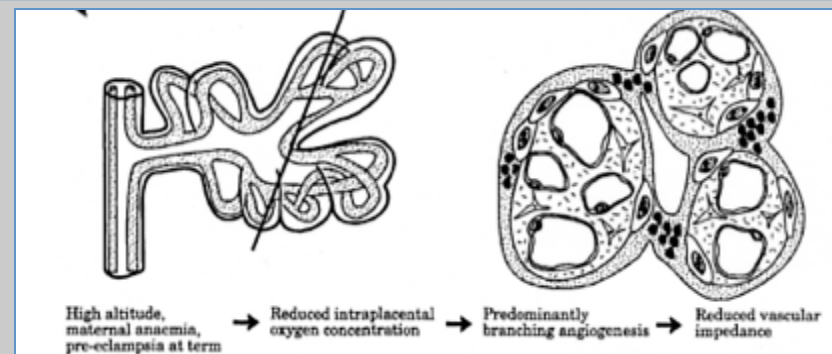


Primary Congenital HCMV

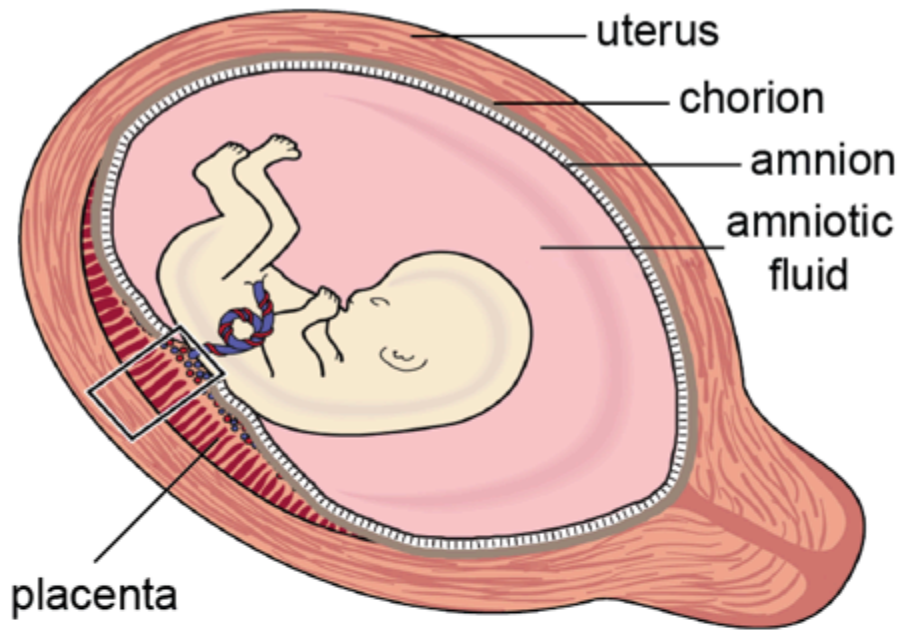
Infected TBPCs impair placental development: leads to fibrosis, avascular villi, reduced perfusion & hypoxia.



Antibody treatment increases: STB surface, new villi with blood vessels, oxygen & nutrient transfer.

