

# The Effect of Nanoparticles in Rats During Critical Periods of Pregnancy

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**Key words:** nanoparticles; quantum dots; pregnancy; embryotoxicity; placental barrier.

**Summary.** Background and Objective. Nanotechnology works with substances at a nanometer scale, and it offers many solutions for biomedicine. Nanoparticles (NPs) have been shown as effective agents for imaging, drug delivery, pathogen detection, etc. However, to date, NP toxicity is poorly known. The aim of our study was to investigate the embryotoxicity and teratogenicity of quantum dots (QDs) at the different stages of rat embryogenesis.

**Materials and Methods.** Wistar rats were injected with CdSe/ZnS or CdTe QDs on the 6th, 13th, and 18th days of embryogenesis. Cyclophosphamide was chosen as a positive control of embryotoxicity. On the 21st day, the number of resorptions, weight, length, and external malformations of the embryos were estimated. Fluorescence spectroscopy and microscopy analysis were used to determine the accumulation of QDs in the tissues.

**Results.** Exposure to cyclophosphamide during the pregnancy decreased the embryonic weight and length when compared with the control group and produced numerous malformations. The effects depended on the stage of embryogenesis. Meanwhile, QDs did not cause any embryotoxic or teratogenic effects. However, CdTe QDs induced necrosis in the tissues of the peritoneal cavity. The necrotic tissues contained QDs with altered spectroscopic properties. Spectroscopic and microscopic tissue examination revealed that QDs accumulated in the placenta, but no penetration to the embryonic tissues was observed.

**Conclusions.** QDs did not cause any direct embryotoxic or teratogenic effects, but they had adverse effects on the maternal organism. The observed QD effects and the long-term accumulation of QDs in the maternal organism may increase the risk of adverse effects on embryo development.

## Introduction

Nanoparticles (NPs) are the substances ranging from 1 to 100 nm in size. A rapid development of nanotechnologies has given rise to applications of NPs in biomedicine. Nanomaterials, such as nano-silica particles (nSPs), titanium dioxide (TiO<sub>2</sub>) nanoparticles, and nanowires, have been already applied in electronics, food industry, cosmetics (creams, make-up products, toothpastes, and sunscreens) (1, 2). NPs are promising agents for fluorescence and magnetic resonance imaging, drug delivery systems, hyperthermal effects, and other applications in medicine (3–5). The possibilities of NPs are expanding, and the real potential of nanomedicine has not been fully discovered yet. However, in order to optimize the beneficial effects of NP applications, it is essential to understand the fundamental interactions of NPs with biological systems. On the other hand, NPs may be harmful to human health, especially during the formation of the embryo. Therefore, comprehensive knowledge about

biological fate and toxicity of NPs is needed.

Our group is interested in the biomedical application of quantum dots (QDs), because they are semiconductor NPs ranging from 2 to 10 nm in size and have superior optical properties when compared with conventional organic dyes. QDs are rapidly being applied to existing and emerging technologies, and become popular in biomedicine for fluorescence imaging and drug delivery systems (6, 7). QDs are a perfect model for investigating the interactions of NPs with a biological system because their size and surface properties can be easily adjusted for the desired experimental purpose by a chemical modification. In this study, two types of QDs were used: CdSe/ZnS QDs coated with polyethylene glycol, which are used for vascular imaging (8), and CdTe QDs coated with mercaptopropionic coating, which might be unstable in a biological environment (9).

In vitro studies suggest that the certain types of QDs may be cytotoxic. For instance, CdTe QDs coated with mercaptopropionic acid (MPA) and cysteamine were cytotoxic to rat pheochromocytoma cell (PC12) cultures at a concentration of 10 µg/mL. Uncoated CdTe QDs were cytotoxic at a concentra-

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tion of 1  $\mu\text{g}/\text{mL}$ . Cell death was characterized as chromatin condensation and membrane blebbing, symptomatic of apoptosis (10). Other study showed that QDs induced necrosis and the malformations of the yolk sac and tail in a zebra fish model, and the toxic effects depended on the size and surface coating of QDs (11).

However, NP toxicity *in vivo* has been studied to a lesser extent. The knowledge about embryotoxicity is of great importance because it is a necessary part of the toxicological profile that must be established for any new biologically active substance relevant to human safety. The studies in this field have been already carried out by many authors (12–15), but the detailed effects of NPs still pose many questions.

Embryos are most sensitive to the harmful factors in the critical periods of embryogenesis when mortality or different congenital anomalies are highly possible. The classical critical periods of fetus formation are as follows: implantation and early organogenesis (6th–9th days), placenta formation and active organogenesis (10th–13th days), and the time before birth, when placental activity decreases (18th day) (16).

Therefore, the aim of our study was to investigate the embryotoxicity and teratogenicity of CdSe/ZnS and CdTe QDs at the different stages of rat embryogenesis. The results were compared with the effects induced by cyclophosphamide, a cytostatic drug, which is known as an embryotoxic compound.

## Materials and Methods

### *Animals and Treatment Schedule*

The study of QD effects on the embryo formation was carried out on 426 fetuses (114 in the control group). Albino Wistar rats (9–11 weeks old) were obtained from the State Scientific Research Institute of Innovative Medical Center (Vilnius, Lithuania). The animal husbandry and experiments on animals were carried out according to the national and European regulations and were approved by the Lithuanian Animal Care and Use Committee (permission No. 0019).

Animals were housed under conditions of constant temperature, humidity, and standard light/dark cycle. Food and fresh drinking water were available *ad libitum*. After being acclimated for at least 7 days, female rats were mated overnight with males of the same strain. Vaginal smears from each female rat were collected and subjected to microscopic examination on the following morning in order to determine the estrous cycle and the presence of sperm. The day of sperm detection in vaginal smears was designated as day 0 of gestation.

