Cytoskeletal and ultrastructural alterations induced by TiO_2 and ZnO nanoparticles into antral follicular cells

R. Santacruz-Márquez[1], S. González-Pozos[2], S.P. García-Zepeda[1], A. Solorio-Rodriguez[1], A. De Vizcaya-Ruiz[1], I. Hernández-Ochoa [1]

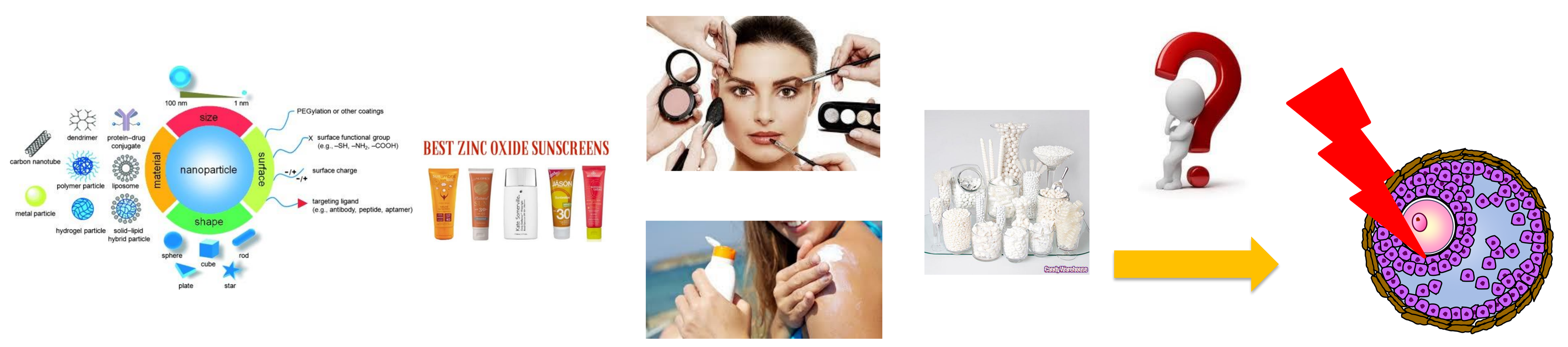
[1]Department of Toxicology, Cinvestav–IPN, Mexico, [1]Unidad de Microscopia Electronica (LaNSE), Cinvestav–IPN, Mexico

59th Annual Meeting & TOXEXPO • March 15–19, 2020
Anaheim, California
Abstract No.

