

Dr. Michael McMaster

Hosted By:

U.S. Department of Health and Human Services
National Institutes of Health

Trophoblast invasion in pregnancies complicated by trisomy 21: a spectrum of abnormalities

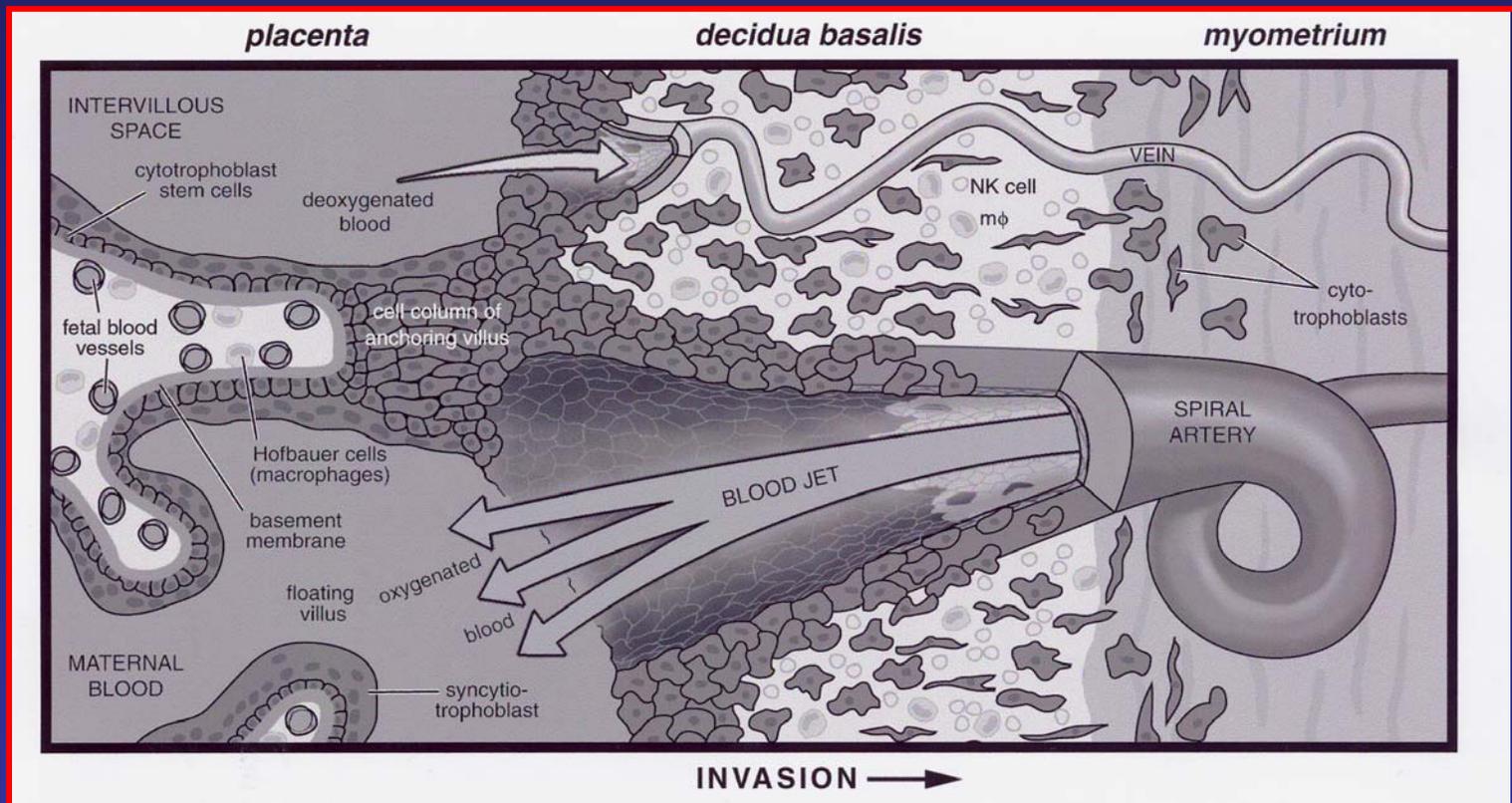
Michael McMaster, Alexi Wright and Susan Fisher
University of California San Francisco

NICHD workshop, "Role of Genomic Imprinting, Confined Placental Mosaicism and Uniparental Disomy in Fetal Growth and Beyond"

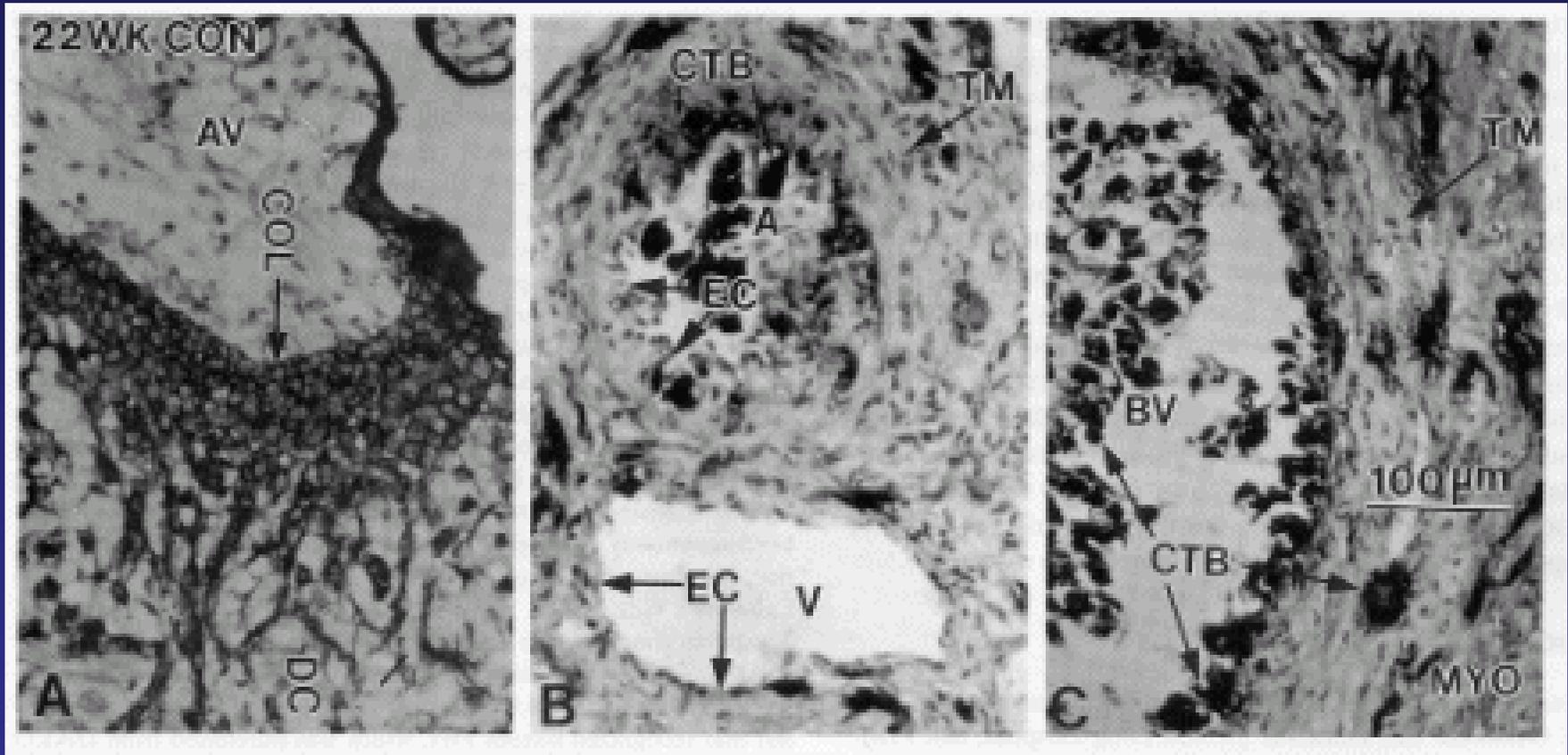
May 15-16, 2003

The placenta is composed of floating and anchoring villi

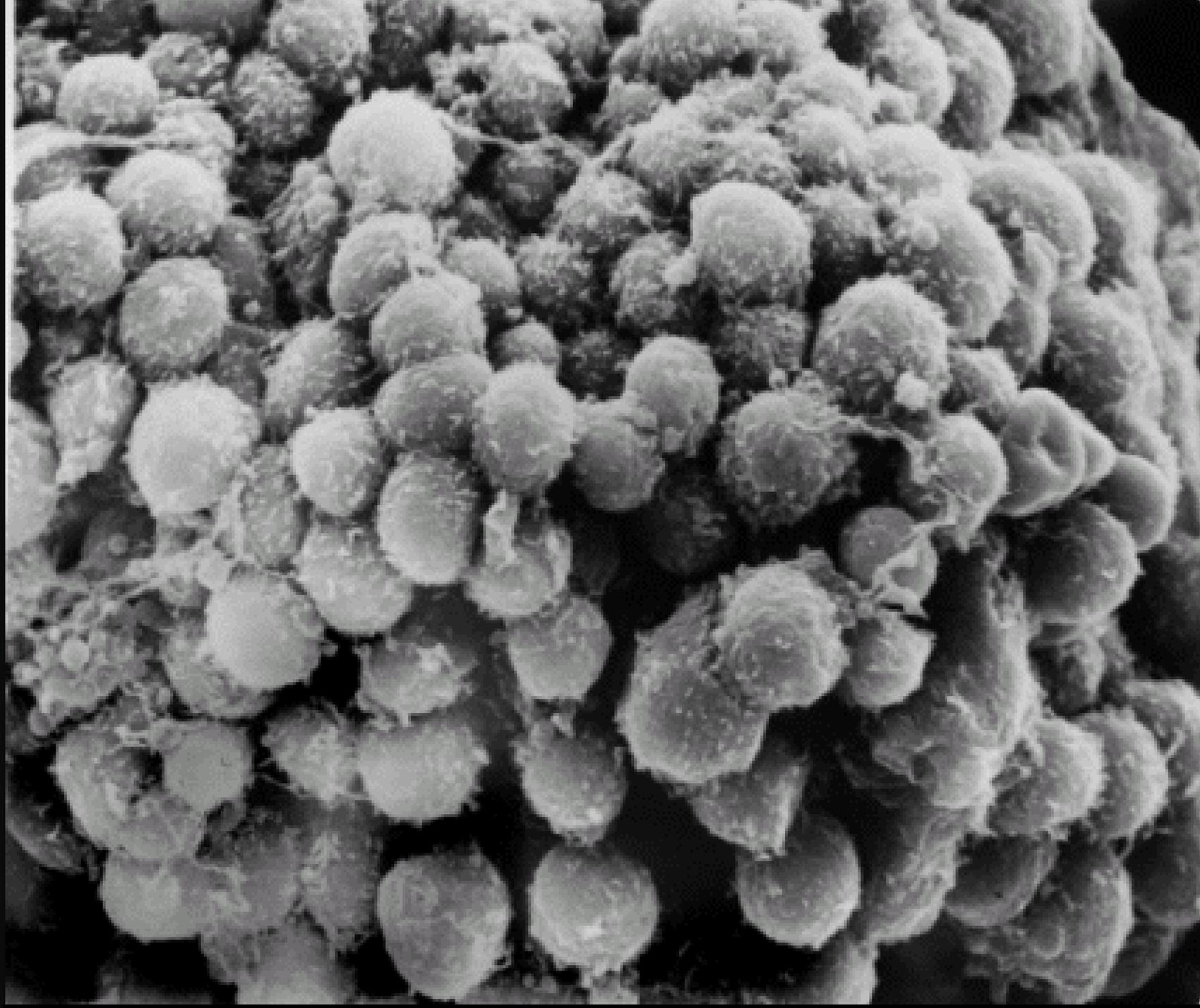
Villi- stromal core containing fetal blood vessels covered by a layer of cytotrophoblasts and syncytiotrophoblasts

