Chapter 6

Applications of Fluorescent Quantum Dots for Reproductive Medicine and Disease Detection

Sapna Jain, Seong B. Park, Shreekmar R. Pillai,
Peter L. Ryan, Scott T. Willard and Jean M. Feugang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72978

Abstract

Understanding the mechanisms associated with fertility and disease management in animals remains challenging. Continuing advances in nanotechnology provide new tools and alternative approaches for the investigation of these mechanisms. Fluorescent quantum dot nanoparticles, for example, have unique physicochemical properties, which allow for *in vivo* and *in vitro* imaging in various areas of life sciences. Traditional quantum dots contain heavy metal semiconductor cores, which have raised concern over their potential for toxicity. The majority of available quantum dots today prevent heavy metal release with additional chemical and polymer layers for safe water solubility. In this chapter, the most widely used quantum dots made of cadmium selenide, which possess great potential for real-time imaging in disease detection and reproductive medicine, are discussed.

Keywords: quantum dots, spermatozoa, *in vivo* imaging, real-time imaging, luminescence, fertility

1. Introduction

Since their discovery by Alexie Ekimov and Louis Brus in the 1980s, quantum dot (QD) nanoparticles have been categorized as a novel class of fluorescent particles [1]. Fluorescent nanoparticles exhibit distinct energy levels and size-dependent fluorescent emission [2]. The QD sizes range from 2 to 10 nm (10–50 atoms) in diameter, with the smaller size corresponding to the larger bandgap [3]. Each QD absorbs white light and then reemits a specific color associated with the material's bandgap, from blue to red or near-infrared (NIR) as the QD crystals increase in size [4]. The variety of fluorescence emission is very useful for both *in vitro*

and *in vivo* multiplex bioimaging as multiple QDs can be used in one subject or field of view to image a variety of targets under a single excitation.

QDs have unique advantages over traditional dyes and fluorescent proteins such as a high quantum yield, extreme brightness, tunable emission wavelength, long fluorescence duration, exceptional photostability and resistance to photobleaching [5]. In addition, their high extinction coefficient makes them ideal for optical applications and transport. Since QDs wavelengths are tunable based on size, their conducting properties can be very well controlled to suit various applications. Zinc sulfide (ZnS)-coated cadmium selenide (CdSe) nanocrystals are the most commonly studied QDs for bioapplications due to their wide bandgap and easily tunable emission in the visible range [6]. These qualities make them especially useful for various industrial, agricultural, and biomedical applications [7]. Another reason for the popularity of the CdSe QDs is their well-established synthesis and characterization protocols [8]. In this chapter, the synthesis, toxicity, and surface modification of CdSe QDs in bioanalytics and biomedical diagnostics are discussed.

1.1. Synthesis of QDs

Quantum dots can be prepared by formation of nanosized semiconductor particles through colloidal chemistry or by epitaxial growth and/or nanoscale patterning [9]. Preparation of QDs designed for biological applications has four basic steps: core synthesis, shell growth, aqueous solubilization, and biomolecular conjugation or biofunctionalization.

1.1.1. Core-shell protocol

QDs core is generally made from heavy metal semiconductors of group II–VI (CdSe, CdS, CdTe, HgS, ZnS, ZnSe), III–V (GaAs, GaN, InP, InAs, InGaAs), IV–VI (PbS, PbSe, PbTe, SnTe), and group III–V (InP and InGaP) (**Table 1**). The most common method for preparation of QDs core consists of a rapid injection of semiconductor or organometallic precursors (e.g., Cd precursor and TOPSe) into hot and vigorously stirred specific coordinating solvent (e.g., thiol stabilizers). Coordinating solvents stabilize the bulk semiconductors and avoid aggregation as the QDs grow [10]. Thereafter, the semiconductor core material (e.g., CdSe) must be protected from degradation and oxidation to optimize QDs performance. Hence, an external layer or protective shell (e.g., ZnS) is usually synthesized to cover the QD semiconductor core to enhance stability, while increasing its photoluminescence [11]. Due to their synthesis in nonpolar organic solvents, the inorganic core-shell semiconductor QDs (e.g., CdSe) are typically hydrophobic, which prevents their solubility and enhances the formation of aggregates or precipitates in water-based solutions. This property limits biological applications of core-shell QDs, requiring additional modifications of their surfaces to achieve biocompatibility or solubility in biological or water-based fluids.

1.1.2. Aqueous solubilization

The aqueous dispersal of core-shell QDs is controlled by the chemical nature of their surface coating. Numerous effective methods have been established to create hydrophilic QDs, which can be divided into two main categories [12]. *The first route*, commonly designated as

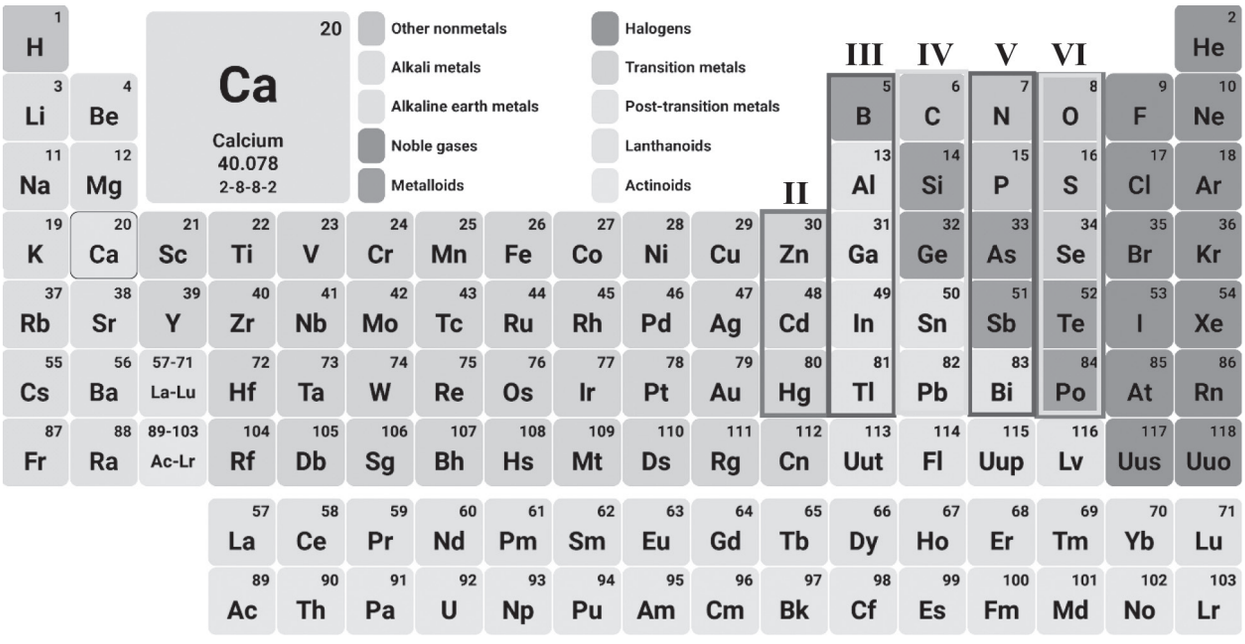

Table 1. Semiconductor elements—groups II to VI—within the periodic table.