Two types of QDs were used in the experiments (Fig. 1): CdSe/ZnS QDs coated with polyethylene glycol (Qtracker-655, nonfunctionalized, Invitrogen

Inc) and CdTe QDs coated with mercaptopropionic acid (PlasmaChem GmbH). The stock solution of CdSe/ZnS was diluted up to 0.8  $\mu\text{M}$  in saline representing the dose, which was applied for the fluorescence imaging studies. CdTe QDs were supplied in powder; therefore, a higher QD concentration of 5 mg/kg could be achieved. This dose was chosen according to the previous report of Chu et al., who reported adverse effects on embryogenesis (13). The toxicity of QDs was compared with the effects of cyclophosphamide, an anticancer drug (dose of 15 mg/kg), the cytotoxicity of which is well known. The control group was injected with saline.

The groups were divided into 3 subgroups according to the stage of embryogenesis when the xenobiotic was injected, namely, on the 6th, 13th, and 18th days of gestation. The solutions were injected intraperitoneally.

### *Embryotoxicity and Teratogenicity Analysis*

All rats were subjected to the caesarean section under neuroleptanalgesia (0.5 mL of Calipsol per one rat) on the 21st day of gestation. The uteruses were excised, and the number of the sites of implantations, resorptions, and dead embryos or live fetuses was recorded in the uterine horn for the determination of postimplantation mortality indices. The fetuses were weighed, measured, and fixed in the Buen's solution for the detection of external malformations. In order to render the skeleton visible, the soft tissues were macerated using caustic soda, stained with alizarin red, and cleared with glycerin (17).

Statistical analysis of the weights and the lengths of the embryos were performed using the two-tailed, unpaired Student *t* test: a *P* value of 0.05 was considered as significant.

### *QD Accumulation in the Tissues*

In order to determine QD accumulation in the embryos and maternal tissues, CdSe/ZnS QDs (500  $\mu\text{L}$  at 0.8  $\mu\text{M}$ ) were injected intraperitoneally on the 13th day of gestation. Three hours after injection, the animals were sacrificed, and the tissues with potential QD accumulation were excised and analyzed by fluorescence spectroscopy and afterward underwent histological tissue examination (3 rats). The control animals (2 rats) were not injected with QDs.

Fluorescence spectroscopy was performed using a Cary Eclipse spectrometer (Varian, USA) with a fiber optics module in order to determine QD accumulation in the embryos and maternal tissues. The spectra of placentas, embryos, and uteruses of animals were recorded. The light of 450 nm was used for tissue excitation. The spectra were analyzed and compared with the autofluorescence of the control

tissues of the uninjected animals. Additional spectroscopic analysis was performed on the damaged and healthy tissues of the peritoneal cavity after CdTe QD administration when the lesions were observed.

In order to visualize QD localization in the tissues, the samples were sliced by cryomicrotomy and investigated under a fluorescence microscope Eclipse TE-2000U (Nikon, Japan) with a confocal scanning system C1. The unstained slices were used to avoid QD bleaching and screening effects of the histological dyes.

Additionally, the tissue samples were fixed in 10% neutral buffered formalin for 24 hours and embedded in paraffin. The sections 4  $\mu$ m in thickness were made with a Leica RM2145 microtome. The tissue slides were deparaffinized and stained with hematoxylin-eosin (HE) to visualize a tissue structure.

## Results

In order to investigate the embryotoxic effects of QDs, the results were compared with those of the control group and the group treated with cyclophosphamide, a cytostatic compound. As the transplacental transfer of compounds depends on the physiological state of the placental barrier, the effects were investigated on different days of embryogenesis.

### *The Effects of Cyclophosphamide on Embryogenesis*

Cyclophosphamide injection during the implantation period, i.e., on the sixth day of embryogenesis, showed high toxicity: resorptions accounted for 62% of all implantations (Table). The embryos also exhibited a significantly reduced weight ( $2.7 \pm 0.4$  g) and length ( $2.7 \pm 0.7$  cm) when compared with the control group ( $3.4 \pm 0.2$  g and  $3.4 \pm 0.2$  cm, respectively) (Fig. 2). The malformations were observed in 31% of the embryos survived.

After the drug injection on the 13th day of embryogenesis, the toxic effects were different from the effects at the earlier developmental stage, and only a single resorption was observed. The weight and body length were significantly lower compared with the control group. Moreover, a decrease in weight was more pronounced and significantly differed from that on the sixth day. The embryonic malformations were more abundant and were observed in 54% of the live embryos. The most common birth

defects were as follows: intra-abdominal hernia, brain abnormalities, impaired limb, and skull bone formation (i.e., micrognathia) (Fig. 3).

The results showed that on the 13th day of gestation, cyclophosphamide had a stronger impact on the fetal development (decreased body weight and increased number of malformations), but the mortality was lower when compared with the sixth day of embryogenesis.

Drug injection on the 18th day of embryogenesis did not contribute to embryonic mortality. Body weight and length were decreased in the cyclophosphamide group when compared with the control group. However, a decrease in both parameters was affected least when compared with other stages of embryogenesis. No malformations were observed. These results indicate that the adverse effects of cyclophosphamide on the embryogenesis were least pronounced on the 18th day of gestation.