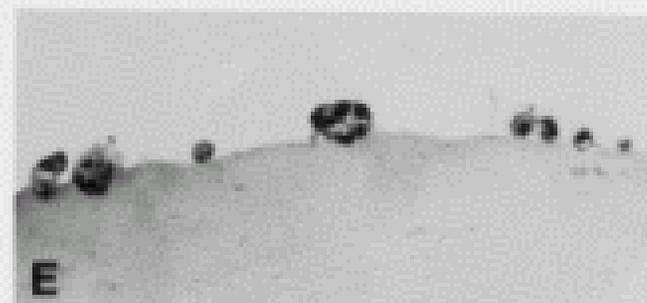
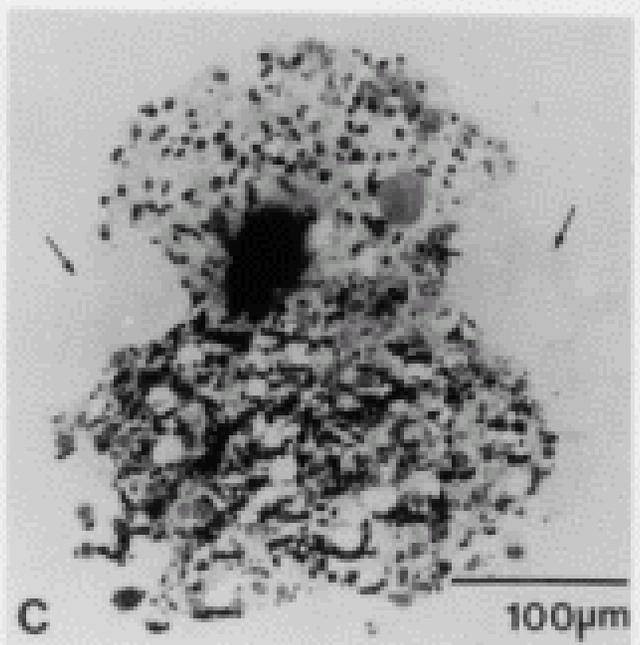
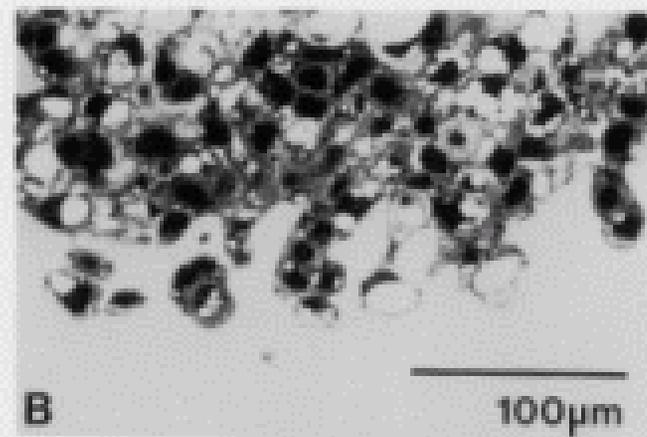
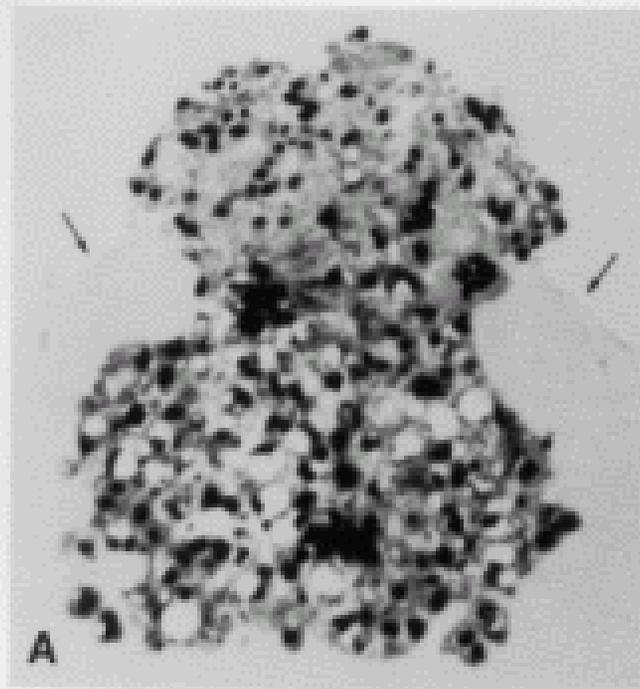


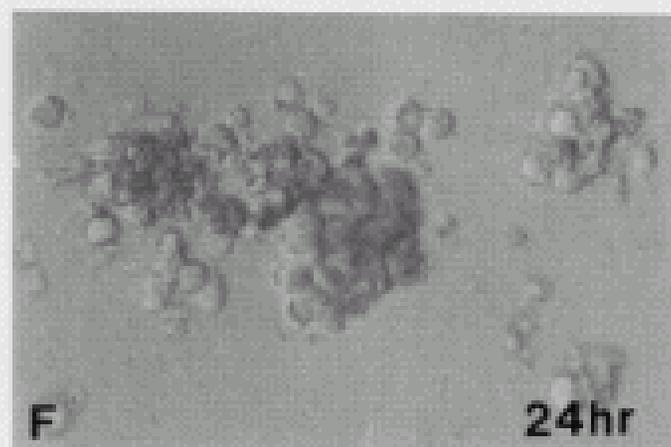
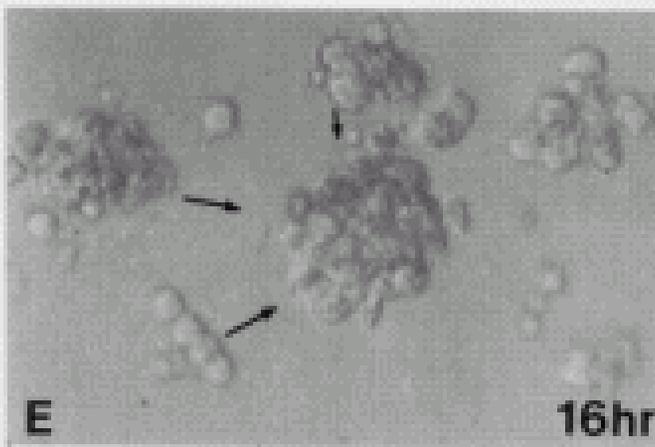
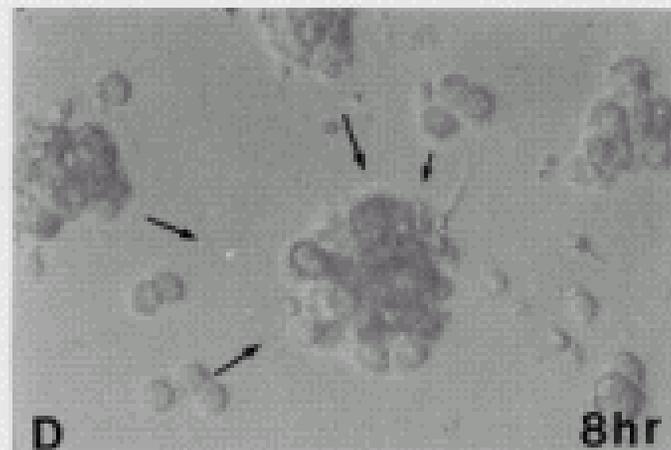
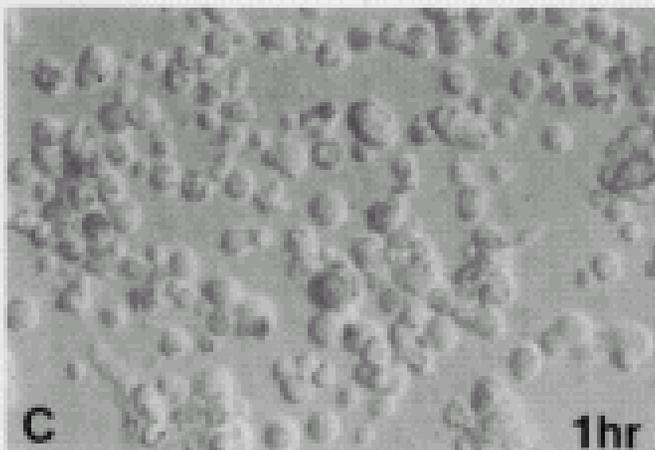
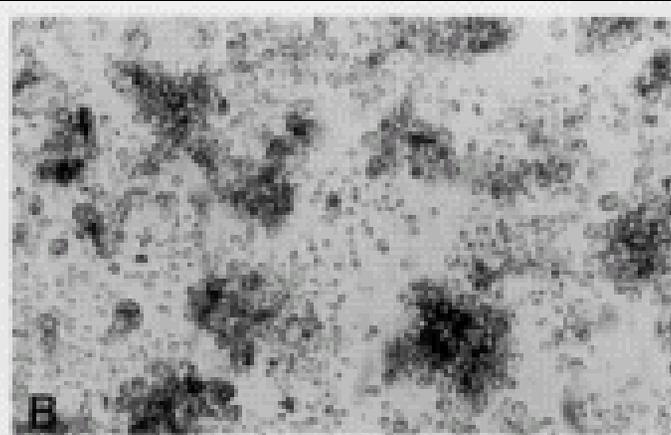
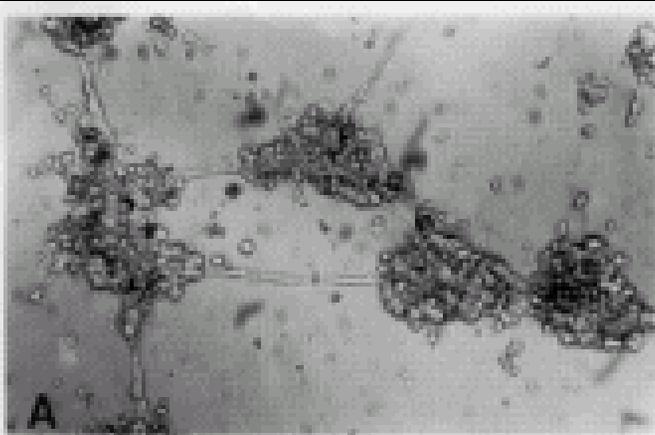
Human cytotrophoblasts line maternal blood vessels.