"cap exchange procedure," consists of a complete replacement of the hydrophobic layer of organic solvent by bioactive molecules containing soft acidic and hydrophilic groups pointing outwards, from the QDs surface to surrounding bulk water molecules [13, 14]. This route allows electrostatic stabilization of inorganic core shell of QDs through their interactions with small charged ligands (e.g., amines, cystamine, cysteine, 2-mercaptoethanol, ethylamine, or mercaptopropionate) or charged surfactants to form a new external coating layer that encapsulates QDs. *The second route* allows steric stabilization through modification of the native coordinating organic ligands on the QDs surface with "bulky" uncharged polymeric surface ligands such as the polyethylene glycol or PEG [15, 16]. *Alternatively to electrostatic and steric stabilizations*, bulky and charged ligands (e.g., polyelectrolytes or polyethyleneimine), amphiphilic inorganic shell (e.g., silica added to QDs during polycondensation) or solid lipid nanoparticles composed of high biocompatible lipids of physical and chemical long-term stability have been successfully tested for further stabilization of QDs [17–19]. All aforementioned coating strategies are useful for QDs solubilization while allowing further addition of polymers or bioactive molecules for cell labeling and imaging.

1.1.3. Biofunctionalization

Biofunctionalization refers to the ability to successfully attach or conjugate bioactive molecules (e.g., oligonucleotides, proteins, polysaccharide, and peptides) to water-dispersed QDs. This process can be achieved by binding to polyhistidine tags, electrostatic (e.g., avidin-biotin) or covalent interactions. This later is typically accomplished by activated 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling amine and carboxyl groups and catalyzed maleinimide (SMCC) linking amine to sulfhydryl groups [20, 21]. It is important to mention that these processes remain challenging due to surface chemistry of QDs, control of attachment

orientation of biomolecules [19, 21, 22], and determination of conjugation efficacy. QDs have greater surface area-to-volume ratio allowing several types of biomolecules to be attached to a single QD to provide multifunctionality of the conjugate [23]. Only few protocols for biofunctionalization are available, and systematic studies are needed for functional evaluation of conjugated or biofunctionalized QDs [14, 20, 21].

2. *In vitro* and *in vivo* toxicity of QDs

Due to their heavy metal semiconductor cores, QDs are considered toxic when the cores are not adequately contained by an outer shell, such as the ZnS shells mentioned above. Without containing the cores, potential damage to biological systems can occur, which composes a challenge to surmount for medical and other *in vivo* applications. The core of the most widely used and studied QDs consists of cadmium selenide (CdSe) or telluride (CdTe) given their quantum confinement region spanning the entire optical spectrum [24]. Cadmium ions (Cd^{2+}) have been identified as the primary cause of QDs cytotoxicity due to their overtime leaking, upon illumination or oxidation [25, 26]. Leaked Cd^{2+} is able to bind to thiol groups of key molecules of mitochondria and cause enough stress and damage leading to cell death [27].

Moreover, the cytotoxicity of QDs appears directly related to the protective inorganic surface layers [25]. Additional surface coatings may be needed to substantially reduce or eliminate the release of Cd^{2+} [26]. The utilization of gelatin during the production of CdTe QDs has resulted in reduced toxicity of particles [28]. In the case of CdSe QDs, it is believed that properly prepared closed (ZnS) or multiple (e.g., ZnS/SiO_2, ZnS/PEG hydrophilic coating) shells render cadmium leakage less likely [29, 30]. However, oxidized QDs surface may unintendedly react with intracellular components, causing formation and release of reduced Cd that results in apoptosis within primary hepatocytes isolated from rats [31–33]. In addition, the charge and chemical reactivity of QDs play dominant roles in their biocompatibility, independent of their size [32]. Various studies have demonstrated the crucial roles of multiple positive charges and size-dependent polycationic materials of QDs in cytotoxic mechanisms of nanoparticles [34, 35].

Majority of *in vitro* studies use transformed cell lines to demonstrate the cytotoxicity of QDs that may not fully reflect the response cascade in normal cells [12]. Nonetheless, the use of these cell types allows for many generalizations to be made regarding the toxicity related to specific QDs features (i.e., size, protective shell, and surface chemistry), experimental dosage, and exposure conditions. **Table 2** summarizes few studies exemplifying the complexity of investigating QDs nanotoxicity due to multiple variables such as their size, shell components and surface chemistry that should be taken into account when designing an experiment. The comparable size of QDs with certain cellular components may facilitate their passage through many biological barriers and accumulation in different tissues to cause adverse effects after long-term exposure [36]. At equal concentrations and positive charges, smaller QDs (i.e., 2–3 nm) display high cytotoxicity than larger ones (i.e., >5 nm), with liver and kidneys often being main target organs due to their blood filtering function [37].