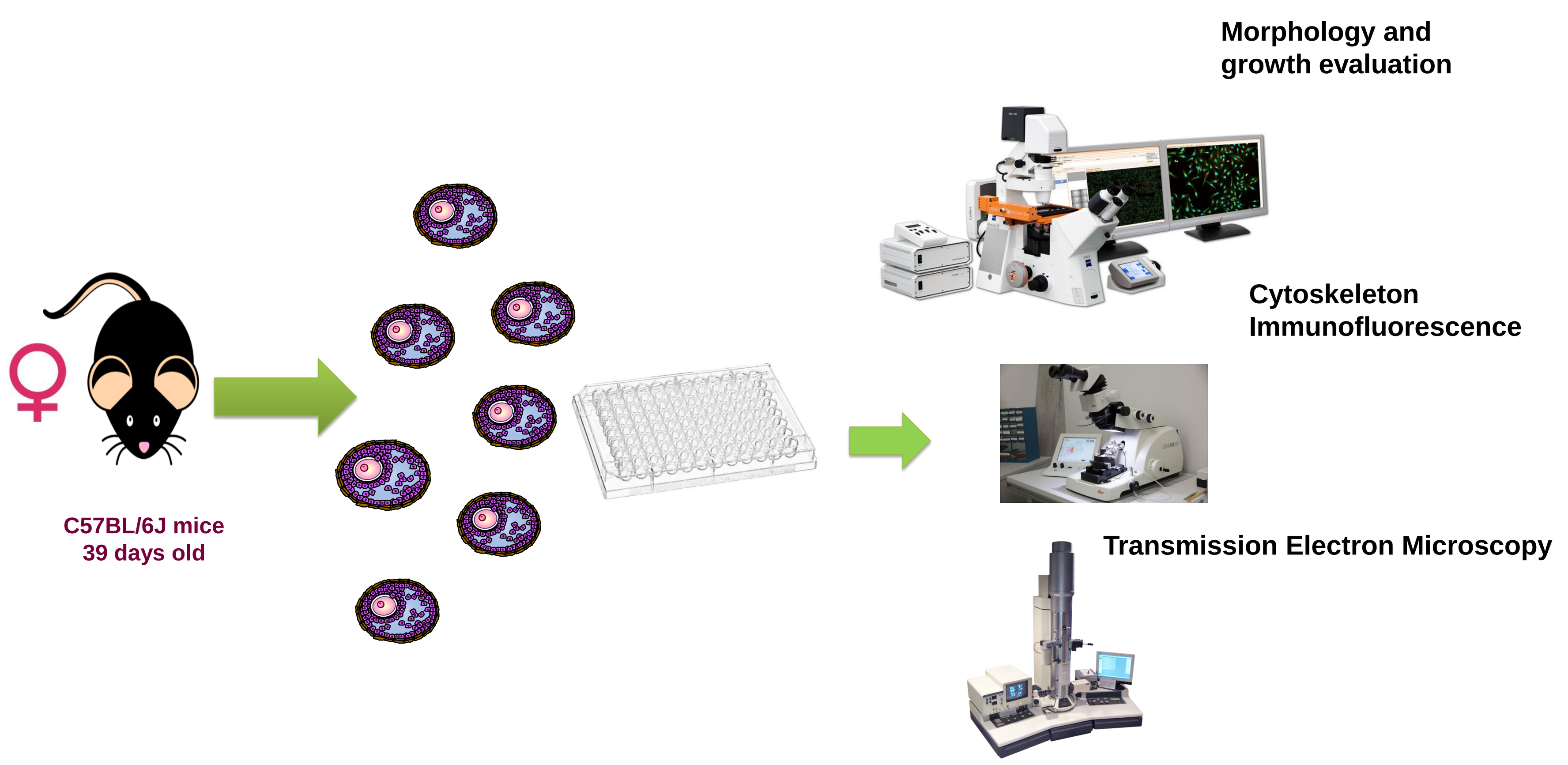
Abstract

Titanium dioxide nanoparticles (TiO_2-NP) and zinc oxide nanoparticles (ZnO-NP) are metallic NP widely used in several personal care products, cosmetics, textiles and food. TiO_2-NP have been detected in ovaries from exposed mice and studies have found that TiO_2-NP and ZnO-NP exposure alters hormone levels, mating and pregnancy rates. However, no studies have evaluated a direct interaction of NP with ovarian cells. Antral follicles are the functional units of the ovary. They possess steroidogenic activity and are responsible for growing, housing and promoting competence of the oocyte to be fertilized. Previously, we characterized both NP in culture media, and we found impaired antral follicle growth and morphological alterations. This work aimed to evaluate ovarian follicle alterations in the cytoskeleton arrangement and ultrastructure after TiO_2-NP and ZnO-NP exposure. Antral follicles from C57BL6/J mice were cultured with TiO_2-NP (0, 5, 25 and 50 µg/mL) or ZnO-NP (0, 5, 15 and 25 µg/mL) for four days. After culture, immunofluorescence and transmission electron microscopy were performed to determine cytoskeletal and ultrastructural alterations, respectively. Our results show alterations in the cytoskeletal organization in cells from exposed antral follicles, as evidenced by the loss of microtubule boundless irradiating to the periphery and by the appearance of hole-like structures in microtubule such as disorganization in microfilaments. TiO_2-NP were mainly internalized into antral follicles using endocytosis and macropinocytosis transport systems. TiO_2-NP were localized at every follicular cell type but the oocyte. ZnO-NP were not observed in follicular cells because they may dissolve in the culture media. Finally, both NP affected mitochondrial ultrastructure, as well as the trans-zonal projections in the oocyte, which communicate it with surrounding cumulus. Our results suggest that NP elicit toxicity in ovarian follicles via different mechanisms of toxicity, possibly due to their differentiated fate in the culture.

Introduction



Methods



Supported by Fondo de Investigación Cinetífica y Desarrollo Tecnológico del Cinvestav (grant No. 189).

