

## Full-length article

# Cytotoxic effect of CdSe quantum dots on mouse embryonic development

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## Key words

quantum dots; blastocyst; apoptosis; development

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#### Abstract

Aim: The aim of this study was to examine the cytotoxic effect of quantum dots (QD), a novel luminescent material, on early post-implantation embryonic development. Methods: Mouse blastocysts were incubated in medium with or without CdSe-core QD (250 or 500 nmol/L) for 24 h. Cell apoptosis was analyzed by terminal deoxynucleotidyl transferase-mediated digoxigenin-dUTP nick-end labeling assay and Annexin V/propidium iodide staining, and proliferation was investigated by dual differential staining. Pre-implantation and post-implantation development was assessed by *in vitro* and *in vivo* analyses, respectively. **Results:** The apoptotic staining analysis showed that CdSe-core QD induced apoptosis in mouse blastocysts in a dose-dependent manner. Pretreatment of blastocysts with CdSe-core QD inhibited cell proliferation, primarily in the inner cell mass. CdSecore QD also inhibited post-implantation embryonic development; fewer CdSecore QD-pretreated blastocysts reached the later stages of development compared to the controls. The pre-implantation development of morulas into blastocysts was also inhibited by CdSe-core QD. Furthermore, CdSe-core QD at 500 nmol/L were associated with resorption of post-implantation blastocysts and a decrease in fetal weight. The cytotoxicity of CdSe QD in embryonic development was significantly reduced by the addition of a ZnS coating. Conclusion: Our results show that CdSe-core QD induce apoptosis in mouse blastocysts, inhibit cell proliferation, retard early post-implantation blastocyst development, and increase early-stage blastocyst death in vitro and in vivo.

### Introduction

Quantum dots (QD) are colloidal nanocrystalline semiconductors that have unique light-emitting properties and can be used as a novel luminescent material. Typical QD are 1–12 nm in diameter and contain a small number of atoms in a discrete cluster<sup>[1]</sup>. QD absorb irradiated energy at wavelengths greater than their lowest energy transition, and then convert the irradiated energy to a narrow bandwidth emission. QD are ideal for development as luminescent probes due to the advantages of broadband excitation, narrow bandwidth emission, high intensity of emitted light, resistance to quenching, and photochemical stability. These properties make QD applicable for biochemical assays, in particular immunofluorescence staining. A previous study showed that CdSe-core QD induce cell death<sup>[2,3]</sup>, but the regulatory mechanisms underlying this effect have not been elucidated.

Apoptosis, which is widely observed in different cells of various organisms, is the process of programmed cell death involving several morphological patterns, including chromatin condensation, membrane blebbing, and DNA fragmentation<sup>[4]</sup>. Apoptosis plays an important role in embryogenesis and homeostasis of multicellular organisms. The impairment of apoptotic function has been associated with several human diseases, including neurodegenerative disorders and cancer<sup>[5]</sup>. Although apoptosis contributes to normal embryonic development<sup>[6–8]</sup>, teratogens can induce abnormal apoptosis during early embryogenesis, leading to disruption of the developmental process<sup>[9–13]</sup>. We recently

reported the capacity of several apoptotic inducers to trigger apoptosis in embryonic stem cells and/or embryos of ICR Swiss Webster mice<sup>[10,11,14–16]</sup>. The effects of CdSe-core QD on human embryonic development are unclear. Our findings provide important new insights regarding the use of CdSe-core QD as biological tracers in mouse embryos.

To examine the effect of CdSe-core QD on pre-implantation development, we exposed mouse blastocysts to CdSecore QD and examined apoptosis and cell proliferation in the inner cell mass (ICM) and trophectoderm (TE). We also investigated the effect of CdSe-core QD on the development of morulas into blastocysts. The effect of CdSe-core QD on post-implantation development was investigated by means of *in vivo* analyses of transferred embryos.