QDs (core and protective layers)	Concentration	Exposure	Toxic effect	References
CdSe/ZnS-SSA	0.1–0.4 mg/mL	0–24 h	0.1 mg/mL altered cell growth; most cells nonviable at 0.4 mg/mL	[38]
CdSe/ZnS-SSA	0.1 mg/mL QDs per 5×10^7 cells	2 h to 7 days	No toxicity in mice *in vivo*	[38]
CdSe/ZnS conjugates: NH_2, OH, OH/COOH, NH_2/OH, COOH	1–2 μM	12 h	2-μM QD-COOH-induced DNA damage upon 2 h of exposure	[39]
CdSe/ZnS/MUA	0–0.4 mg/mL	24 h	0.2 mg/mL, Vero; 0.1 mg/mL, HeLa; 0.1 mg/mL, hepatocytes	[40]
CdTe	0.01–100 μg/mL	2–24 h	10 μg/mL cytotoxic	[41]
CdSe-MAA, TOPO QDs	62.5–1000 μg/mL	1–8 h	62.5 μg/mL cytotoxic under oxidative/photolytic conditions	[25]
			No toxicity on addition of ZnS cap	
QD micelles: CdSe/ZnS QDs in (PEG-PE) and phophatidylcholine	1.5–3 nL of 2.3-μM QDs injected, approx. 2.1×10^9 to 4.2×10^9 QDs/cell	Days	5×10^9 QDs/cell: cell abnormalities, altered viability and motility	[42]
			No toxicity at 2×10^9 QDs/cells	
CdSe/ZnS amp-QDs and mPEG QDs	Injections, approx. 180-nm QD, approx. 20-pmol QD/g animal weight	15-min cells incubation, 1–133 days *in vivo*	No signs of localized necrosis at the sites of deposition	[43]
CdSe/ZnS-DHLA	400–600 nM	45–60 min	No effect on cell growth	[44]
Avidin-conjugated CdSe/ZnS QDs	0.5–1.0 μM	15 min	No effect on cell growth and development	[44]
CdSe/ZnS-amphiphilic micelle	60-μM QD/g animal weight, 1-μM and 20-nM final QD concentration	Information not provided	Mice showed no noticeable ill effects after imaging	[45]
CdSe/ZnS-DHLA QDs	100 μL of B16F10 cells (approx. 2×10^5 to 4×10^5) used for tail vein injection	4–6 h cell incubation, mice sacrificed at 1–6 h	No toxicity observed in cells or mice	[46]
CdSe/ZnS-MUA QDs; QD-SSA complexes	0.24 mg/mL	2 h	0.4 mg/mL MUA/SSA-QD complexes did not affect viability Vero cells	[47]
CdSe/ZnS	10-pmol QDs/1×10^5 cells (approx. 10 nM)	10 days (cell culture)	10 nM QD had minimal impact on cell survival	[48]
CdTe aqQDs	300–600 nM	3 days	Nearly completely inhibited cell growth even from the very beginning	[49]
CdTe-gelatinized/ nongelatinized	1–100 nM	72 h	At 1 nM, did not initiate any detrimental effects; at 100 nM, resulted in the death of all PC12 cells	[50]

QDs (core and protective layers)	Concentration	Exposure	Toxic effect	References
CdTe, CdTe/CdS, CdTe/CdS/ZnS	0.2–0.3 μM	0–48 h	Cells treated with CdTe and CdTe/CdS QDs were mostly nonviable by 48 h (for all concentrations tested)	[51]
CdSe/ZnS-PEG (EviTag T1 490 QD)	0.84–105 μM	0–24 h	Commercially available QDs demonstrated low cytotoxicity but induced cell detachment	[52]
CdSe	1, 10, and 20 nM	24 h	1 nM QD for 24 h showed no decreased in cell viability; in contrast, cells treated with 10 and 20 nM QDs for 24 h showed decreases in cell viability in the order of 20 and 30%	[53]

Table 2. Inconsistent considerations on QDs toxicity evaluation (modified from [3]).

Due to the complexity to characterize the cytotoxicity of nanoparticles, the US National Cancer Institute and several other US health agencies have created the Nanotechnology Characterization Laboratory (NCL) for efficacy and toxicity testing of nanoparticles, including fluorescent QDs. As part of the process, the NCL will describe physical attributes of nanoparticles, their *in vitro* biological properties and their *in vivo* biocompatibility.

3. Bioapplications

Despite the reported and controversial cytotoxicity, QD nanoparticles remain excellent candidates for numerous bioapplications. Compared to organic dyes, QDs display narrow, symmetrical, and tunable emission spectra and contingency for their size and material composition [54]. Various QD sizes have closer but nonoverlapping emission wavelengths [20], which excitation through a single light source leads to a photostable and broad absorption spectra [52].

3.1. QDs labeling

The brightness of CdSe QDs fluorescence has made them the widely labeled nanoparticles for various biosensing (e.g., oligonucleotides, organic dyes) and single or multiplex labeling (e.g., antibodies, peptides) [22, 23, 31, 55–57]. Yet, the localization (intracellular or extracellular), expression level, and environment (oxidizing or reducing) of the target molecule should be considered during the QDs labeling protocol. For example, the intracellular targeting may pose additional challenges requiring the need of cell-penetrating peptides (e.g., polyarginine, polylysine) for effective intracellular delivery of QDs conjugates, while maintaining the homeostasis and osmotic balance of cells. Reproductive studies have shown the ability of porcine gametes to interact with self-illuminated CdSe/ZnS QDs [58, 59] with the necessity to determine the suitable sperm-to-QDs ratio, avoiding or limiting QDs toxicity to sperm function, as observed in previous studies using various nanoparticles [58–65]. *In vitro* matured

oocytes appeared to accumulate higher levels of QDs compared to spermatozoa, which, instead, exhibited stronger membrane labeling (**Figures 1** and **2**). Reduced QDs internalization within the spermatozoa was attributed to sperm membrane specificities, whereas the limitations of QDs as compared to organic dyes may not be ruled out [66]. The conjugation of self-illuminated CdSe/ZnS QDs with anti-plasminogen antibody (for specific targeting) revealed stronger signals within the porcine oocyte than the nonconjugated QDs, applied for plain targeting (**Figure 2**). The use of these CdSe/ZnS QDs also provided opportunity for *ex vivo* imaging of cultured porcine ovarian follicles (**Figure 3**), which would, in the near future, permit real-time monitoring of key molecules having role(s) during folliculogenesis.

3.1.1. QDs labeling for cell imaging and disease detection

Effective labeling of fluorescent QDs is crucial for extracellular and intracellular tracking of target molecules in their native environment. QDs functionalized with antibody are optimal for extracellular targeting of cell-surface membrane proteins (e.g., receptors) and subsequent targeted imaging [20, 22, 31], which practice will create opportunities for precise assessments of cellular and molecular mechanisms of diseases (e.g., cancer) and their treatments. Near-infrared QDs (e.g., CdSe, CdTe) emit in the wavelength range of 650–900 nm to overcome the optical property variations and endogenous autofluorescence of tissues under *in vivo* conditions [67], permitting tumor localization and visualization while offering a new mean for cancer prevention and treatment.