Results

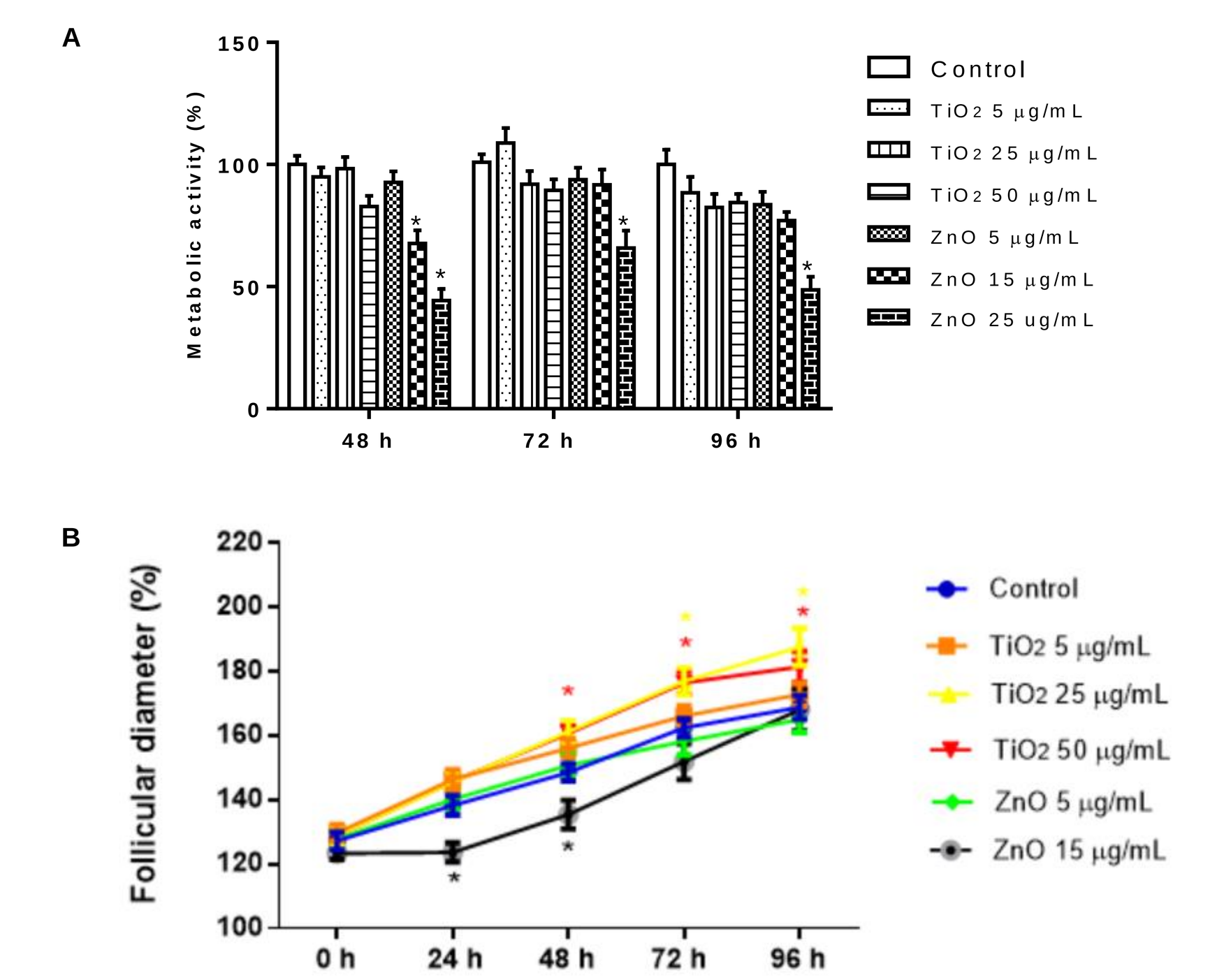


Figure 1. Effects of TiO_2 and ZnO NP on follicular viability and growth. Antral follicles were individually cultured. At day 3 of culture, TiO_2 (5, 25, and 50 µg/mL) or ZnO (5, 15, and 25 µg/mL) NP were added. Follicular viability was determined at different time points (A), and follicular diameters were recorded every 24 h. Data are presented as mean ± SEM from 12-15 antral follicles per treatment group in 3 independent experiments. * Denotes significant differences compared to the control at same time point ($p \leq 0.05$).

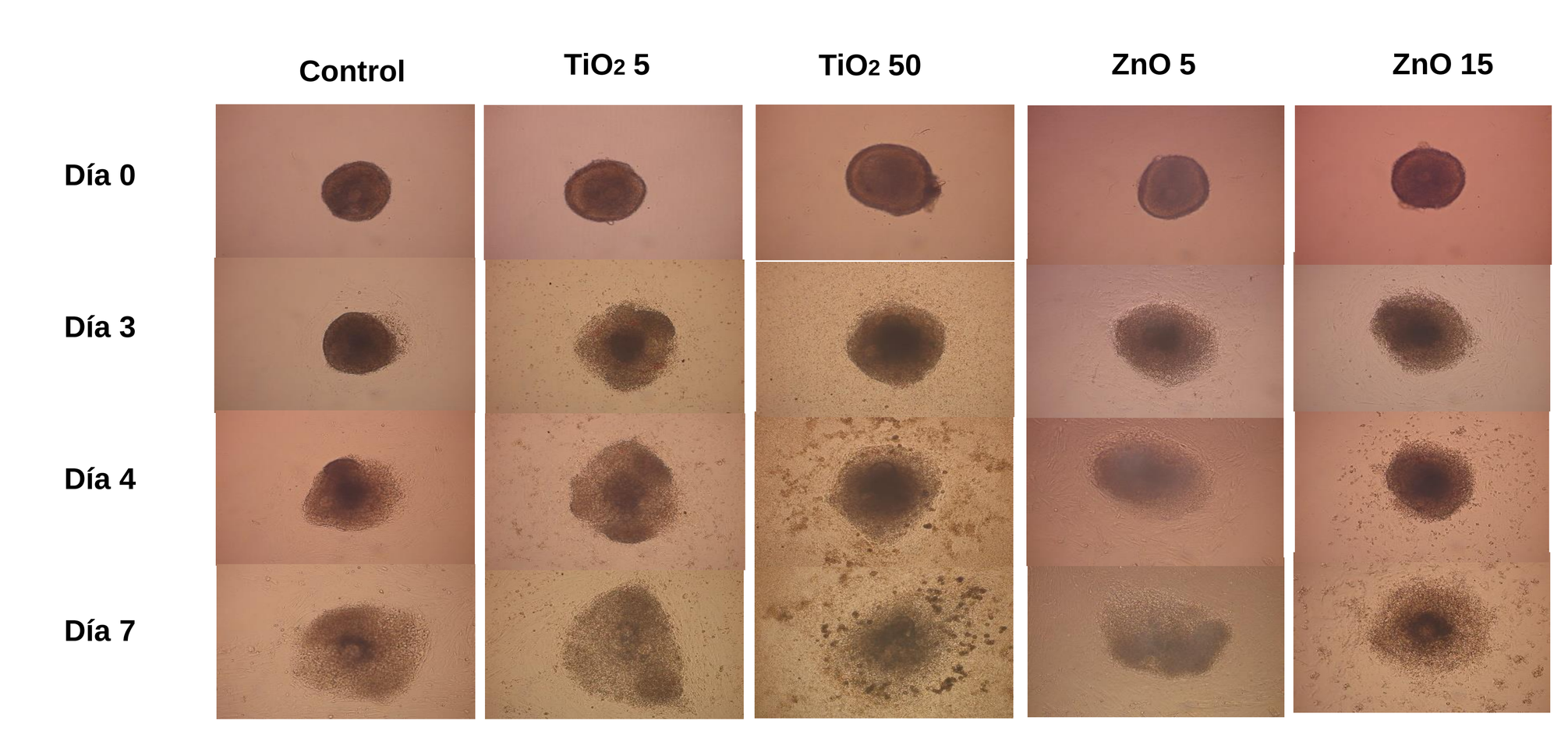


Figure 2. Effects of TiO_2 and ZnO NP on antral follicle morphology. Antral follicles were isolated and cultured and antral follicle morphology alterations were analyzed and recorded using an inverted microscope with a digital camera. Magnification: 20x.