### *The Effects of Quantum Dots*

QD injection on the sixth day of the embryogenesis induced no resorption either in the CdSe/ZnS group or in the CdTe QD group. The weight in the CdSe/ZnS and CdTe groups was slightly lower when compared with the control group ( $3.0 \pm 0.2$  g and  $3.2 \pm 0.3$  g vs.  $3.4 \pm 0.2$  g) (Fig. 2). The body length was lower in the CdSe/ZnS group ( $3.3 \pm 0.1$  cm), but not in the CdTe group ( $3.4 \pm 0.2$  cm). These results indicate that the exposure to QDs during pregnancy had an effect on the embryo development, but did not cause any embryonic mortality.

After QD injection on the 13th day of embryogenesis, the body weight and length were significantly lower in the CdTe QD group when compared with the control group ( $3.2 \pm 0.3$  g vs.  $3.4 \pm 0.3$  g and  $3.3 \pm 0.2$  cm vs.  $3.4 \pm 0.3$  cm, respectively). Nevertheless, there were no significant differences in these parameters when the control and CdSe/ZnS QD groups were compared. There was no embryonic mortality after the injection of any type of QDs. The malformations were not characteristic of any embryo in both QD groups indicating that NPs did not induce teratogenicity.

The weight and length of the embryos after CdTe QD injection on the 18th day was  $3.2 \pm 0.2$  g and  $3.0 \pm 0.3$  cm and appeared to be significantly lower than the corresponding parameters in the control

Table. The Embryotoxic and Teratogenic Effects of Cyclophosphamide and Quantum Dots After Exposure to Pregnant Rats on the Sixth Day of Gestation

Experimental group	Animals, n	Implantations, n	Resorptions, n (%)	Malformations, n (%)
Cyclophosphamide	5	42	26 (62)	5 (31)
CdSe/ZnS quantum dots	3	34	0	0
CdTe quantum dots	4	38	0	0
Control	5	40	1 (3)	0

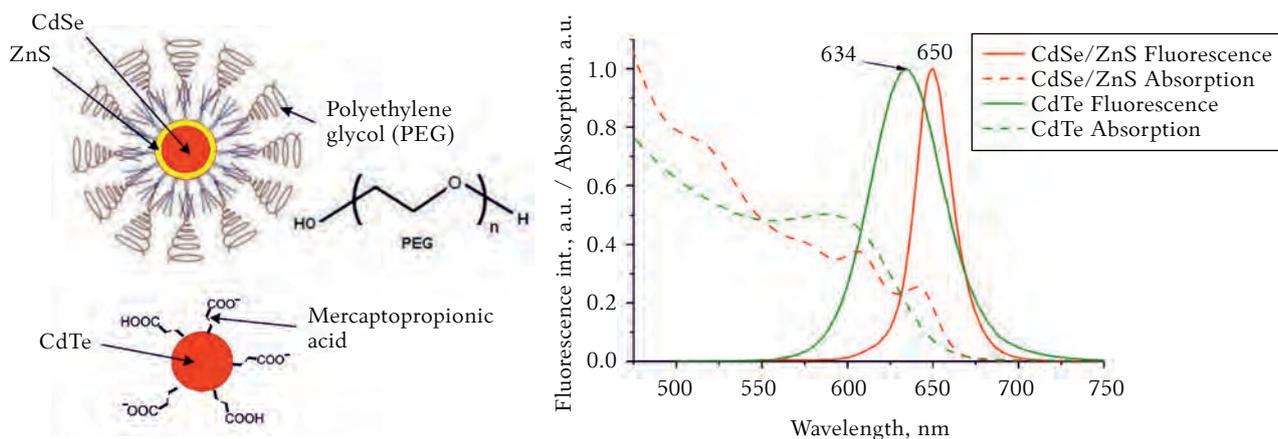


Fig. 1. The structure and spectroscopic properties of CdSe/ZnS and CdTe quantum dots used in the experiments

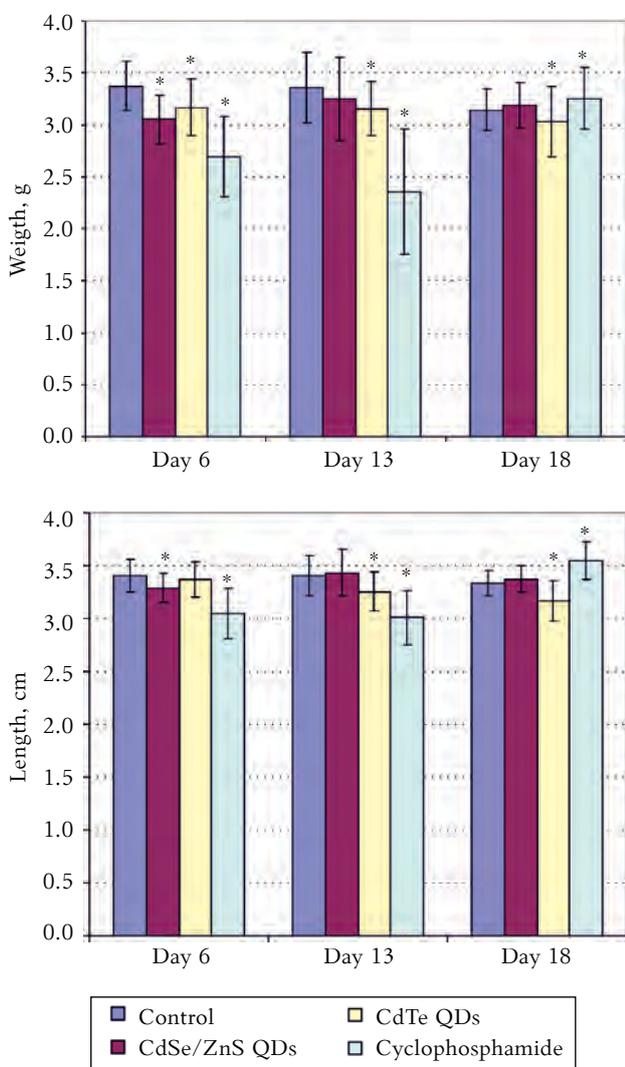


Fig. 2. The body length and weight of the embryos on the 21st day of gestation after the treatment of pregnant rats with CdSe/ZnS and CdTe quantum dots (QDs) and cyclophosphamide on the 6th, 13th, and 18th days of gestation  
\* $P < 0.05$  as compared with the control group.

