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Placenta Perfusion System: a Human ex vivo Model System to Study the Maternal – Fetal Barrier Capacity for Nanosized Materials

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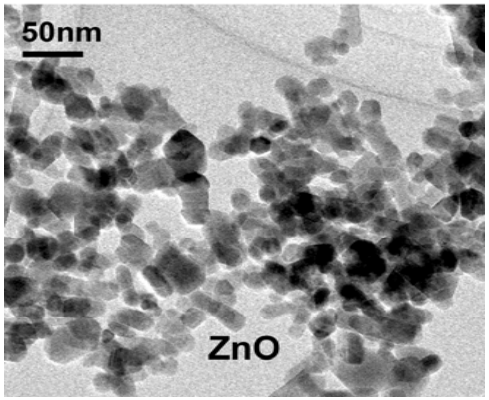
13th ETH Conference on Combustion Generated Nanoparticles
June 22 -24, 2009

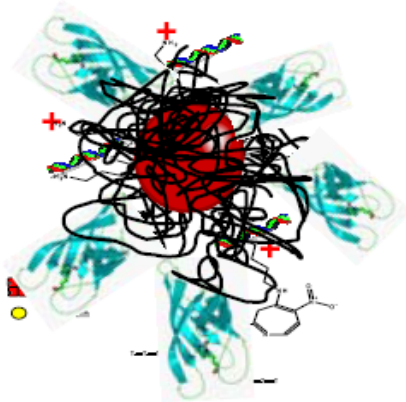
Exposure to NP

- wood fire
- volcano

- combustion derived
  (e.g. diesel exhaust)
- engineered particles

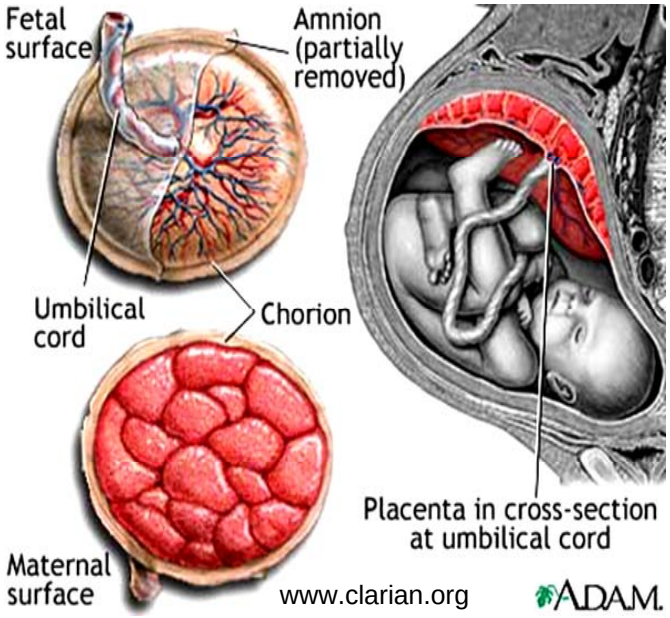
- medical applications (injected)
  - SPIONs
  - Imaging agents
  - NP drug delivery systems
  - NP vaccines
  - ...





Aim of the study

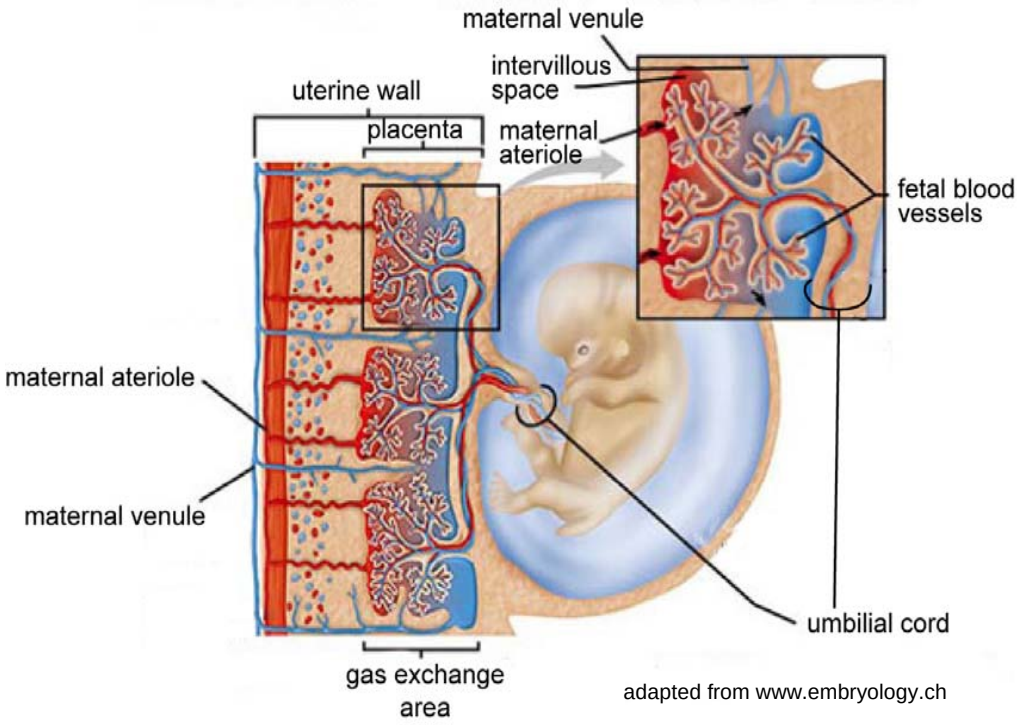
- Determination of the barrier capacity of placental tissue for nanoparticles
  (model particles: fluorescent polystyrene)
- Localization of the PS beads
- Analysis of histological and ultra structural changes of the tissue after perfusion
- Determination of the influence on viability and functionality of placental tissue after perfusion



Function and physiology of the human Placenta

function:

- exchange of oxygen / carbon dioxide
- exchange of nutrients and waste products
- exchange surface 5 – 12 m^2
- separating the two individual blood systems
- suppression of rejection