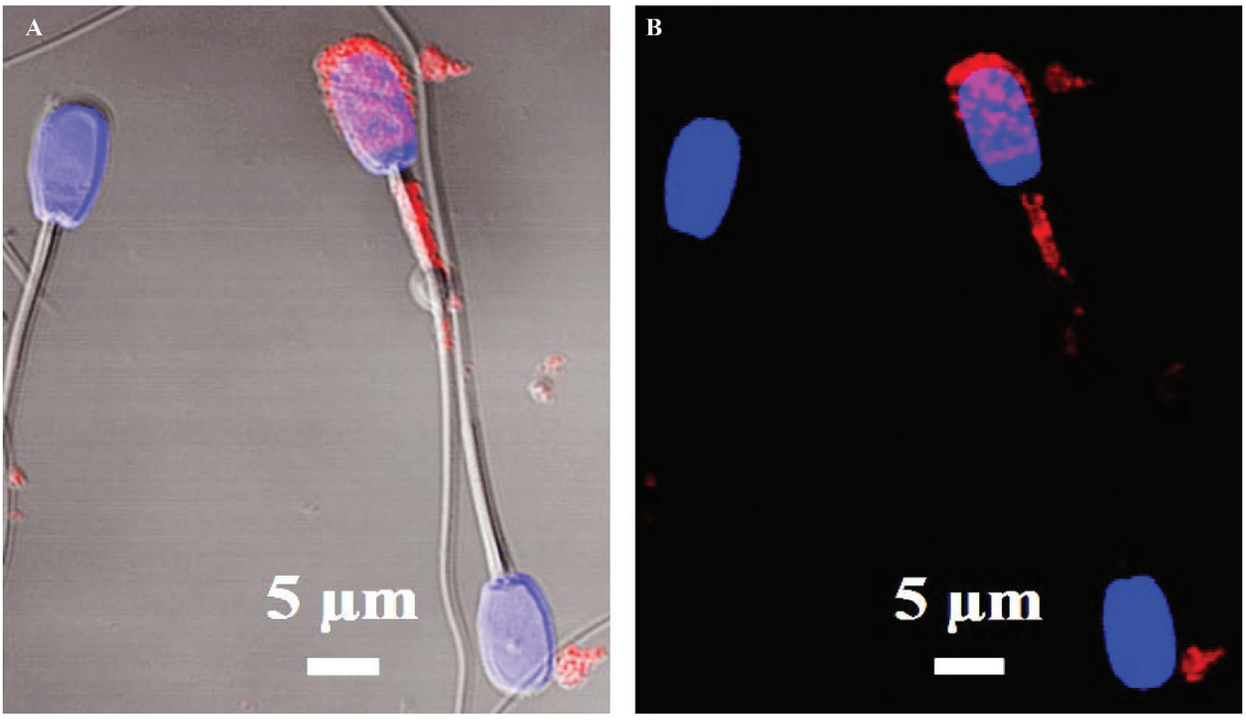

Figure 1. Confocal microscope imaging of mature boar spermatozoa labeled with CdSe QDs 655 nm. Labeled spermatozoa revealed major localizations of QDs (red spots) in the head and mid-piece regions. Sperm nuclei are counterstained in blue with DAPI. Micrograph A = overlay of 3 lights (visible, blue DAPI and red QDs 655 nm); Micrograph B = overlay of DAPI and QD 655 nm.

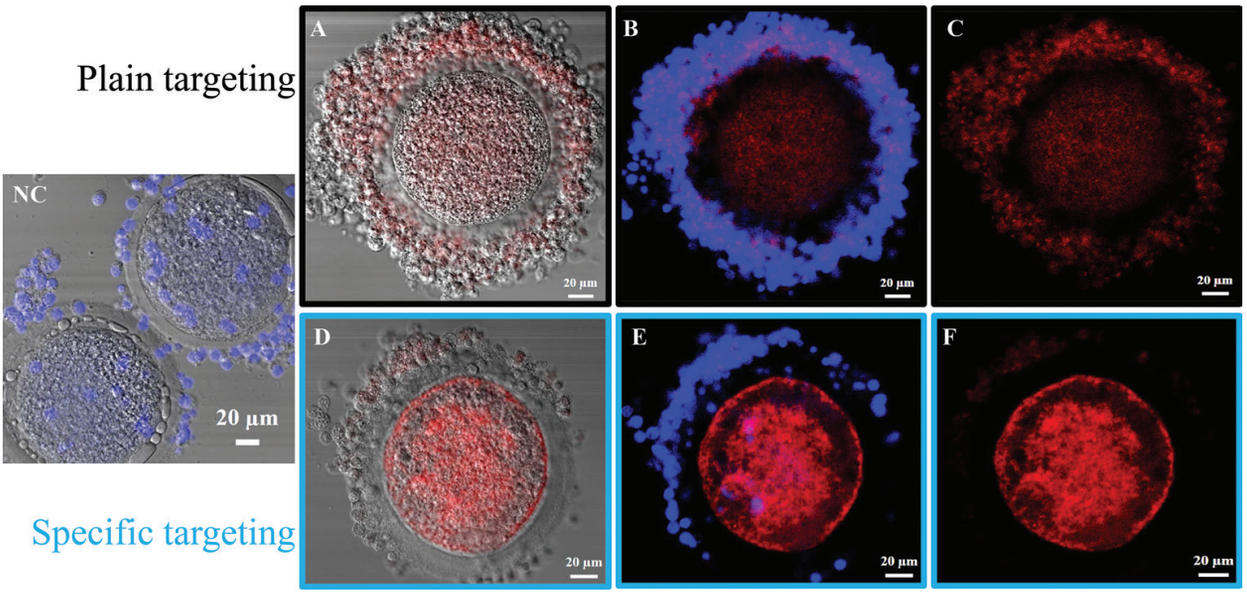

Figure 2. Confocal microscope imaging of porcine oocytes matured in the presence of QDs 655 nm. Cumulus-oocyte complexes were matured in the presence of QD alone (plain targeting; micrographs A/B/C) or QD conjugated with anti-plasminogen antibody (specific targeting: micrographs D/E/F). Micrograph NC = Control without QDs; A and D = Overlays of visible light and QDs 655 nm filter; B and D = overlays of DAPI and QDs 655 nm, and C and F = QDs 655 nm filter alone. The stronger and differential distribution of the red signal can be seen following QDs conjugation.