## Materials and methods

**Chemicals and reagents** Pregnant mare's serum gonadotropin (PMSG) and sodium pyruvate were purchased from Sigma (St Louis, MO, USA). Human chorionic gonadotropin (hCG) was obtained from Serono (NV Organon Oss, the Netherlands). The Annexin V-FLUOS staining kit and TUNEL *in situ* cell death detection kit were obtained from Roche (Mannheim, Germany) and the CMRL-1066 medium was from Gibco Life Technologies (Grand Island, NY, USA).

QD preparation Nanocrystals comprising a CdSe core and a ZnS shell were synthesized by Prof Chuan-hsin LU and coworkers at the Department of Chemical Engineering, National Taiwan University. Briefly, appropriate amounts of trioctylphosphine oxide (TOPO), cadmium oxide, and tetradecylphosphonic acid were heated to 180 °C under zargon, and dried and degassed under a vacuum. The reaction temperature was then increased to 330 °C; selenium (Se) precursor solution in trioctylphosphine (TOP) was injected into the reaction flask, and the mixture was allowed to cool to 240 °C. Zn and S stock solutions prepared with bis (trimethylsilyl)sulfide in TOP, along with a dimethyl zinc solution, were added drop-wise with vigorous stirring until a final mole ratio of 1:4 (Cd/Se:Zn/S) was achieved in the reaction. The reaction mixture was cooled to room temperature, and the nanocrystals were precipitated with anhydrous methanol, collected by centrifugation, and washed 3 times with anhydrous methanol for the removal of residual TOPO and the unreacted reagents. The precipitate was dissolved in anhydrous chloroform or tetrahydrofuran for the experiments. For water solubilization, the CdSe QD were surface coupled with mercaptoacetic acid and then suspended in phosphate-buffered saline (PBS) (the modification was performed by Prof Ruoh-chyu RUANN and coworkers at the Department of Chemical and Materials Engineering, National Central University, Taiwan). A particle sizer was used to measure the CdSe QD, which were found to be approximately 3.5 nm in diameter.

Animals and collection of embryos ICR mice were purchased from the National Laboratory Animal Center (Taiwan). This research was also approved by the Animal Research Ethics Board of Chung Yuan Christian University (Taiwan). ICR virgin albino mice (6-8 weeks old) were induced to superovulate by an injection of 5 IU PMSG followed by an injection of 5 IU hCG 48 h later. The mice were then mated overnight with a single fertile male of the same strain. The female mice with vaginal plugs were separated and used for the experiments. All of the mice were maintained on breeder chow and kept under a 12-h day/12-h night regimen with food and water available ad libitum. All of the animals received humane animal care, as outlined in the Guidelines for Care and Use of Experimental Animals (Canadian Council on Animal Care, Ottawa, Canada, 1984). The day a vaginal plug was found was defined as d 0 of pregnancy. Morulas were obtained by flushing the fallopian tubes on the afternoon of d 3, and blastocysts were obtained by flushing the uterine horn on d 4; in both cases, the flushing solution consisted of CMRL-1066 culture medium containing 1 mmol/L glutamine and 1 mmol/L sodium pyruvate.

**QD treatment** The embryos were collected in uncoated plastic 35 mm culture dishes and washed at least 3 times. Expanded blastocysts from different females were pooled and randomly selected for the experiments. The blastocysts were incubated at 37 °C for 24 h in CMRL-1066 medium with and without 0–500 nmol/L QD, and were then used for further experiments as described later.

