# Mini-placentas: Promising tools for studying early pregnancy and its complications

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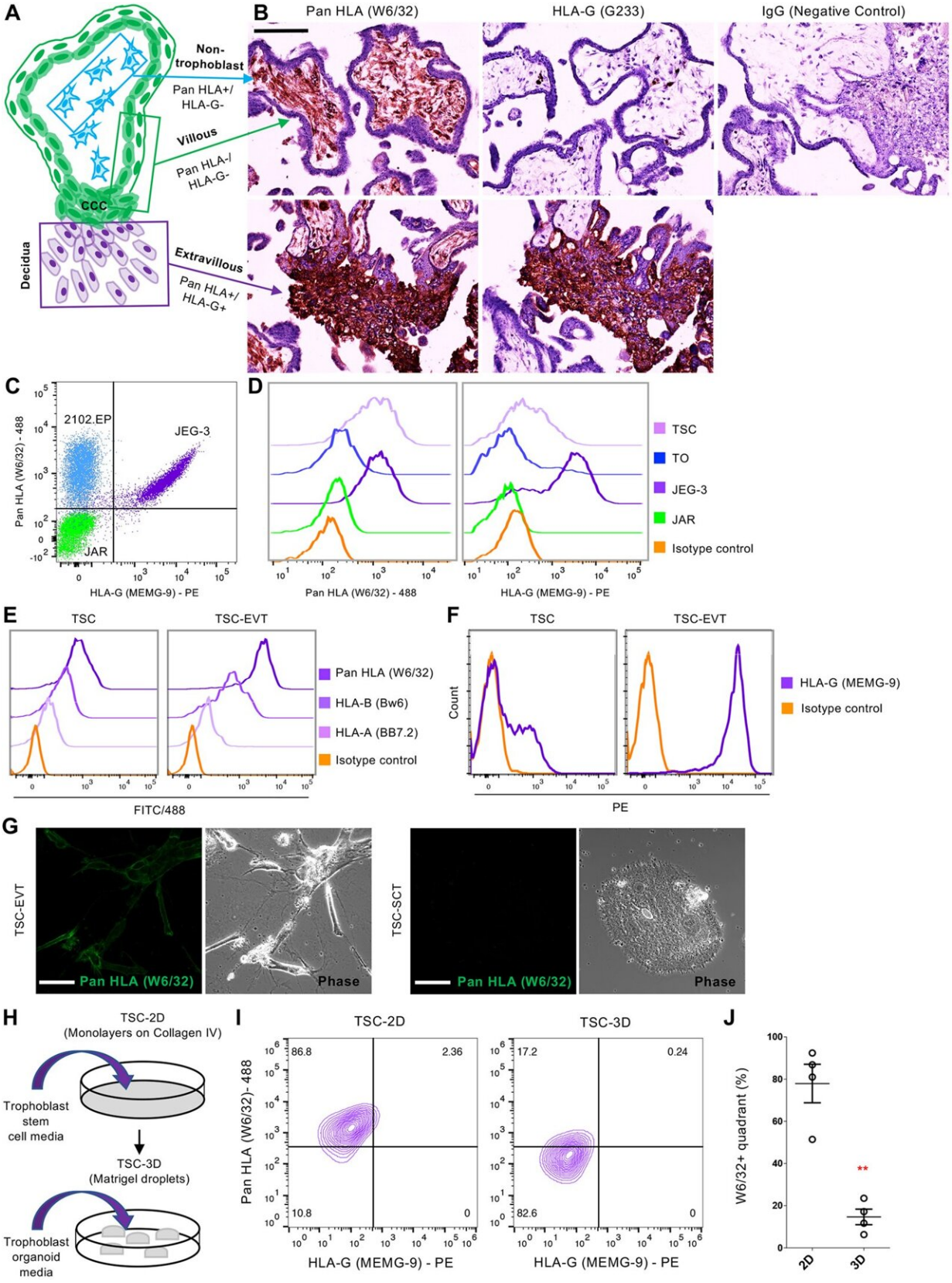
A
Non-trophoblast
Pan HLA+/ HLA-G-
Villous
Pan HLA-/ HLA-G-
CCC
Decidua
Extravillous
Pan HLA+/ HLA-G+
B
Pan HLA (W6/32)
HLA-G (G233)
IgG (Negative Control)
C
2102.EP
JEG-3
JAR
Pan HLA (W6/32) - 488
HLA-G (MEMG-9) - PE
D
TSC
TO
JEG-3
JAR
Isotype control
Pan HLA (W6/32) - 488
HLA-G (MEMG-9) - PE
E
TSC
TSC-EVT
Pan HLA (W6/32)
HLA-B (Bw6)
HLA-A (BB7.2)
Isotype control
FITC/488
F
TSC
TSC-EVT
Count
HLA-G (MEMG-9)
Isotype control
PE
G
TSC-EVT
Pan HLA (W6/32)
Phase
TSC-SCT
Pan HLA (W6/32)
Phase
H
TSC-2D
(Monolayers on Collagen IV)
Trophoblast stem cell media
TSC-3D
(Matrigel droplets)
Trophoblast organoid media
I
TSC-2D
TSC-3D
86.8
2.36
10.8
0
17.2
0.24
82.6
0
Pan HLA (W6/32)- 488
HLA-G (MEMG-9) - PE
J
W6/32+ quadrant (%)
**
2D
3D