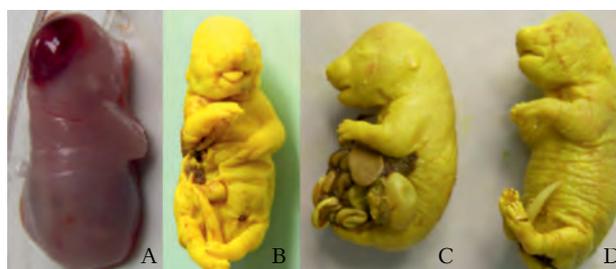


Fig. 3. The embryos of pregnant rats treated with cyclophosphamide on the 13th day of embryogenesis. The embryos were prepared on the 21st day of embryogenesis. A, exencephaly; B, micrognathia and limb deformations; C, intra-abdominal hernia; D, control (unexposed animal).

group ( $3.3 \pm 0.1$  g and  $3.1 \pm 0.2$  cm). There were no significant changes in the CdSe/ZnS QD group. There was one dead embryo in the CdTe group, but no death occurred in the CdSe/ZnS QD group. Teratogenicity was not pronounced in any group.

Interestingly, after CdTe QD injection on the 13th and 18th day of embryogenesis, 3 rats (out of 6 rats) had brown spots in the peritoneal tissues, which were identified as the necrotic areas by histological examination (Fig. 4). The localization of necrosis was different among animals: one rat had necrosis in the abdominal muscle; another one, in the omentum (Fig. 4). Two rats had lesions on the embryos. One of them also had lesions in the placenta, and the corresponding embryo was dead. According to the body length (3.1 cm) and weight (2.0 g), the embryo died within 18th–21st days of embryogenesis, i.e., after the injection of CdTe QDs. However, other embryos in the same or other rats had necrosis or other signs of toxicity. Necrosis or dead embryos were not observed in any other group (control, cyclophosphamide, or CdSe/ZnS QD). The necroses exhibited strong red fluorescence under UV illumination (Fig. 4C–D, right). Therefore,

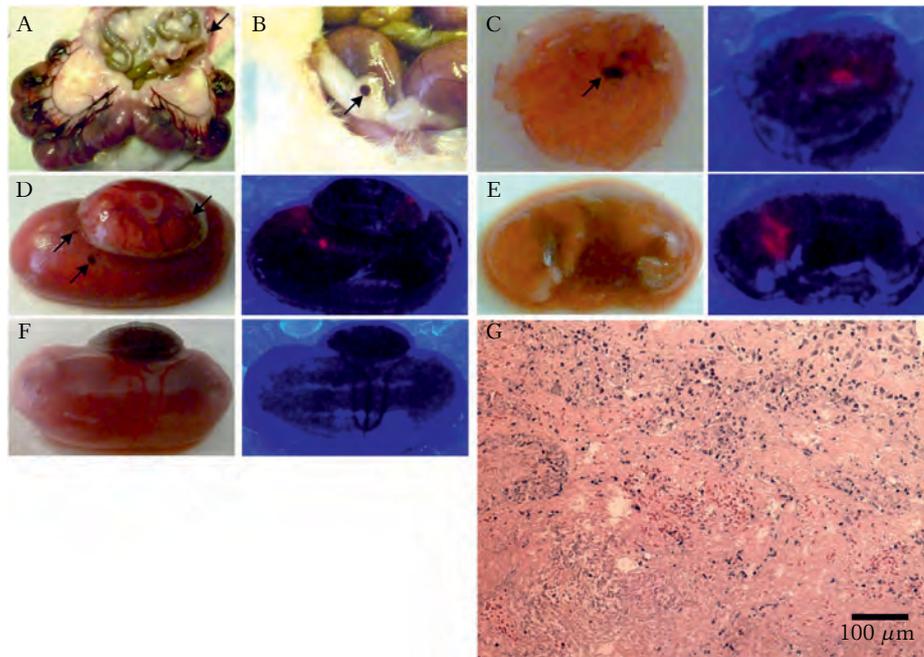


Fig. 4. The photograph images of excised rat (A and B) and abdominal muscle tissue (C) with necrotic lesions (brown spots). These lesions were also observed on a single embryo and its placenta (D). The same embryo had damaged tissues in the neck area (E). All lesions exhibited red fluorescence under UV illumination (C–E, right). The healthy embryos exhibited no red fluorescence (F). The histological analysis showed necrosis in the damaged tissues (G).