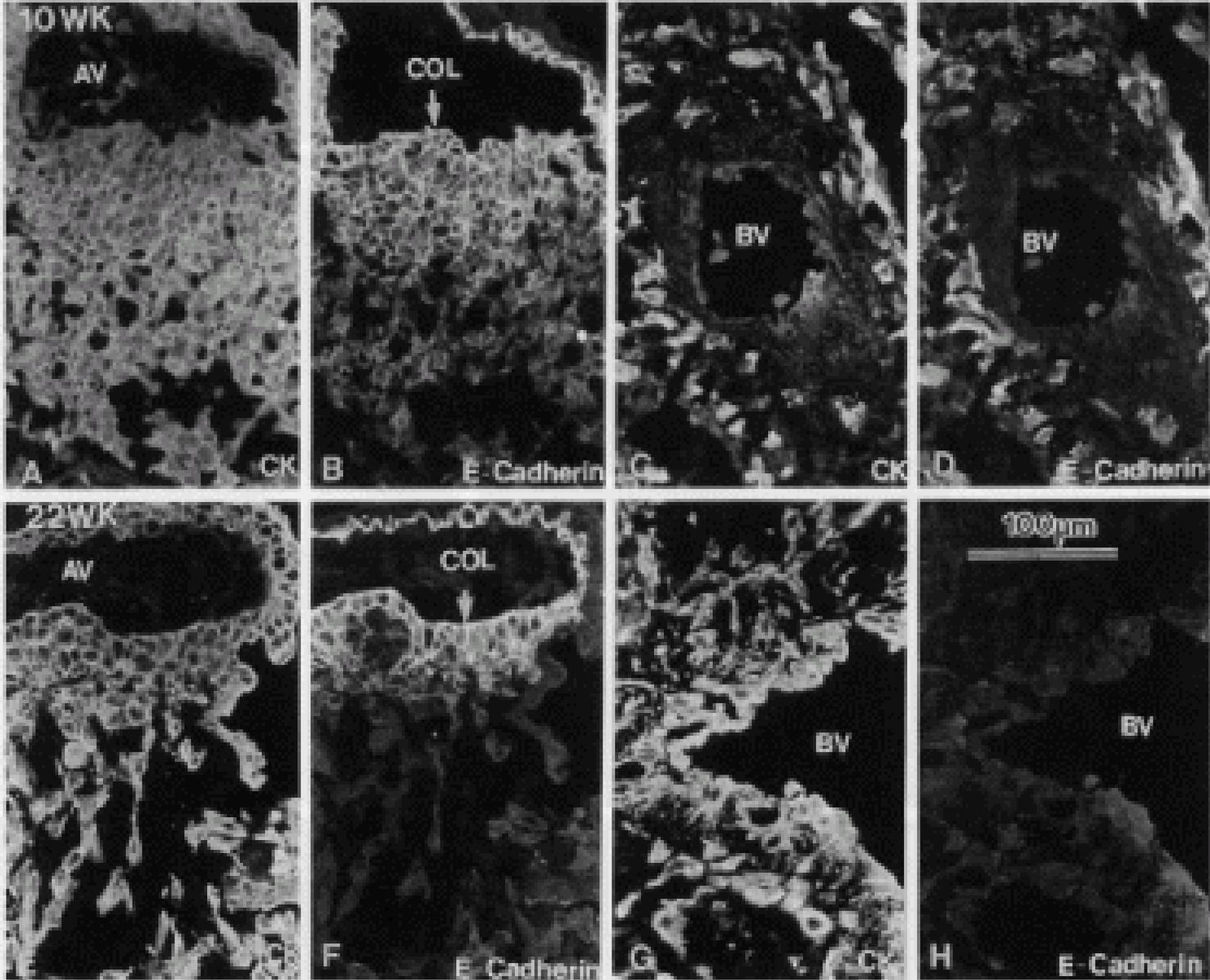




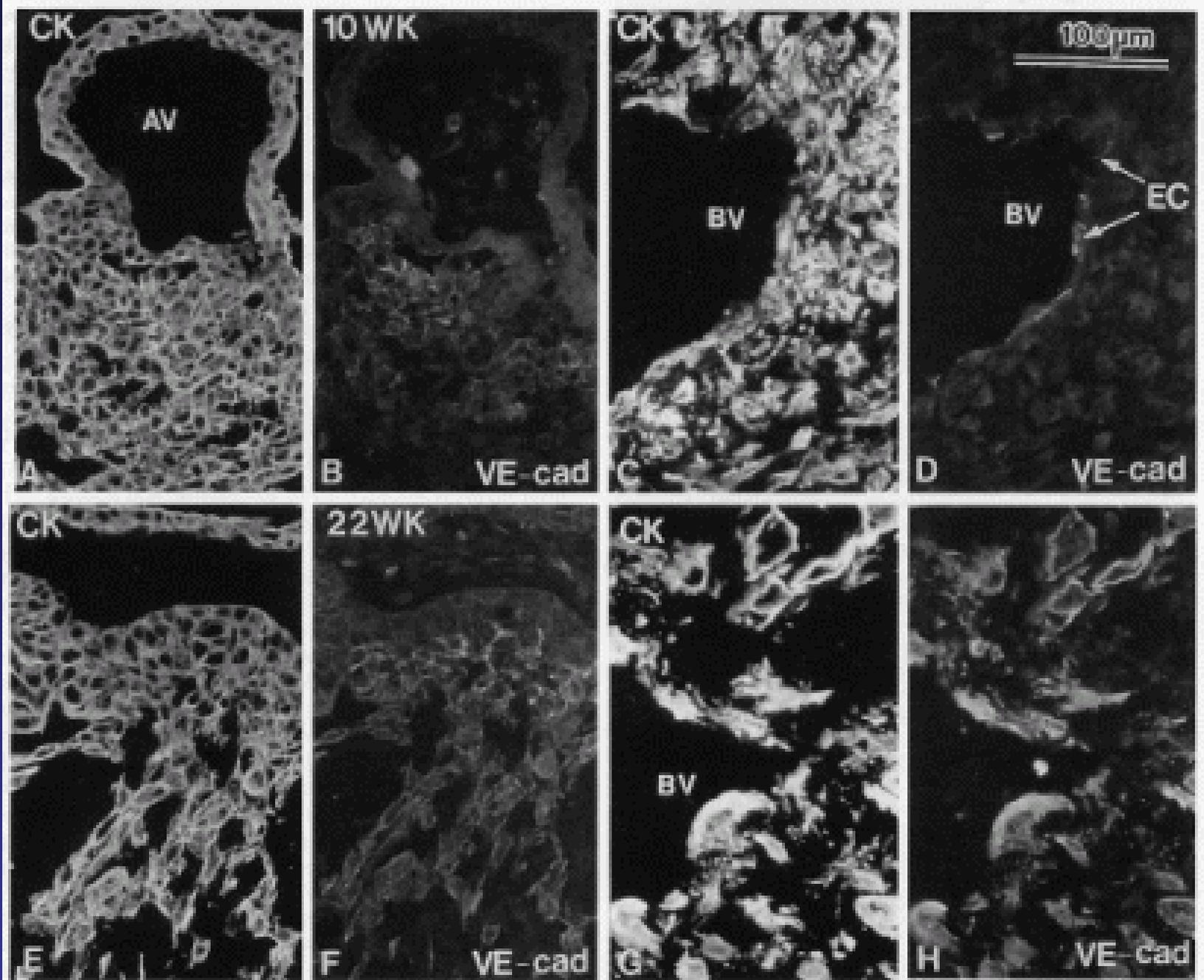




E-cadherin



VE-cadherin

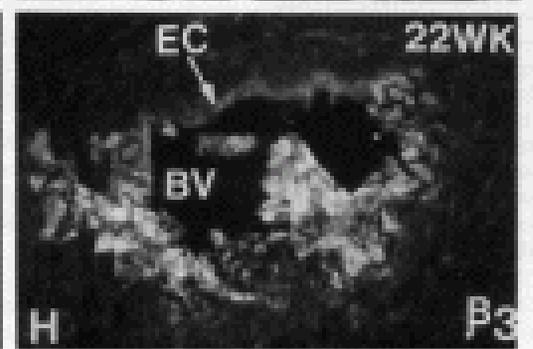
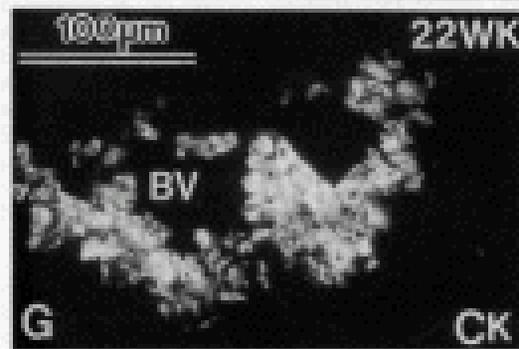
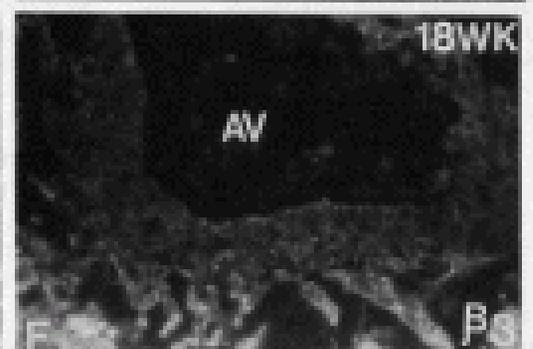
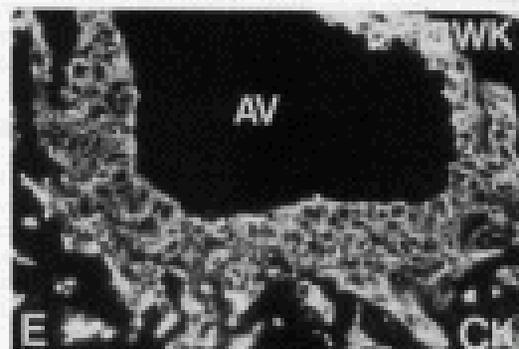
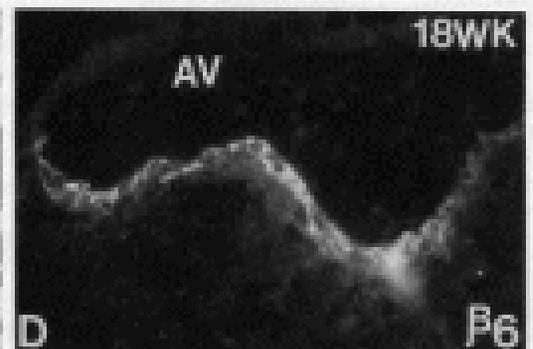
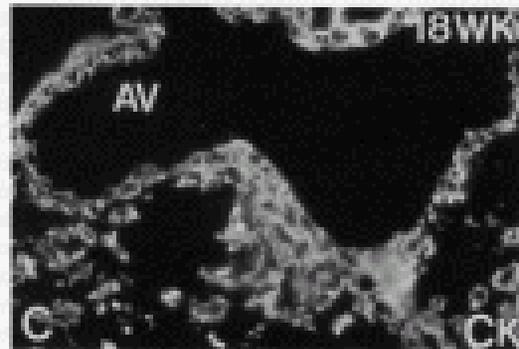
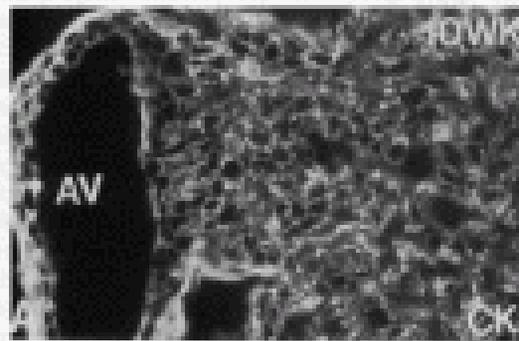


αV family
integrin switch

$\beta 5, \beta 6$



$\beta 3$



- **What is the influence of genotype on the differentiation of interstitial and endovascular cytotrophoblasts?**
 - **Normal human placentas contain aneuploid populations of cells and exhibit genetic mosaicism.**
 - **Very little is known about the genotype of human invasive cytotrophoblasts.**
 - **The effects of karyotypic abnormalities on cytotrophoblasts' phenotype are largely unknown.**
 - **What is the interplay of genotype and phenotype during human placental development?**

