Safe-by-design nanoparticles show reduced risk for female fertility

Antonella Camaioni

Associate Professor of Histology and Embryology
Department of Biomedicine and Prevention
University of Rome Tor Vergata

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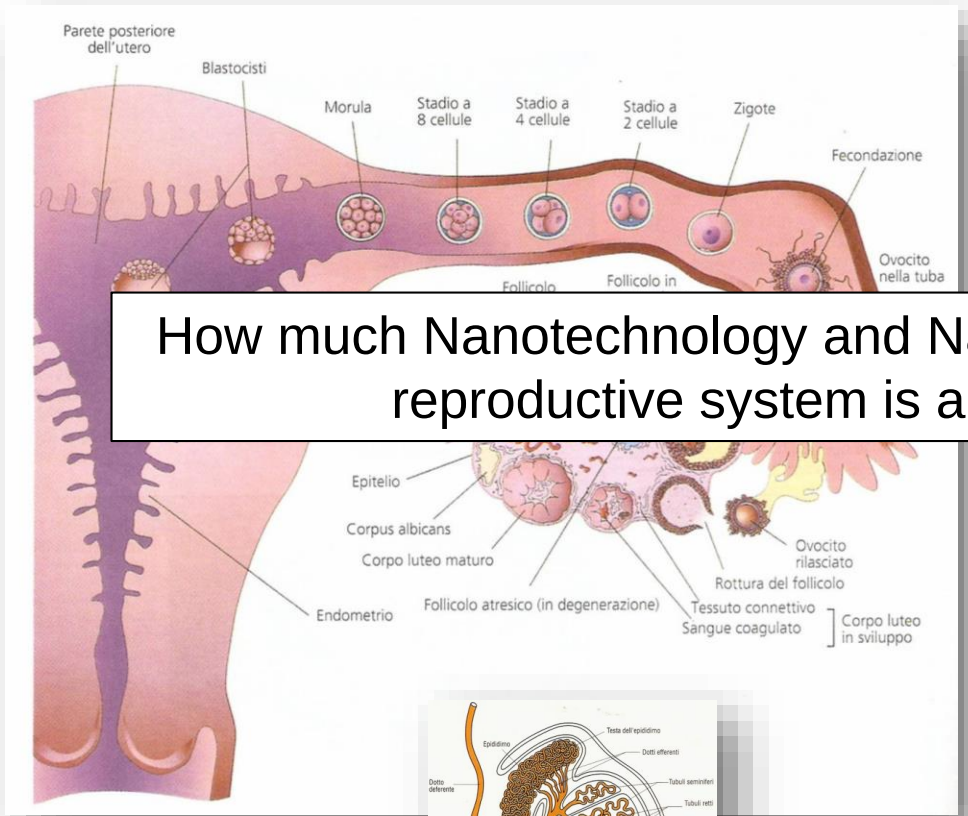
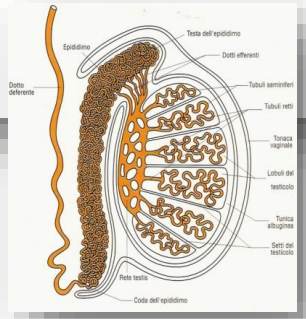
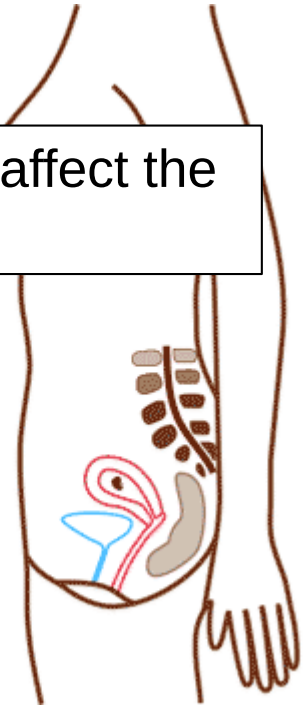
Biology of reproduction

Gamete maturation → fertilization → pre-implantation embryo development → implantation → placentation → post-implantation embryo development



How much Nanotechnology and Nanomaterials affect the reproductive system is an open issue





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for example…..

- Metal oxide nanoparticles (NPs) are increasingly used for industrial and biomedical applications (cosmetics, food packaging and additives, paint formulation, sunscreens, etc.)

- Therefore, the interest of the scientific community on the effect of NPs exposure on reproductive apparatus and on embryonic development is now growing; however available data in the literature are still scant and fragmentary

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- Therefore, the interest of the scientific community on the effect of metal NPs exposure on reproductive apparatus and on embryonic development is now growing; however available data in the literature are still scant and fragmentary

PLoS One. 2013; 8(4):e59378

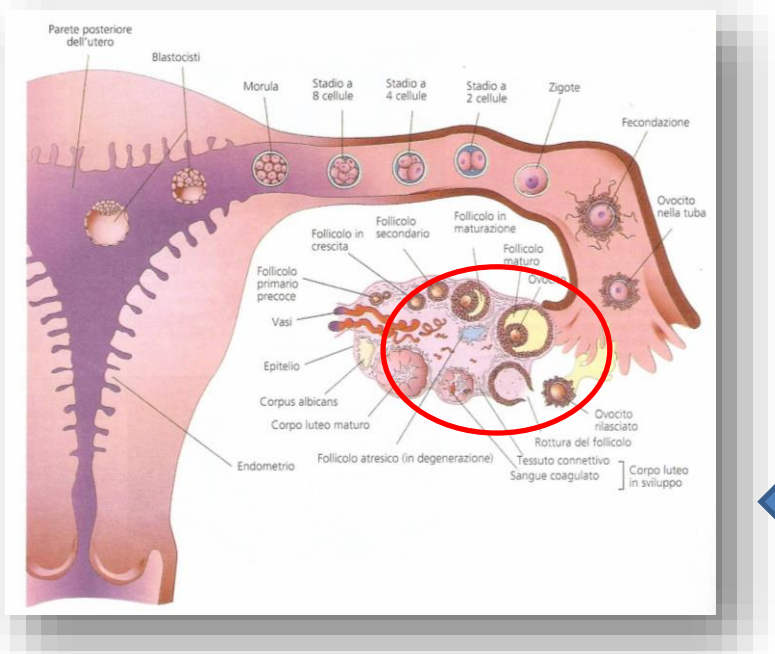
Nanosized TiO_2-induced reproductive system dysfunction and its mechanism in female mice.

Zhao X, Ze Y, Gao G, Sang X, Li B, Gui S, Sheng L, Sun Q, Cheng J, Cheng Z, Hu R, Wang L, Hong F.

Nanotoxicology. 2016; 10(1):111.

Cerium dioxide nanoparticles affect in vitro fertilization in mice.

Preaubert L, Courbiere B, Achard V, Tassistro V, Greco F, Orsiere T, Bottero JY, Rose J, Auffan M, Perrin J.



Analyst. 2014; 139(5):931

Reprotoxicity of gold, silver, and gold-silver alloy nanoparticles on mammalian gametes.

Tiedemann D, Taylor U, Rehbock C, Jakobi J, Klein S, Kues WA, Barcikowski S, Rath D.