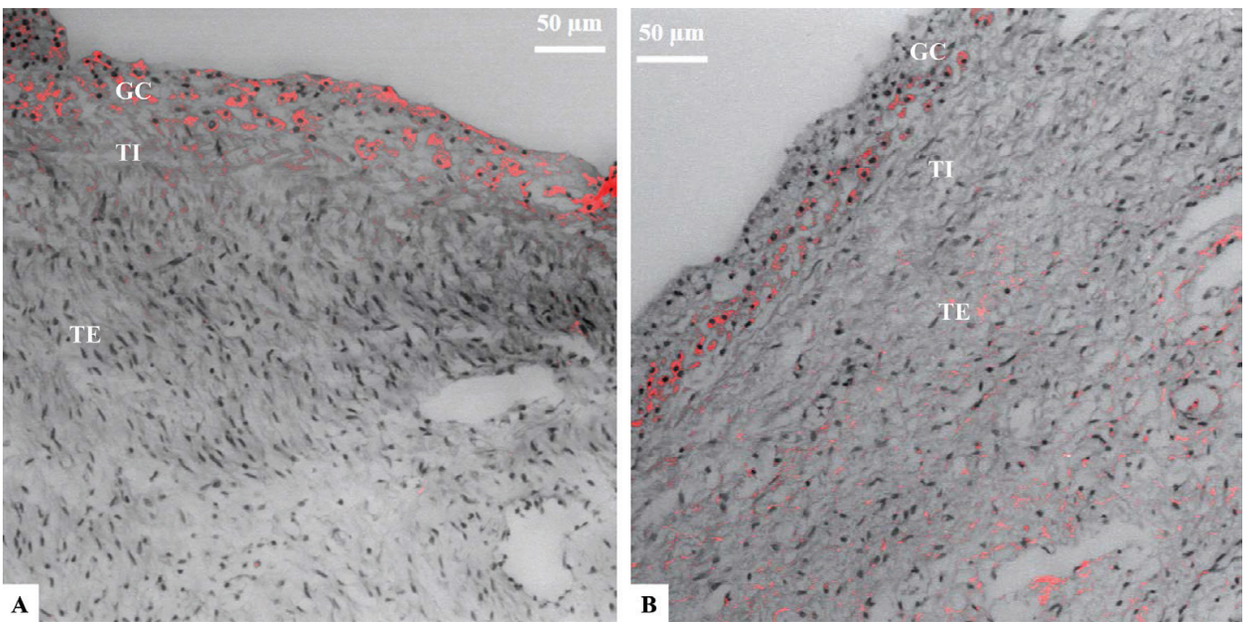

Figure 3. Confocal microscope imaging of porcine follicles microinjected with nonconjugated QDs 655 nm. Dissected antral follicles were microinjected, cultured for 1 (A) or 3 (B) days, then prepared for histology slides and imaging. QDs (red sports) are mainly visible within the layer of granulosa cells (GC) after 1 day of culture (A) and then throughout the theca interna (TI) and externa (TE) after 3 days of culture (B).

The intracellular localization of selective biomolecules for targeting presents additional challenges associated with QDs conjugates delivery within the cells. Available methods for QDs delivery are composed of, but not limited to, positively charged peptides or cell-penetrating peptides on QDs, microinjection, electroporation, or nonspecific or receptor-mediated endocytosis

[57, 67–69]. Electroporation technique has shown robust and highly efficient delivery of both monomer and aggregate QDs to the cells due to induced electrical pulses that temporarily permeabilize the plasma membrane [70]. It has been used for *in vivo* imaging of cancer cells through active intracellular delivery of QDs [67]. Electroporation of QDs in lung (NCI-H460) and ovary (SK-OV-3) cancer cells revealed high and longer (over a month) QDs retention inside the cells, allowing observation of the entire process of subcutaneous tumor growth and cancer cell dissemination at late stages of metastasis in a natural tissue environment [67]. It is important to mention that biofunctionalized QDs have been used for imaging in many other diseases, including the brain tissue [71, 72].

3.1.2. QDs labeling for cell imaging in reproductive biology

The small size (2–10 nm in diameter) and unique physicochemical properties of QDs, especially their tunable size-dependent fluorescence emission, make them excellent candidates for applications in the reproductive field. For example, the multicolor detection of various QDs permits spectral multiplexing for simultaneous detection and quantification of different biomolecules in *in vitro* bioassays [23, 54, 73–75], which may be crucial in understanding the complexity of mammalian gamete maturation in their native environment.

Additionally, signal amplification of enzymatic reactions could be achieved through QDs emitting localized and bright fluorescence in bioassays. This later could be illustrated by the novel QD-BRET (Bioluminescent Resonance Energy Transfer), a luciferase-doped QDs which enzymatic reaction with its substrate (luciferin or coelenterazine) produces energy (480 nm) that is immediately absorbed by the CdSe QDs to emit brighter and long-lasting NIR fluorescence [76]. Numerous *in vivo*, *in vitro*, and *ex situ* studies have successfully applied the QD-BRET for imaging of somatic and reproductive (mammalian gametes and ovarian follicle; **Figures 1–3**) cells [58, 59, 77].

The use of spectral multiplexing and signal amplification in reproductive biology has potential for fast diagnostics of gamete quality through direct (e.g., fluoroimmunoassays) or indirect (lab-on-chip arrays) evaluations. The proposed arrays should contain various QDs sizes that are biofunctionalized to target various key biomarkers of reproductive cells.

In addition to above-mentioned applications, there is potential to use QDs conjugates for targeted labeling, tracking, and imaging of ovarian follicle cells (during folliculogenesis) or spermatozoa (during intrauterine migration). Moreover, a recent study using amphibians reported the ability of living tadpoles to accumulate QD (655 nm) nanoparticles, likely impeding with their development [78].

4. Future outlook

QDs applications have been explored for molecular and pharmaceutical fields, but are rapidly expanding to other research areas. It is expected that QDs will be used for (1) categorizing various types of biological processes, (2) localizing and identifying molecular mechanisms of disease, (3) developing novel drug-action mechanisms, (4) applications in intracellular and extracellular compartments and (5) innovative approaches for biochemical assays. Ventana

Medical Systems has just begun publicizing their QDs Map family of immunohistochemistry reagent kits for automated slide processing and fluorescent detection of fixed specimens (www.ventanadiscovery.com). The increased commercial offering of QDs products reflects the desirability of QDs photophysical properties, namely, photostability, single source excitation, narrow emission, multiplexing capabilities, and high quantum yield.

Unfortunately, the lack of reliable and reproducible techniques to conjugate a variety of biomolecules such as antibodies, protein markers, DNA, and RNA to QDs in a methodical way with control over their ratio, orientation, and avidity remains to hinder their ongoing use in clinical diagnostics [3]. In the future, it is to be expected that more commercial products integrating QDs for clinical, diagnostic, and research purposes will be released for public use and manipulation, which will likely give rise to more reliable conjugation techniques as they are further investigated. The outcomes of current research combining nanotechnology and reproductive biology clearly indicate nanoparticles as promising tools for both basic and applied research in animal reproduction [79–84].