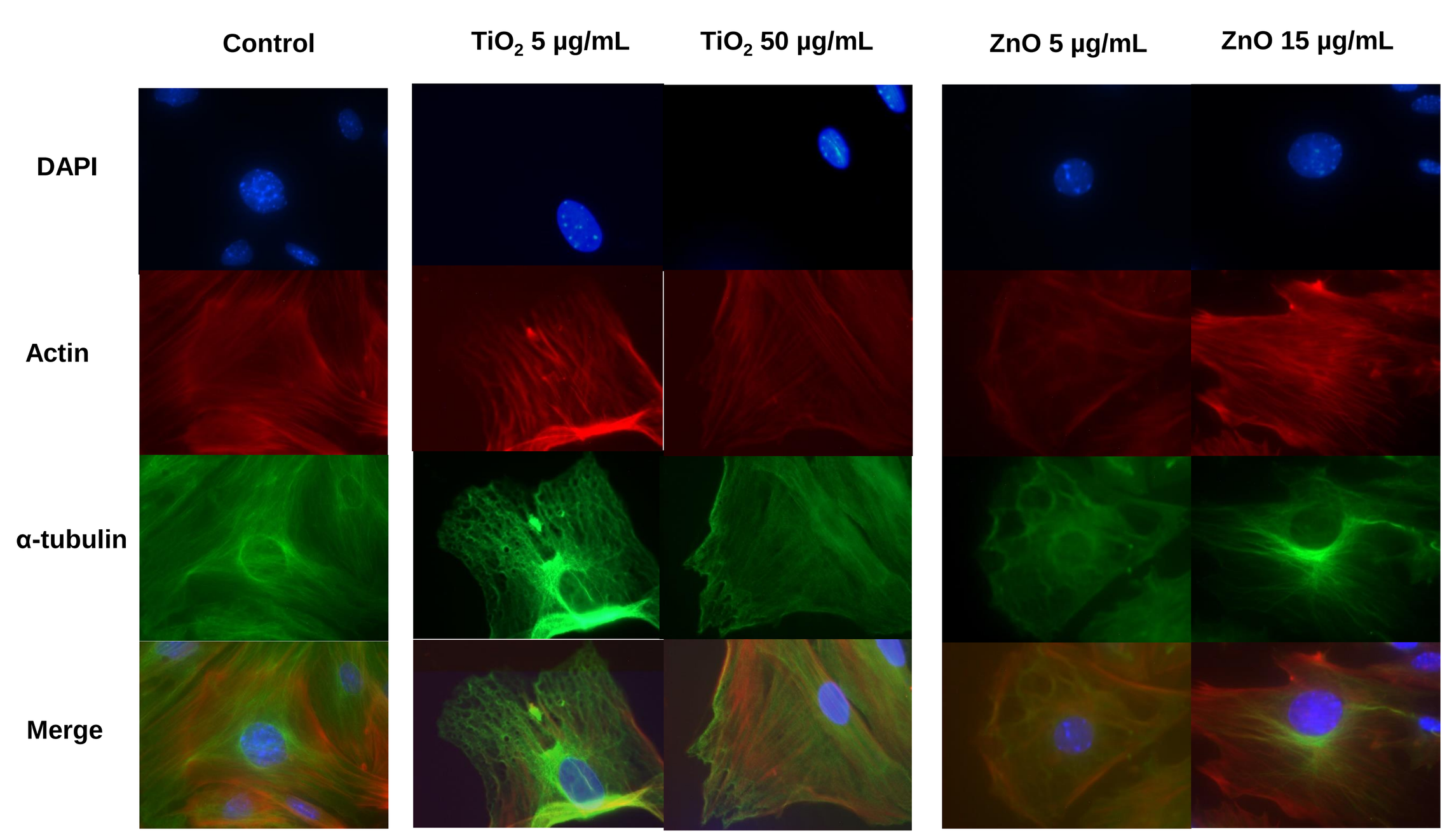


Figure 3. Effect of TiO_2 and ZnO NP in cytoskeletal organization. Antral follicles were exposed to NP and processed for immunofluorescence to evaluate α-tubulin and F-actin organization within theca cells. Representative micrographs of TiO_2 and ZnO NP effect into the cytoskeletal organization are presented. DAPI was used to stain nuclei.

