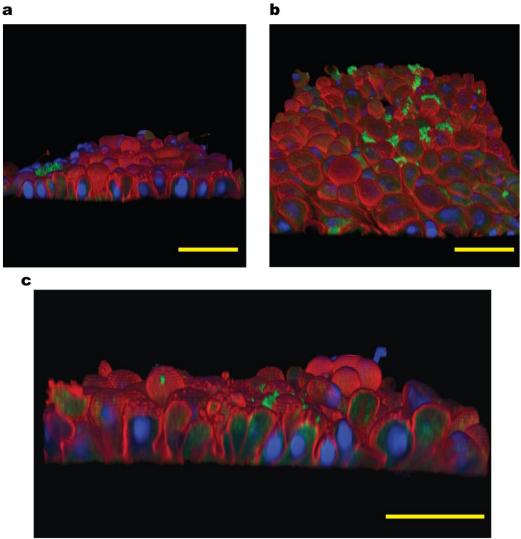
Supplementary Information for "An oviduct-on-a-chip provides an enhanced *in vitro* environment for zygote genome reprogramming" by Ferraz et al.

Supplementary methods

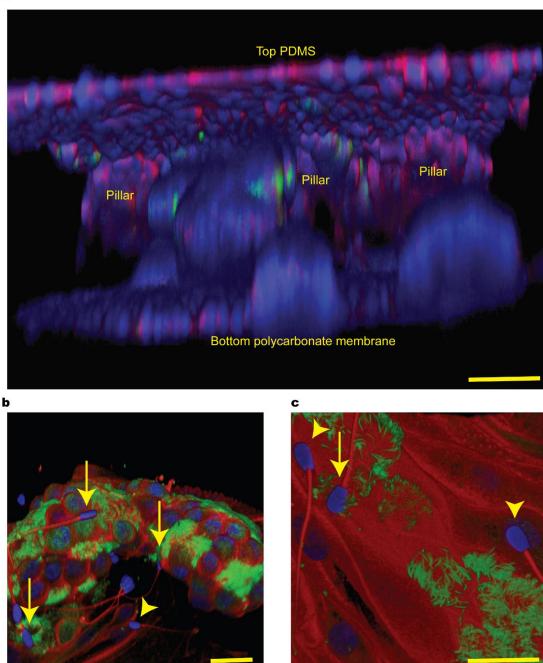
We used two Aladdin Syringe Pumps (WPI, Germany) to deliver solutions into the microfluidic devices. Solutions were transferred into 1 ml plastic syringes (BD Biosciences, Breda, The Netherlands) and Tygon® microbore tubing (0.020" ID x 0.060" OD, Cole-Parmer, Schiedam, The Netherlands) was connected using EFD Precision tips (23 gauge, EFD Nordson, Maastricht, The Netherlands). The other end of the tubing was connected to inlets on the microfluidic device using stainless steel pins (23 gauge, New England Small Tube Co., Litchfield, NH, USA) as shown below.



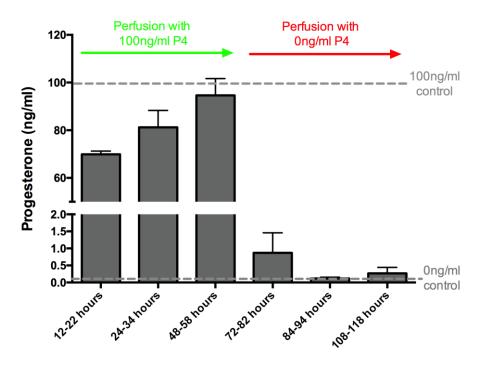


3D reconstruction of confocal immunofluorescence (IF) images for cilia (acetylated alphatubulin, green), nuclei (HOECHST33342, blue) and actin filaments (phalloidin, red). In (a, b and c) 3D reconstruction of part of the apical chamber, showing that BOECs lost their cuboid to columnar morphology, and started having apical protrusions, becoming a blebbing balloon shape. Scale Bars = $25 \,\mu$ m.