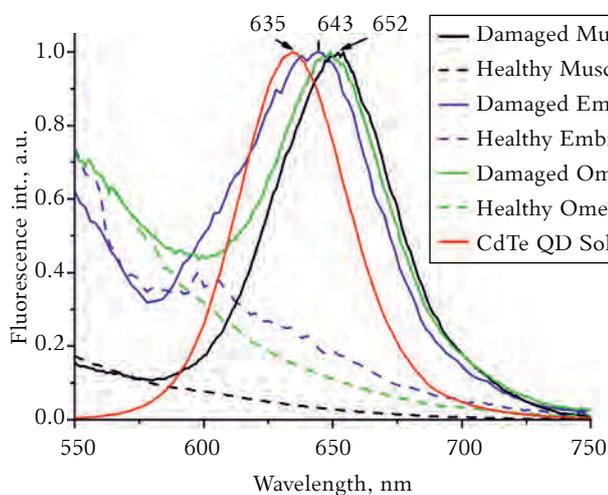


Fig. 5. The fluorescence spectra of necrotic muscle tissues (Fig. 4B) and embryonic tissues in the neck area (Fig. 4D)

The band of CdTe quantum dots (QDs) in the tissues is red-shifted when compared with the spectrum of quantum dots in distilled water. Healthy tissues exhibited no specific fluorescence band in the investigated spectral region ( $\lambda_{\text{excitation}}=480 \text{ nm}$ )

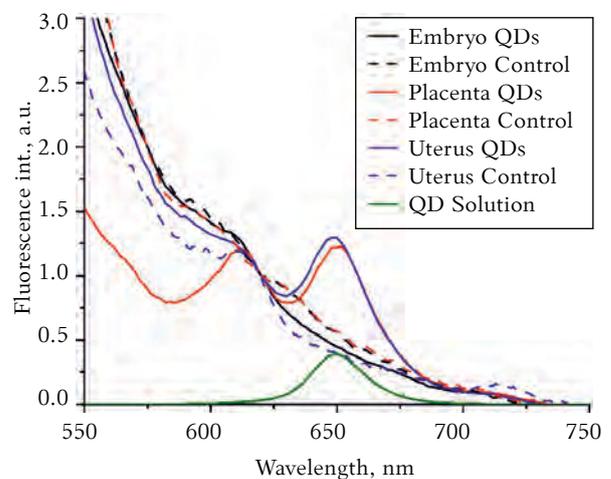


Fig. 6. The averaged fluorescence spectra of rat tissues indicating the presence of CdSe/ZnS quantum dots (QDs) (fluorescence band peak at 650 nm) in the maternal tissues (uterus and placenta), but not in embryonic tissues 3 hours after QD injection, 13th day of embryogenesis ( $n_{\text{rats}}=3$ ,  $n_{\text{embryos}}=10$ )

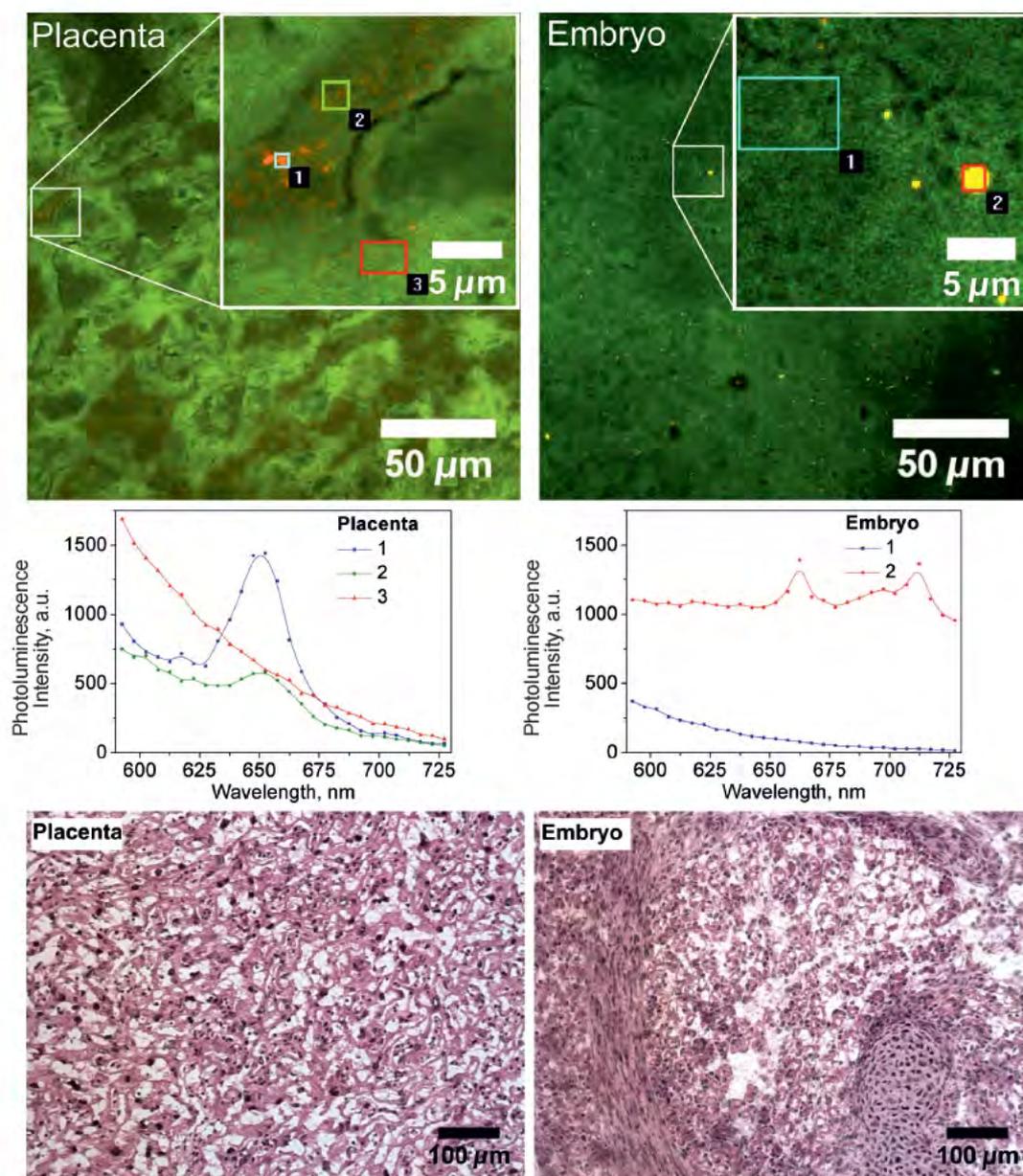


Fig. 7. Confocal microscopy images (top) of rat placenta (labyrinthine zone) and embryo tissue 3 hours after the injection of quantum dots (cryotomy slices)