Down Syndrome

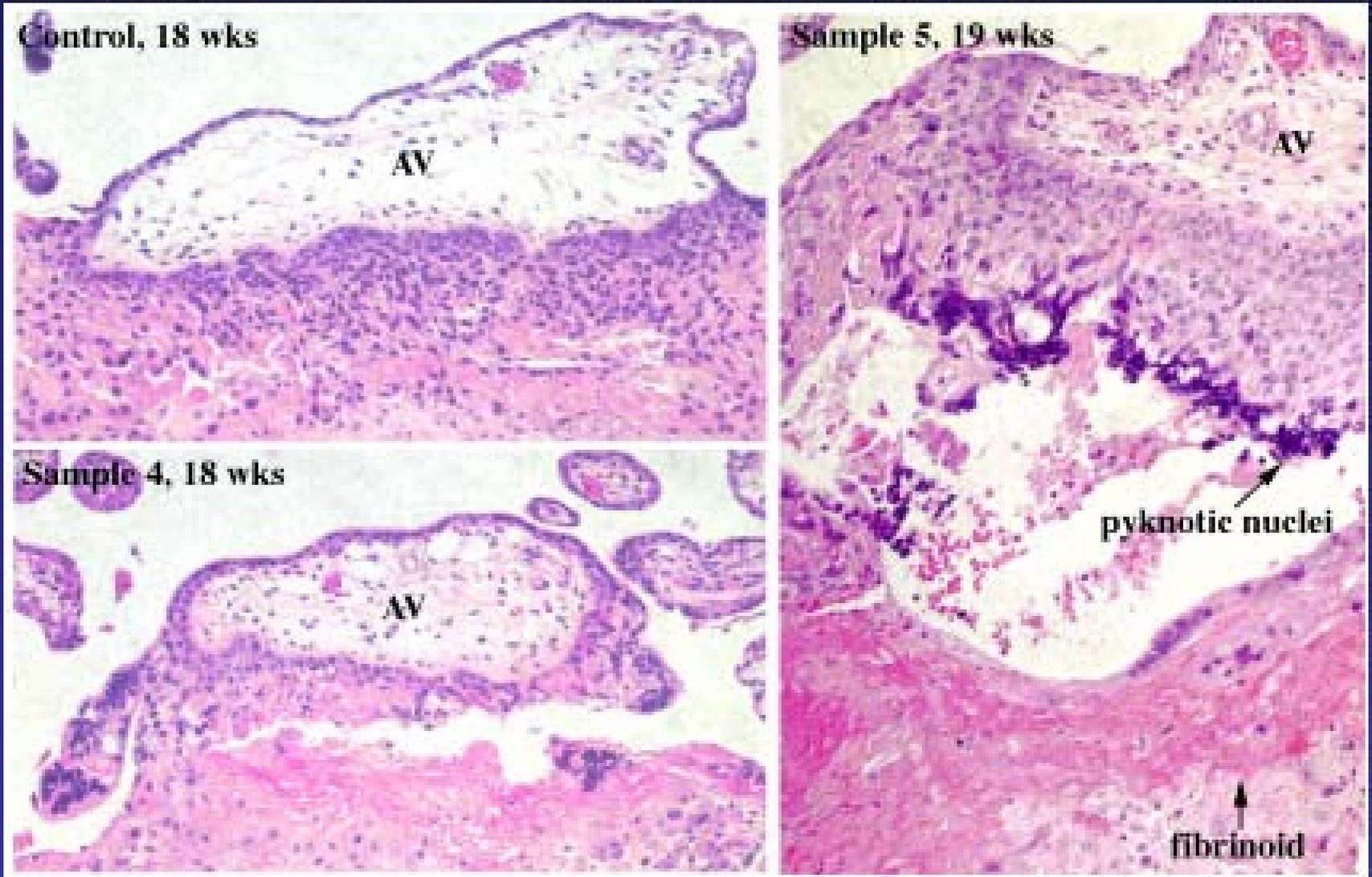


Photo: Down Syndrome WWW page

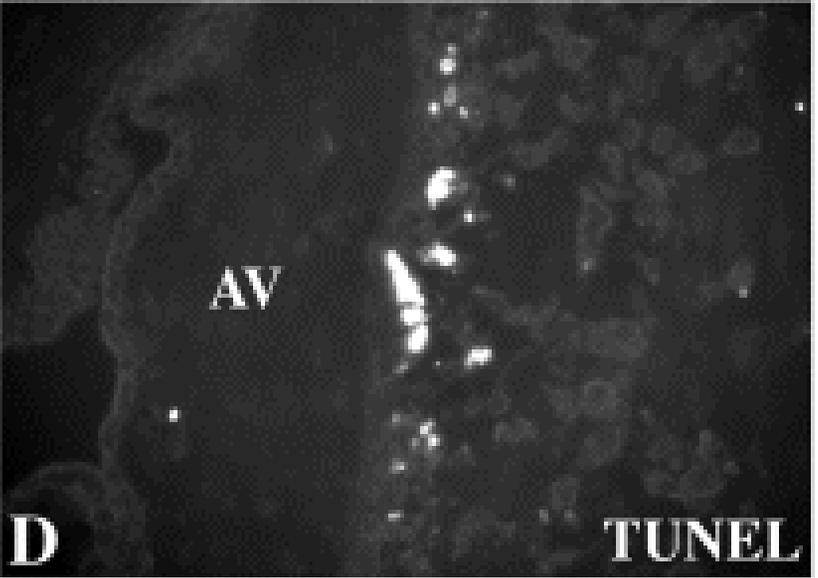
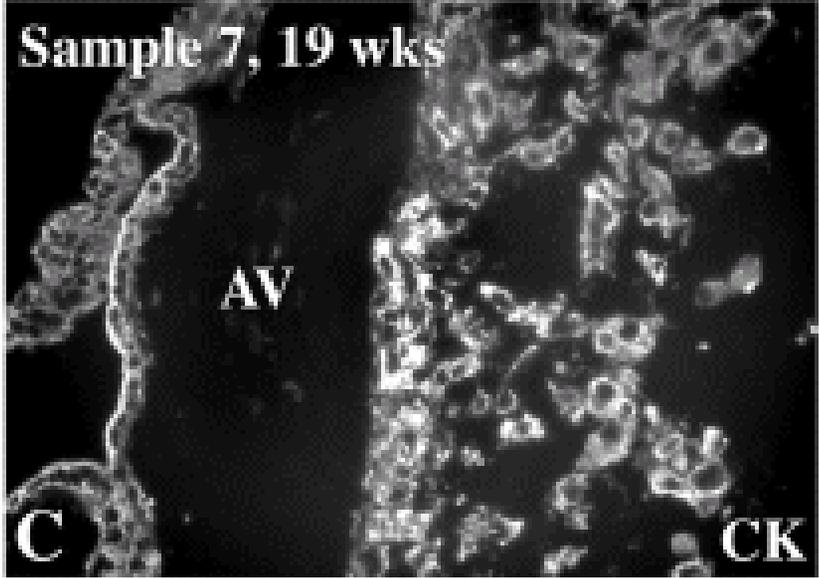
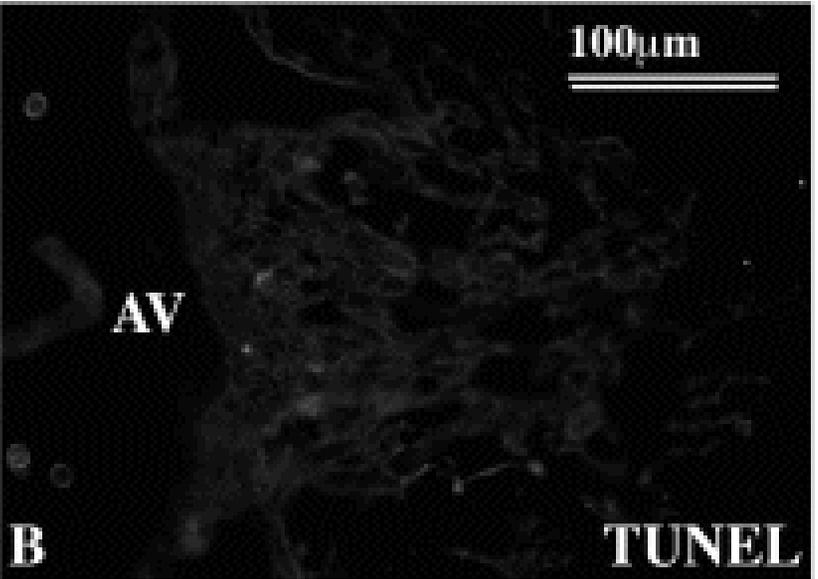
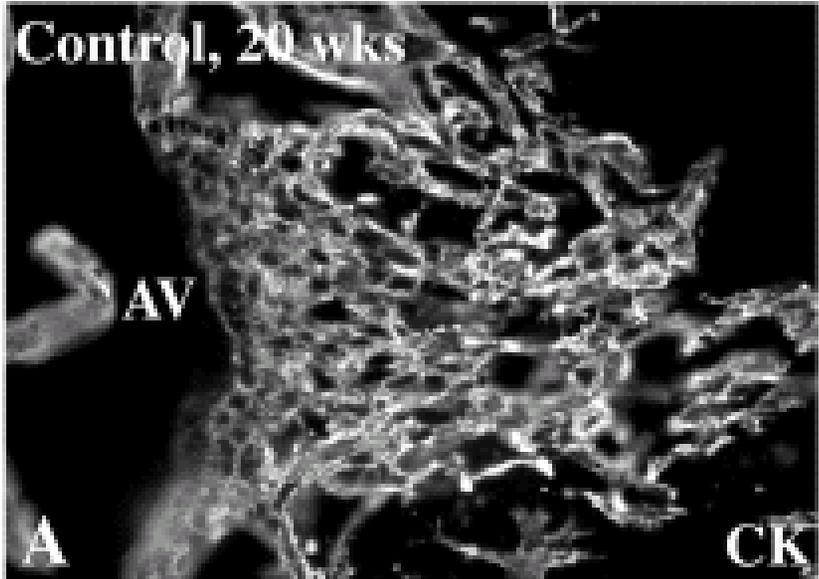
- **30% spontaneous abortion rate in utero.**
- **Variable phenotype.**
- **Mental retardation and hypotonia usually present.**
- **May also include congenital heart disease, GI anomalies, others.**

- Established a tissue bank of genetically abnormal placentas including trisomy 21 cases.
- Analyzed cytotrophoblasts by FISH with probes for chromosomes X, Y, and 21.
- Histology with H&E
- Apoptosis with TUNEL
- Immunolocalization to assess expression of stage-specific antigens during CTB differentiation/invasion *in situ* and *in vitro* (trisomy 21 placentas vs. normal controls).