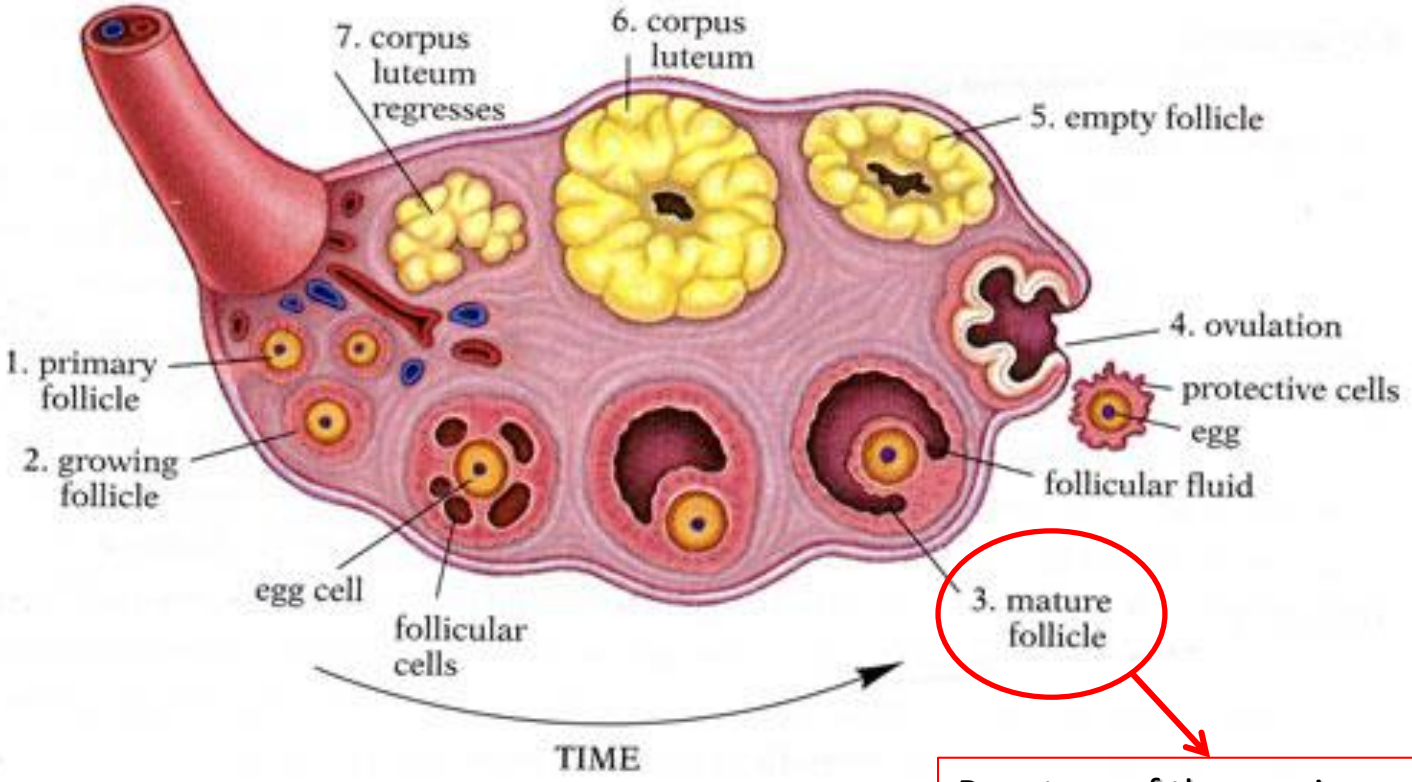
Theranostics. 2012; 2(7):734

The Invasion and Reproductive Toxicity of QDs-Transferrin Bioconjugates on Preantral Follicle in vitro.

Xu G, Lin S, Law WC, Roy I, Lin X, Mei S, Ma H, Chen S, Niu H, Wang X.