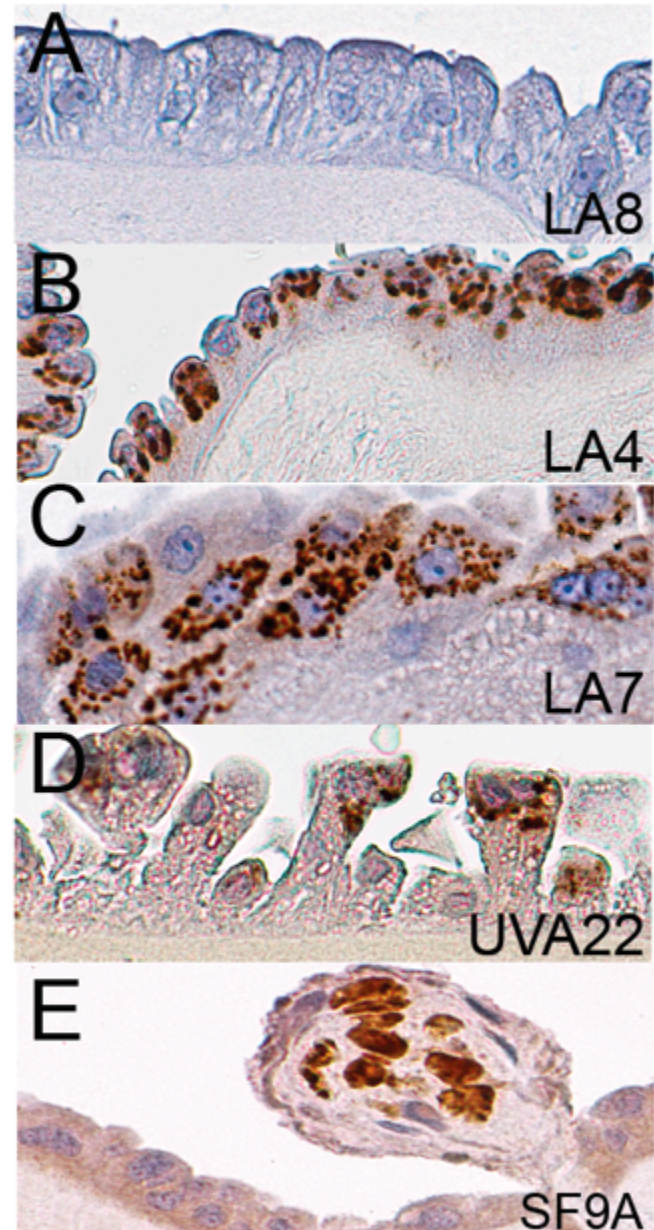


HCMV proteins in amniotic epithelium

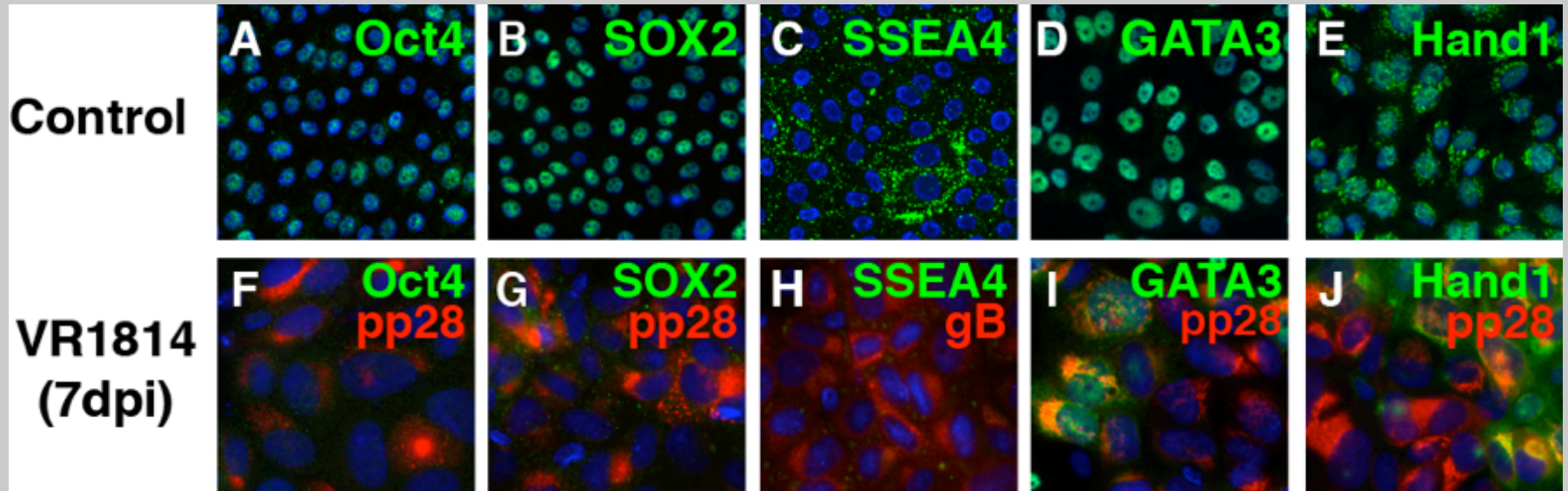


Amniotic Fluid Contacts Epithelium

- A) Seronegative.
- B) Asymptomatic recurrent infection.
- C) Asymptomatic recurrent infection.
- D) Symptomatic congenital infection.
- E) Congenital infection with IUGR.