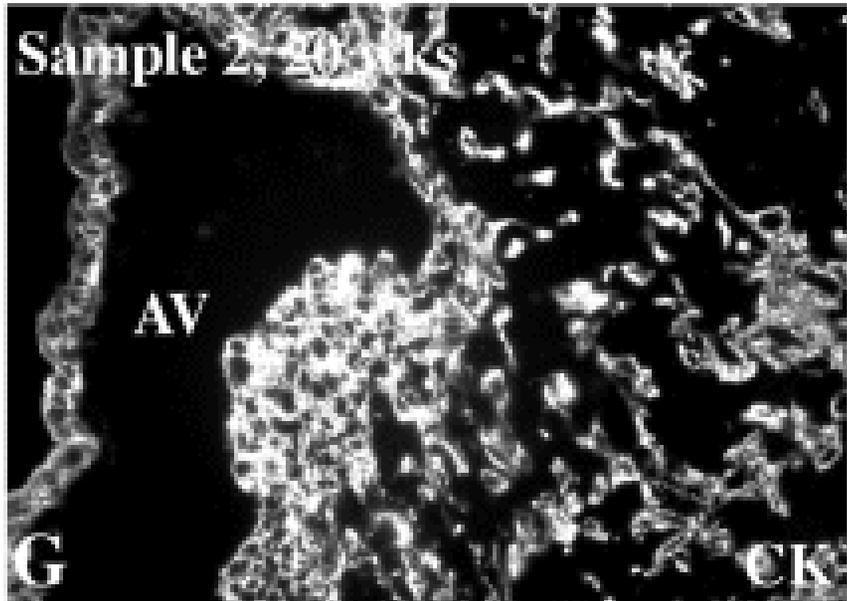
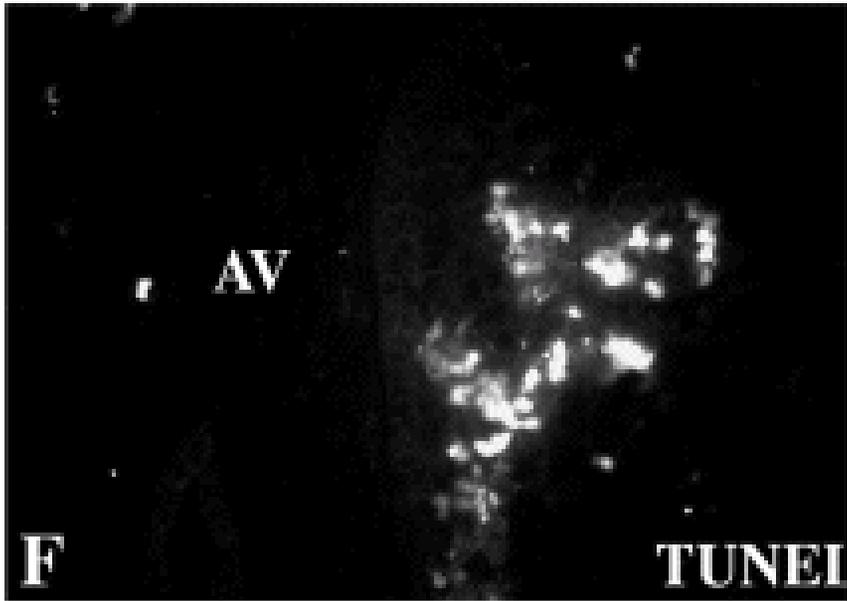
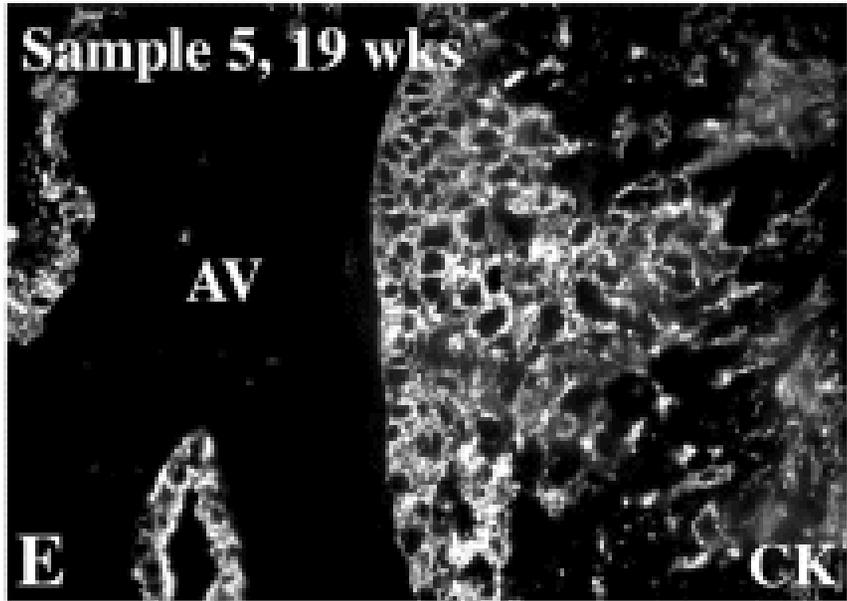
Histopathology of trisomy 21 placentas at the maternal-fetal interface



Apoptosis of interstitial CTBs in trisomy 21 placentas



Apoptosis of interstitial CTBs in trisomy 21 placentas



CTB apoptosis in trisomy 21 placentas

Sample	Karyotype	Degree of CTB apoptosis by compartment		
		Floating Villi	Cell Column	Uterine Wall
1	46,XX	-	+/25%	-
2	46,XY/47,XY,21	+	2+/50%	3+
3	47,XX,21	1+	2+/75%	1+
4	47,XX,21	1+	2+/50%	1+
5	47,XX,21	1+	3+/100%	1+
6	47,XY,21	1+	3+/100%	1+

Degree of apoptosis/Percent of compartment affected

- = <1%

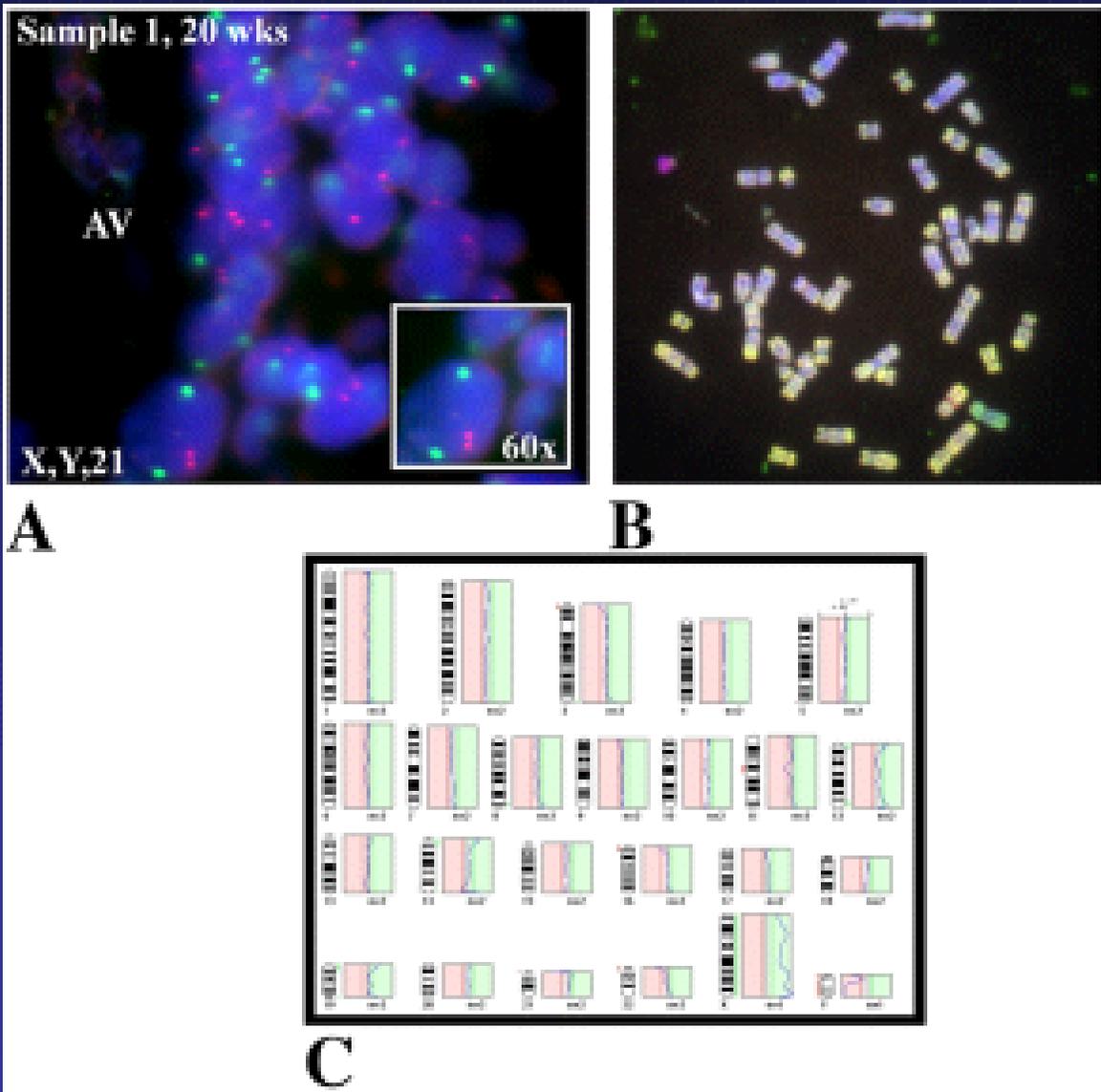
+ = 1-5%

1+ = 5-15%

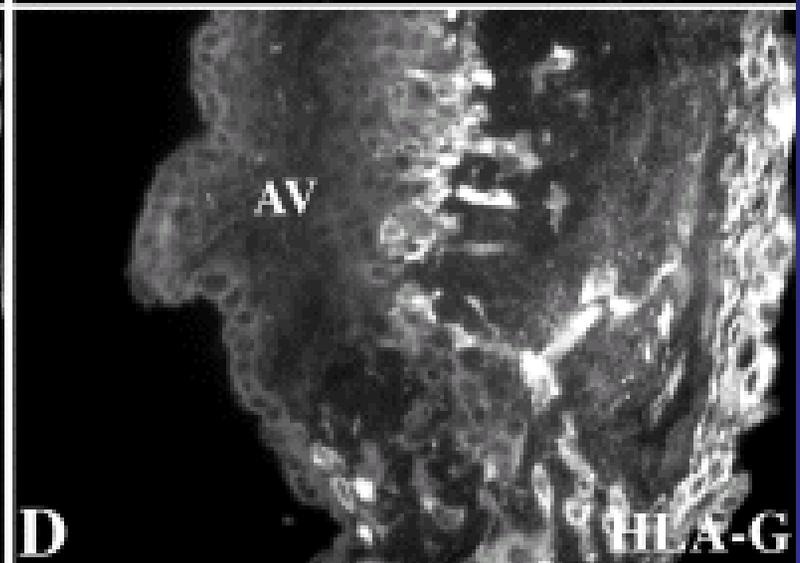
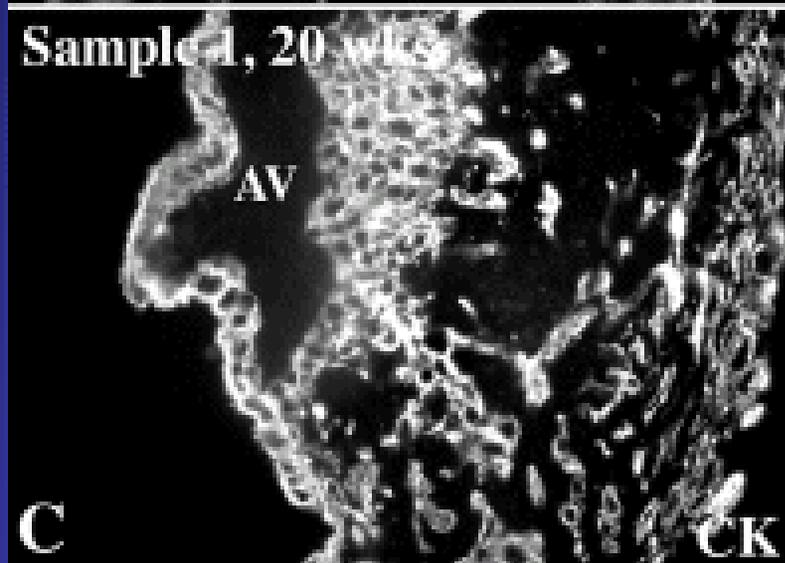
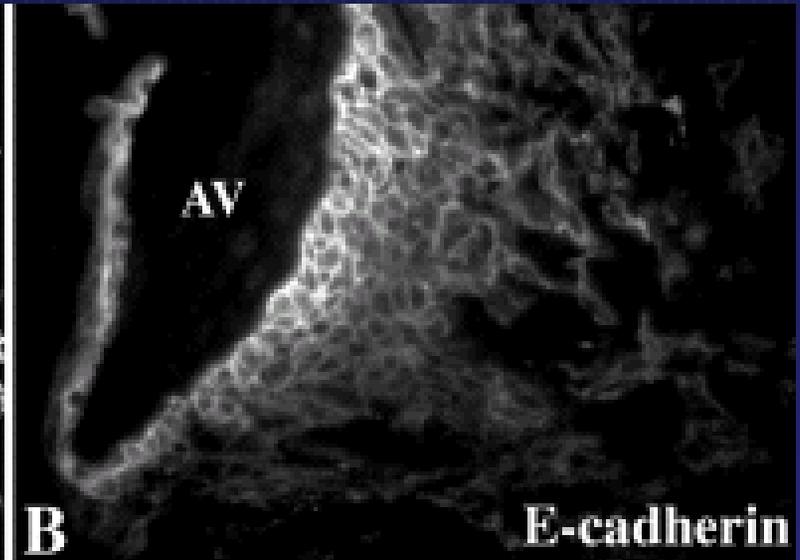
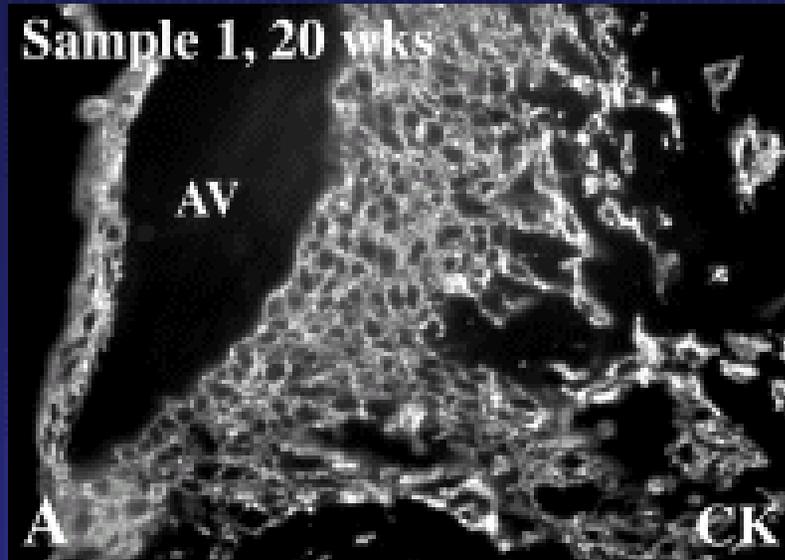
2+ = 15-30%