Blastocyst cell counting To investigate the effect of CdSe-core QD on cell proliferation in embryos, we treated the mouse blastocysts (180 blastocysts in each group) with or without CdSe-core QD and then analyzed proliferation using differential staining. The blastocysts were incubated with culture medium containing 0-500 nmol/L QD for 24 h, washed with QD-free medium, and then dual differential staining was used to facilitate counting of cell numbers in the ICM and  $TE^{[17]}$ . The blastocysts were incubated in 0.4% pronase in M2 medium containing 0.1% bovine serum albumin (M<sub>2</sub>-BSA medium) for the removal of the zona pellucida. The denuded blastocysts were exposed to 1 mmol/L trinitrobenzenesulphonic acid in BSA-free M<sub>2</sub> medium containing 0.1% polyvinylpyrrolidone at 4 °C for 30 min, and then washed with M<sub>2</sub> medium<sup>[18]</sup>. The blastocysts were further treated with 30 µg/mL antidinitrophenol-BSA complex antibody in M2-BSA at 37 °C for 30 min, and then with M2 medium supplemented with 10% whole guinea pig serum as a source of complement,  $20 \mu g/mL$  bisbenzimide, and  $10 \mu g/mL$  propidium iodide (PI) at 37 °C for 30 min. The immunolysed blastocysts were gently transferred to slides and protected from light before observation. Under UV light excitation, the ICM cells (which take up bisbenzimidine, but exclude PI) appeared blue, whereas the TE cells (which take up both fluorochromes) appeared orange-red. As multinucleated cells are not common in pre-implantation embryos<sup>[19]</sup>, the number of nuclei was considered to represent an accurate measure of the cell number.

Detection of cell apoptosis To study the apoptotic effects of CdSe-core QD on embryos, we treated mouse blastocysts (200 blastocysts in each group) with 0-500 nmol/L CdSe-core QD at 37 °C for 24 h, and then measured cell apoptosis. For terminal deoxynucleotidyl transferase-mediated digoxigenin-dUTP nick-end labeling (TUNEL) staining, the embryos were washed in QD-free medium, fixed, permeabilized, and subjected to TUNEL labeling using an in situ cell death detection kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's protocol. Photographic images were taken using a fluorescence microscope under bright-field illumination (Olympus BX 51, Tokyo, Japan). For Annexin V staining, the blastocysts were incubated in 0-500 nmol/L QD for 24 h, washed with QD-free culture medium, and then stained with an Annexin V-FLUOS staining kit (Roche, Germany), according to the manufacturer's instructions. Briefly, the blastocysts were incubated in M<sub>2</sub>-BSA for the removal of the zona pellucida, washed well with PBS plus 0.3% BSA, and then incubated for 60 min with a mixture of 100 µL binding buffer, 1 µL fluorescein isothiocyanate-conjugated Annexin V, and  $1 \,\mu L$  PI. After incubation, the embryos were washed and photographed using a fluorescence microscope under fluorescent illumination. The cells staining Annexin V<sup>+</sup>/PI<sup>-</sup> were considered apoptotic, while those staining Annexin V<sup>+</sup>/PI<sup>+</sup> were considered necrotic.

**Observation of** *in vitro* **implantation and post-implantation development** The blastocysts were cultured according to a modification of the previously reported method<sup>[11]</sup>. Briefly, the embryos were cultured in 4-well multidishes at 37 °C. For group culture, 3 embryos were cultured per well. The basic medium consisted of CMRL-1066 supplemented with 1 mmol/L glutamine and 1 mmol/L sodium pyruvate plus 50 IU/mL penicillin and 50 mg/mL streptomycin (hereinafter called culture medium). For the treatments, the embryos were cultured with the indicated doses of QD for 24 h without serum supplementation. Thereafter, the embryos were cultured for 3 d in culture medium supplemented with 20%

fetal calf serum, and for 4 d in culture medium supplemented with 20% heated-inactivated human placental cord serum for a total culture time of 8 d from the onset of treatment. The embryos were inspected daily under a phase contrast microscope (Olympus IMT-2, Tokyo, Japan), and the developmental stages were classified according to established methods <sup>[20]</sup>. Developmental parameters, such as hatching through the zona pellucida, attachment to the culture dish, trophoblastic outgrowth, and differentiation of the embryo proper were recorded daily. To decrease observer bias, all data were analyzed using the following previously published criteria<sup>[9]</sup>: an implanted blastocyst was defined by attachment to the culture dish, followed by outgrowth; an early egg cylinder (EEC) was defined as an embryo that had reached stage 9 or 10 by d 4; a late egg cylinder (LEC) was defined as an embryo that had reached stage 11, 12, or 13 by d 6 of culture; and an early somite (ESS) was defined as an embryo that had reached stage 14 or 15 by d  $8^{[9]}$ .