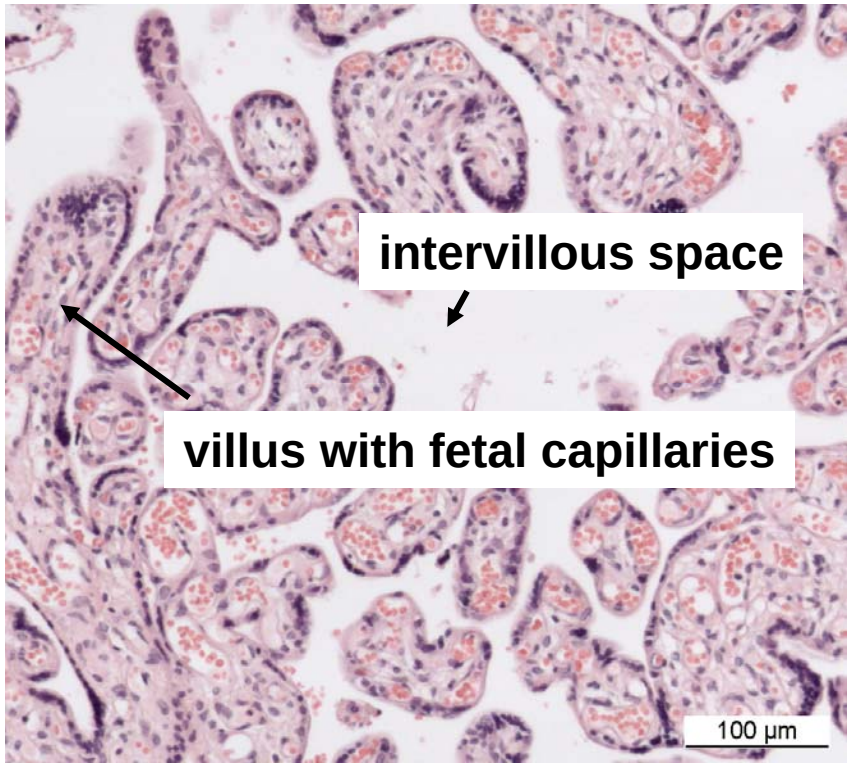


adapted from www.embryology.ch

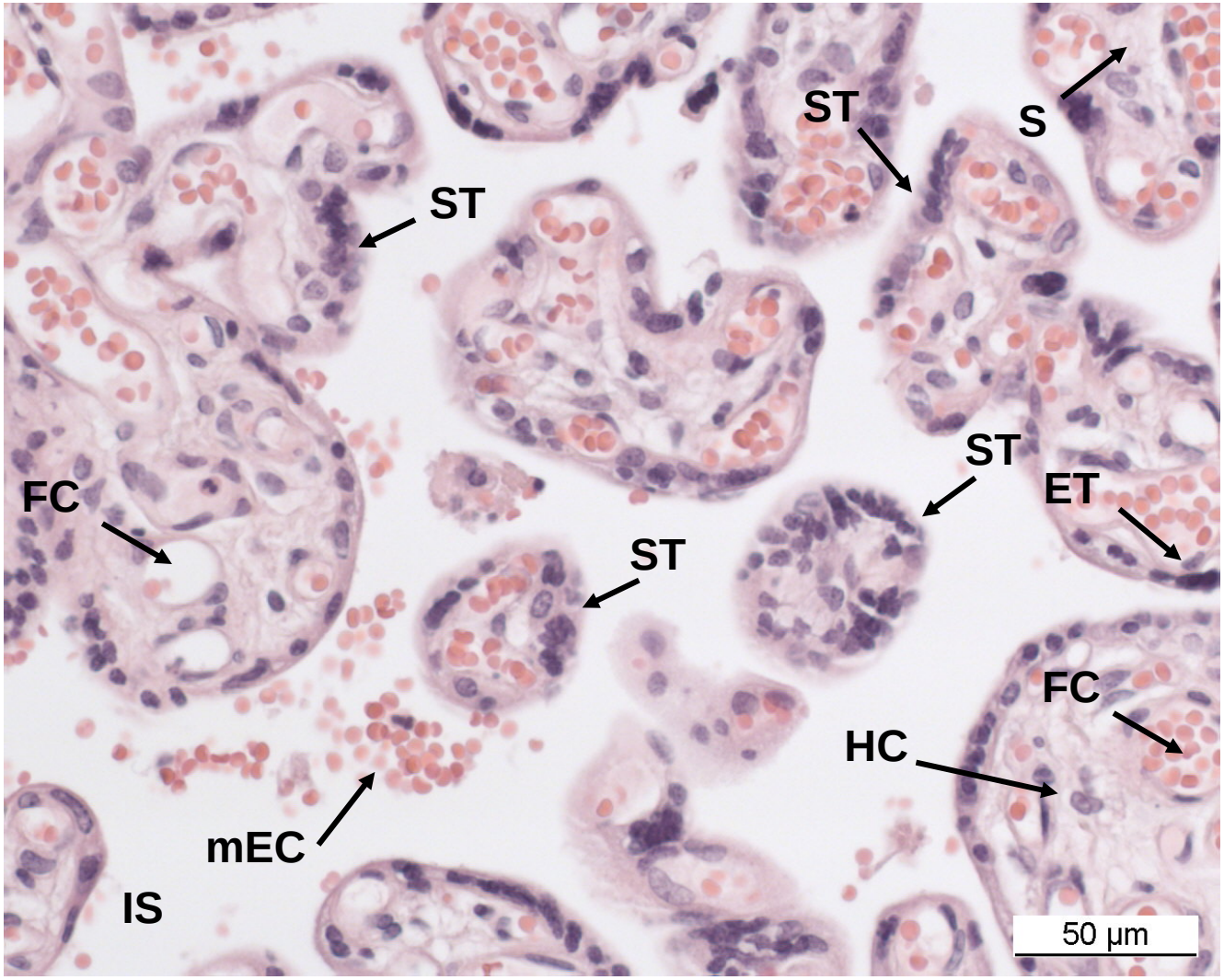
Function and physiology of the human Placenta

physiology:

- placenta is an embryonic tissue
- maternal blood flow open circuit
- unique for humans
- animal model such as mice and rats not comparable with human placenta
- four types of transport across placenta
  - diffusion
  - active transport
  - biotransformation through metabolic enzymes
  - phago- and pinocytosis



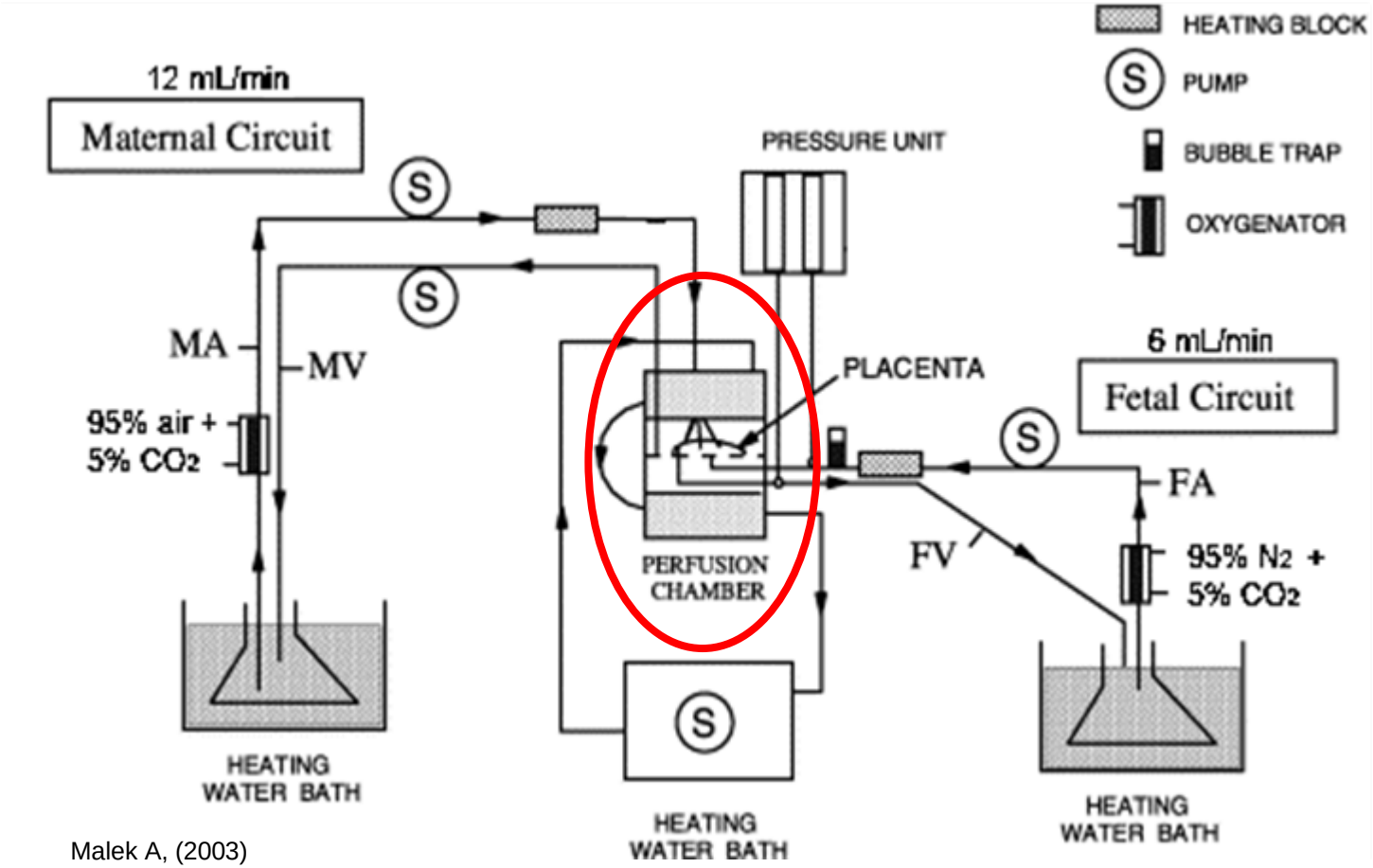
Placental morphology before perfusion



ET
endothelial cell
FC
fetal capillary
HC
Hofbauer cell
IS
intervillous space
mEC
maternal erythrocyte
ST
syncytio-
throphoblast
S
stroma of the villus

Hämalaun / Eosin staining

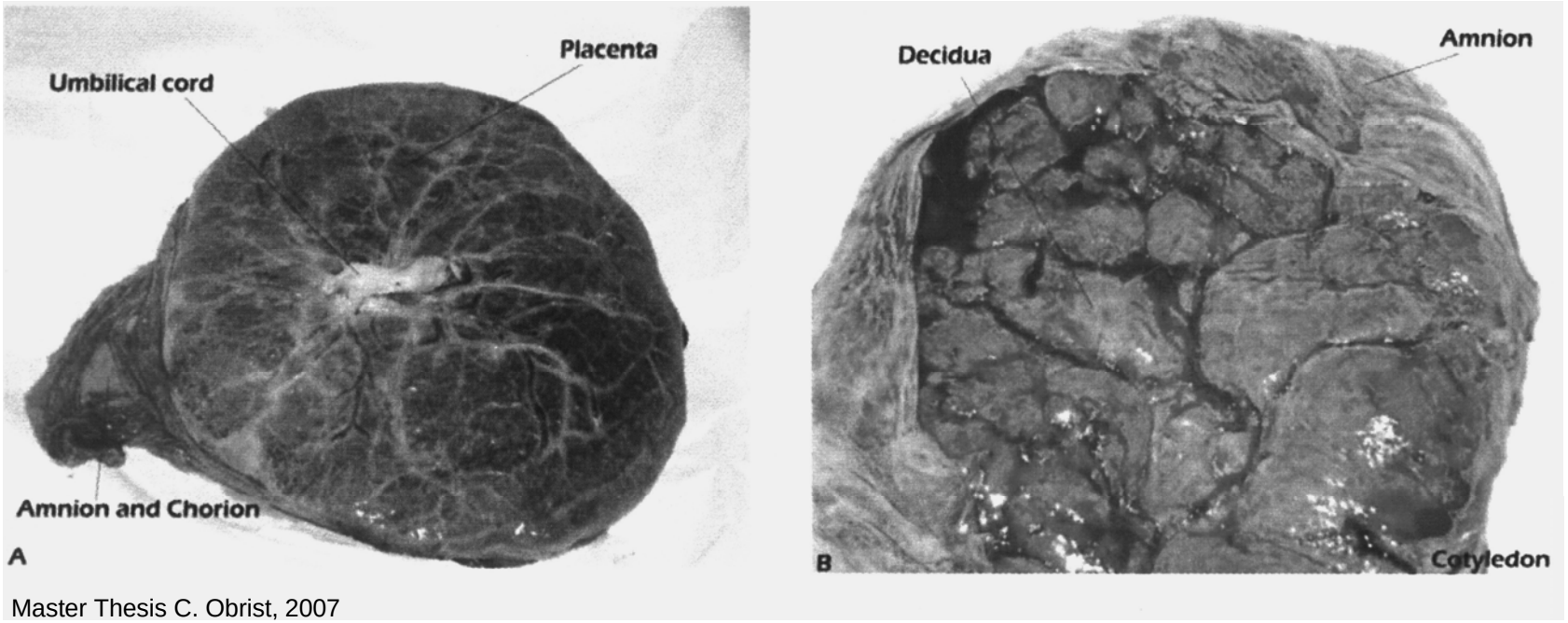
Re-circulating placenta perfusion model



Malek A, (2003)