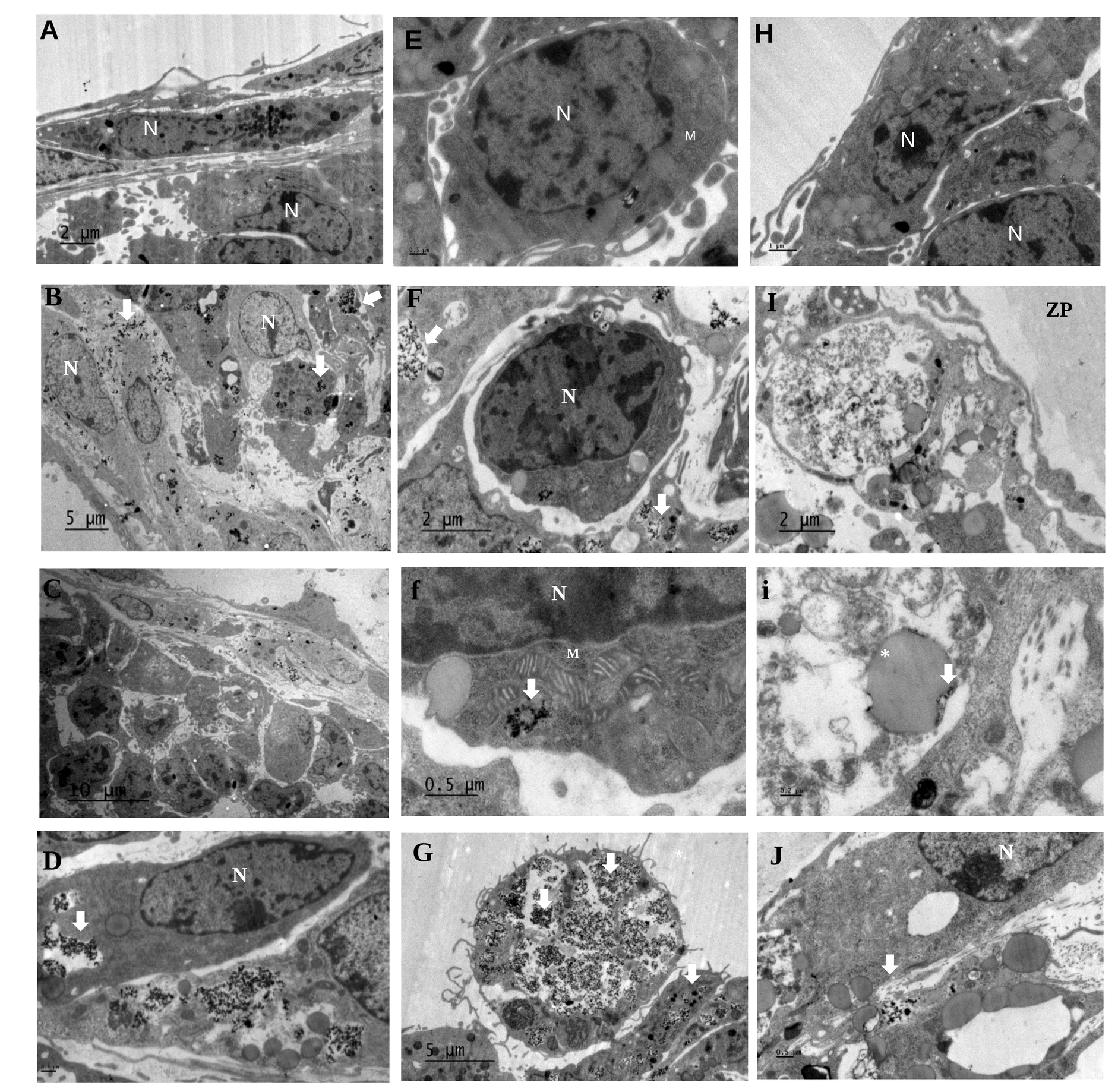


Figure 4. Analysis of TiO_2 NP internalization into the follicle. Antral follicles were cultured and exposed to TiO_2 NP (50 µg/mL). After 96 h of exposure, antral follicles were processed for TEM as described in materials and methods. Representative micrographs of control follicular cells (A, E and H) and TiO_2 NP exposed theca (B, C, and D), granulosa (F and G) and cumulus cells (I and J) are presented with magnifications of granulosa (f) and cumulus (i) cells. White arrow indicates NP localization in cells. N; nucleus, M; mitochondria, *: lipid droplets, ZP; zona pellucida.