Blastocyst development following the embryo transfer To examine the ability of expanded blastocysts to implant and develop in vivo, the generated embryos were transferred to recipient mice. ICR females (white skin color) were mated with vasectomized males (C57BL/6J; black skin color; from the National Laboratory Animal Center, China) to produce pseudopregnant dams as recipients for the embryo transfer. To ensure that all fetuses in the pseudopregnant mice came from the embryo transfer (white color) and not from fertilization by C57BL/6J (black color), we examined the skin color of the fetuses at d 18 post-coitus. To assess the impact of QD on post-implantation growth in vivo, the blastocysts were exposed to 0 and 500 nmol/L QD for 24 h, and then 8 embryos were transferred in parallel to the paired uterine horns of d 4 pseudopregnant mice. The surrogate mice were killed on d 18 post-coitus, and the frequency of implantation was calculated as the number of implantation sites per number of embryos transferred. The incidence rates of resorbed and surviving fetuses were calculated as the number of resorptions or surviving fetuses, respectively, per number of implantations. The weights of the surviving fetuses and placentae were measured immediately after dissection.

**Statistics** The data were analyzed using one-way ANOVA and *t*-tests, and are presented as the mean±SD. A *P*-value <0.05 was considered significant.

## Results

Effect of CdSe-core QD on cell apoptosis in mouse blastocysts TUNEL staining revealed that 250 and 500 nmol/L CdSe-core QD induced apoptosis in mouse blastocysts in a dose-dependent manner (Figure 1A). Quantitative analyses



**Figure 1.** CdSe-core QD induce apoptosis in mouse blastocysts. (A) mouse blastocysts were treated with or without CdSe-core QD (CdSe; 125, 250, or 500 nmol/L) or ZnS-coated CdSe QD (ZnS; 500 nmol/L) for 24 h. Apoptosis was examined by TUNEL staining followed by light micros-copy under bright-field illumination, showing positive cells in black. (B) mean number of Apoptotic (TUNEL positive) cells per blastocyst. (C) mean number of Annexin V-positive/PI-negative cells per blastocyst. Data are presented as a percentage, and are based on at least 200 samples in each group. Values are presented as mean $\pm$ SD of 5 determinations. <sup>c</sup>P<0.01 versus the control group.

revealed that 250 and 500 nmol/L CdSe-core QD increased the number of apoptotic cells in blastocysts 4.2- and 6.6-fold, respectively, above the controls (Figure 1B). Annexin V and PI staining revealed a higher ratio of Annexin V<sup>+</sup>/PI<sup>-</sup> cells in the ICM of blastocysts exposed to QD than in the

ICM of the controls. The ratio of Annexin V<sup>+</sup>/PI<sup>-</sup> cells was similar in the TE and controls, suggesting that the TE is unaffected by exposure to QD (Figure 1C). These results show that CdSe-core QD potently induce apoptosis in the ICM of mouse blastocysts.

Effect of CdSe-core QD on blastocyst cell number The effect of CdSe-core QD on cell proliferation was investigated by differential staining. The results showed that blastocysts exposed to 250 or 500 nmol/L of CdSe-core QD contained fewer cells than the control blastocysts, an effect that was most pronounced in the ICM (Figure 2).



**Figure 2.** Effect of CdSe-core QD on blastocyst viability. Mouse blastocysts were treated with or without CdSe-core QD (CdSe; 125, 250, or 500 nmol/L) or ZnS-coated CdSe QD (ZnS; 500 nmol/L) for 24 h. Total number of cells per blastocyst and cell numbers in the inner cell mass and trophectoderm lineages were counted. Data are presented as a percentage, and are based on 180 samples in each group. Values are presented as mean±SD of 5 determinations.  $^{c}P$ <0. 01 versus the control group.