HLA class I expression in the human placenta and in vitro models of human trophoblast. (A) Illustration of a first-trimester anchoring villus. HLA-null villous cells [SCT, VCT and the base of the cell columns (CCC)] are in green; HLA-C, E and G+ EVT are in purple; and HLA-A+, B+, C+ and HLA-G− nontrophoblast cells of the villous core are in blue. (B) Immunohistochemical staining for HLA class I molecules on acetone-fixed first-trimester placental sections. Here, the pan-class I antibody W6/32 (binds all HLA class I molecules) stains the villous core (nontrophoblast) and the EVT in the cell columns but not VCT or SCT. Staining with G233, specific for HLA-G, increases as cells move away from the villi into the cell column, whereas the entire villus remains negative. (C) HLA profile (using W6/32 and MEMG-9, an HLA-G-specific antibody) of the cell lines used as controls for HLA expression by FACS: JEG-3 (control for HLA profile of extravillous trophoblast); JAR (control for villous trophoblast); and 2102Ep (nontrophoblast control). (D) FACS analysis of W6/32 and MEMG-9 in JAR, JEG-3, TOs and TSCs. TOs have the profile of villous trophoblast (W6/32−/MEMG-9−; n=4), whereas TSCs have neither villous nor extravillous profiles [W6/32+/MEMG-9−; n=5]. (E) FACS analysis of TSCs grown under proliferative conditions and when differentiated to EVT (n=2). Allele-specific antibodies were used to assess HLA-A (BB7.2) and HLA-B (Bw6) expression in a HLA-genotyped TSC line (BTS5). Undifferentiated TSCs express HLA-A and HLA-B, which is maintained after EVT differentiation. (F) FACS analysis demonstrating upregulation of HLA-G in TSCs following EVT differentiation (n=3). (G) Live staining for W6/32-Alexa488 on TSCs differentiated to either EVT or SCT (n=2). Distinct membrane staining is seen when differentiated to EVT and is absent in SCT. (H) Experimental set-up of the different trophoblast culture conditions. (I) FACS analysis of TSCs grown in 2D versus 3D (passaged more than six times in 3D, n=4) with W6/32 and MEMG-9. The number of cells that are W6/32+/MEMG-9− significantly decreases when cultured in 3D. (J) Quantification of the percentage of cells in the W6/32+/MEMG-9− quadrant under 2D or 3D conditions (data are mean±s.e.m., paired two-tailed Student's t-test, **P=0.0019). Scale bars: 50 µm in G; 150 µm in B. Credit: DOI: 10.1242/dev.199749

Despite its crucial role in healthy pregnancies, the placenta is one of the

least understood organs in the human body. In a new study, Margherita Yayoi Turco and her colleagues compared the two main experimental models of the human placenta. The findings suggest that 3D clusters of placental cells called trophoblast organoids are best suited for investigating interactions between the mother and the fetus, hormone secretion or pathogens that infect the fetus in the womb. Understanding such processes could reveal new clues to pregnancy complications.

About four to five days after fertilization, the human embryo is a hollow ball of cells surrounding an inner cell mass. The inner cells grow into the fetus, while the outer layer of cells, called trophectoderm, gives rise to the trophoblast, the main cell type of the placenta. As the placenta starts to form, some trophoblast stem cells differentiate into extravillous trophoblast, which infiltrate the uterus and open up maternal blood vessels to provide blood supply to the fetus. Stem cells also differentiate to form the syncytiotrophoblast, a cell layer that is in contact with maternal blood and is the principal site of nutrient and oxygen exchange.

By nourishing the fetus in the womb, the placenta plays a key role in a healthy pregnancy. However, studying placental formation in humans has been difficult due to a scarcity of good experimental models. Now, FMI group leader Margherita Yayoi Turco and her colleagues at the University of Cambridge, Megan Sheridan and Ashley Moffett, compared the two main models of the human placenta: 2D cultures of trophoblast stem cells and 3D clusters of placental cells called trophoblast organoids.

Both models are derived from placental tissues, grown in a laboratory dish, and resemble normal first-trimester placentas. However, unlike trophoblast stem cells, trophoblast organoids undergo spontaneous differentiation into syncytiotrophoblast and mimic the portion of the placenta that mediates the exchange of nutrients, hormones and oxygen between the mother and the fetus. In contrast, two-dimensional

trophoblast cultures resemble more closely cells located in the region from where extravillous trophoblasts are derived.

The researchers also found that, unlike trophoblast stem cells, trophoblast organoids maintain the expression of important cell-surface molecules responsible for interacting with the immune system. This suggests that mechanical cues are key for placental development, and that culturing trophoblast in three dimensions allows the cells to maintain important characteristics that they would have in the womb.

The findings, published in *Development*, will help scientists to choose the best model to study how the placenta develops and functions, and what happens when something goes wrong, the researchers say. The results also suggest that trophoblast organoids are best suited for studying maternal-fetal interactions. Understanding such processes will help investigate pregnancy complications that can lead to miscarriage and other conditions.

**More information:** Megan A. Sheridan et al, Characterization of primary models of human trophoblast, *Development* (2021). DOI: 10.1242/dev.199749

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