3+ = 30-50%

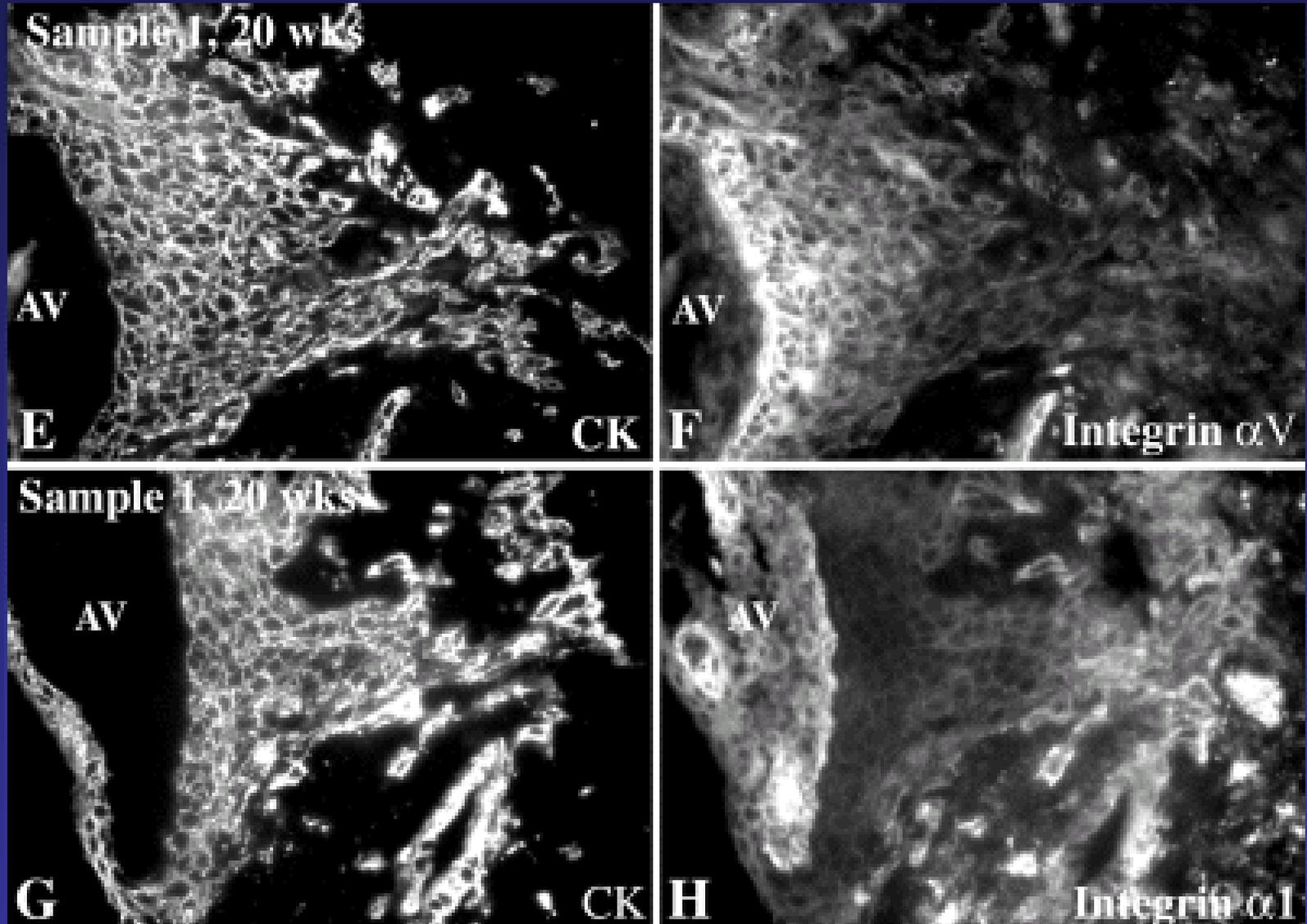
Sample 1: 46,XX karyotype from trisomy 21 placenta



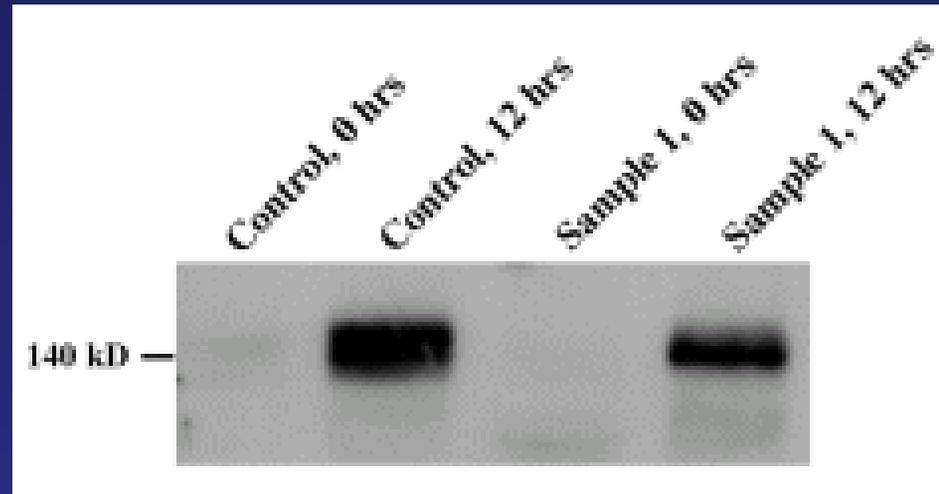
Expression of stage-specific antigens in 46XX tissue from a 20 week trisomy 21 placenta



Expression of stage-specific antigens in 46XX tissue from a 20 week trisomy 21 placenta



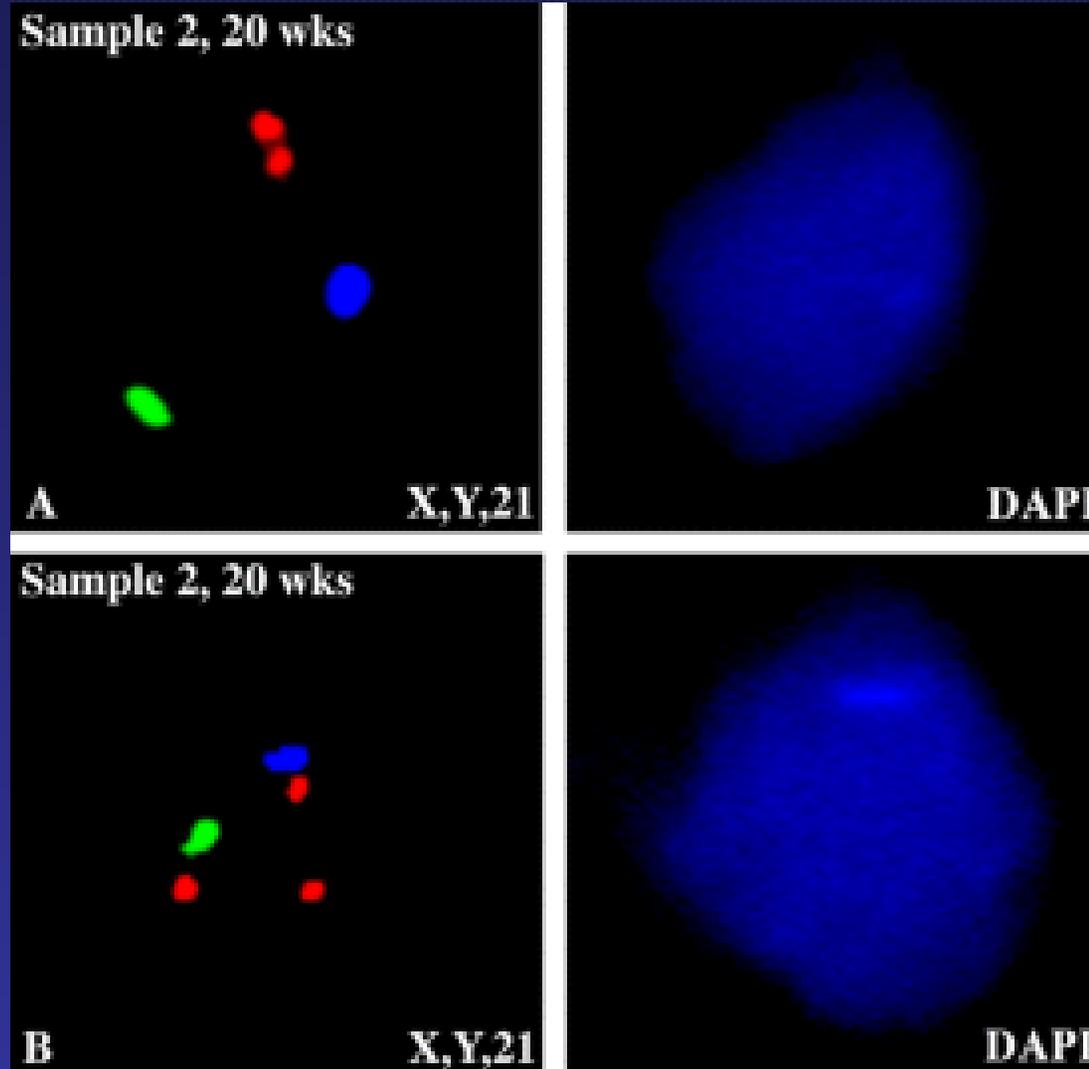
Expression of VE-cadherin in 46XX CTBs differentiating *in vitro*