Effect of CdSe-core QD on implantation and post-implantation development The blastocysts treated with 125, 250, or 500 nmol/L CdSe-core QD (240, 200, and 250 blastocysts, respectively) showed reduced formation of the 2-layer ICM and ectoplacental cones. Also, fewer embryos developed to the advanced egg cylinder stages (LEC and ESS stages) compared to the controls (Figure 3A). When morulas were exposed to 500, 250, or 125 nmol/L CdSe-core QD, 41%, 55%, and 78.7% developed into blastocysts, respectively. By comparison, 85% of control morulas developed into blastocysts (Figure 3B).

**Determination of blastocyst developmental potential by embryo transfer** The effect of CdSe-core QD on post-implantation development was investigated by transferring mouse blastocysts exposed to CdSe-core QD into a host mother and examining the embryos 13 d post-transfer (d 18 post-coitus). The implantation ratios for the CdSe-core QDpretreated blastocysts and controls were ~65% (130 of 200 embryos in 25 recipients) and ~70% (140 of 200 embryos in 25 recipients), respectively (Figure 4A). The proportion of



**Figure 3.** *In vitro* development of mouse embryos exposed to CdSecore QD at the blastocyst or morula stages. (A) mouse blastocysts were treated with or without CdSe-core QD (CdSe; 125, 250, or 500 nmol/L) or ZnS-coated CdSe QD (ZnS; 500 nmol/L) for 24 h. Development of blastocysts *in vitro* was observed for 7 d post-treatment. Morphological assessment of hatched, EEC, LEC, and ESS embryos was made according to established methods. (B) mouse morulas were treated with or without CdSe-core QD (CdSe; 125, 250, or 500 nmol/L) or ZnS-coated CdSe QD (ZnS; 500 nmol/L) for 24 h. Treated morulas were cultured for a further 24 h at 37 °C. Blastocysts were counted, and the percentage of morulas developing into blastocysts was calculated. Values are presented as mean±SD of 5 determinations. °*P*<0.01 versus the control group.

implanted embryos that failed to develop normally was significantly higher for the CdSe-core QD-pretreated blastocysts (105 of 130 implanted embryos; 80.8%) compared to the controls (50 of 140 implanted embryos; 35.7%). The embryos that implanted but fail to develop subsequently resorbed. The embryos pretreated with CdSe-core QD had a higher resorption rate and a lower surviving fetus rate than the controls (Figure 4A). There was no difference in placental weight between the CdSe-core QD-pretreated and the control groups (Figure 4B). By comparison, the fetal weight was lower in the CdSe-core QD-pretreated group versus the controls (477±61 mg vs 583±58 mg). Fetal weight is an important indicator of successful embryonic and fetal development. Of the fetuses in the CdSe-core QD-pretreated group, only 13% weighed over 600 mg. By comparison, 35% of the control fetuses exceeded this threshold (Figure 4C). Collectively, these results show that exposure to CdSe-core QD at the blastocyst stage reduces post-implantation development potential.

ZnS coating decreases the cytotoxic effect of CdSe QD



**Figure 4.** Effect of CdSe-core QD treatment of mouse blastocysts on implantation, resorption, fetal survival, and fetal weight. (A) mouse blastocysts were treated with or without CdSe-core QD (CdSe; 500 nmol/L) for 24 h. Implantation, resorption, and fetal survival was analyzed as described. Percentage of implantations represents the number of implantations per number of transferred embryos×100. Percentage of resorptions or surviving fetuses denotes the number of resorptions or surviving fetuses per number of implantations×100. (B) placental weights of 25 recipient mice were measured. (C) weight distribution of surviving fetuses on d 18 postcoitus. Surviving fetuses were obtained by the embryo transfer of control and CdSe-core QD-pretreated blastocysts, as described (200 total blastocysts across 25 recipients).  $^bP$ <0.05,  $^cP$ <0.01 vs controls.