Author details

Sapna Jain[1], Seong B. Park[2], Shreekmar R. Pillai[1], Peter L. Ryan[2], Scott T. Willard[2] and Jean M. Feugang[2]*

*Address all correspondence to: jn181@ads.msstate.edu

1 Alabama State University, Alabama, USA

2 Mississippi State University, Mississippi, USA

References

[1] Maiti A, Bhattacharyya S. Quantum dots and applications in medical science. International Journal of Chemical Science and Chemical Engineering. 2013;**3**(2):37-42

[2] Wu M, Wang X, Wang K, Guo Z. An ultrasensitive fluorescent nanosensor for trypsin based on upconversion nanoparticles. Talanta. 2017;**174**(supplement C):797-802

[3] Valizadeh A, Mikaeili H, Samiei M, Farkhani SM, Zarghami N, Kouhi M, Akbarzadeh A, Davaran S. Quantum dots: Synthesis, bioapplications and toxicity. Nanoscale Research Letters. 2012;**7**(1):480

[4] Liu Y, Bose S, Fan W. Effect of size and shape on electronic and optical properties of CdSe quantum dots. Optik—International Journal for Light and Electron Optics. 2018;**155**(supplement C):242-250

[5] Xu R, Huang B, Wang T, Yuan Y, Zhang L, Lu C, Cui Y, Zhang J. Bright and high-photostable inner-Mn-doped core/giant-shell quantum dots. Superlattices and Microstructures. 2017;**111**(supplement C):665-670

[6] Borah P, Siboh D, Kalita PK, Sarma JK, Nath NM. Quantum confinement induced shift in energy band edges and band gap of a spherical quantum dot. Physica B: Condensed Matter. arXiv preprint arXiv:1705.10343 (2017)

[7] Zhang H, Gao X, Liu S, Su X. One-pot synthesis of stable water soluble Mn: ZnSe/ZnS core/shell quantum dots. Journal of Nanoparticle Research. 2013;**15**(6):1749

[8] Wang J, Li Q, Zhou J, Wang Y, Yu L, Peng H, Zhu J. Synthesis, characterization and cells and tissues imaging of carbon quantum dots. Optical Materials. 2017;**72**(supplement C):15-19

[9] Bodas D, Khan-Malek C. Direct patterning of quantum dots on structured PDMS surface. Sensors and Actuators B: Chemical. 2007;**128**(1):168-172

[10] Talapin DV, Rogach AL, Kornowski A, Haase M, Weller H. Highly luminescent monodisperse CdSe and CdSe/ZnS nanocrystals synthesized in a hexadecylamine−trioctylphosphine oxide−trioctylphosphine mixture. Nano Letters. 2001;**1**(4):207-211

[11] Peng X, Schlamp MC, Kadavanich AV, Alivisatos AP. Epitaxial growth of highly luminescent CdSe/CdS core/shell nanocrystals with photostability and electronic accessibility. Journal of the American Chemical Society. 1997;**119**(30):7019-7029

[12] Medintz IL, Mattoussi H, Clapp AR. Potential clinical applications of quantum dots. International Journal of Nanomedicine. 2008;**3**(2):151

[13] Jorge P, Martins MA, Trindade T, Santos JL, Farahi F. Optical fiber sensing using quantum dots. Sensors. 2007;**7**(12):3489-3534

[14] Wang H-Q, Zhang H-L, Li X-Q, Wang J-H, Huang Z-L, Zhao Y-D. Solubilization and bioconjugation of QDs and their application in cell imaging. Journal of Biomedical Materials Research Part A. 2008;**86**(3):833-841

[15] Hao Y, Gan Z, Xu J, Wu X, Chu PK. Poly(ethylene glycol)/carbon quantum dot composite solid films exhibiting intense and tunable blue–red emission. Applied Surface Science. 2014;**311**(Supplement C):490-497

[16] Zhang C, Kuai Y, Cheng H, Liu X, Ma L. Covalent bonding of grafted polymer brushes of poly(poly(ethylene glycol) monomethacrylate) on surface of silicon quantum dots and the activation of the end hydroxyls. Arabian Journal of Chemistry. 2017

[17] Liu W, He Z, Liang J, Zhu Y, Xu H, Yang X. Preparation and characterization of novel fluorescent nanocomposite particles: CdSe/ZnS core-shell quantum dots loaded solid lipid nanoparticles. Journal of Biomedical Materials Research. 2008;**84**(4):1018-1025

[18] Koole R, van Schooneveld MM, Hilhorst J, de Mello DonegÃ¡ C, Hart DCÊ, van Blaaderen A, Vanmaekelbergh D, Meijerink A. On the incorporation mechanism of hydrophobic quantum dots in silica spheres by a reverse microemulsion method. Chemistry of Materials. 2008;**20**(7):2503-2512

[19] Zhu M-Q, Chang E, Sun J, Drezek RA. Surface modification and functionalization of semiconductor quantum dots through reactive coating of silanes in toluene. Journal of Materials Chemistry. 2007;**17**(8):800-805

[20] Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, Ge N, Peale F, Bruchez MP. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nature Biotechnology. 2003;**21**(1):41-46

[21] Zhang Y, Clapp A. Overview of stabilizing ligands for biocompatible quantum dot nanocrystals. Sensors. 2011;**11**(12):11036-11055

[22] Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Quantum dot bioconjugates for imaging, labelling and sensing. Nature Materials. 2005;**4**(6):435-446

[23] Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, AM W, Gambhir SS, Weiss S. Quantum dots for live cells, *in vivo* imaging, and diagnostics. Science. 2005;**307**(5709):538-544

[24] Gerion D. Fluorescence imaging in biology using nanoprobes. Nanotechnologies for the Life Sciences. 2006

[25] Derfus AM, Chan WCW, Bhatia SN. Probing the cytotoxicity of semiconductor quantum dots. Nano Letters. 2004;**4**(1):11-18

[26] Wang L, Nagesha DK, Selvarasah S, Dokmeci MR, Carrier RL. Toxicity of CdSe nanoparticles in Caco-2 cell cultures. Journal of Nanobiotechnology. 2008;**6**(1):11

[27] Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metals toxicity and the environment. EXS. 2012;**101**:133-164

[28] Byrne SJ, Williams Y, Davies A, Corr SA, Rakovich A, Gun'ko YK, Rakovich YP, Donegan JF, Volkov Y. "Jelly dots": Synthesis and cytotoxicity studies of CdTe quantum dot-gelatin nanocomposites. Small. 2007;**3**(7):1152-1156