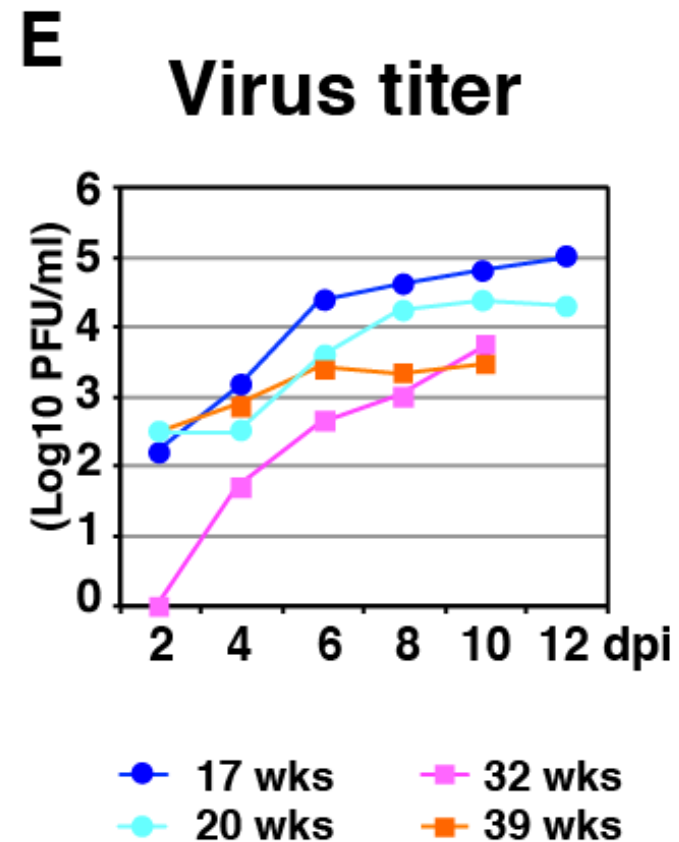
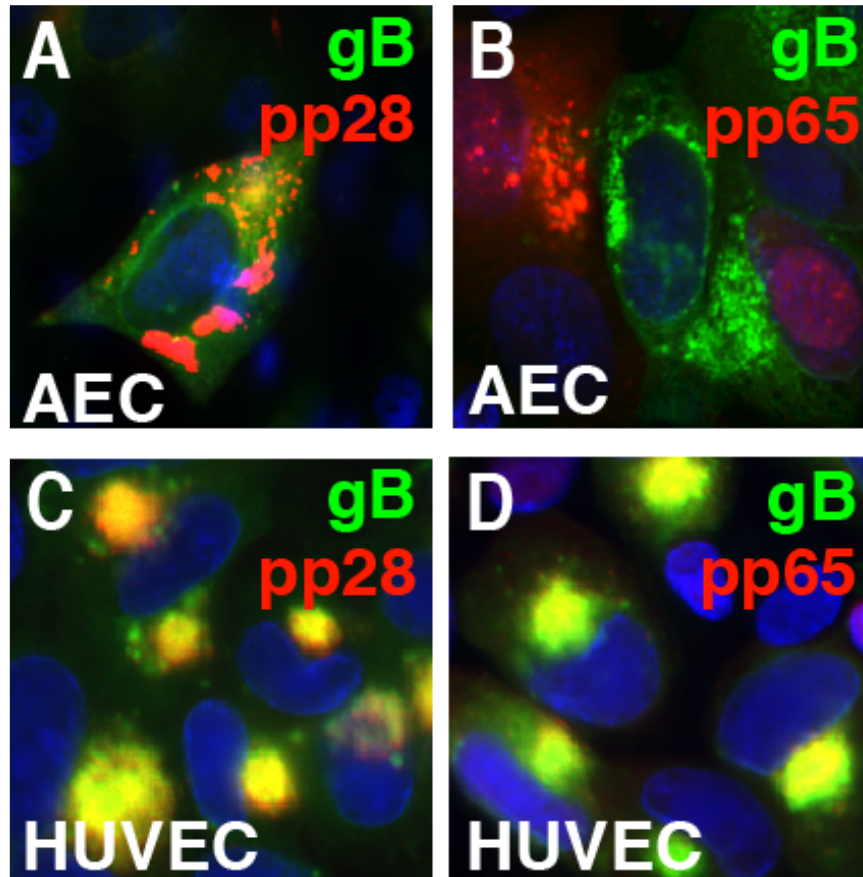