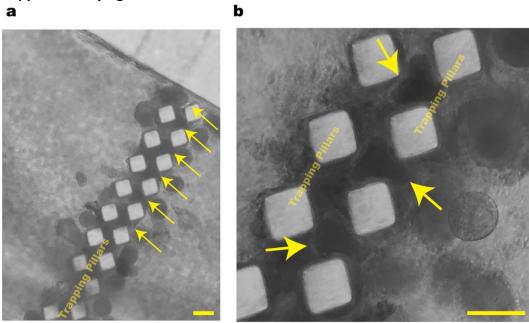




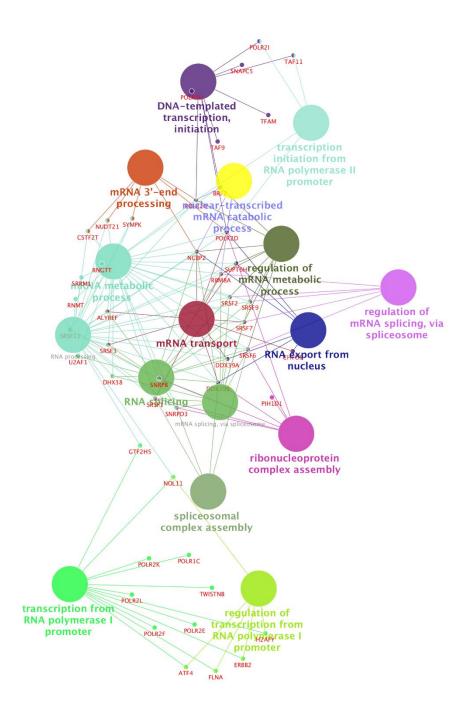
3D reconstruction of confocal immunofluorescence (IF) images for cilia (acetylated alphatubulin, green), nuclei (HOECHST33342, blue) and actin filaments (phalloidin, red) with and without sperm cells. (a) 3D reconstruction of part of the apical chamber, showing that BOECs grew on the overlying PDMS, on the trapping pillars and on the underlying polycarbonate membrane. Note the formation of villus-like structures, mimicking oviduct mucosal folding. (b) Closer look at a villus-like structure with attached sperm cells. Sperm cells were bound to ciliated (arrows) and non-ciliated cells (arrow heads). (c) Closer look at sperm-epithelium binding: sperm cells were bound to ciliated (arrows) and non-ciliated cells (arrow heads). Scale Bars = $50 \mu m$ (a) and $10 \mu m$ (b and c).



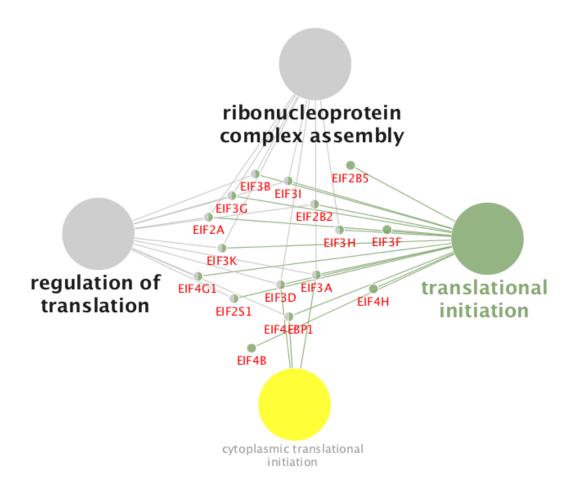
Progesterone levels (in ng ml⁻¹) measured in the fluids that passed cell free PDMS chips (n=5). The basolateral compartment was perfused at a speed of 5 uL hour⁻¹ with medium containing 100 ng mL⁻¹ P4 (green arrow) for 60 hours. This was followed by the same medium containing no progesterone (red arrow). The fluids that came through the PDMS chips were collected in the given perfusion (total collected volume was 50 uL). The P4 levels of the collected fluids were measured for each time point in duplo (15 uL each). Mean \pm SD are provided, the dashed line provides triplo measurements of the input fluids of both the 100 ng/mL and the 0 ng/mL containing media used for this perfusion experiment.



Phase-contrast images of cumulus-oocyte-complexes (COCs) inside the oviduct-on-a-chip. Note the COCs trapped (arrows) between the trapping pillars in (a) and (b). Scale bars = 100 μ m.



Functionally grouped gene ontology (GO) terms for genes up-regulated in G2 zygotes. The CytoScape plugin ClueGO was used to group the genes into functional GO terms of "molecular processes" and "biological processes" using genes related to transcription.



Supplementary figure 6: Functionally grouped gene ontology (GO) terms for genes upregulated in G2 zygotes. The CytoScape plugin ClueGO was used to group the genes into functional GO terms of "molecular processes" and "biological processes" using genes related to translation initiation.

Supplementary figure 7 b а Up-regulated GO pathways in Group 2 Up-regulated GO pathways in Group 1 histone ubiguitination BAR1 TRMT61A ATregative positive regulation of TRMT2A HEMKI METTL17 RNF20 egulation of histone TYWE histope modification e acetylation H3F3A protein TRMT1 methylati C SWARKS RNM histone H3-K9 ASH2 pos CHD METTL1 Micedification SATB1 HDACI NAD1 histone thylation SETD1A tion/of WDR4 RNF4 modification DNMT1 SFPQ RNA methylation KMT2 . ALPHILE DMRTC2 TET2 chromatin KIMAN NSUN5 SMARCB1 rethy TRMT10C CARM1 rganization GNMT NOP2 TRMTS NSUN2 chror ARMT1 SETD1B orgai gene/silencing macromolecule KDM6histon ATE7IP TET3 KMT2E methylation DIMITIA deacetyl histone H3-K9 modification of modification import ZFP57 BCDIN3D TRIMEDNA methylation to nucleus GATAD2B H1FOQ GATAD2A DNA methylation tion of ... to DNA ncing demethy TDR TDRD5 TDRD9 res otic cell caege silencing by dar timulus **RNA DNA** methylation **DNA** methylation DNA involved in involved in demethylation PABPC1 embryo gamete gene silencing by RBM3 TDC development RNA generation

Functionally grouped gene ontology (GO) terms for genes up-regulated in G1 and G2 zygotes. The CytoScape plugin ClueGO was used to group the genes into functional GO terms of "molecular processes" and "biological processes" using genes related to (de)methylation and (de)acetylation. In (a) up-regulated GO pathways in G1 zygotes; in (b) up-regulated GO pathways in G2 zygotes.