Università di Roma Tor Vergata

The ovarian cycle



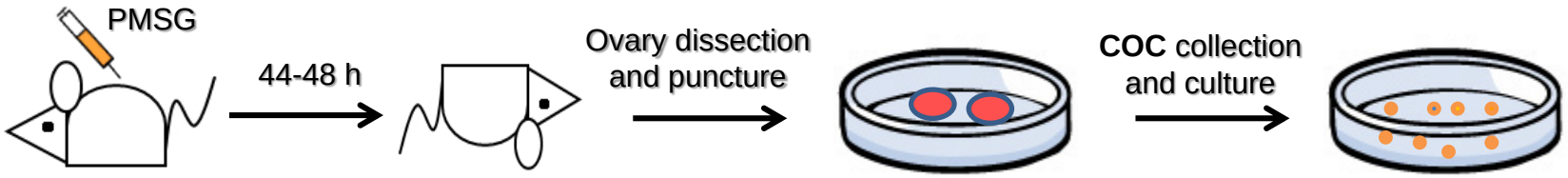
hormonal stimulation

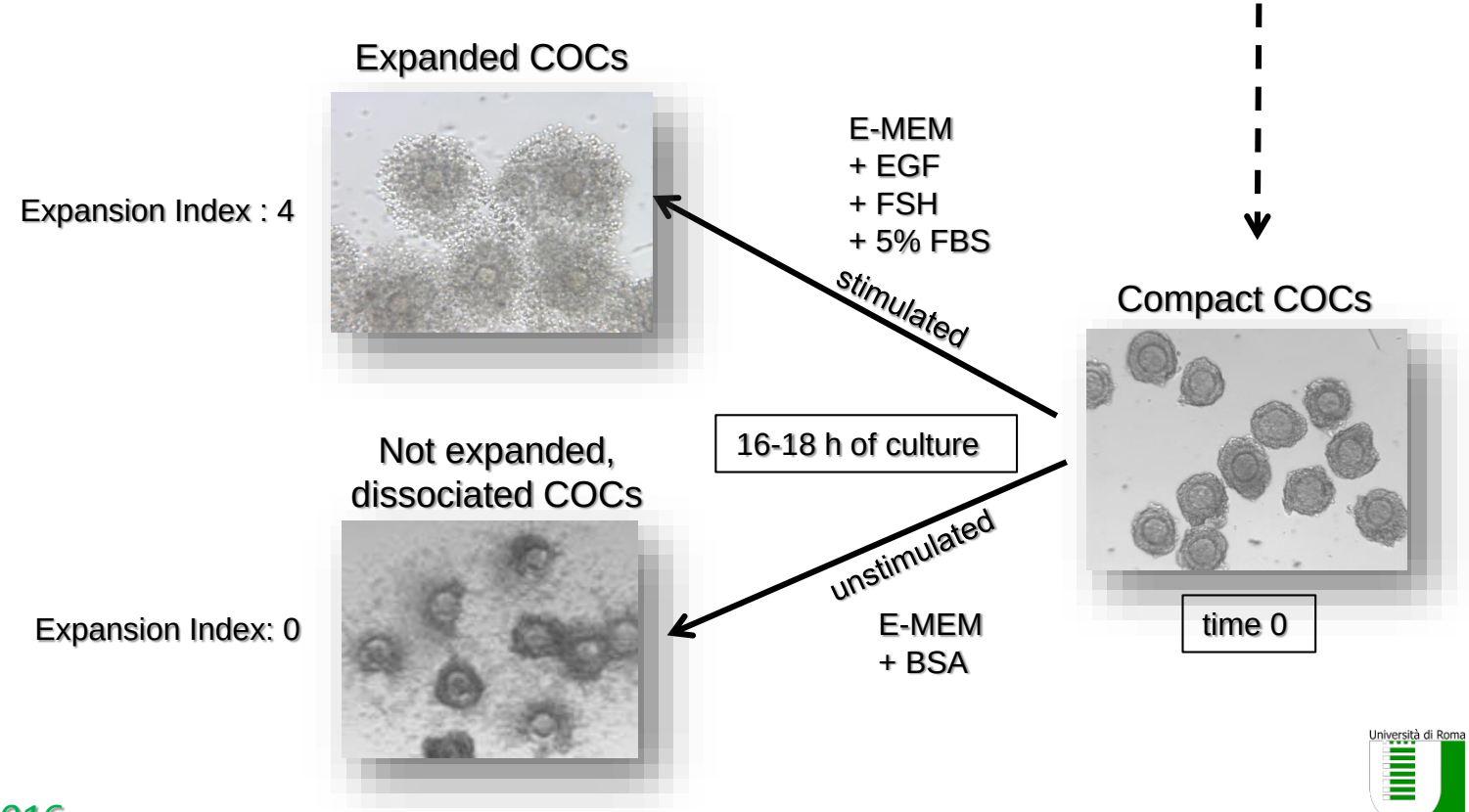
Puncture of the ovarian surface

Collection of cumulus cells-oocyte complexes (COCs)

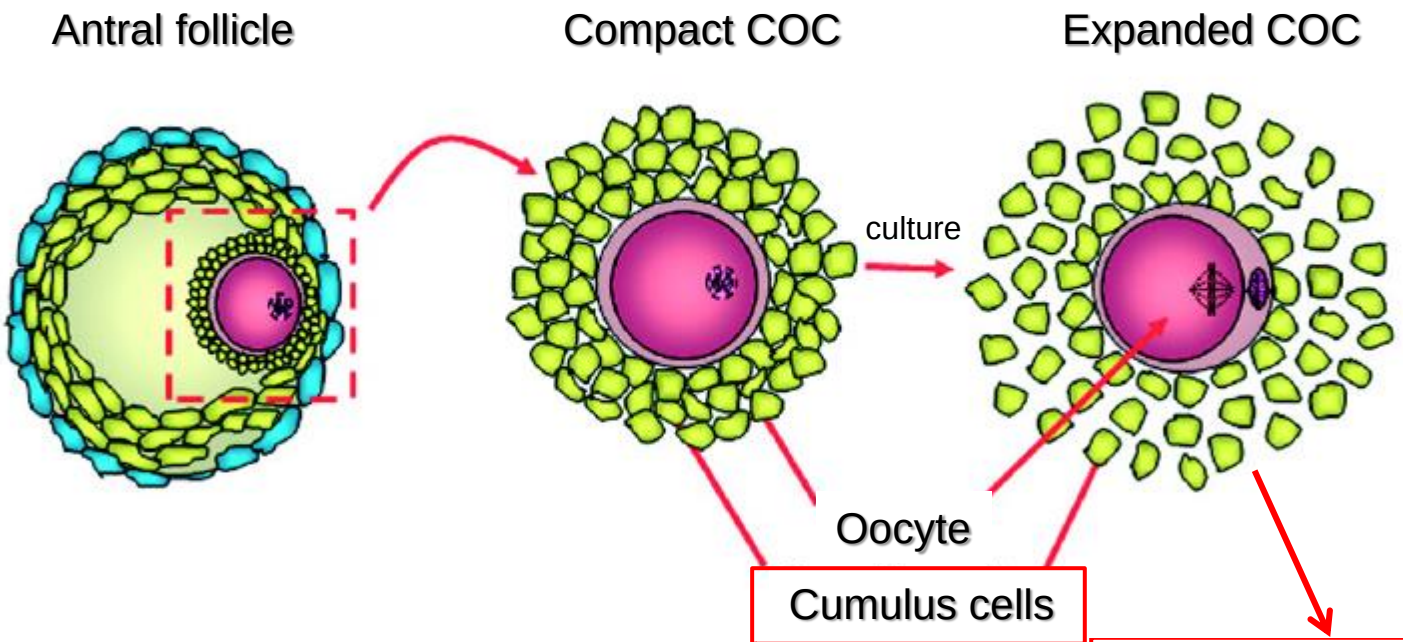
Experimental set-up:

Cumulus cell-oocyte complexe (COC) collection and maturation *in vitro*





Culture conditions allow the final maturation of COCs by mimicking what occurs physiologically in antral follicles before ovulation

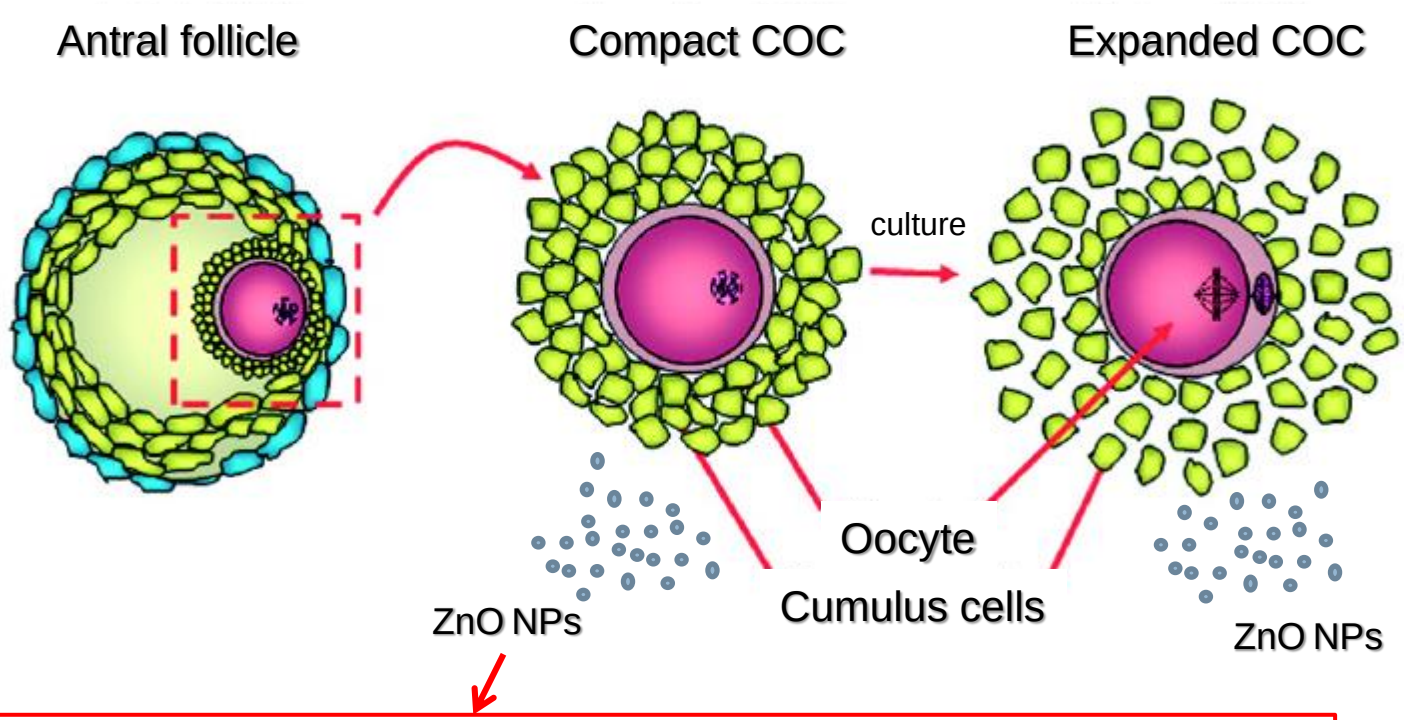


Interact with the oocyte in different ways, through:

- direct contact
- typical ligand-receptor binding
- paracrine signalling pathways
- gap junctions and other junctional contacts like desmosomes and zonulae adherentes

Hyaluronan (HA)
Proteoglycans
Pentraxin 3
Hyaluronan synthase 2
CD44 (HA receptor)

During the culture time ZnO nanoparticles (NPs) are added to the medium



from:
Joint Reserch Center (JRC) Repository, uncoated and coated

in-house prepared at Harvard School of Public Health, uncoated and coated

Zinc is important in reproduction?

Zinc deficiency leads to:

- In males, poor semen quality in term of sperm motility, vialibility and head morphology:
  Croxford et al., J Nutr 2011
  Sorensen et al., *Human Reprod* 2009

- In female, oocyte meiotic resumption impairment.
  Bernhardt ML et al. *Biol of Reprod* 2011
  Kim et al., *Nat Chem Biol* 2010

- Developmental problems throughout pregnancy:
  Uriu-Adams et al., *Birth defects Res B Dev Reprod Toxicol* 2010
  Keen et al., *J Nutr* 2003

Can zinc ions overloading be toxic for the cell?

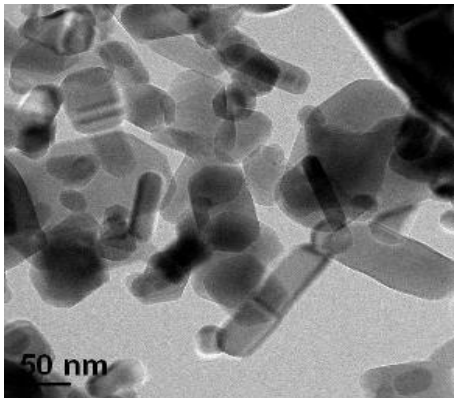
Oocyte and cumulus cells have zinc transporters, both for efflux and influx of Zn^{+}

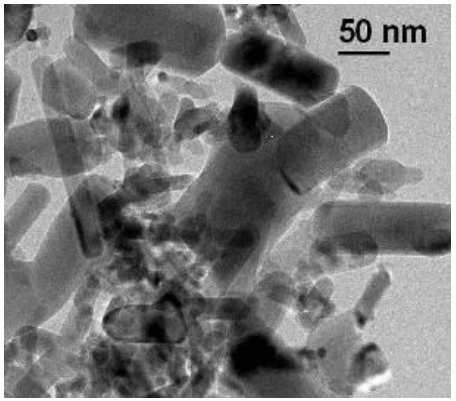
Cumulus cells are important for oocyte zinc homeostasis
Lisle RS et al., *Reproduction* 2013

Previous studies demonstrated that nano sized ZnO particles exert toxicity in mammalian cells like alveolar epithelial cells, macrophages and neuronal cell as measured by lysosomal damage, mitochondrial perturbation, reactive oxygen species generation, DNA damage, induction of pro-inflammatory cytokine release, and cell death….

…. no information is available on the effects of ZnO NPs on the reproductive system

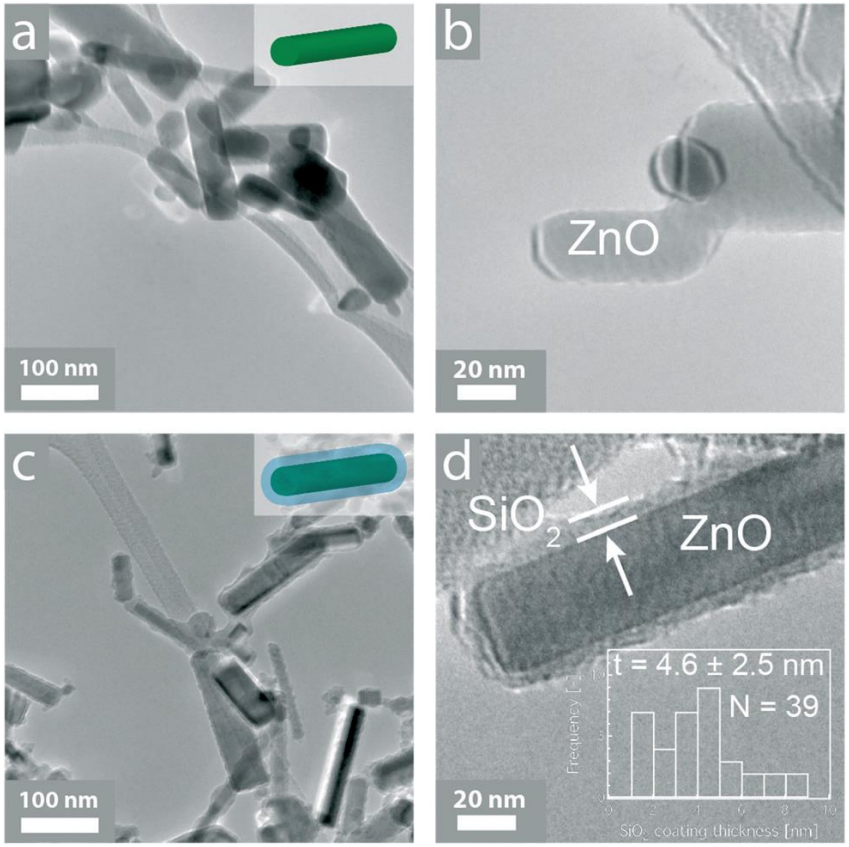
JRC ZnO Nanoparticles





	Average crystal size:
NM110 uncoated	42 nm
NM111 coated with triethoxycaprylsilane	34 nm

Home-made prepared ZnO NPs (Sotiriou et al. *Environ Sci.: Nano* 2014)



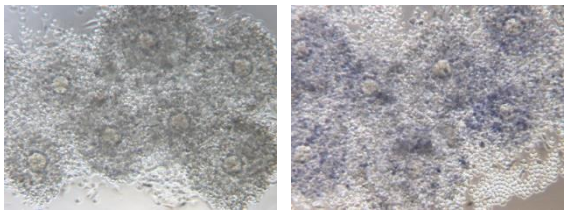
Average crystal size:

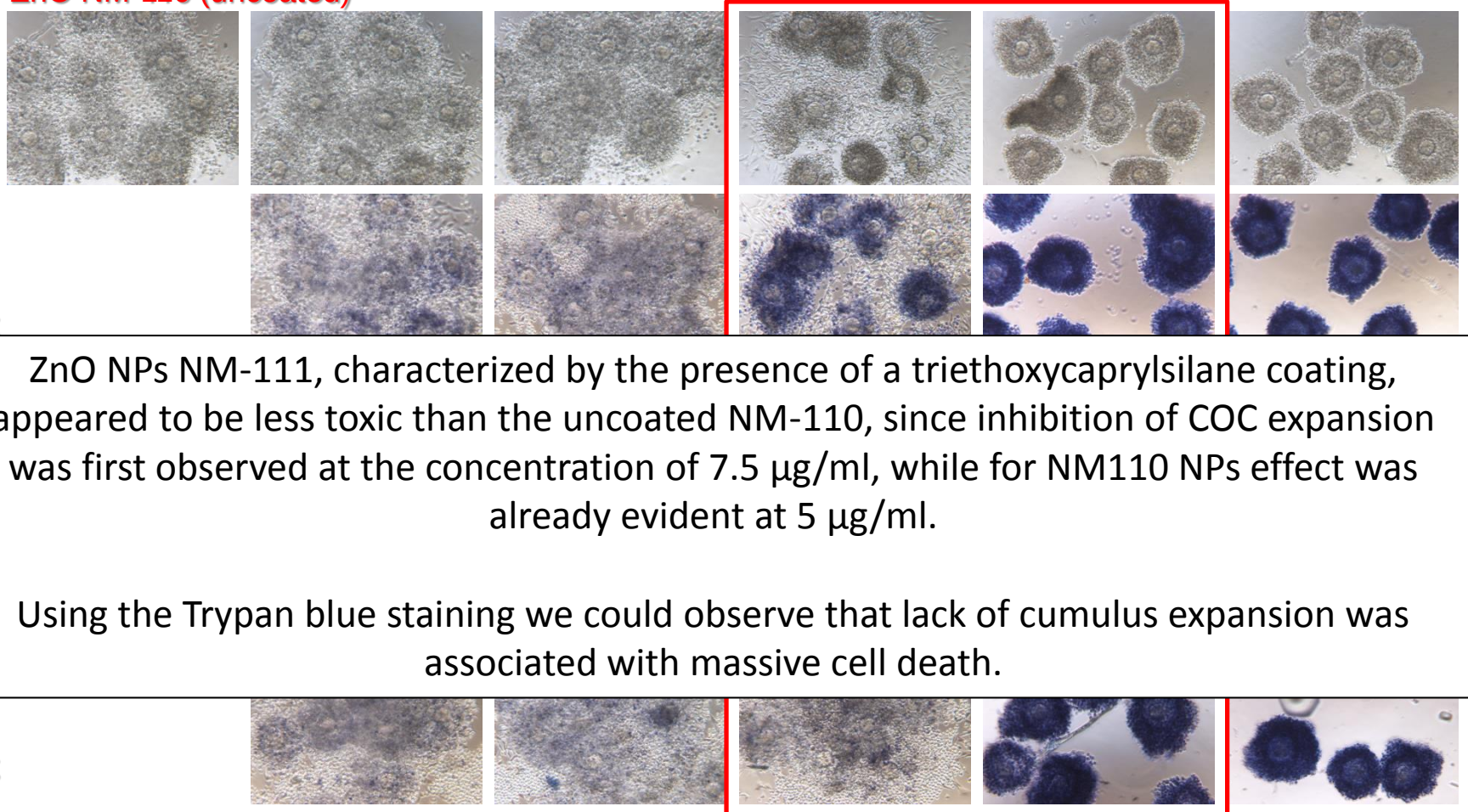
ZnO NPs uncoated — ~ 30 nm

ZnO NPs SiO_2-coated — ~ 30 nm

Coat: a nanothin amorphous SiO_2 shell of 4.6 ± 2.5 nm encapsulating the core particles

Effect of ZnO NPs (JRC) on COC expansion





ZnO NPs NM-111, characterized by the presence of a triethoxycaprylsilane coating, appeared to be less toxic than the uncoated NM-110, since inhibition of COC expansion was first observed at the concentration of 7.5 µg/ml, while for NM110 NPs effect was already evident at 5 µg/ml.

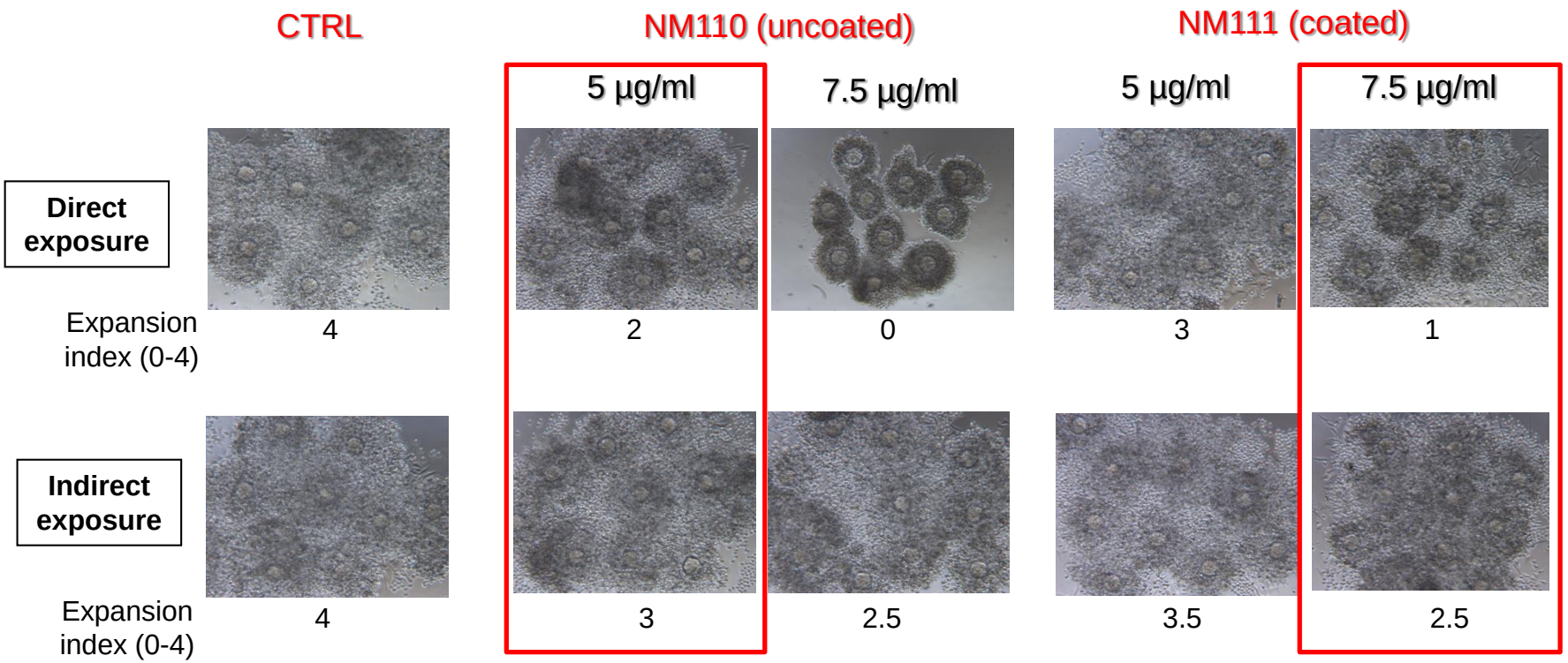
Using the Trypan blue staining we could observe that lack of cumulus expansion was associated with massive cell death.