Fetal-derived AECs express stem cell proteins

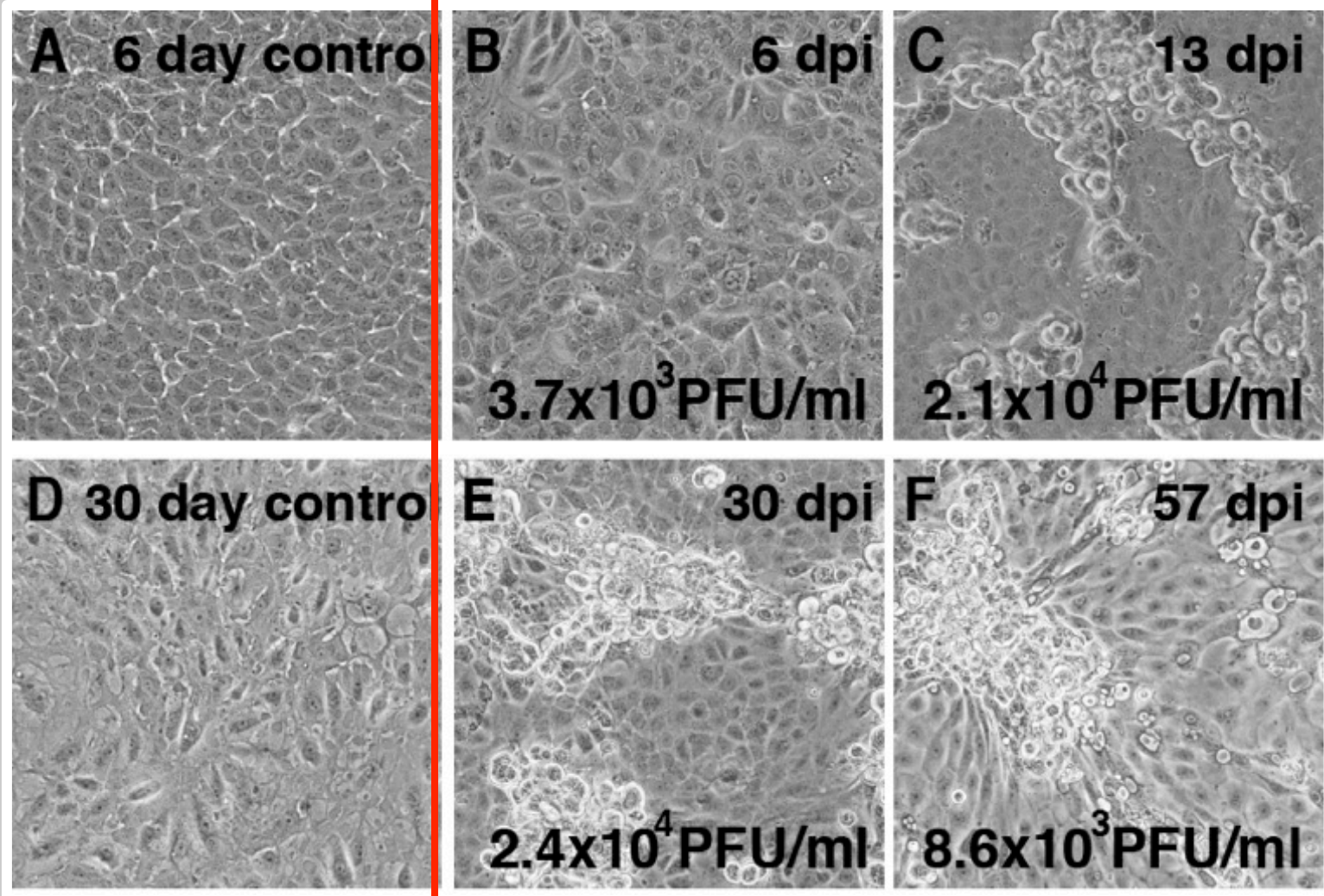


Immunosuppressive properties,
used for transplantation,
& grafts for reconstruction of ocular surface