The fluorescence of quantum dots (at 650 nm) was identified by spectral imaging (middle) in the placenta, but not embryonic tissues. The QD distribution (red color) in the placental tissue resembles the network of maternal blood sinuses, which can be seen in the histological slices stained with hematoxylin-eosin (bottom).

the samples were investigated spectroscopically to evaluate the origin of the fluorophores. The analysis revealed that fluorescence spectra of the lesions had one fluorescence band, and the band peak position varied among the specimens in the range of 643–652 nm (Fig. 5). These bands were red-shifted in respect to a fluorescence band of CdTe QD solution at 635 nm in distilled water. There was also an increase in the full width at half maximum (FWHM) of the spectrum from 50 nm in distilled water up to 70 nm in the damaged muscle tissue.

#### **QD Penetration Through the Placental**

#### **Barrier**

The direct embryotoxicity of NPs first depends on the ability of the compound to cross the maternal-fetal barrier and to accumulate in the embryonic tissues. In our study, fluorescence spectroscopy and confocal microscopy to investigate the penetration of CdSe/ZnS QDs through the placenta on the 13th and 18th days of embryogenesis were used, when the placental structures can be clearly identified by histological examination.

Fluorescence spectroscopy of the tissues excised 3 hours after CdSe/ZnS QD injection revealed that

a characteristic fluorescence band of QDs could be detected in the uterus and placenta tissues (Fig. 6). However, QD fluorescence was not registered in the embryonic tissues: the embryo, yolk sac placenta, and umbilical cord.

The prepared tissue slides were used to investigate QD localization using a confocal microscope coupled with a spectral detector, which enables the discrimination of the QD photoluminescence from the tissue autofluorescence with a spectral resolution. This technique revealed that QDs were accumulated in the labyrinthine zone of the placenta (Fig. 7). QDs appeared to be distributed in the samples not homogeneously, but patterned, indicating QD accumulation in the areas with a lower autofluorescence background (represented green). In the labyrinthine zone, the maternal blood sinuses had a strong absorbance and lack of endogenous fluorophores, which were more abundant in the connective fetal tissue. Therefore, maternal blood gives the autofluorescence of lower intensity and is seen as darker green areas in the image when compared with fetal tissue. In this way, QDs were mostly distributed in the maternal blood sinuses.

The examination of the embryo tissues showed no appearance of QDs in the samples. There were seen some yellowish endogenous objects, but they exhibited fluorescence spectra, which were not characteristic of QDs. The results of fluorescence microscopy and fluorescence spectroscopy showed that QDs were found in the labyrinthine zone of the placenta, but there was no QD accumulation in the embryos. This finding suggests that QDs did not penetrate through the placental barrier.

### Discussion

Our results showed that cyclophosphamide injection during the pregnancy contributed greatly to embryotoxicity. The adverse effects highly depended on the stage of embryonic development. Cyclophosphamide injection on the sixth day of gestation caused high level of resorptions and a significant decrease in weight (20%) and length (10%). At this stage, the superficial layer of the embryo is composed of specialized trophoblast cells, which later give rise to the fetal part of the placenta (18). At this time, the embryonic protective mechanisms are not fully developed, and therefore, harmful substances can easily enter the embryonic tissues. As this day of embryogenesis is the onset of implantation, the disturbance of this process leads to the damage of uterine and fetal tissues causing an unsuccessful implantation, cancellation of proliferation, and embryotoxicity (62% of resorptions). As there is still no placenta developed, the survived embryos are strongly affected by the cytostatic agent, and this effect is reflected in the decreased embryonic body weight and length.

Cyclophosphamide injection on the 13th day caused the different effects on embryogenesis: there was no mortality of the embryos, but the impact on the body weight (30%) and length (12%) was greater when compared with the implantation stage. On the 13th day of embryogenesis, the placental barrier is completely formed, and the transfer of substances that enter fetal circulation is tightly controlled by the mechanisms of active transport, biotransformation by metabolic enzymes, phagocytosis, and pinocytosis in addition to passive diffusion, which dominates on the sixth day of gestation (15). The placental barrier protects the embryo from the various chemical agents and other foreign substances in the body. However, the passage of xenobiotic molecules through the placental barrier is not completely prevented, and cyclophosphamide affects fetal cell proliferation, embryonic growth, and organ formation. The most rapidly proliferating tissues become most sensitive to the cytotoxic agents. Therefore, the organs that are intensively developing at the time of cyclophosphamide exposure are damaged mostly. This effect leads to numerous malformations.

On the 18th day of embryogenesis, the embryos are least sensitive to the effect of cyclophosphamide when compared with other stages. There were no fetal mortality and malformations, and the body weight and length was affected to a lesser extent than on the 6th or 13th days of gestation. This should be primarily caused by the complete formation of internal organs, the protective functioning of placenta, and shorter action time of cyclophosphamide until the excision.