**on blastocysts** The capacity of a ZnS coating on CdSe QD to reduce the cytotoxicity of CdSe QD on embryos was investigated. TUNEL assays and a proliferation analysis revealed that ZnS-coated CdSe QD had no significant cytotoxic effect on blastocysts (Figures 1,2). Furthermore, ZnS-coated CdSe QD did not inhibit post-implantation development (Figure 3). Collectively, these findings show that CdSe QD coated with ZnS have no significant cytotoxic effect on embryonic development.

### Discussion

The induction of cell death by CdSe-core QD under certain conditions correlates with the release of free Cd<sup>2+</sup> from the CdSe lattice. This effect is significantly reduced by adding a ZnS coating to the CdSe-core QD<sup>[2]</sup>. Photoluminescent semiconductor QD are novel nanometer-size probes for the bioimaging of immunostained cells<sup>[21]</sup>, and fluorescent QD probes are useful bioimaging tools for tracing target cells over time in mouse models<sup>[22]</sup>. Fluorescent QD probes have the potential for development as biotracers for human disease diagnosis. For this reason, it is essential to fully investigate the cytotoxic capacity of QD. In the present study we show that CdSe-core QD-induced apoptosis and the inhibition of embryonic development is effectively reduced by a ZnS coating (Figures 1-3). Our results suggest that QD are likely to have latent cytotoxicity in the event of disruption of the ZnS coatings in vivo.

Cadmium (Cd) is a significant environmental and occupational toxic metal. Cd can have pro- or anti-apoptotic effects, depending on the cell type, dosage, and exposure period. Cd induces apoptosis in T lymphocytes<sup>[23]</sup>, pig proximal tubule epithelial cells (LLC-PK1 cells)<sup>[24]</sup>, canine proximal tubules<sup>[25]</sup>, and rat testicular tissue<sup>[26]</sup>. The induction of apoptosis by Cd occurs by caspase-dependent<sup>[27]</sup> and -independent<sup>[28]</sup> pathways. Cd<sup>2+</sup> also blocks apoptosis induced by a variety of agents<sup>[29]</sup>. Recent studies show that Cd<sup>2+</sup> activates Ca2+/calmodulin-dependent protein kinase II in mouse mesangial cells and triggers both apoptotic and necrotic cell death<sup>[30]</sup>. Cd can also induce reactive oxygen species (ROS) generation (ie oxidative stress) and trigger apoptosis via a caspase-dependent pathway<sup>[31]</sup>. However, the mechanisms underlying Cd-induced apoptosis, cell cytotoxicity, and carcinogenesis remain unclear.

Se is an important dietary essential trace element for human life. There are at least 8 Se-containing proteins, including glutathione peroxidase and thioredoxin reductase. Dietary Se supplementation can protect cells from oxidative injury<sup>[32]</sup> and inhibit apoptosis<sup>[33,34]</sup>. Conversely, Se can also trigger cell apoptosis<sup>[34,35]</sup>. Further investigation is required to unravel the mechanisms underlying the protective and apoptotic effect of Se.

Embryonic development is a complex process during which chemical injury can lead to abortion or embryonic malformation. Thus, it is important to examine the possible teratogenic effects of commonly used nanoparticle fluorescence biotracers. The present study shows for the first time that CdSe-core QD decrease the viability of mammalian blastocysts by inducing apoptosis (Figure 1). Our results reveal that apoptosis in mouse blastocysts exposed to 250 and 500 nmol/L CdSe-core QD increases 4.2- and 6.6-fold, respectively, above the controls in a dose-dependent manner. CdSe-core QD at 125 nmol/L had no cytotoxic effect, as assessed by TUNEL staining (Figure 1A,1B). Annexin V/PI staining revealed significant CdSe-core QD-induced apoptosis in the ICM, but not in the TE (Figure 1C). While the blastocysts exposed to CdSe-core QD maintained the capacity to implant *in vitro*, post-implantation development was retarded, lead-ing to embryonic death.