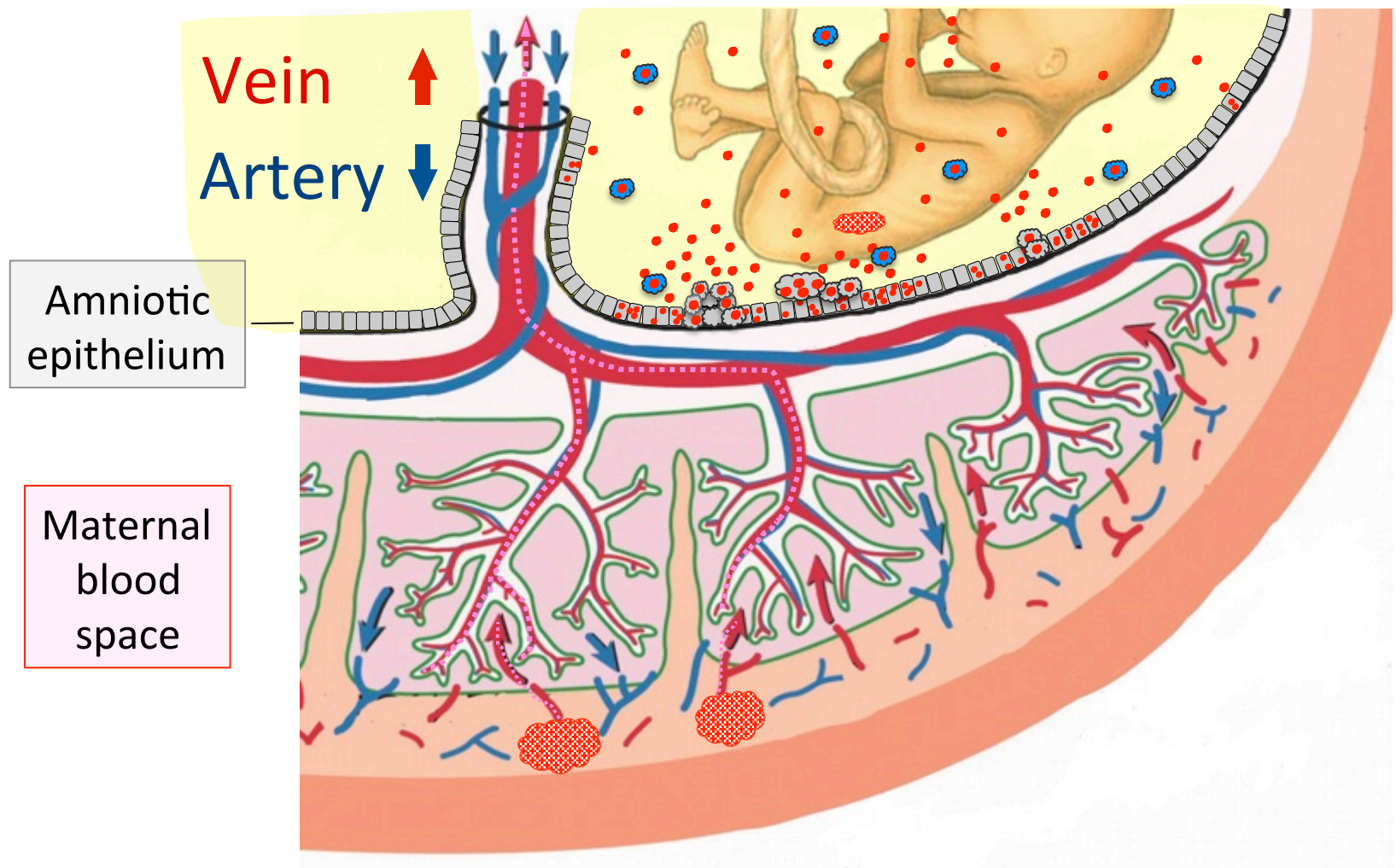
AECs infected with VR1814 fail to make Viral Assembly Compartment & progeny reduced



AECs become persistently infected, survive
& continuously release low virus titers



Amniotic epithelial infection amplifies viral load



Implications for prenatal diagnosis & outcome

Pereira Lab, University of California San Francisco

Analysis of Congenitally Infected Placentas & Serology

Matthew Petitt, June Fang-Hoover

Molecular Mechanisms of Infection in Trophoblast Progenitor Cells

Takako Tabata, Martin Zydek

Antibody Protection

Takako Tabata, Matthew Petitt, Mitsuru Tsuge, June Fang-Hoover, Martin Zydek

Susan Fisher Lab: Isolation of Human Trophoblast Progenitor Cells

Olga Genbacev, Mirhan Kapidzik

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