However, direct evidence of the embryotoxic or teratogenic effects of CdSe/ZnS or CdTe QDs were not observed at any stage of embryogenesis. A decrease in the embryonic weight in the groups treated with both types of QDs was mostly expressed after the QD injection on the sixth day of embryogenesis (CdSe/ZnS QDs, 9.6%; CdTe QDs, 6.2%). These results correlate with the effects of cyclophosphamide and can be attributed to the absence of the protective placental mechanism. Still, the observed changes in the embryonic weight and length were less pronounced when compared with the effect of cyclophosphamide. After the QD injection on the 13th day of gestation, when the placenta was completely formed, a decrease in weight was significant only in the CdTe QD group (5.9%), and on the 18th day of gestation, there were no significant changes in both the groups. The changes in the embryonic length were expressed even to a lesser extent. The observed changes in the size of embryonic body after QD exposure were not sufficient to confirm an adverse effect of QDs on embryonic development.

Yamashita et al. have shown that NPs (nSP and TiO<sub>2</sub>s 70 nm and 35 nm in diameter, respectively)

can cross the placental barrier in pregnant mice and cause neurotoxicity in their offspring (19). They showed that the NPs were found in the placenta, fetal liver, and fetal brain. Moreover, they found that the mice treated with these NPs had smaller uteri and fetuses than the untreated controls. The authors concluded that these detrimental effects were linked to structural and functional abnormalities in the placenta on the maternal side and were abolished when the surfaces of the silica NPs were modified with carboxyl and amine groups.

Other authors argue that some NPs (CdSe and CdTe/CdS) in different sizes, at different dosages, and with different outer capping materials can increase the rate of early-stage blastocyst death in mice and potentially can be transferred across the placenta to the fetuses (12, 15).

These studies show that NPs can enter the embryo through the placenta, which is a natural barrier for a large variety of organic substances with diverse molecular structures. The NPs, which appear in the maternal body during pregnancy, can cross the placental barrier and may even cause developmental deformities. More to add, NP transfer may depend on the physiological state of maternal-fetal barrier (20).

Our results showed that the injection of CdTe QDs induced necrosis in the tissues of the peritoneal cavity. The spectroscopic analysis of these lesions revealed that the fluorescence band of QDs in these tissues had a peak in range of 643–652 nm and the FWHM of 70 nm. Meanwhile, the fluorescence spectra of QDs in distilled water had a peak at 635 nm and the FWHM of 50 nm. The differences of the spectroscopic properties imply the structural changes of the NPs in the biological environment. The spectroscopic alterations might be associated with the reorganization of superficial ligand molecules, QD aggregation (9, 21), or even QD disruption (22, 23) because of the interaction with surrounding molecules. The fluorescence band shift of QDs in vivo was observed earlier and was attributed to the proteolytic degradation or pH-induced changes (22, 23). The changes of spectroscopic properties indicate that QDs interact with biological molecules, and this process leads to the tissue damage.

The exact mechanisms of NP toxic activity remain unknown. However, QD toxicity in vivo was reported earlier, and it is mainly attributed to the degradation of an inorganic core and the release of cytotoxic Cd<sup>2+</sup> into the tissue (13, 24). King-Heiden et al. observed QD-induced necrosis and malformations in a zebra fish larva model, but the effects were not characteristic of Cd<sup>2+</sup>-evoked effects indicating that the nanostructure of the particles determined new features that were not typical of particle components (11). A recent review by Hoshino et al. has

highlighted that QD-capping material, rather than the core metalloid complex, is responsible for the majority of QD toxicity and biological activity (7). These opinions complement each other and show that there is a high need for additional studies and better understanding of NP effects in the biological systems. An integrative approach is also of great importance for the interpretation of reported results from different research groups.

The tissue damage was not observed in the CdSe/ZnS QD group by us. These nanoparticles differ from CdTe QDs not only in the composition of the core and the shell, but also in the superficial coating, which is known to play a key role in biological effects (7, 25). CdSe/ZnS QDs are chemically conjugated with an amphiphilic polymer and methoxy polyethylene glycol (PEG) for the NP stabilization, biocompatibility, and protection from the interaction with biomolecules of the organism. Meanwhile, the core of CdTe QD does not have a protective shell, and the surface is covered only with small molecules of mercaptopropionic acid. Therefore, CdTe QDs are expected to be more sensitive to the proteolytic and other chemical activity of biomolecules. Moreover, these QDs were found in the peritoneum following 2 weeks postinjection indicating an inefficient clearance, which might lead to a further disruption and increased toxicity in the longer perspective. The localized accumulation of CdTe QDs could be caused by the colloidal instability, aggregation, and/or protein adhesion, which lead to decreased NP diffusion in a biological milieu and inefficient uptake into blood circulation. In contrast, CdSe/ZnS QDs were not found to be accumulated in a certain tissue indicating a more homogenous distribution in the organism. These differences could be determined by the QD surface as the PEG coating minimizes the aggregation of NPs and interaction with proteins (25).

It was shown that QD absorption from the injection site was rather slow due to a low penetration through blood vessels (4). It explains that in our case, the toxic effects appeared in the peritoneal tissues, as the QD solution was injected intraperitoneally, and the surrounding tissues were exposed to the highest dose. The embryotoxic and teratogenic effects might be unexpressed due to a low QD concentration in the blood circulation and, consequently, in the embryos. The dose of CdTe QDs (5 mg/kg) was chosen according to the previous report of Chu et al. as these authors observed a dose-dependent embryotoxicity of CdTe QDs in mice after the intravenous injection. The authors synthesized QDs by themselves (13). The different results of our study could be caused by a different synthesis protocol of QDs, their stability, and route of administration. Moreover, the results of spectro-

scopic and microscopic tissue analysis have shown that QDs accumulate in the placenta and uterus, but they are not observed in the embryonic tissues, which implies that QDs do not penetrate through the placental barrier. The inability of QDs to accumulate in the embryonic tissues minimizes the risk of direct embryotoxic and teratogenic effects.