developed by Panigel M, et 1967 and improved by Schneider et al 1972

Human placenta shortly after delivery

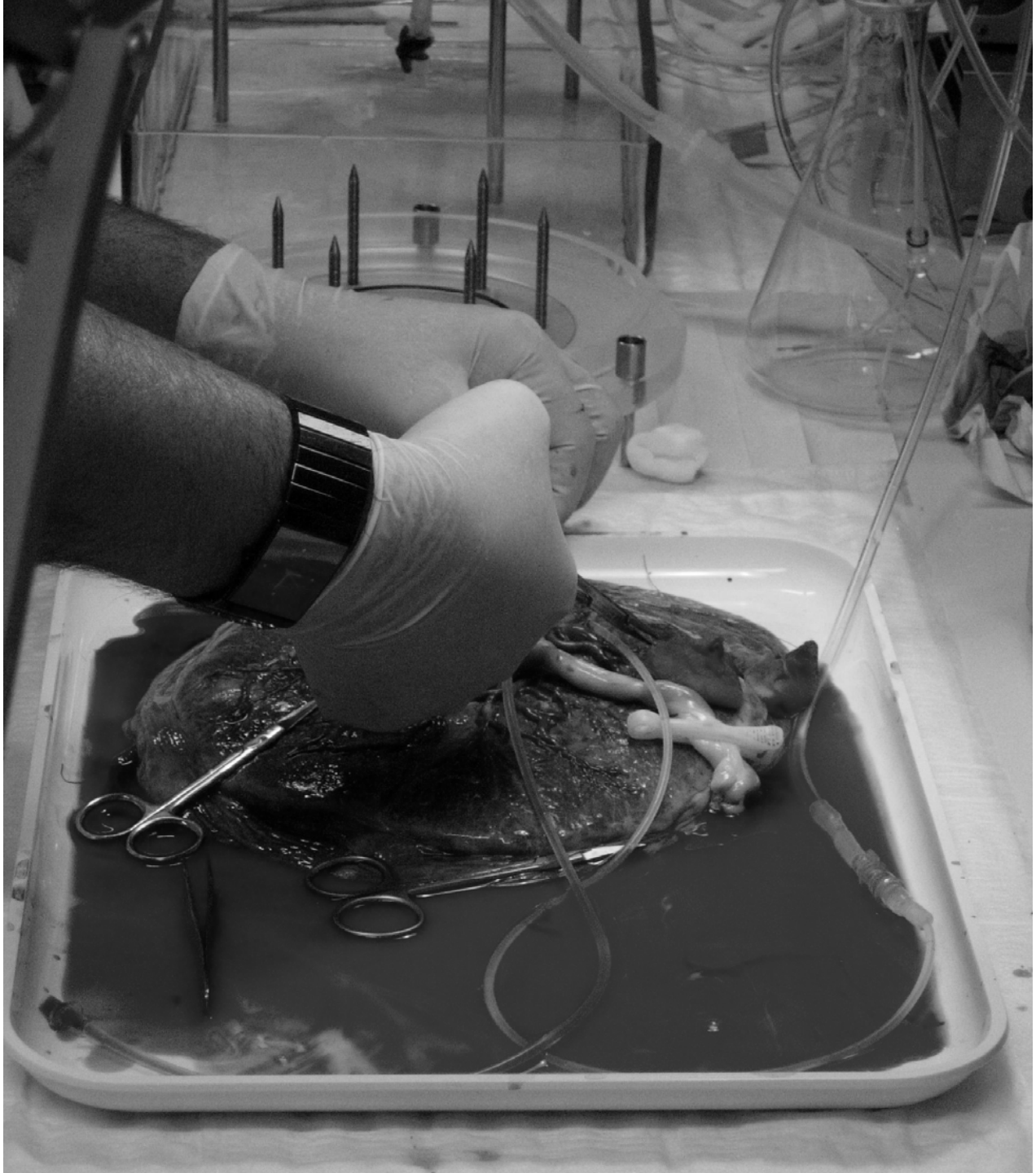


Master Thesis C. Obrist, 2007

Fetal surface with umbilical cord

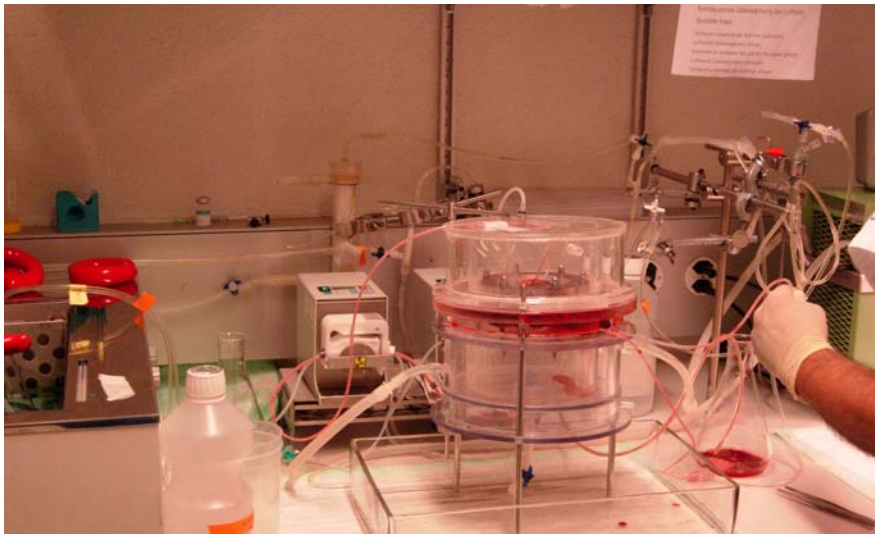
Maternal surface with *decidua basalis*

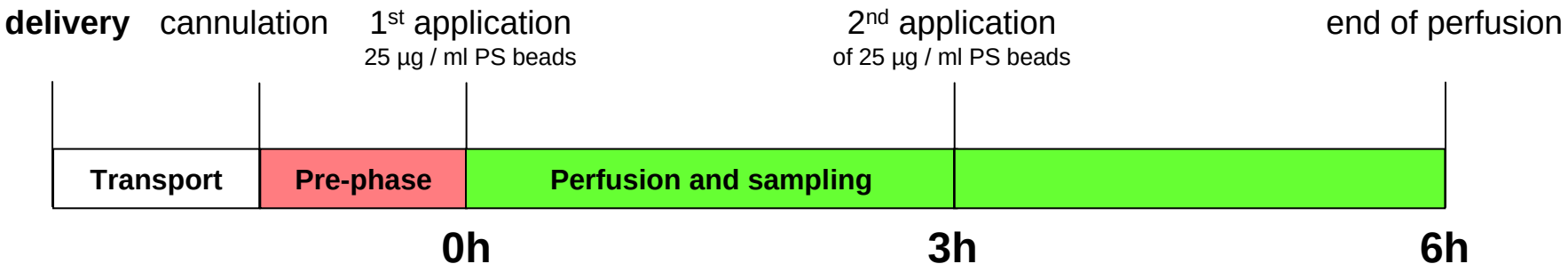
Intact placenta were obtained from uncomplicated term pregnancies either after vaginal or cesarean delivery and has to be cannulated within minutes




Fortdauernde Überwachung der Luftfalle
(bubble trap)

Work procedure for placenta perfusion assay





Quality criteria for a successful perfusion

Visual control:
intact membranes, no lesions, no disruption of the placenta
Measurable control:
Leakage of fetal circuit < 4 ml / h
^{14}C-antipyrine values

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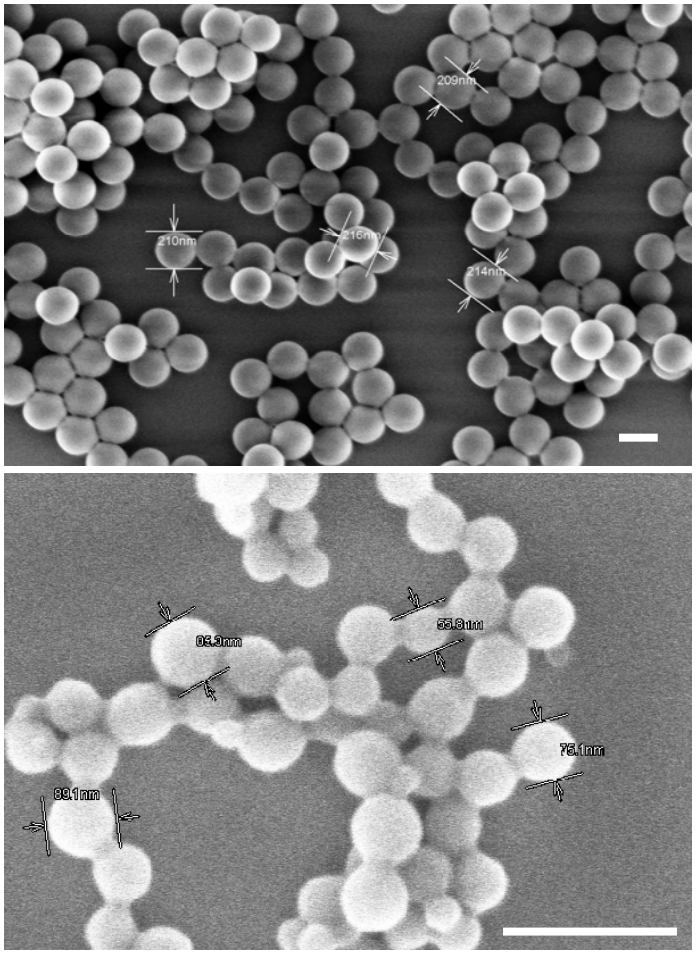
Fluorescent PS beads used for perfusion assay