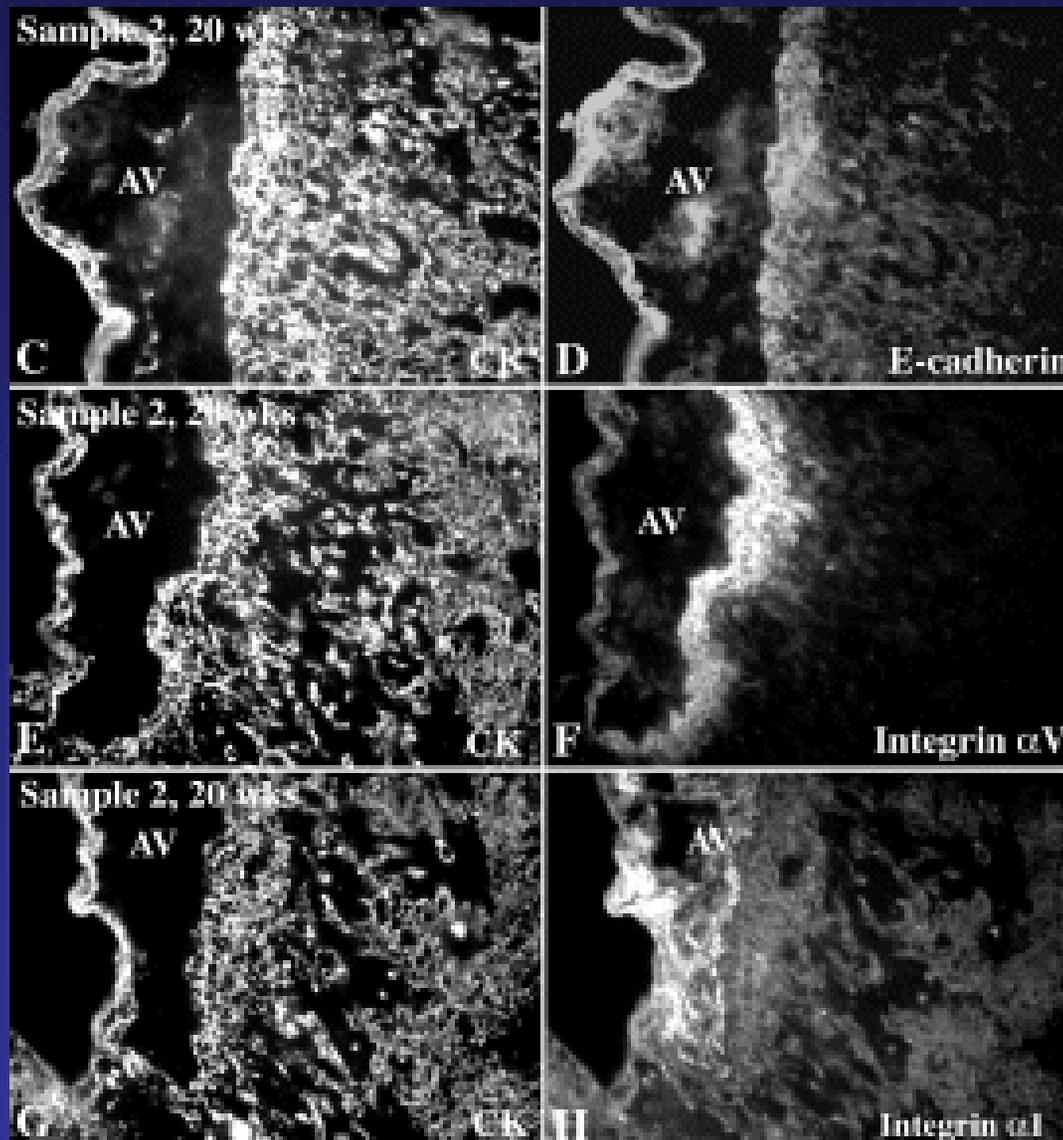
- 46,XX CTBs isolated from a trisomy 21 placenta express 40% less VE-cadherin *in vitro* compared to controls

Karyotypic analysis of sample 2: 46,XY/47,XY,21 mosaicism

Green: X
Blue: Y
Red: 21



Differentiation markers in sample 2 CTBs (47,XY,21)

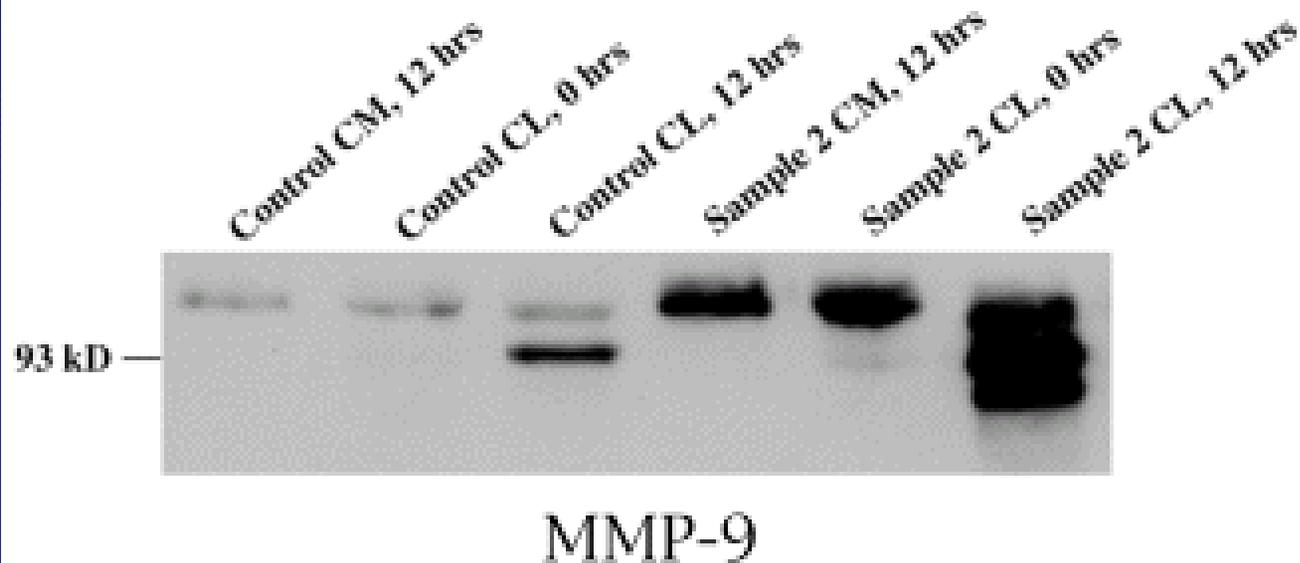
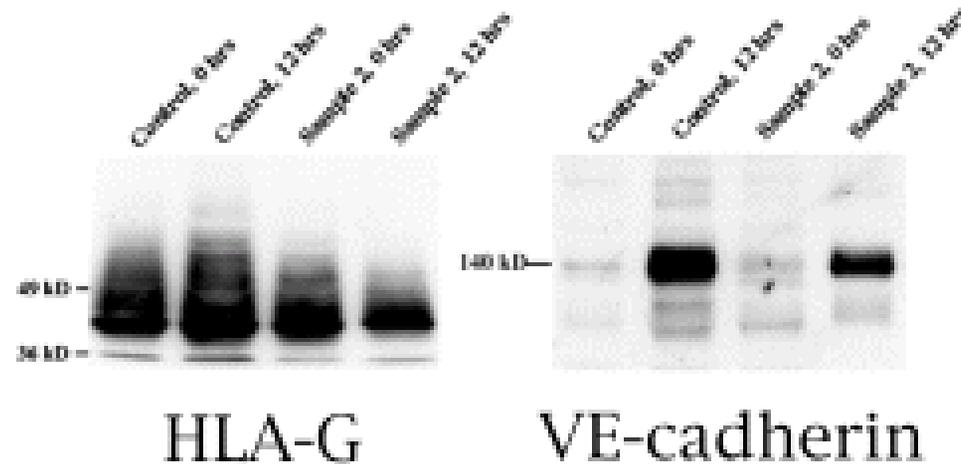


+/-

+

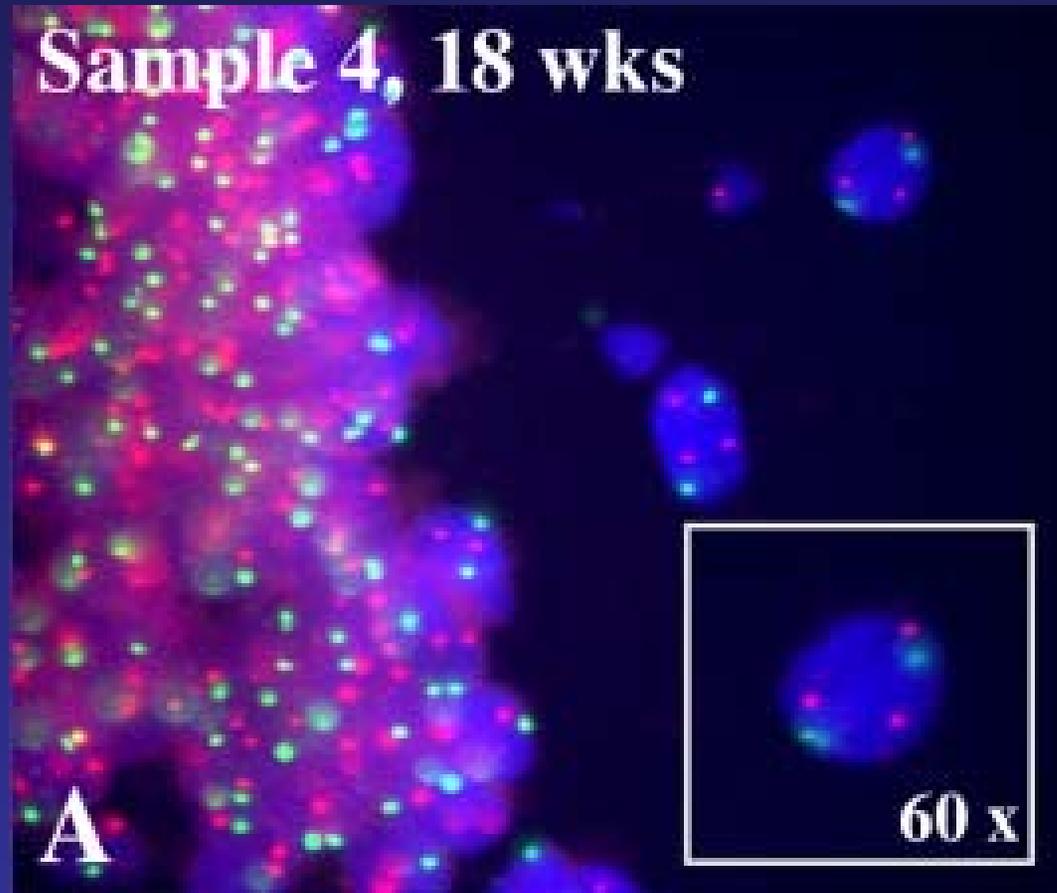
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Expression of markers by 46,XY/47,XY,21 cytotrophoblasts differentiating in vitro