[29] Zhang A, Dong C, Li L, Yin J, Liu H, Huang X, Ren J. Non-blinking (Zn)CuInS/ZnS quantum dots prepared by *In Situ* interfacial alloying approach. Scientific Reports. 2015;**5**:15227

[30] Kirchner C, Liedl T, Kudera S, Pellegrino T, Muñoz Javier A, Gaub HE, Stölzle S, Fertig N, Parak WJ. Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. Nano Letters. 2005;**5**(2):331-338

[31] Mashinchian O, Johari-Ahar M, Ghaemi B, Rashidi M, Barar J, Omidi Y. Impacts of quantum dots in molecular detection and bioimaging of cancer. BioImpacts: BI. 2014;**4**(3):149

[32] Lee KP, Kelly DP, Schneider PW, Trochimowicz HJ. Inhalation toxicity study on rats exposed to titanium tetrachloride atmospheric hydrolysis products for two years. Toxicology and Applied Pharmacology. 1986;**83**(1):30-45

[33] Hoet PHM, Bruske-Hohlfeld I, Salata OV. Nanoparticles-known and unknown health risks. Journal of Nanobiotechnology. 2004;**2**(1):12

[34] Hoet PHM, Gilissen L, Nemery B. Polyanions protect against the *in vitro* pulmonary toxicity of polycationic paint components associated with the Ardystil syndrome. Toxicology and Applied Pharmacology. 2001;**175**(2):184-190

[35] Hoet PH, Gilissen LP, Leyva M, Nemery B. *In vitro* cytotoxicity of textile paint components linked to the "Ardystil syndrome". Toxicological Sciences: An Official Journal of the Society of Toxicology. 1999;**52**(2):209-216

[36] Su Y, Peng F, Jiang Z, Zhong Y, Lu Y, Jiang X, Huang Q, Fan C, Lee S-T, He Y. *In vivo* distribution, pharmacokinetics, and toxicity of aqueous synthesized cadmium-containing quantum dots. Biomaterials. 2011;**32**(25):5855-5862

[37] Jin S, Hu Y, Gu Z, Liu L, Wu H-C. Application of quantum dots in biological imaging. Journal of Nanomaterials. 2011;**2011**:13

[38] Hoshino A, Hanaki K-I, Suzuki K, Yamamoto K. Applications of T-lymphoma labeled with fluorescent quantum dots to cell tracing markers in mouse body. Biochemical and Biophysical Research Communications. 2004;**314**(1):46-53

[39] Hoshino A, Fujioka K, Oku T, Suga M, Sasaki YF, Ohta T, Yasuhara M, Suzuki K, Yamamoto K. Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. Nano Letters. 2004;**4**(11):2163-2169

[40] Shiohara A, Hoshino A, Hanaki K, Suzuki K, Yamamoto K. On the cytotoxicity caused by quantum dots. Microbiology and Immunology. 2004;**48**(9):669-675

[41] Lovrić J, Cho SJ, Winnik FM, Maysinger D. Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death. Chemistry & Biology. 2005;**12**(11):1227-1234

[42] Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A. *In vivo* imaging of quantum dots encapsulated in phospholipid micelles. Science. 2002;**298**(5599): 1759-1762

[43] Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS. Noninvasive imaging of quantum dots in mice. Bioconjugate Chemistry. 2004;**15**(1):79-86

[44] Jaiswal JK, Mattoussi H, Mauro JM, Simon SM. Long-term multiple color imaging of live cells using quantum dot bioconjugates. Nature Biotechnology. 2002;**21**:47

[45] Larson DR, Zipfel WR, Williams RM, Clark SW, Bruchez MP, Wise FW, Webb WW. Water-soluble quantum dots for multiphoton fluorescence imaging *in viv*o. Science. 2003;**300**(5624):1434

[46] Voura EB, Jaiswal JK, Mattoussi H, Simon SM. Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. Nature Medicine. 2004;**10**:993

[47] Hanaki K-I, Momo A, Oku T, Komoto A, Maenosono S, Yamaguchi Y, Yamamoto K. Semiconductor quantum dot/albumin complex is a long-life and highly photostable endosome marker. Biochemical and Biophysical Research Communications. 2003;**302**(3):496-501

[48] Chen F, Gerion D. Fluorescent CdSe/ZnS nanocrystal-peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells. Nano Letters. 2004;**4**(10):1827-1832

[49] Chen N, He Y, Su Y, Li X, Huang Q, Wang H, Zhang X, Tai R, Fan C. The cytotoxicity of cadmium-based quantum dots. Biomaterials. 2012;**33**(5):1238-1244

[50] Prasad BR, Nikolskaya N, Connolly D, Smith TJ, Byrne SJ, Gérard VA, Gun'ko YK, Rochev Y. Long-term exposure of CdTe quantum dots on PC12 cellular activity and the determination of optimum non-toxic concentrations for biological use. Journal of Nanobiotechnology. 2010;**8**(1):7

[51] Su Y, He Y, Lu H, Sai L, Li Q, Li W, Wang L, Shen P, Huang Q, Fan C. The cytotoxicity of cadmium based, aqueous phase—Synthesized, quantum dots and its modulation by surface coating. Biomaterials. 2009;**30**(1):19-25

[52] Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP. Semiconductor nanocrystals as fluorescent biological labels. Science. 1998;**281**(5385):2013-2016

[53] Tang M, Xing T, Zeng J, Wang H, Li C, Yin S, Yan D, Deng H, Liu J, Wang M, Chen J, Ruan D-Y. Unmodified CdSe quantum dots induce elevation of cytoplasmic calcium levels and impairment of functional properties of sodium channels in rat primary cultured hippocampal neurons. Environmental Health Perspectives. 2008;**116**(7):915-922

[54] Resch-Genger U, Grabolle M, Cavaliere-Jaricot S, Nitschke R, Nann T. Quantum dots *versus* organic dyes as fluorescent labels. Nature Methods. 2008;**5**(9):763-775

[55] Wang Y, Hu R, Lin G, Roy I, Yong K-T. Functionalized quantum dots for biosensing and bioimaging and concerns on toxicity. ACS Applied Materials & Interfaces. 2013;**5**(8):2786-2799

[56] Lu ZS, Li CM. Quantum dot-based nanocomposites for biomedical applications. Current Medicinal Chemistry. 2011;**18**(23):3516-3528

[57] Liu BR, Huang Y-W, Chiang H-J, Lee H-J. Cell-penetrating peptide-functionized quantum dots for intracellular delivery. Journal of Nanoscience and Nanotechnology. 2010;**10**(12):7897-7905