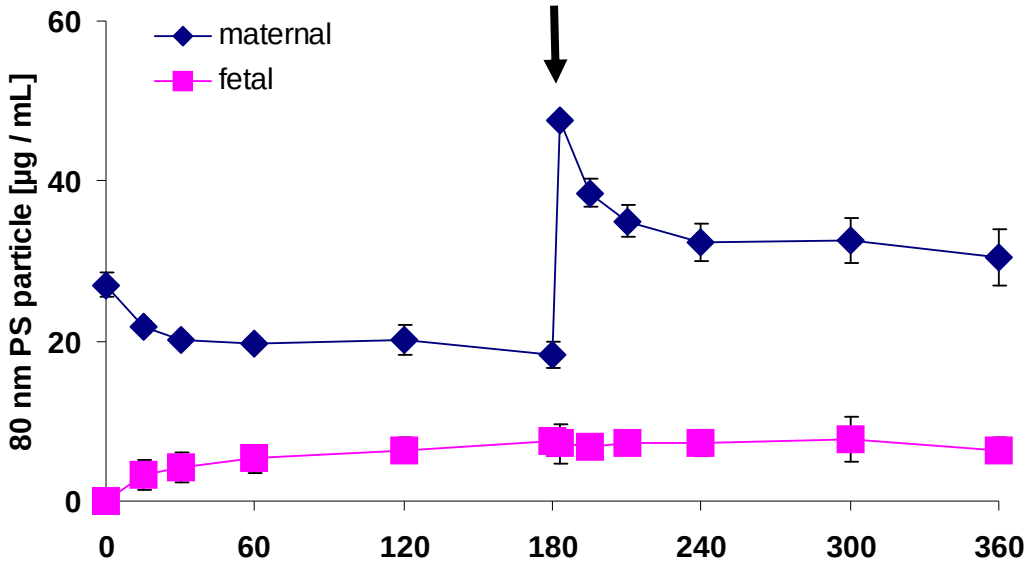


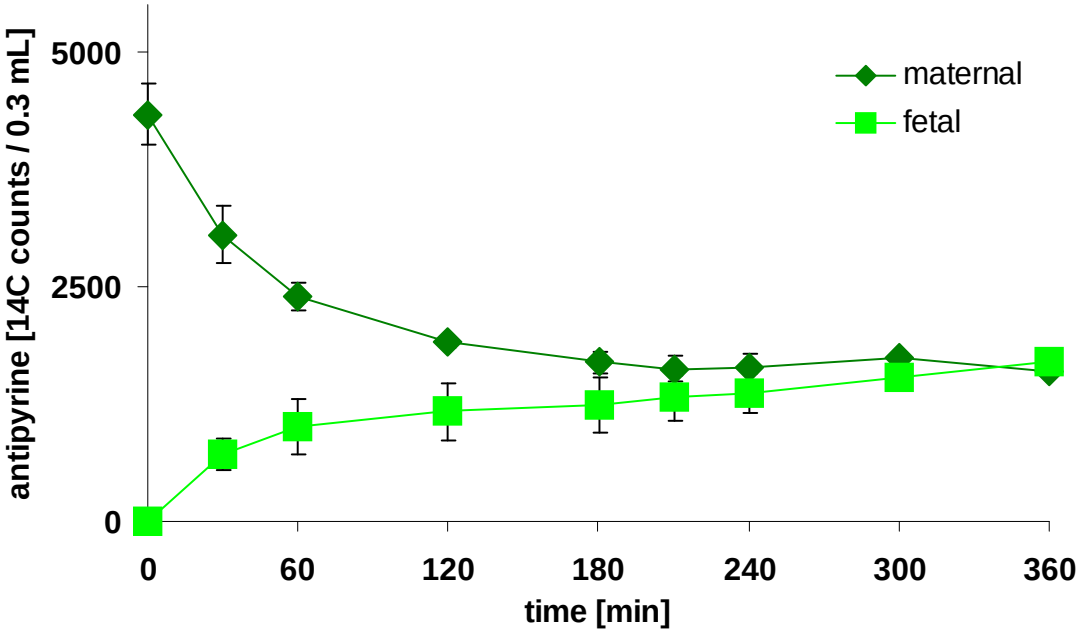
Scale bar 200nm

Advantages:

- easy to detect (detection limit ~1 µg / ml with ELISA plate reader)
- spherical, reduced agglomeration
- known as biocompatible
- uncharged
- commercially available in different sizes
- used: 50, 80, 240 and 500 nm

Perfusion data of 80 nm polystyrene beads





↓ 2nd application of 25 µg / ml beads

Starting concentration of 25 µg / ml represent high dose (overload)

(n=4; mean ± S.E.M.)

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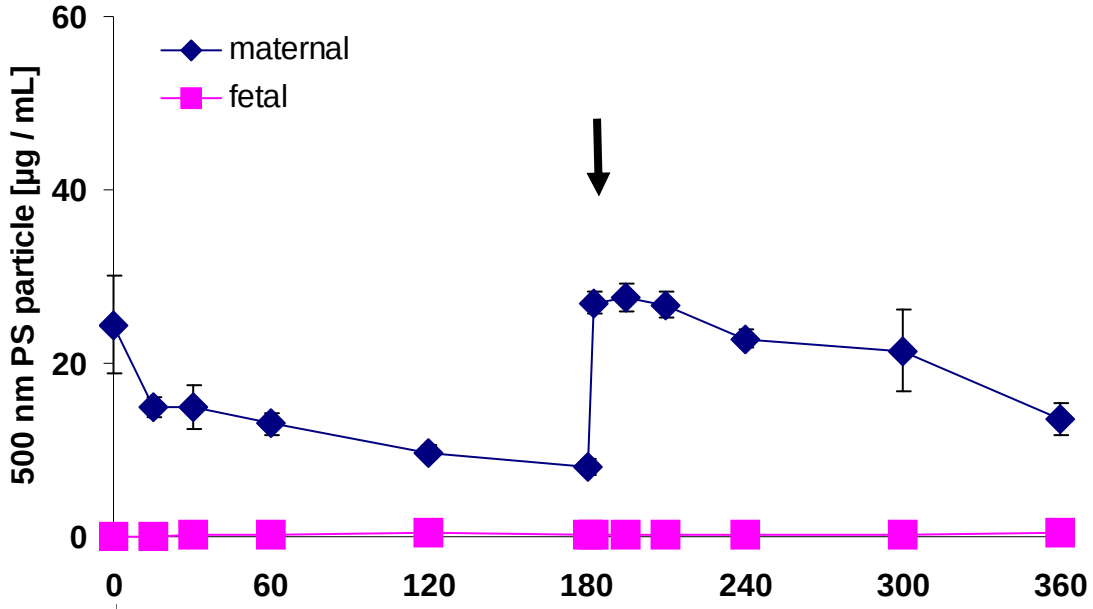
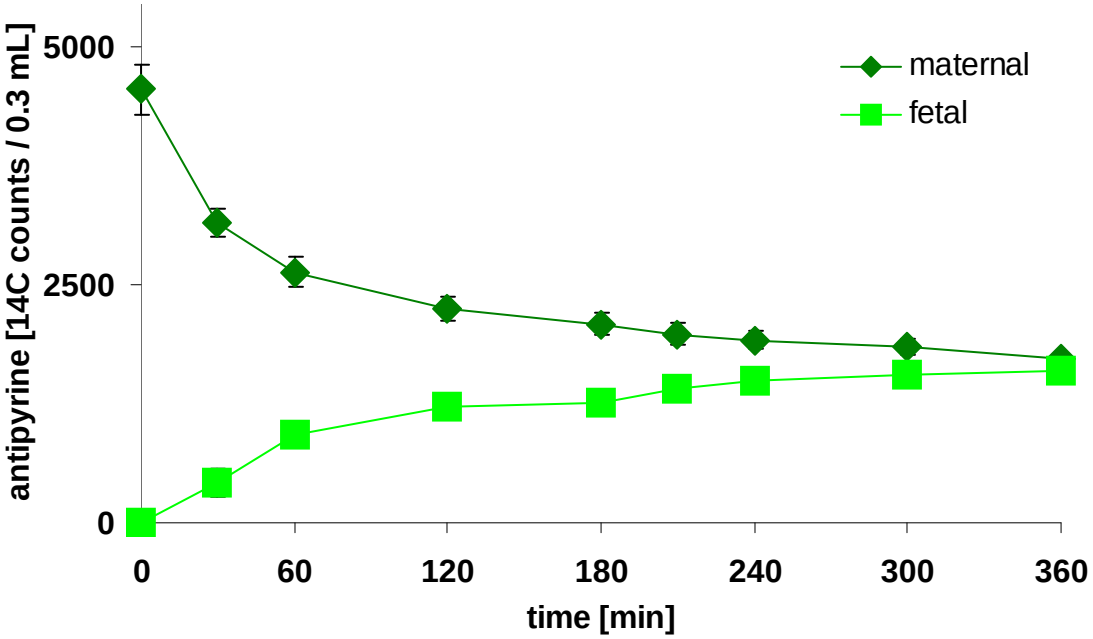
Perfusion data of 500 nm polystyrene beads





↓ 2nd application of 25 µg / ml beads

500 nm polystyrene beads were not able to cross the placenta

(n=4; mean ± S.E.M.)