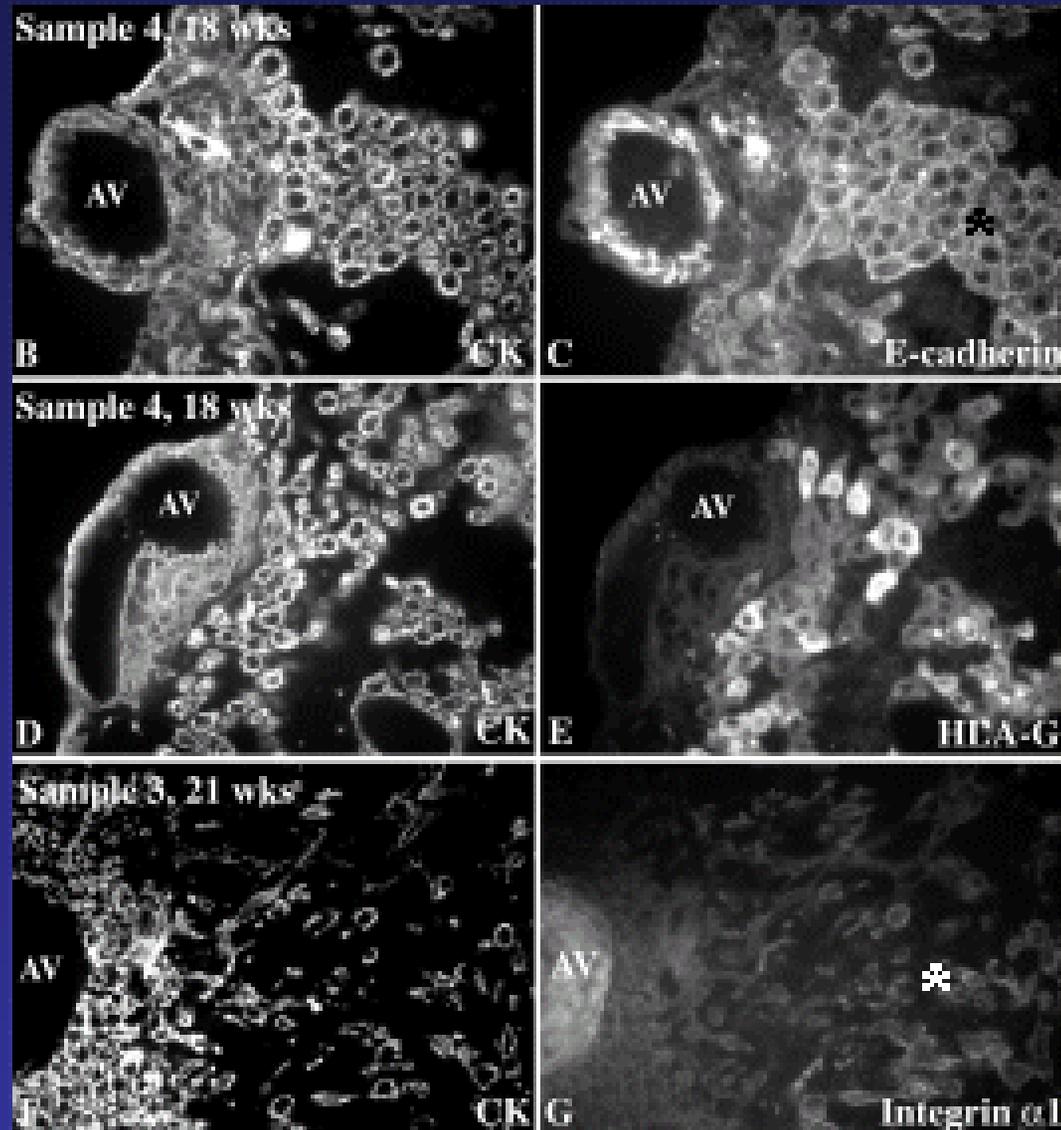


Sample 4: 47,XX,21

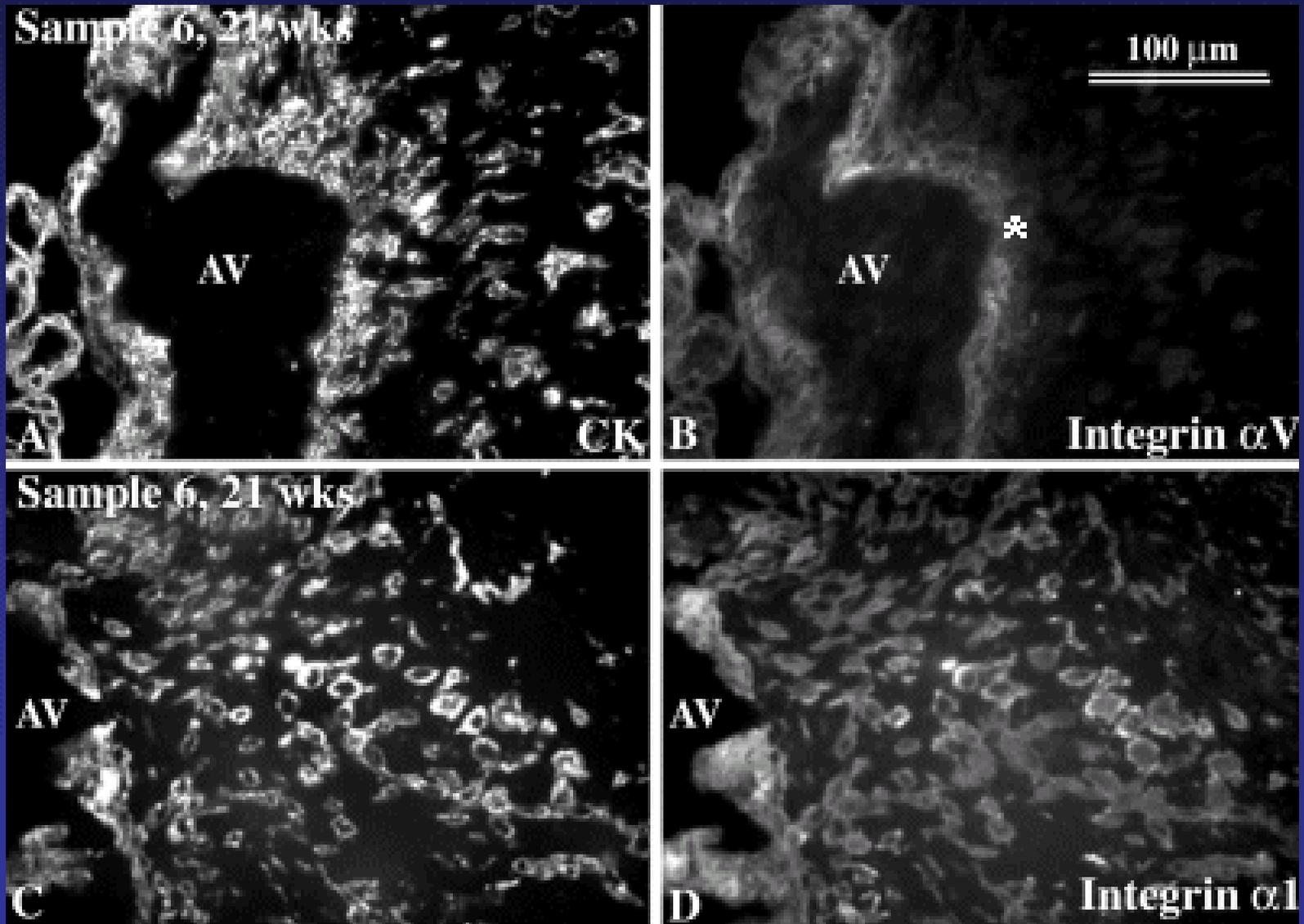
Green: X
Blue: Y
Red: 21



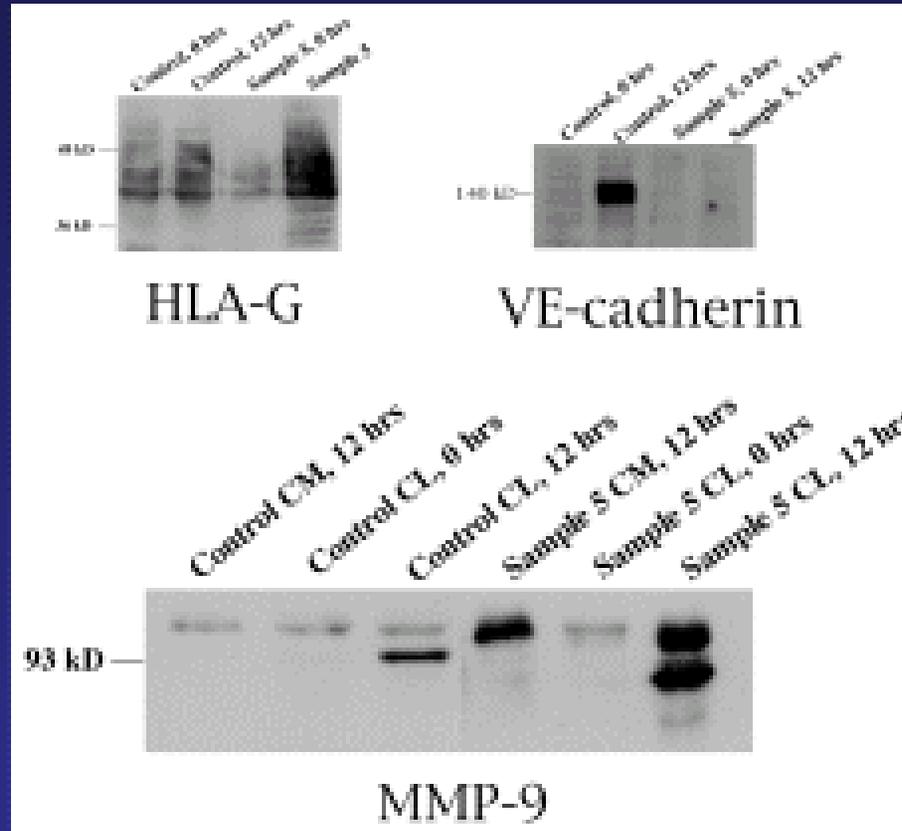
Differentiation markers in sample 4 CTBs (47,XX,21)



Differentiation markers in sample 6 CTBs (47,XY,21)



Expression of markers by 47,XX,21 cytotrophoblasts differentiating *in vitro*



- CTBs from sample 5 express higher levels of HLA-G and MMP-9 than controls, but fail to differentiate.

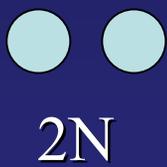
Conclusions

- Trisomy 21 is associated with a range of developmental defects in CTB interstitial and endovascular invasion.
- Trisomy 21 is associated with a variable amount of apoptosis among differentiating/invading CTBs.
- Placentas from pregnancies complicated by trisomy 21 exhibit significant mosaicism in the extraembryonic lineages.
- CTB developmental defects may be severe or mild, mirroring the variable phenotype of Down Syndrome.

- Retinoblastoma knockout mice (1992)
 - Embryonic lethal (E 13.5-15.5)
 - Abnormal neurogenesis, erythropoiesis
 - Ectopic cell cycle entry and apoptosis in CNS, PNS, liver
- What about the placentas in these animals?
 - Drastic disorganization of labyrinth layer
(Wu et al., Nature, 2003)
- Tetraploid rescue of Rb^{-/-} mice
 - De Bruin et al., PNAS, 2003

Tetraploid aggregation

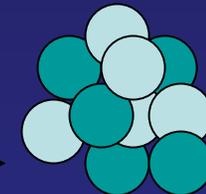
$Rb^{+/+} \times Rb^{+/+}$



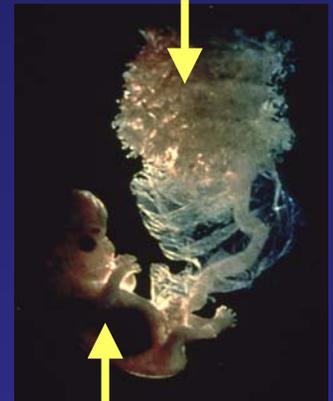
Fusion



$Rb^{+/-} \times Rb^{+/-}$



Normal (4N)



Rb null

Phenotype of $Rb^{-/-}$ mice with tetraploid rescue

- Embryos survive
- Neurological and erythroid abnormalities thought to be responsible for lethality absent
- “Rb function in extraembryonic lineages plays an important role in the survival of neuronal cells and differentiation of erythroid lineage..” De Bruin et al., PNAS, 2003

- Failures of the development/function of extraembryonic tissues can have profound effects on embryonic development via non-cell autonomous mechanisms. In the case of trisomy 21, the degree of mosaicism in the placenta and the consequent effects on CTB differentiation may determine the severity of the phenotype on a continuum from mild to severe or result in spontaneous abortion.