[58] Feugang JM, Youngblood RC, Greene JM, Willard ST, Ryan PL. Self-illuminating quantum dots for non-invasive bioluminescence imaging of mammalian gametes. Journal of Nanobiotechnology. 2015;**13**(1):38

[59] Feugang JM, Youngblood RC, Greene JM, Fahad AS, Monroe WA, Willard ST, Ryan PL. Application of quantum dot nanoparticles for potential non-invasive bio-imaging of mammalian spermatozoa. Journal of Nanobiotechnology. 2012;**10**:45

[60] Wiwanitkit V, Sereemaspun A, Rojanathanes R. Effect of gold nanoparticles on spermatozoa: The first world report. Fertility and Sterility. 2009;**91**(1):e7-e8

[61] Tiedemann D, Taylor U, Rehbock C, Jakobi J, Klein S, Kues WA, Barcikowski S, Rath D. Reprotoxicity of gold, silver, and gold-silver alloy nanoparticles on mammalian gametes. The Analyst. 2014;**139**(5):931-942

[62] Taylor U, Rehbock C, Streich C, Rath D, Barcikowski S. Rational design of gold nanoparticle toxicology assays: A question of exposure scenario, dose and experimental setup. Nanomedicine. 2014;**9**(13):1971-1989

[63] Taylor U, Barchanski A, Petersen S, Kues WA, Baulain U, Gamrad L, Sajti L, Barcikowski S, Rath D. Gold nanoparticles interfere with sperm functionality by membrane adsorption without penetration. Nanotoxicology. 2014;**8**(S1):118-127

[64] Moretti E, Terzuoli G, Renieri T, Iacoponi F, Castellini C, Giordano C, Collodel G. *In vitro* effect of gold and silver nanoparticles on human spermatozoa. Andrologia. 2013;**45**(6):392-396

[65] Amiri G, Valipoor A, Parivar K, Modaresi M, Noori A, Gharamaleki H, Taheri J, Kazemi A. Comparison of toxicity of CdSe: ZnS quantum dots on male reproductive system in different stages of development in mice. International Journal of Fertility & Sterility. 2016;**9**(4):512-520

[66] Chan WCW, Nie S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science. 1998;**281**(5385):2016-2018

[67] Yoo JS, Won N, Kim HB, Bang J, Kim S. *In vivo* imaging of cancer cells with electroporation of quantum dots and multispectral imaging. Journal of Applied Physics. 2010;**107**(12):124702

[68] Liu BR, Li J-F, S-W L, Lee H-J, Huang Y-W, Shannon KB, Aronstam RS. Cellular internalization of quantum dots noncovalently conjugated with arginine-rich cell-penetrating peptides. Journal of Nanoscience and Nanotechnology. 2010;**10**(10):6534-6543

[69] Jeong SH, Kim JH, Yi SM, Lee JP, Kim JH, Sohn KH, Park KL, Kim M-K, Son SW. Assessment of penetration of quantum dots through *in vitro* and *in vivo* human skin using the human skin equivalent model and the tape stripping method. Biochemical and Biophysical Research Communications. 2010;**394**(3):612-615

[70] Si-Han W, YHaC YM. Mesoporous silica nanoparticles as nanocarriers. Chemical Communications. 2011;**47**:9972

[71] Levene MJ, Dombeck DA, Kasischke KA, Molloy RP, Webb WW. *In vivo* multiphoton microscopy of deep brain tissue. Journal of Neurophysiology. 2004;**91**(4):1908-1912

[72] Xu G, Mahajan S, Roy I, Yong K-T. Theranostic quantum dots for crossing blood–brain barrier *in vitro* and providing therapy of HIV-associated encephalopathy. Frontiers in Pharmacology. 2013;**4**

[73] Naji Asfestani M, Rasouli Heikalabad S. A unique structure for the multiplexer in quantum-dot cellular automata to create a revolution in design of nanostructures. Physica B: Condensed Matter. 2017;**512**(supplement C):91-99

[74] Xing Y, Rao J. Quantum dot bioconjugates for *in vitro* diagnostics & *in vivo* imaging. Cancer Biomarkers. 2008;**4**(6):307-319

[75] Pinaud F, Michalet X, Bentolila LA, Tsay JM, Doose S, Li JJ, Iyer G, Weiss S. Advances in fluorescence imaging with quantum dot bio-probes. Biomaterials. 2006;**27**(9):1679-1687

[76] So M-K, Loening AM, Gambhir SS, Rao J. Creating self-illuminating quantum dot conjugates. Natural Protocols. 2006;**1**(3):1160-1164

[77] So MK, Xu C, Loening AM, Gambhir SS, Rao J. Self-illuminating quantum dot conjugates for *in vivo* imaging. Nature Biotechnology. 2006;**24**(3):339-343

[78] Julien A, Park S, Vance C, Ryan P, Willard S, Kouba A, Feugang J. Incorporation and developmental toxicity of quantum dot nanoparticles in amphibian larvae. Reproduction, Fertility and Development. 2017;**29**(1):166-167

[79] Odhiambo JF, DeJarnette JM, Geary TW, Kennedy CE, Suarez SS, Sutovsky M, Sutovsky P. Increased conception rates in beef cattle inseminated with nanopurified bull semen. Biology of Reproduction. 2014;**91**(4):97

[80] Feugang JM. Novel agents for sperm purification, sorting and imaging. Molecular Reproduction and Development. 2017;**9999**:1-10

[81] Durfey CL, Burnett DD, Liao SF, Steadman CS, Crenshaw MA, Clemente HJ, Willard ST, Ryan PL, Feugang JM. Nanotechnology-based selection of boar spermatozoa: Growth development and health assessments of produced offspring. Livestock Science. 2017; **205**(supplement C):137-142

[82] Durfey C, Swistek S, Tan W, Clemente H, Ryan P, Willard S, Feugang J. Beneficial effects of semen purification with magnetic nanoparticles. Mississippi Academy of Science. 2017;**62**(1):164

[83] Durfey C, Liao S, Devost-Burnett D, Dinh T, Crenshaw M, Willard S, Ryan P, Clemente H, Feugang J. Growth and market quality of pigs born from magnetic nanoparticle-treated spermatozoa. Reproduction, Fertility and Development. 2017;**29**(1):141-141

[84] Barkalina N, Jones C, Townley H, Coward K. Functionalization of mesoporous silica nanoparticles with a cell-penetrating peptide to target mammalian sperm *in vitro*. Nanomedicine. 2015;**10**(10):1539-1553