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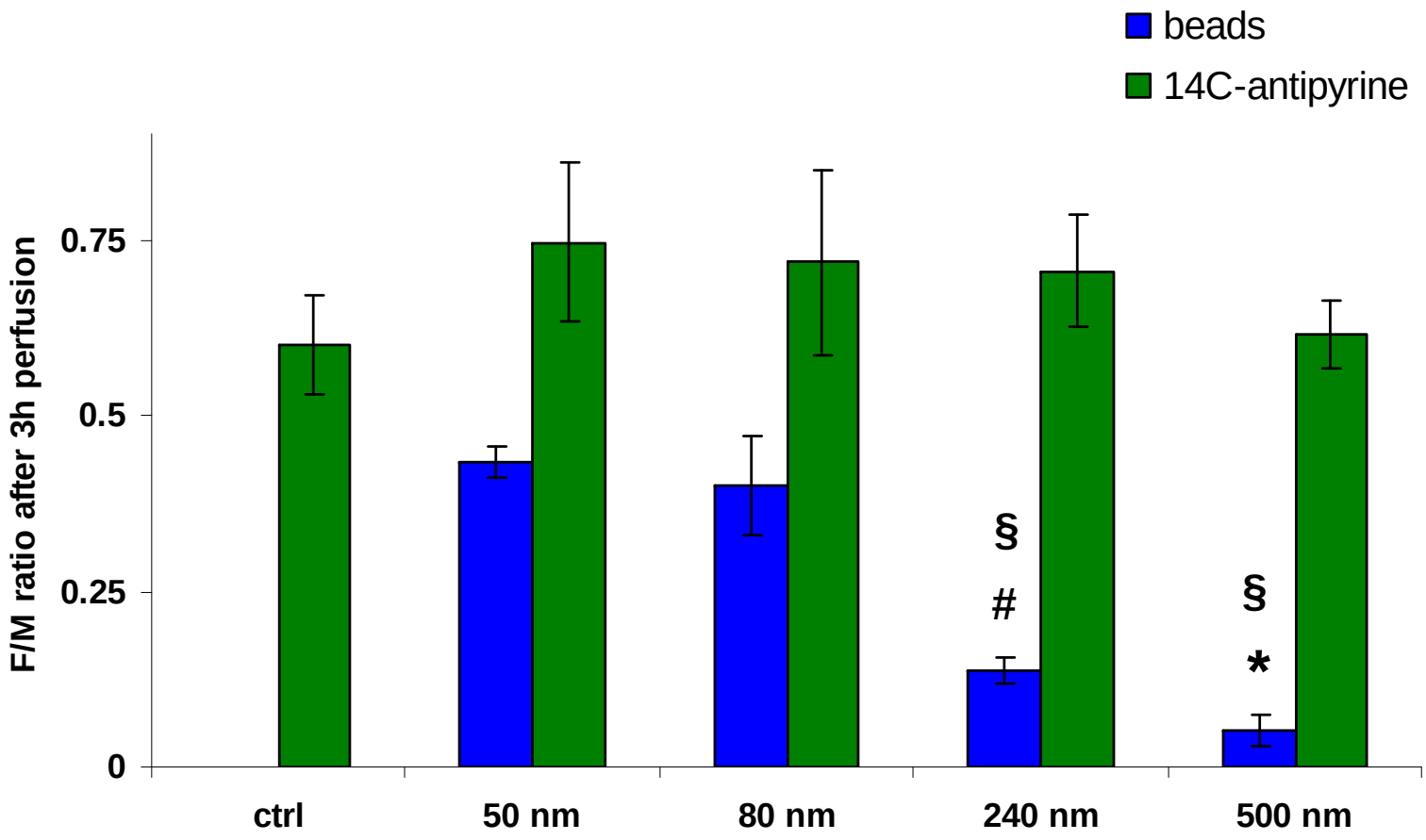
Barrier capacity of the placenta is size dependent



(at least n=4; mean ± S.E.M.)

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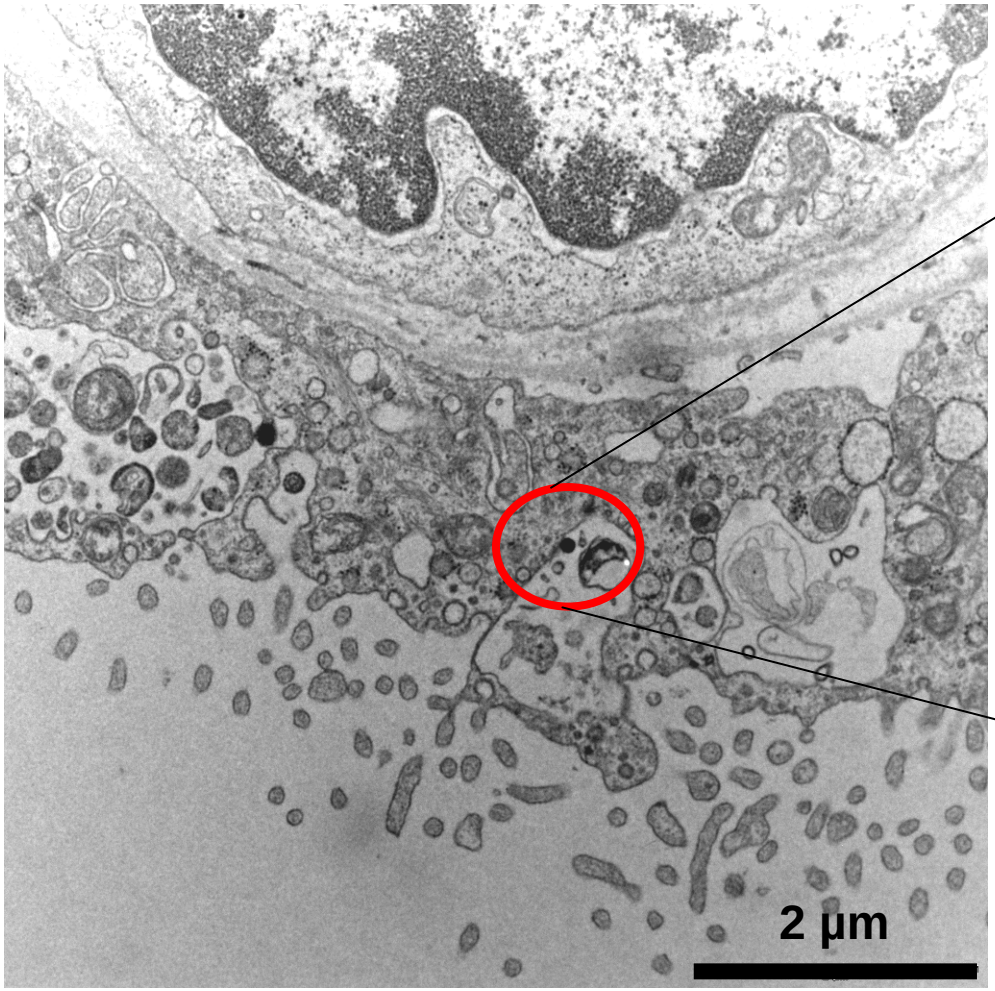
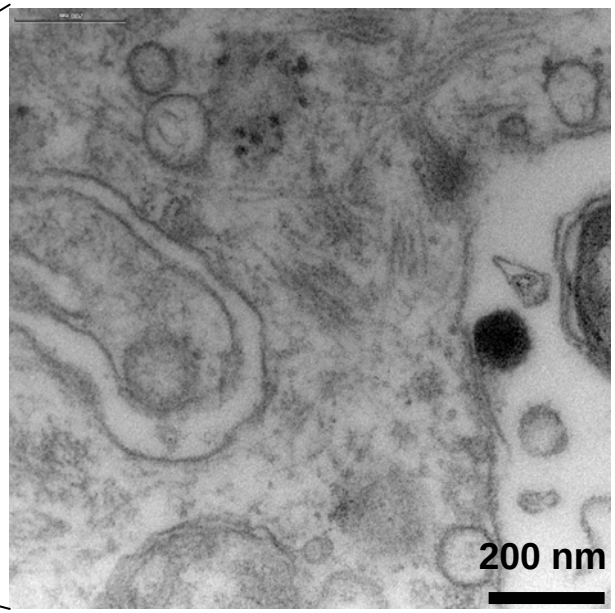
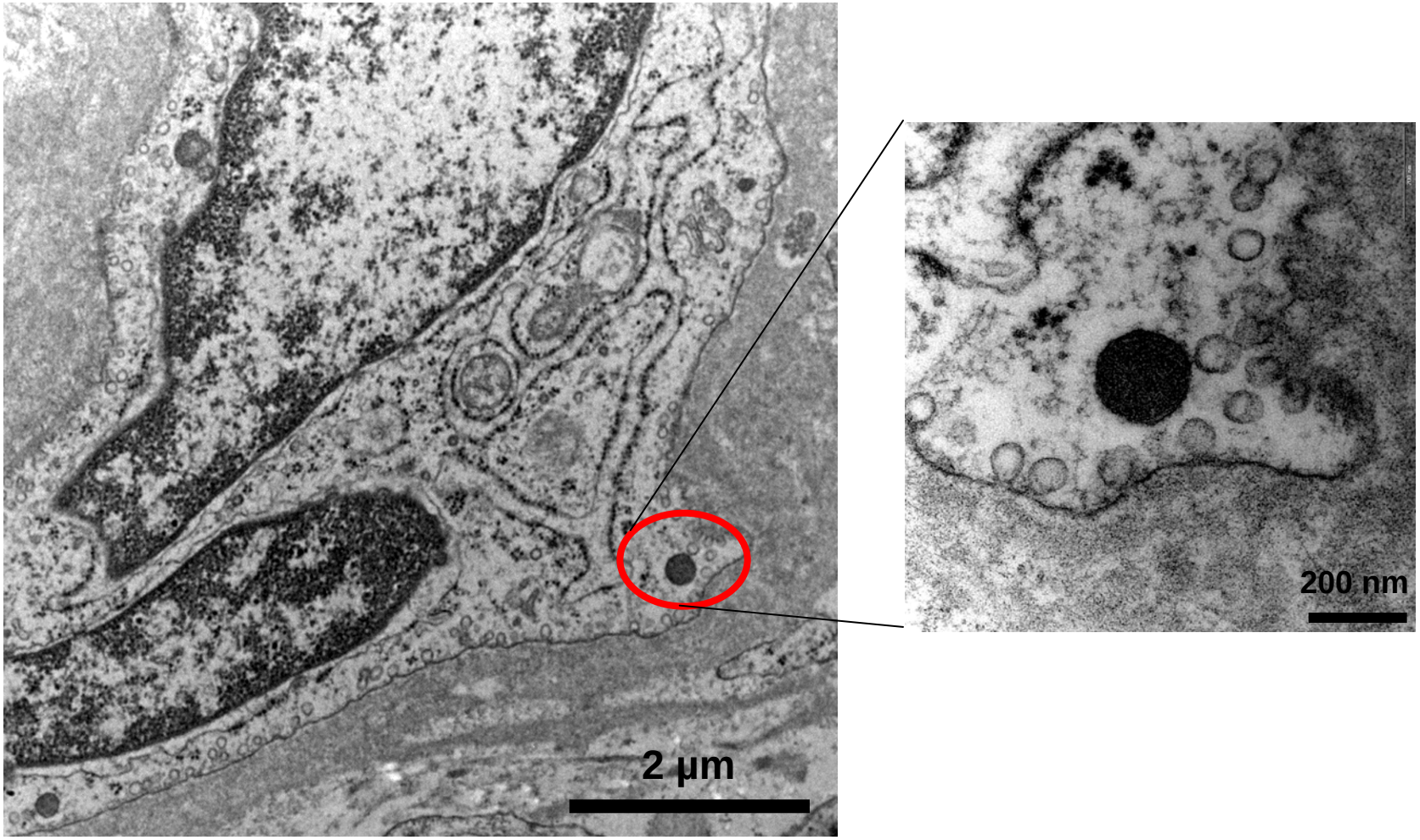
Ultra structural analysis after perfusion with 240 nm beads