In the literature, the reported cytotoxicity associate with ZnO NP exposure is attributed to

✓ the released ions from its rapid dissolution within aqueous solution and

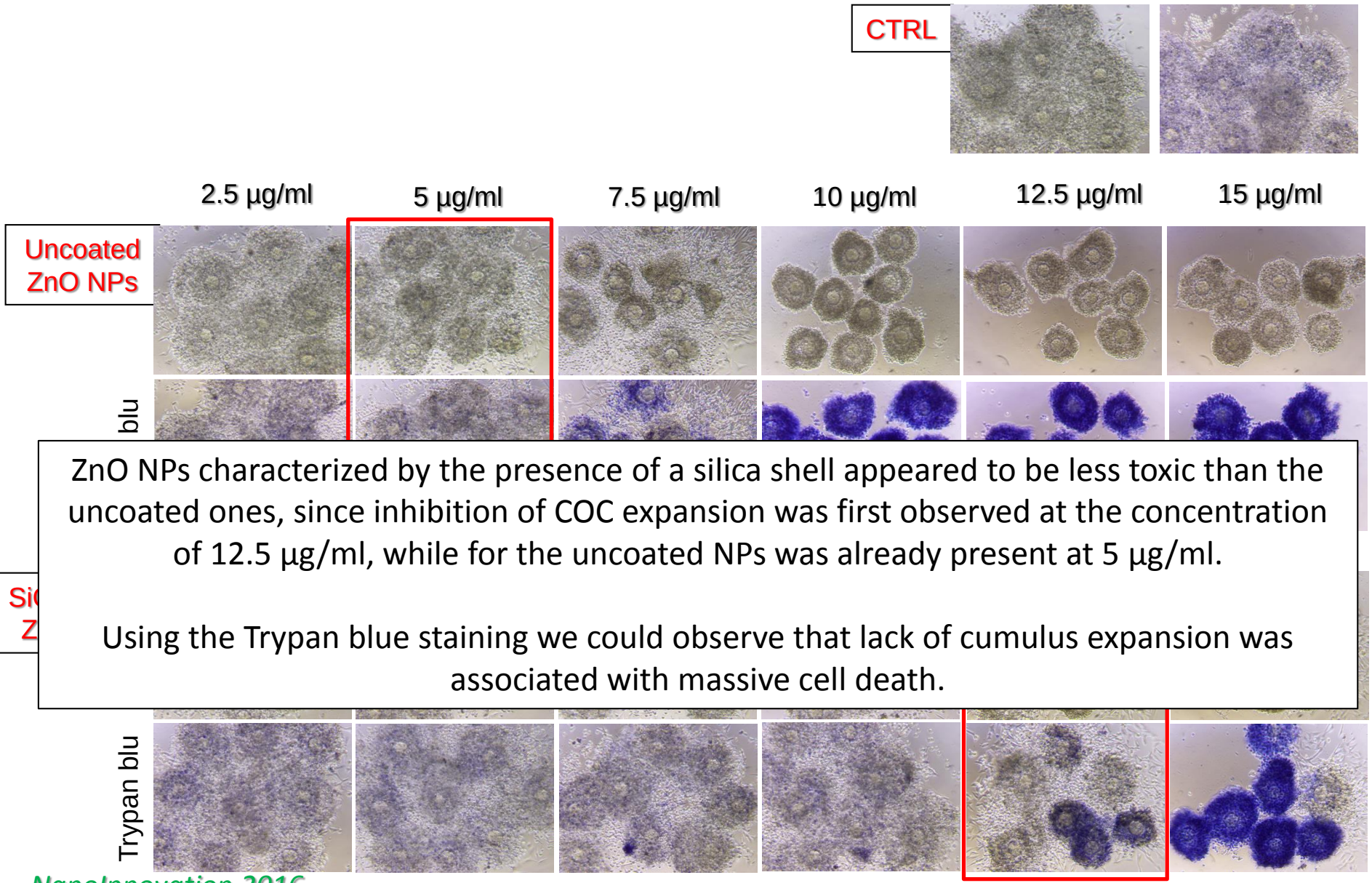
✓ from direct particle interaction with cells at the plasma membrane level, eventually followed by endocytosis events

Comparison beetwen direct exposure and indirect exposure (conditioned medium) to ZnO NPs uncoated and coated



ZnO NP-conditioned medium is not as toxic as direct ZnO NP exposure

Effect of uncoated and SiO_2-coated ZnO NPs (Harvard) on COC expansion

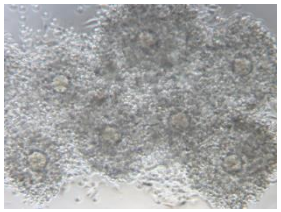


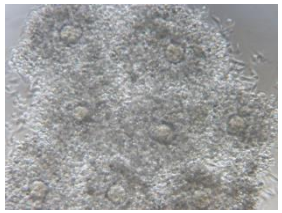
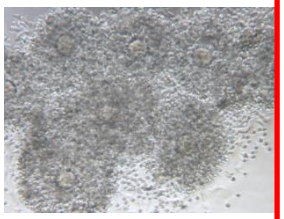
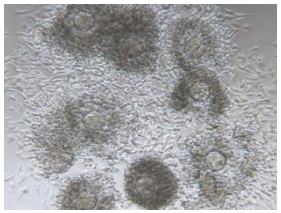
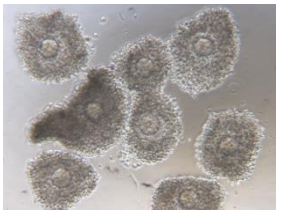
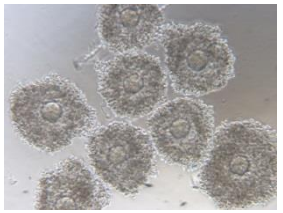
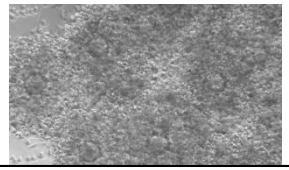
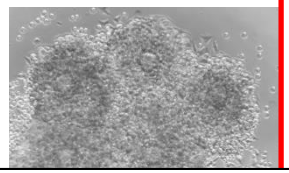
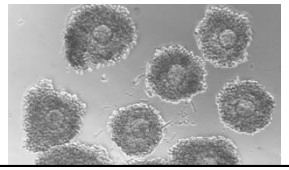
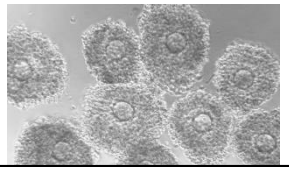
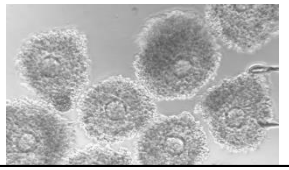
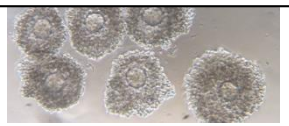
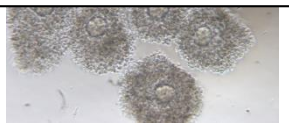
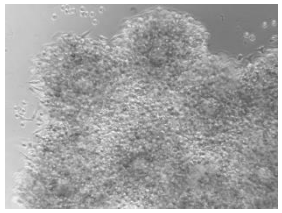
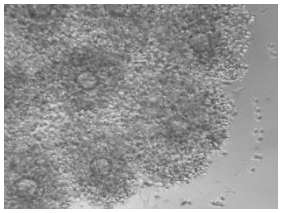
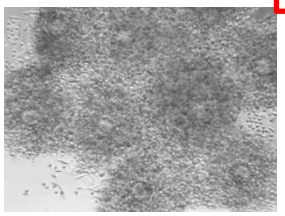
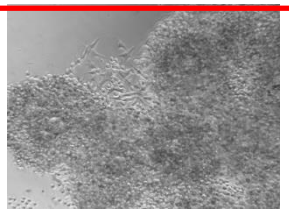
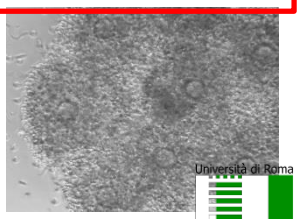
ZnO NPs characterized by the presence of a silica shell appeared to be less toxic than the uncoated ones, since inhibition of COC expansion was first observed at the concentration of 12.5 µg/ml, while for the uncoated NPs was already present at 5 µg/ml.