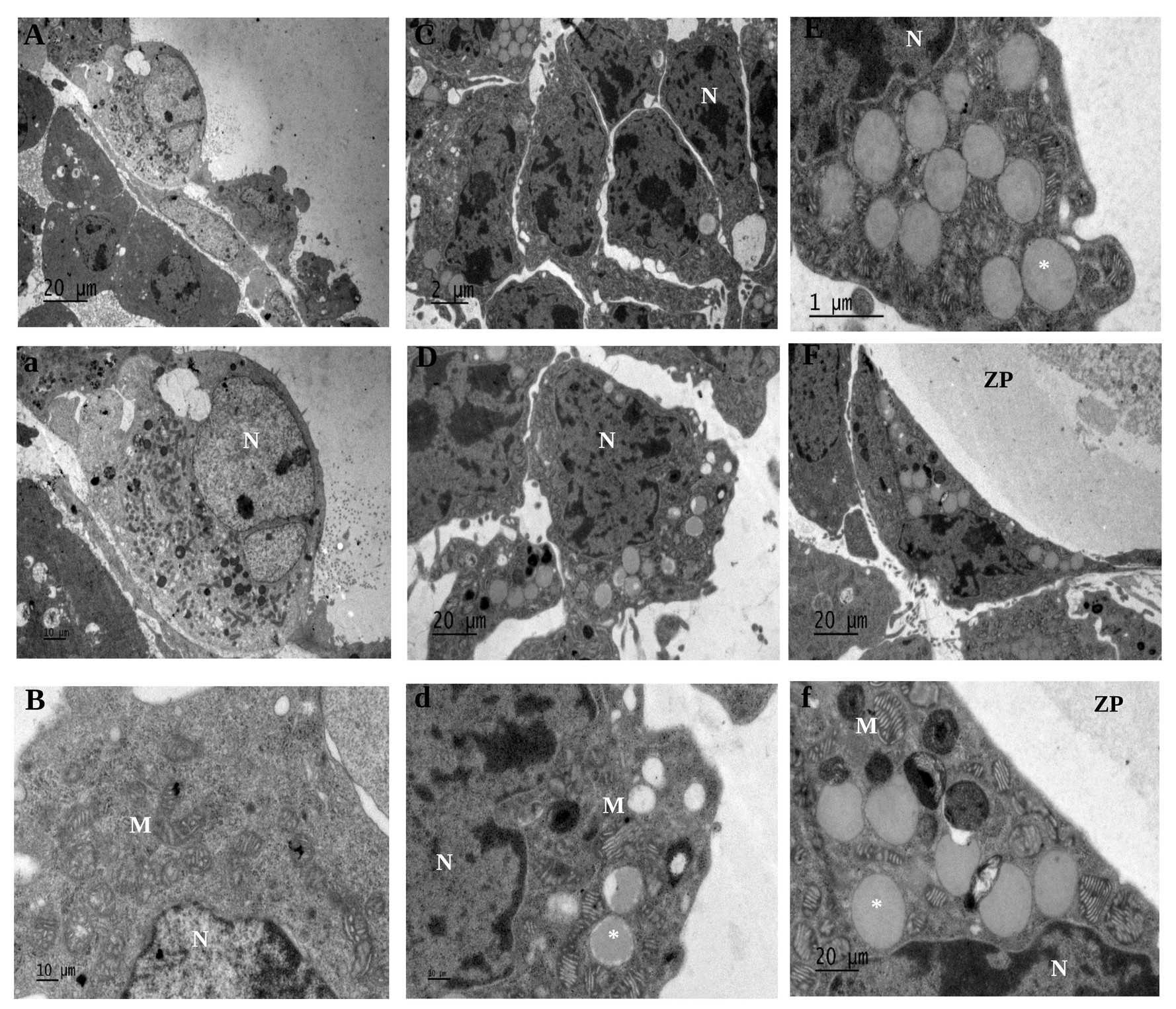


Figure 5. Analysis of ZnO NP internalization into the follicle. Antral follicles exposed to ZnO (15 µg/mL) were processed for TEM analysis. Representative micrographs of theca (A and B), granulosa (C and D) and cumulus (E and F) cells as well as magnifications of granulosa (d) and cumulus (f) cells are presented. There is no obvious presence of ZnO NP into the follicular cells. N; nucleus, M; mitochondria, *: lipid droplets, ZP; zona pellucida.