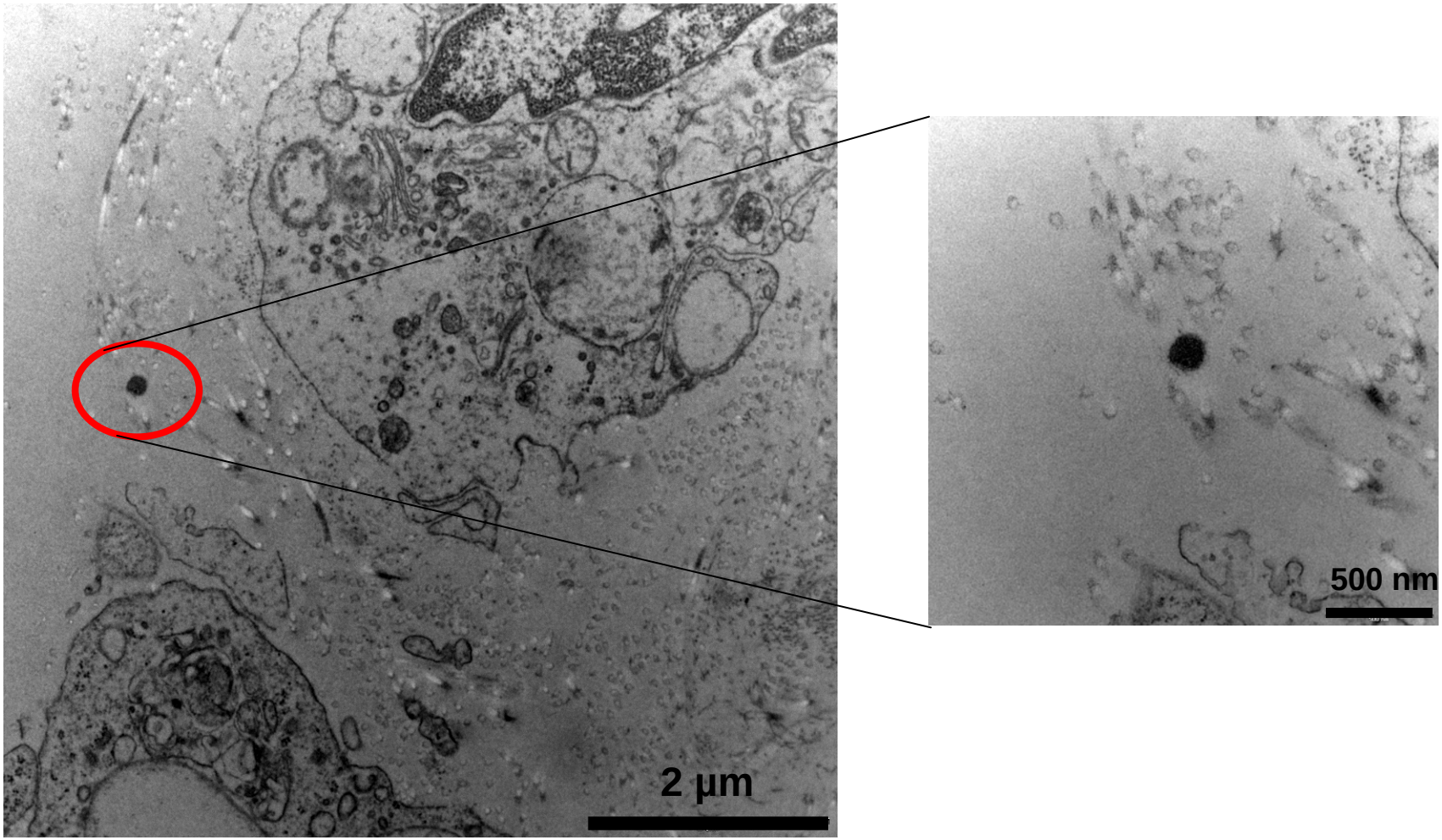
Intervillious space (maternal side)

Ultra structural analysis after perfusion with 240 nm beads



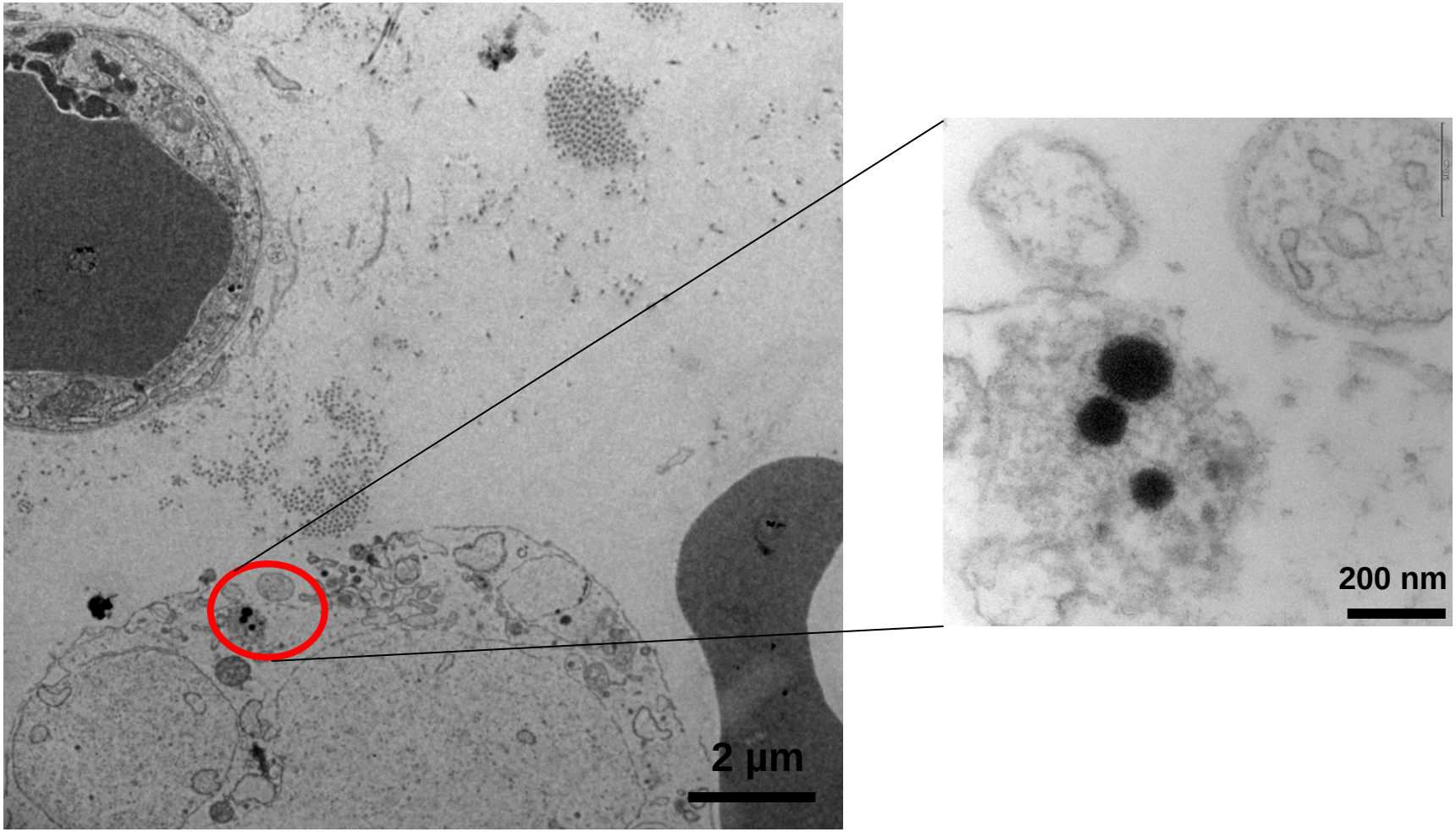
In syncytiothrophoblast

Ultra structural analysis after perfusion with 240 nm beads



Stroma, PS bead crossed syncytiotrophoblast (1st barrier)