Using the Trypan blue staining we could observe that lack of cumulus expansion was associated with massive cell death.

Comparison beetwen the two preparation of ZnO NPs, both uncoated and coated

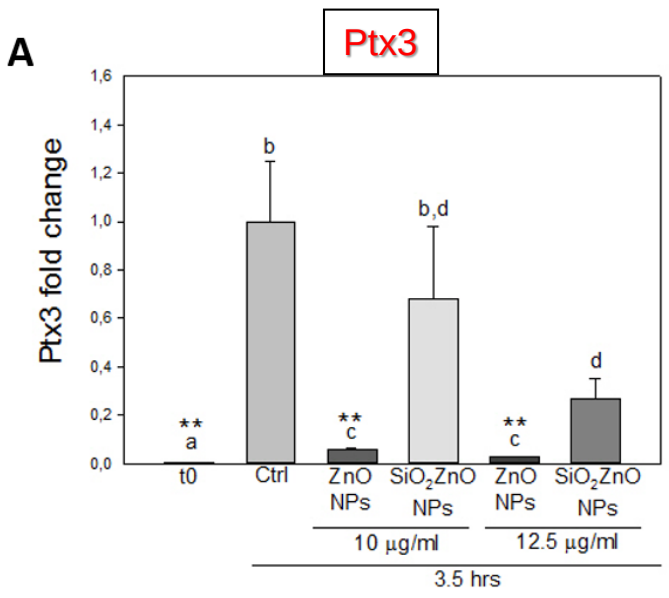
CTRL

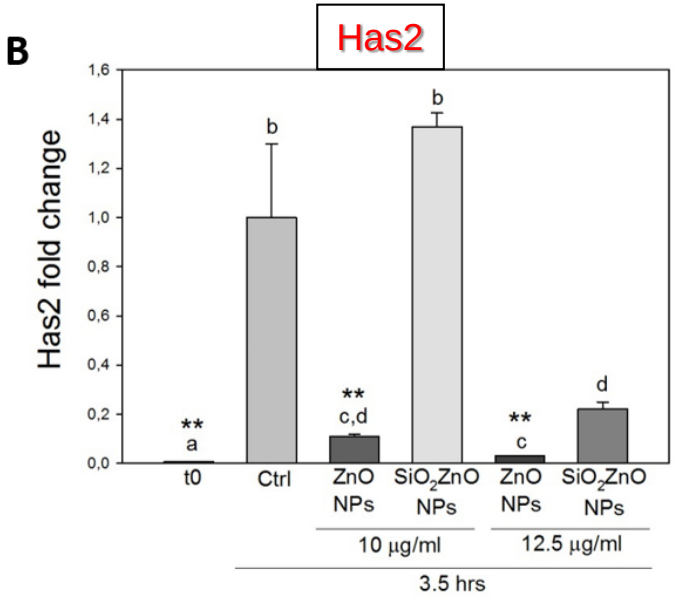


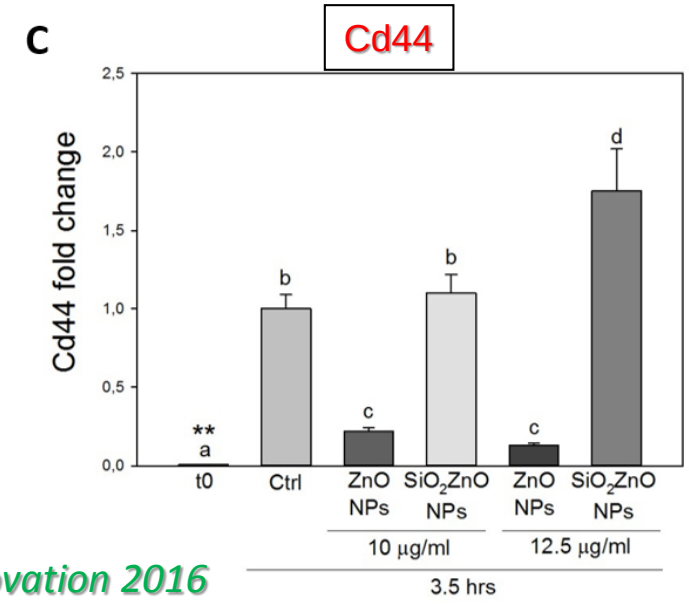
	1 µg/ml	2.5 µg/ml	5µg/ml	7.5 µg/ml	10 µg/ml
Uncoated — ZnO NM-110 (JRC)					
Uncoated — ZnO (Harvard)					
Coated — ZnO NM-111 (JRC)					
Coated — SiO_2 ZnO (Harvard)					

The presence of the silica shell in the in-house prepared Zn NPs (Harvard) prevent toxicity better than the triethoxycaprylsilane coating of the JRC ZnO NPs

Gene expression by quantitative real-time PCR in cumulus cells treated with uncoated and SiO_2-coated ZnO NPs for 3.5 hours







Gene expression by cumulus cell is affected by ZnO NPs in a dose- and coat-dependent manner

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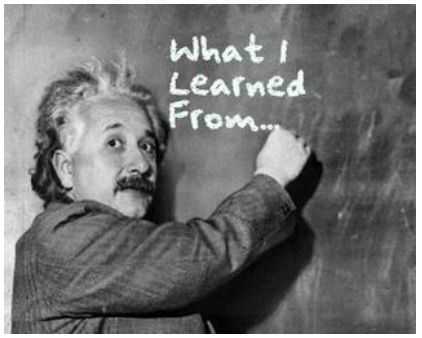
Take-home messages



From the biologist and toxicologist point of view:

- the COC expansion process performed *in vitro* could be a powerful system to assess alterations in the microenvironment of the oocyte and could provide a quite sensitive platform to explore the reproductive effect and toxicological mechanisms of nanomaterials

from the materials science and chemical engineer point of view:

- the encapsulation and coating of NPs with inert or smart material is a powerful tool to minimize risks to the human health and to maintain or possibly enhance the performance of a NP by itself, but they need to be carefully analyzed and compared