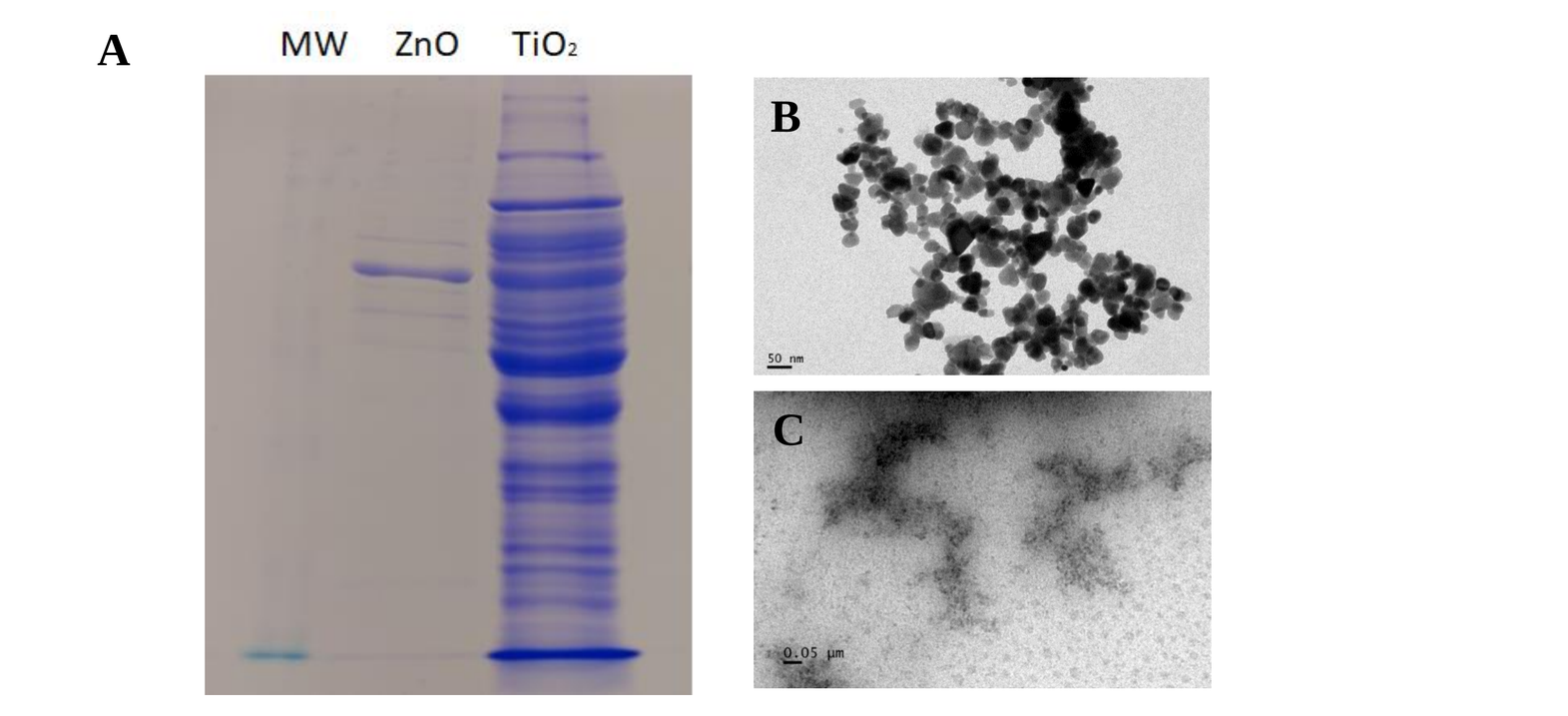


Figure 6. ZnO NP dissolution into the culture media. ZnO NP (15 µg/mL) were suspended as previously described, and then observed in TEM or subjected to a protein corona formation protocol to determine protein binding. Representative micrographs of ZnO NP suspensions in water (B) and in culture media (C) are presented. In a, protein binding pattern are presented for TiO_2 and ZnO NP.

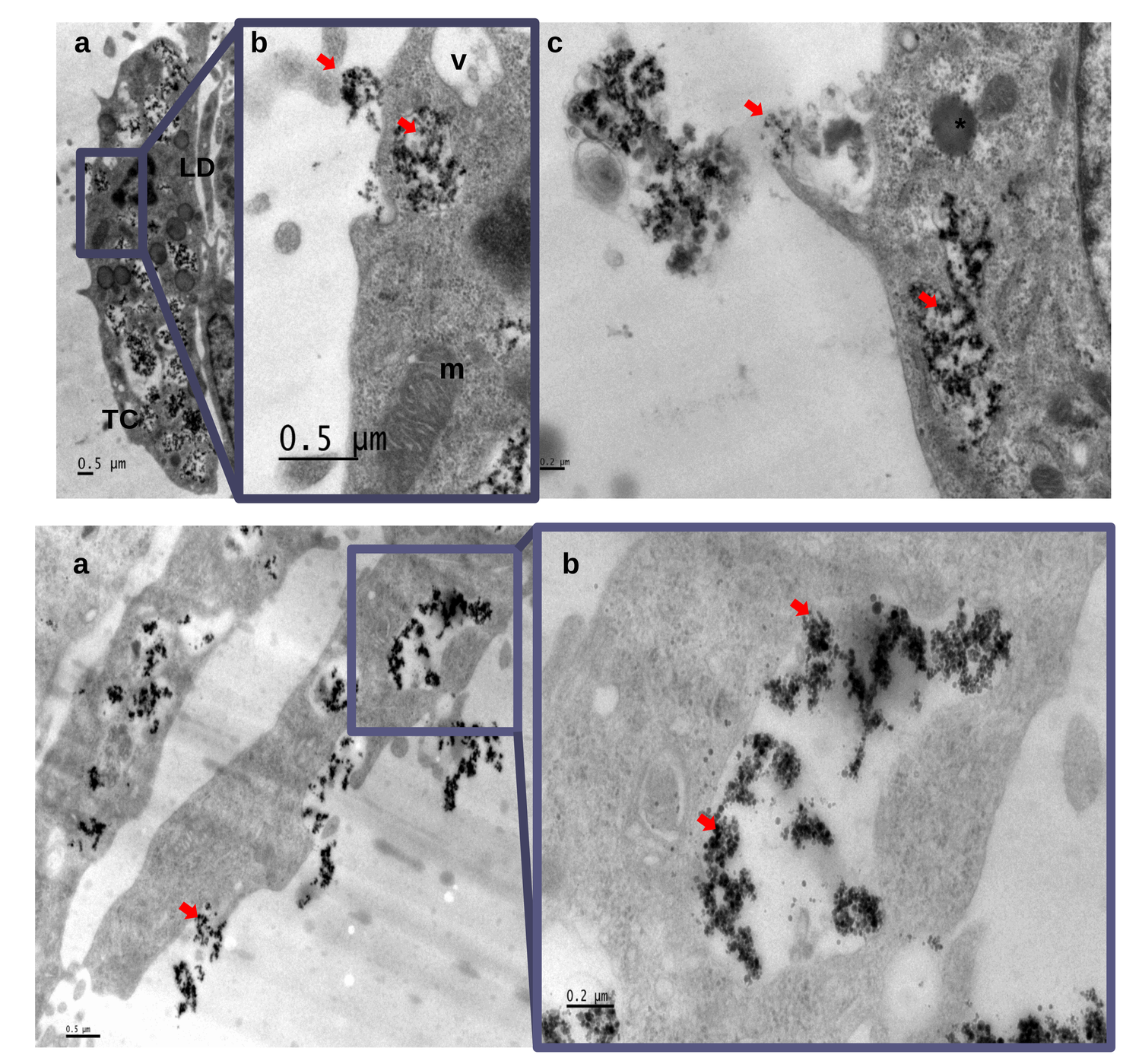


Figure 7. Internalization pathways of TiO_2 NP into follicular cells. Antral follicles were cultured and exposed to TiO_2 NP (50 µg/mL). After 96 h of exposure, antral follicles were processed for TEM. TiO_2 NP can be observed inside theca cells. a; theca cell, b d and e; invaginations formation into theca cell membranes, c; macropinocytosis internalization process. *: lipid droplets, red arrows: TiO_2 NP, m: mitochondria.