Ultra structural analysis after perfusion with 240 nm beads

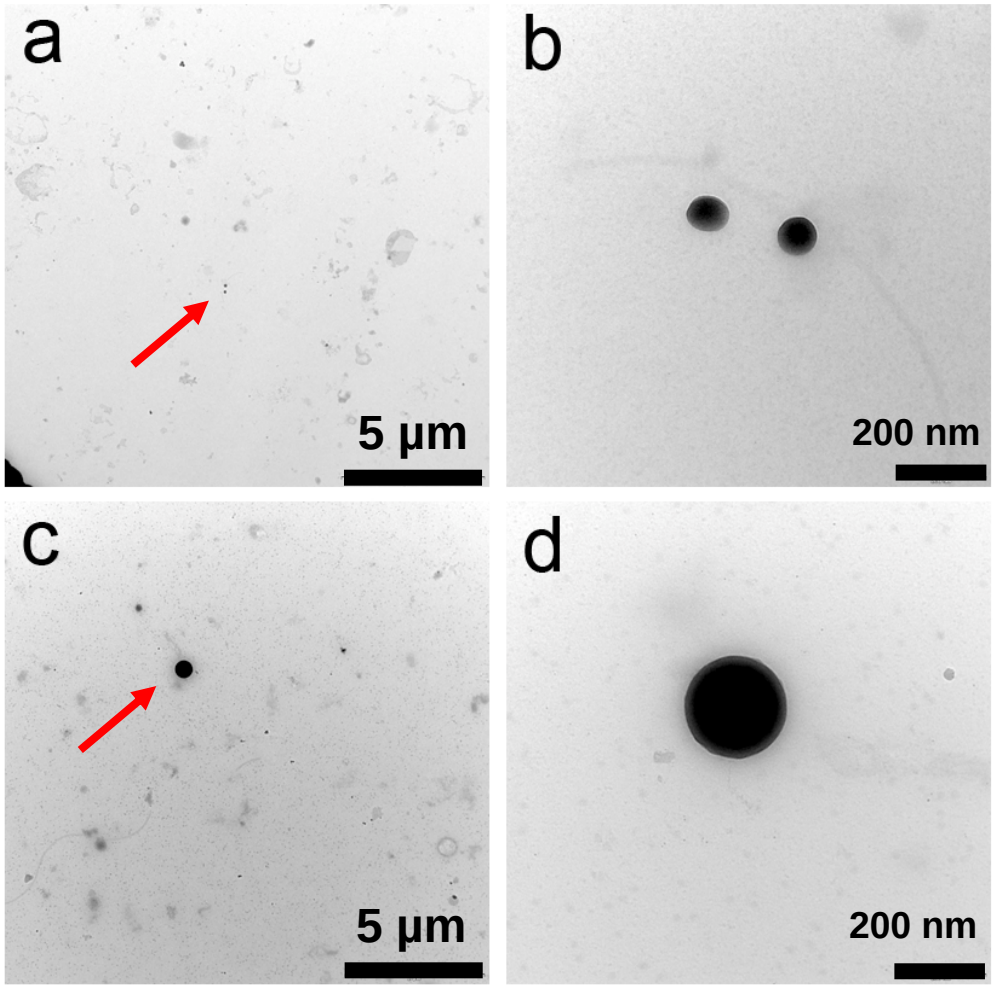


Fibroblast or cytothrophoblast, close to fetal capillary

Detection of PS beads in fetal circuit

80 nm

240 nm



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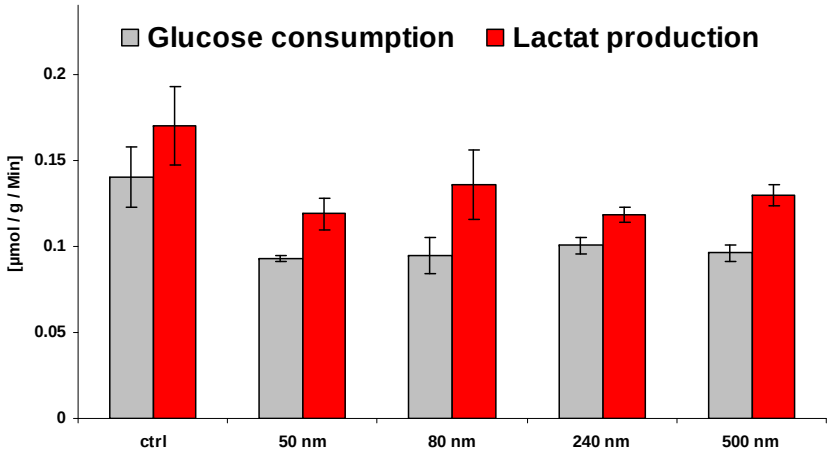
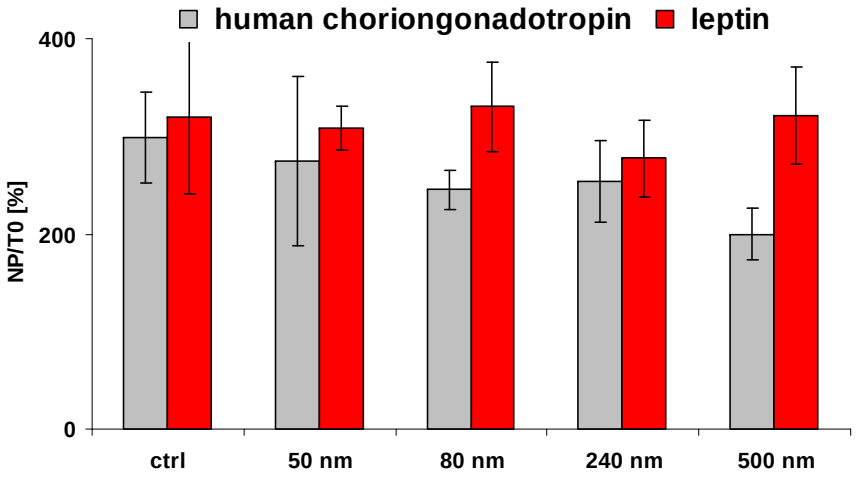
Viability and functionality of the placenta after perfusion





Neither the viability (glucose consumption / lactate production) nor the function of the placenta (hCG / leptin) were affected after the perfusion with polystyrene beads.
(at least n=4; mean ± S.E.M.)

Summary

- Placenta ex vivo model useful for (nano-) toxicological as well as pharmacological studies
- Polystyrene beads < 240 nm were able to cross placenta
- Polystyrene beads found in syncytiothrophoblast, stroma and detected in the fetal circuit
- No morphological changes in placenta tissue observed after perfusion of PS beads
- Viability and functionality of placenta was not affected after perfusion

- This suggests that most nanomaterials have the potential for transplacental transfer and underlines the need for further nanotoxicological studies on this important organ system.

… and you
for your attention

Bundesamt für Umwelt, Wald und Landschaft BUWAL

Bundesamt für Gesundheit

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and team

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Prof. Dr. B. Fadeel
and team

University Hospital Zurich
Prof U. von Mandach
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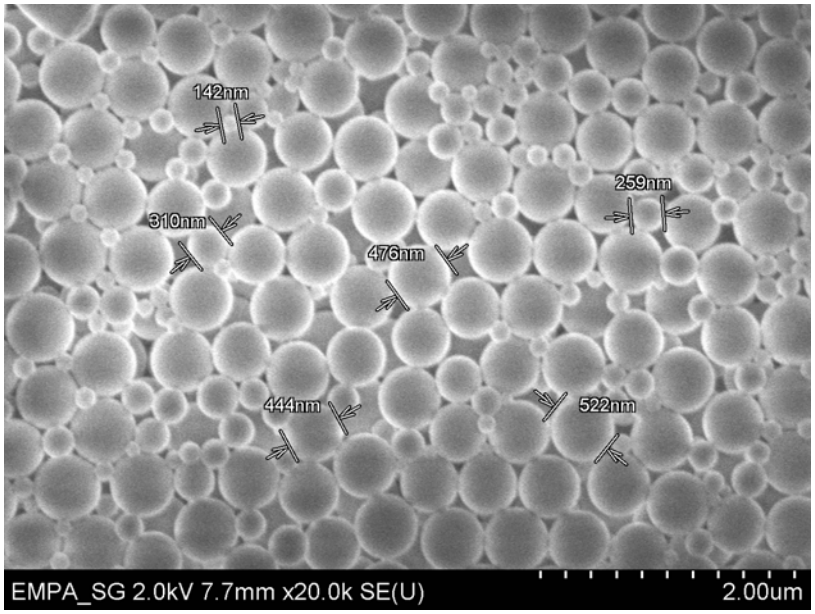
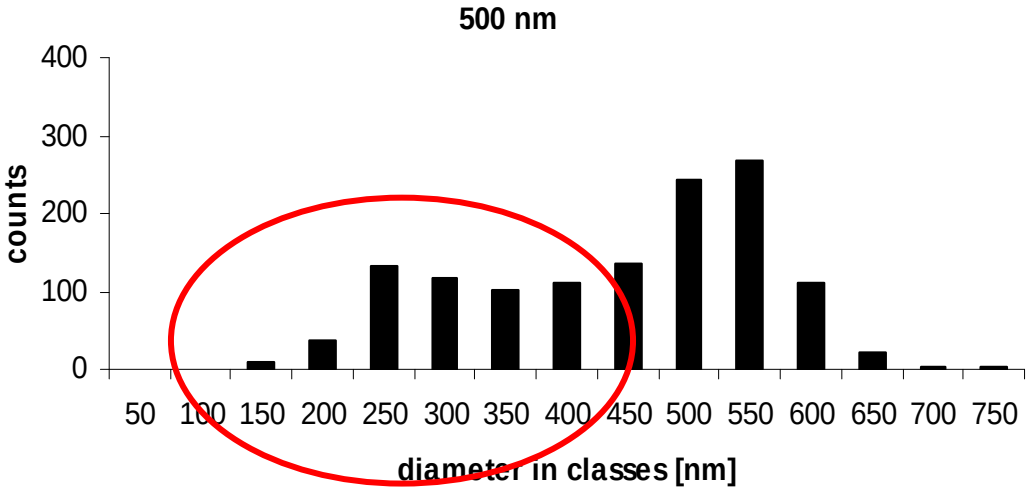
Material characterization prior to use!