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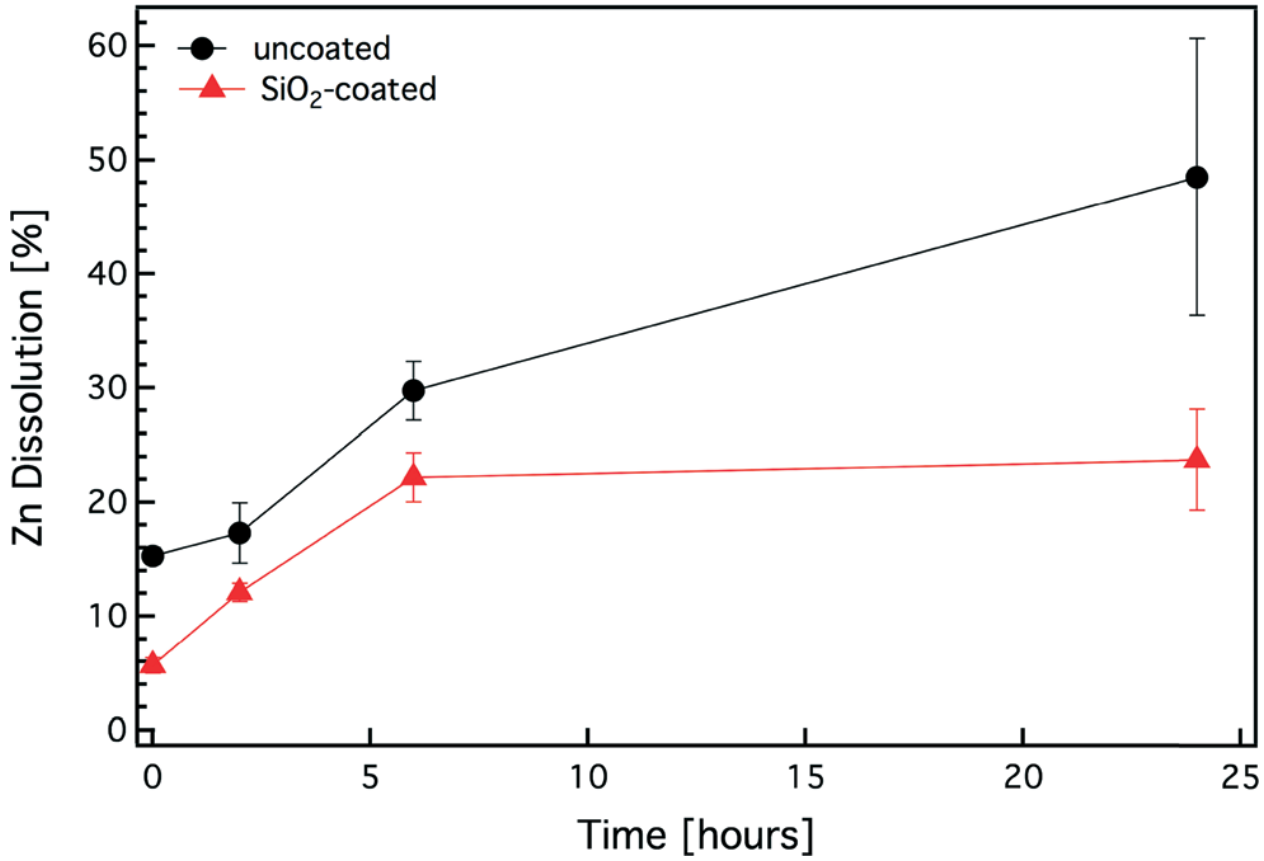
Luisa Campagnolo
Antonietta Salustri
Antonio Pietroiusti
Andrea Magrini

Micol Massimiani
Lucia Vecchione
Diletta Piccirilli

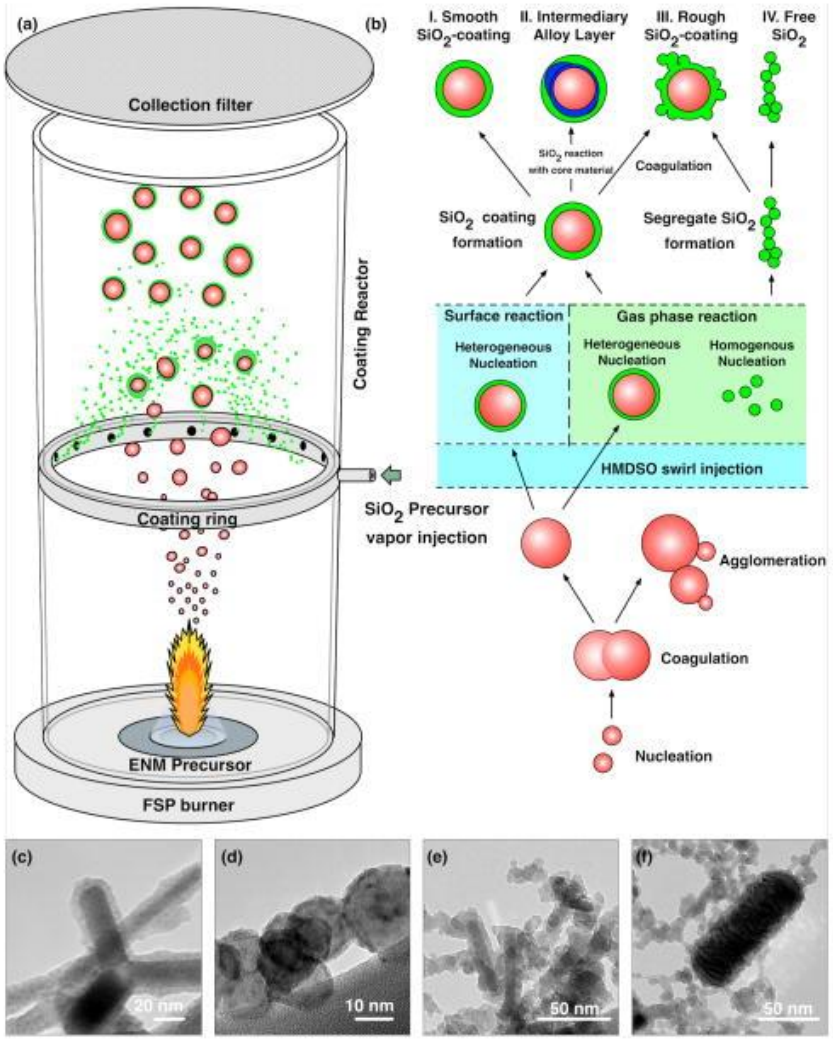
Georgios A. Sotiriou
Philip Demokritou
Center for Nanotechnology and Nanotoxicology
Harvard School of Public Health, Harvard, USA

Flemming Cassee
Dept of Inhalation Toxicology
National Institute for Public Health and the Environment
Bilthoven, The Netherlands



The dissolution kinetics of uncoated (black circles) and SiO_2-coated ZnO nanorods (red triangles) in RPMI-1640 cell culture medium (pH = 7.4) for initial ZnO concentration of 100 mg/L

One step Engineered Nanomaterials (ENM) synthesis and in-flight SiO_2 incapsulation.



Core-shell particles exhibit the surface properties of their amorphous SiO_2 shell while maintaining specific functional properties of their core material.

ACS Sustain Chem Eng. 2013;1(7):843
A Safer Formulation Concept for Flame-Generated Engineered Nanomaterials.
Gass S, Cohen JM, Pyrgiotakis G, Sotiriou GA, Pratsinis SE, Demokritou P.