### Conclusions

Our study shows that the embryotoxic and teratogenic effects of cyclophosphamide depend on the stage of embryogenesis. However, CdSe/ZnS or CdTe quantum dots did not cause any direct embryotoxic or teratogenic effects at any stage of embryogenesis. Quantum dots were efficiently retained by

the placental barrier from passage to the embryonic tissues. CdTe quantum dots accumulated in the abdominal cavity and induced necrosis of the peritoneal tissues. Our results indicate that quantum dots may have adverse effects on the maternal organism. The observed effects and the prolonged accumulation of quantum dots in the maternal organism may increase the risk of adverse effects on embryo development.

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### Statement of Conflict of Interest

The authors state no conflict of interest.

### References

- Bowman DM, van Calster G, Friedrichs S. Nanomaterials and regulation of cosmetics. *Nat Nanotechnol* 2010;5:92.
- Augustin MA, Sanguansri P. Nanostructured materials in the food industry. *Adv Food Nutr Res* 2009;58:183-213.
- Zhao Q, Wang L, Cheng R, Mao L, Arnold RD, Howarth EW, et al. Magnetic nanoparticle-based hyperthermia for head & neck cancer in mouse models. *Theranostics* 2012;2:113-21.
- Kosaka N, Ogawa M, Sato N, Choyke PL, Kobayashi H. In vivo real-time, multicolor, quantum dot lymphatic imaging. *J Invest Dermatol* 2009;129:2818-22.
- Liong M, Lu J, Kovoichich M, Xia T, Ruehm SG, Nel AE, et al. Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *ACS Nano* 2008;2:889-96.
- Jamieson T, Bakhshi R, Petrova D, Pocock R, Imani M, Seifalian AM. Biological applications of quantum dots. *Biomaterials* 2007;28:4717-32.
- Hoshino A, Hanada S, Yamamoto K. Toxicity of nanocrystal quantum dots: the relevance of surface modifications. *Arch Toxicol* 2011;85:707-20.
- Mayes P, Dicker D, Liu Y, El-Deiry W. Noninvasive vascular imaging in fluorescent tumors using multispectral unmixing. *Biotechniques* 2008;45:459-64.
- Kulvietis V, Streckytė G, Rotomskis R. Spectroscopic investigations of CdTe quantum dot stability in different aqueous media. *Lith J Pys* 2011;51:163-71.
- Hardman R. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ Health Perspect* 2006;114:165-72.
- King-Heiden TC, Wicinski PN, Mangham AN, Metz KM, Nesbit D, Pedersen JA, et al. Quantum dot nanotoxicity assessment using the zebrafish embryo. *Environ Sci Technol* 2009;43:1605-11.
- Chan WH, Shiao NH. Cytotoxic effect of CdSe quantum dots on mouse embryonic development. *Acta Pharmacol Sin* 2008;29:259-66.
- Chu M, Wu Q, Yang H, Yuan R, Hou S, Yang Y, et al. Transfer of quantum dots from pregnant mice to pups across the placental barrier. *Small* 2010;6:670-8.
- Menjoge AR, Rinderknecht AL, Navath RS, Faridnia M, Kim CJ, Romero R, et al. Transfer of PAMAM dendrimers across human placenta: prospects of its use as drug carrier during pregnancy. *J Control Release* 2011;150:326-38.
- Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, et al. Barrier capacity of human placenta for nano-sized materials. *Environ Health Perspect* 2010;118:432-6.
- Žalgevičienė V, Burkanas M, Žukienė J, Lapienis J, Sukackaitė A, Graželiene G, Didžiapetrienė J. The influence of photosensitized treatment on development of rat embryo. The 4th International Conference on Medical Physics. Kaunas, Lithuania; 2006. p. 12-5.
- Hayes WA. Principles and methods of toxicology. New York: Raven Press; 1994.
- Rout UK. Valproate, thalidomide and ethyl alcohol alter the migration of HTR-8/SVneo cells. *Reprod Biol Endocrinol* 2006;4:44.
- Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, et al. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nat Nanotechnol* 2011;6:321-8.
- Kulvietis V, Zalgevičienė V, Didžiapetrienė J, Rotomskis R. Transport of nanoparticles through the placental barrier. *Tohoku J Exp Med* 2011;225:225-34.
- Poderys V, Matulionyte M, Selskis A, Rotomskis R. Interaction of water-soluble CdTe quantum dots with bovine serum albumin. *Nanoscale Res Lett* 2011;6:9.
- Karabanovas V, Zakarevicius E, Sukackaitė A, Streckytė G, Rotomskis R. Examination of the stability of hydrophobic (CdSe)ZnS quantum dots in the digestive tract of rats. *Photochem Photobiol Sci* 2008;7:725-9.
- Fitzpatrick JA, Andreko SK, Ernst LA, Waggoner AS, Ballou B, Bruchez MP. Long-term persistence and spectral blue shifting of quantum dots in vivo. *Nano Lett* 2009;9:2736-41.
- Lin CH, Chang LW, Chang H, Yang MH, Yang CS, Lai WH, et al. The chemical fate of the Cd/Se/Te-based quantum dot 705 in the biological system: toxicity implications. *Nanotechnology* 2009;20:215101.
- Schipper ML, Iyer G, Koh AL, Cheng Z, Ebenstein Y, Aharoni A, et al. Particle size, surface coating, and PEGylation influence the biodistribution of quantum dots in living mice. *Small* 2009;5:126-34.

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