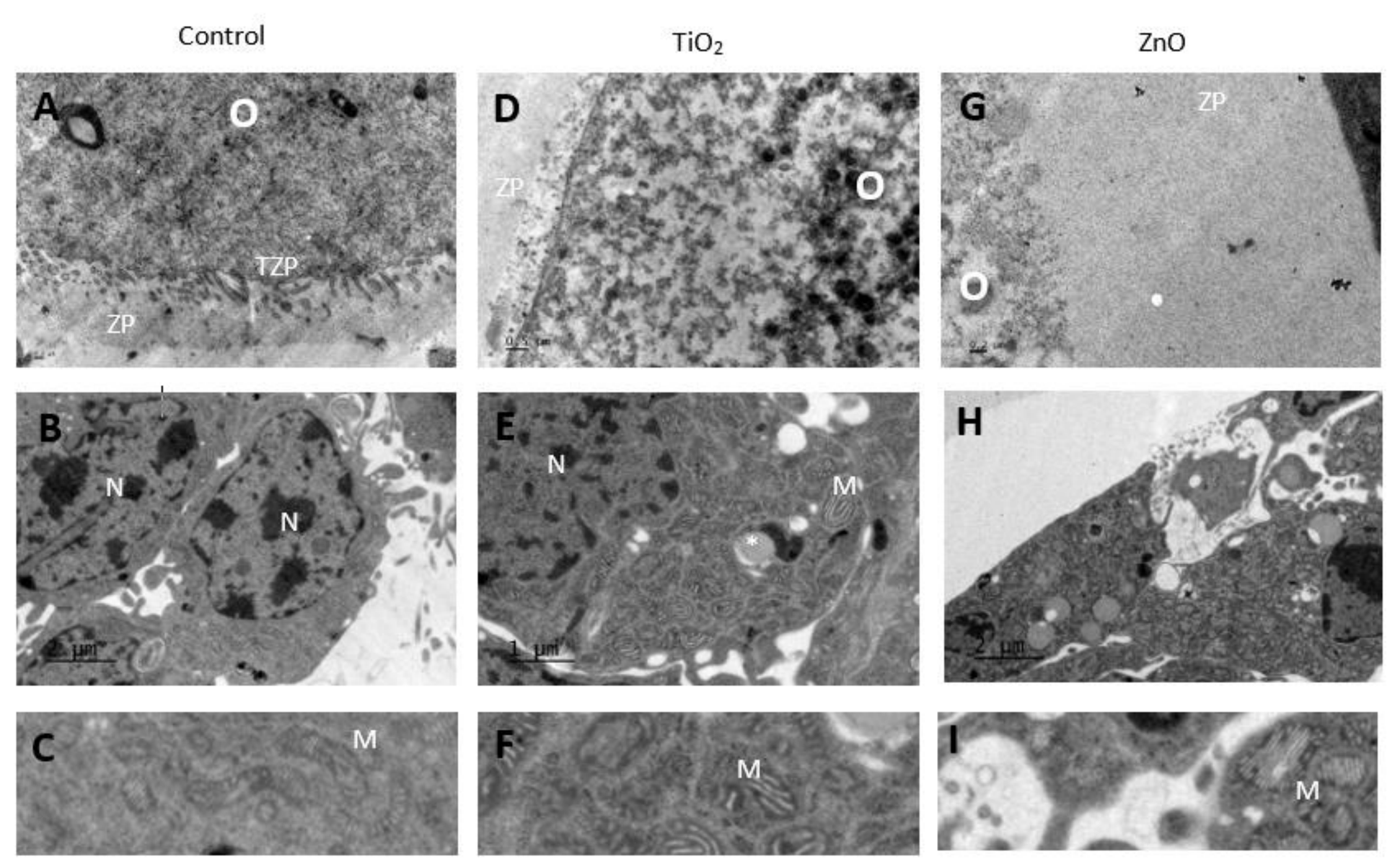


Figure 8. Ultrastructural alterations induced by TiO_2 and ZnO NP in follicular cells. Antral follicles were cultured and exposed to TiO_2 (50 µg/mL) and ZnO (15 µg/mL) NP. After 96 h of exposure, antral follicles were processed for TEM. Representative micrographs of control (A), TiO_2 (D) and ZnO (G) exposed oocytes are shown. Follicular cells showing mitochondrias from control (B and C), from TiO_2 (E and F) and from ZnO (H and I) NP treated are depicted. ZP: zona pellucida, O: oocyte, N: nucleus, M: mitochondria, TZP: transzonal projections.

Conclusions

- TiO_2 and ZnO nanoparticles affect the antral follicle morphology by affecting cytoskeleton arrangement.
- TiO_2 nanoparticles are internalized into the follicular cells by endocytosis and macropinocytosis.
- TiO_2 nanoparticles are internalized and aggregated into all follicular cells, except into the oocyte, located into vesicles and also as ‘free’ into the cytoplasm.
- ZnO nanoparticles were not observed into antral follicular cells, due to their dissolution capability.
- TiO_2 and ZnO nanoparticles affects antral follicle cells ultrastructure as evidenced by the mitochondrial alterations and the oocyte cytoplasmic projections loss.
- TiO_2 and ZnO nanoparticles exposure may have negative impact into the antral follicle functions and oocyte quality.