During embryonic development, cells are often poised between proliferation and apoptosis. While our previous studies showed that CdSe-core QD induced apoptosis, no studies have examined the apoptotic effects of CdSe-core QD during embryonic development. In the present study, we show that the exposure of mouse blastocysts to CdSecore QD decreases cell number, induces apoptosis, delays pre-implantation development *in vitro*, and inhibits postimplantation development, perhaps due to a teratogenic effect.

The TE arises from the trophoblast at the blastocyst stage and subsequently develops into a sphere of epithelial cells surrounding the ICM and the blastocoel; these cells contribute to the placenta and are required for the development of the mammalian conceptus<sup>[36]</sup>. CdSe-core QD did not reduce cell numbers or induce apoptosis in the TE, and implantation *in vitro* was unaffected (Figures 1–3). Further work will be required to assess the effect of CdSe-core QD on differentiation and giant-cell formation *in vitro* and *in vivo*.

Mitochondria acts as important signaling conduits during programmed cell death, and loss of mitochondrial integrity can be promoted or inhibited by many key regulators of apoptosis<sup>[37,38]</sup>. Our previous study showed that CdSe-core QD induced the loss of mitochondrial membrane potential and the mitochondrial release of cytochrome c in IMR-32 cells in a dose-dependent manner<sup>[3]</sup>. That study also revealed a key role for ROS in CdSe-core OD-induced apoptosis in mitochondria, and that c-Jun N-terminal kinase (JNK) is an upstream regulator of the mitochondria-dependent apoptotic pathway<sup>[3]</sup>. These results indicate that the mechanism underlying CdSe-core QD-induced apoptosis involves ROS generation, JNK activation, and mitochondrial-dependent processes in IMR-32 cells. Our previous study also showed that CdSe-core QD-induced apoptosis was associated with reduced protein levels of heat shock protein 90 (HSP90) and the downstream targets, Ras, Raf-1, extracellular signal-regulated kinase-1 (ERK-1), and ERK-2<sup>[3]</sup>. These findings suggest that a CdSe-core QD-induced decrease in HSP90 expression leads to an increase in the proteasome-dependent degradation of Ras and Raf-1, with the decrease in Raf-1 levels resulting in the subsequent downregulation of ERK-1 and ERK-2. Details of the regulatory mechanisms underlying CdSe-core QD-induced apoptosis in blastocysts are unclear and require further investigation.

Cd-induced cytotoxicity is associated with inflammation, fibrosis, organ dysfunction<sup>[39,40]</sup> and the development of various cancers<sup>[41]</sup>. Studies in Hep G2 cells implicate Cd in caspase-dependent apoptosis<sup>[31]</sup>. The cytotoxicity of CdSe QD correlates with the release of free Cd<sup>2+</sup> from the CdSe lattice, which appears to be associated with surface oxidation<sup>[2]</sup>. The addition of a ZnS coating to CdSe QD significantly reduces any associated cytotoxicity<sup>[2]</sup>. For this reason, the capacity of a ZnS coating to reduce CdSe-core QD cytotoxicity during embryogenesis was investigated. Our study shows that a ZnS coating effectively reduces CdSe QD-induced cytotoxicity in blastocysts. We propose that the ZnS coating prevents CdSe-core QD-induced cell death and cytotoxicity by blocking surface oxidation and the subsequent release of Cd<sup>2+</sup> ions. Further studies are required to comprehensively assess the mechanism by which a ZnS coating prevents CdSe-core QD-induced cell death and cytotoxicity.

In summary, the present study shows for the first time that CdSe-core QD decrease the viability of mammalian blastocysts by inducing apoptosis in the ICM. This is the first evidence that CdSe-core QD have a teratogenic effect via the induction of apoptosis. Further work is required to fully elucidate the mechanisms underlying CdSe-core QD-induced cell death and cytotoxicity and the relationship between these processes and teratogenicity.

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