Contamination of smaller (around 250 nm) polystyrene beads within the 500 nm beads

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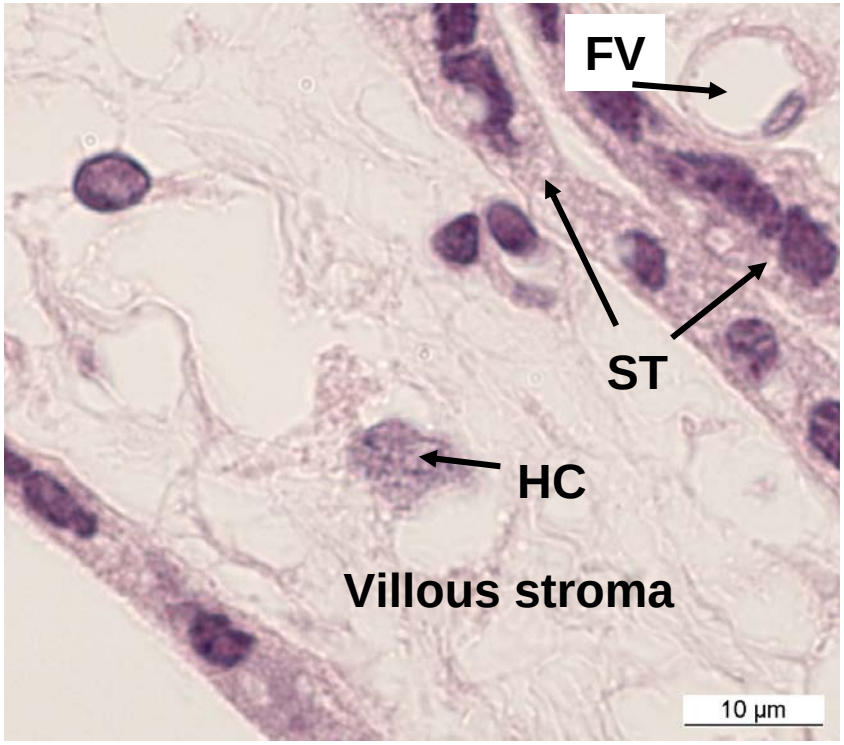
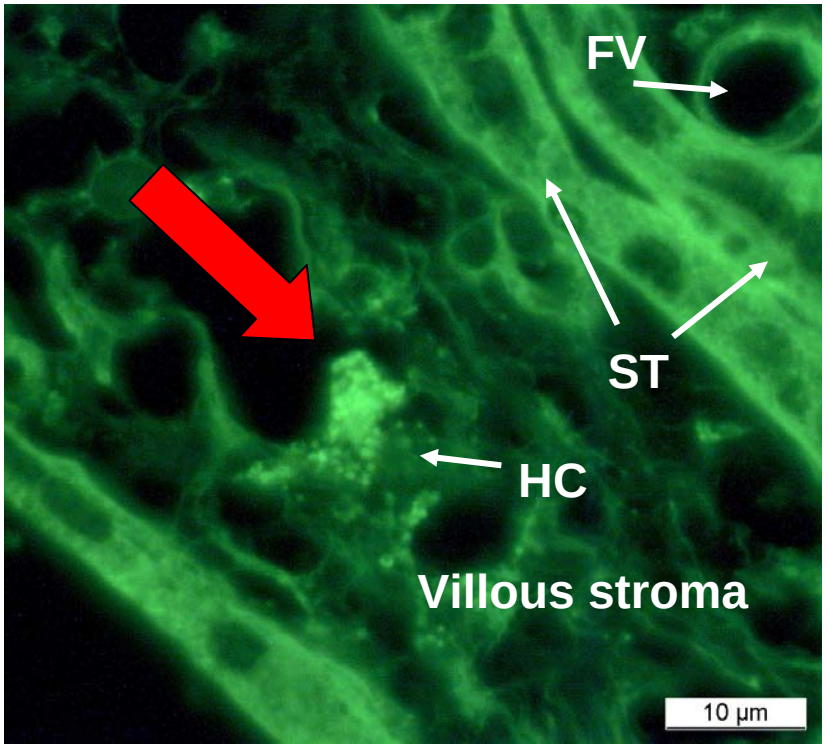
Placental morphology after 6h perfusion with 240 nm beads



Hämalaun / Eosin staining



Fluorescence microscopy

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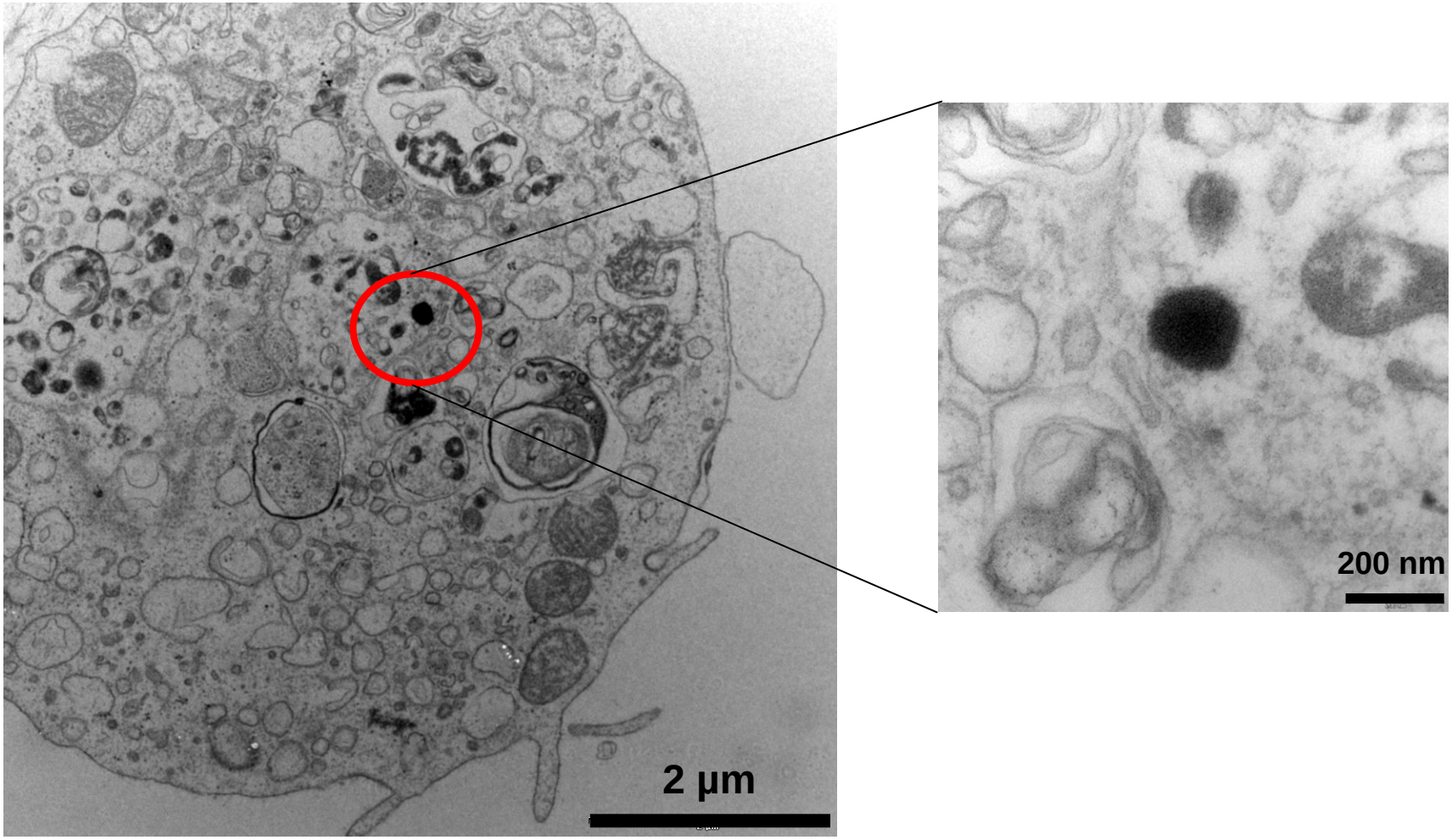
Ultra structural analysis after perfusion with 240 nm beads



Hofbaur cell (macrophage) in Stroma

Placenta cotyledon after 6h of perfusion