Journal of Animal and Feed Sciences, 24, 2015, 179–192
The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jabłonna

Nanobiotechnology in reproduction – pros and cons. A review

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KEY WORDS: nanobiotechnology, reproduction, nanostructures, embryogenesis, drug delivery, nanotoxicity

Received: 27 June 2014
Revised: 17 July 2015
Accepted: 9 September 2015

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ABSTRACT. The phenomenal development of nanobiotechnology in the twenty-first century has opened the door to exciting progress in medical nanoapplications. Nanotechnology promises a revolution in medicine to improve or create novel therapies in such areas as reproduction. Nanomaterials are used as active agents, drug delivery systems, and diagnostic molecules to treat and prevent diseases at the systemic, cellular and molecular level. Such broad implementation of nanoobjects is possible due to their unique properties resulting from their extremely small size. In this mini-review we discuss documented predictions and concerns associated with intentional or unintentional application of or exposure to nanostructures in reproduction and embryogenesis.

Nanotechnology – what is it?

Nanotechnology is defined as a production process that encompasses the use of technology to achieve very high precision and extremely small size (at the 1 nm level) (Taniguchi, 1974).

It is currently a multidisciplinary integrative achievement of natural sciences such as biology, chemistry, physics, mathematics, computer science and engineering, dedicated to creating and testing a variety of structures with sizes ranging from 0.1 to 100 nm (Koopmans and Aggeli, 2010). The unrivalled potential of structures in a *nano* scale, which are comparable in size to many biological molecules, has been predicted since 1959 (Feynman, 1960). Nevertheless, the true development of nanotechnology dates from the discovery of scanning tunneling microscopy (Binnig and Rohrer, 1982) and atomic force microscopy (Binnig et al., 1986), which allowed the visualization processing of very small structures with unique precision. Since then, thousands of nano-sized materials have been obtained and used to make objects of various forms. Their common characteristic is having at least one side with a dimension of less than 100 nm. On this scale, the physical, chemical and biological properties of nanomaterials basically differ from the properties of single atoms or of bulk solid matter and are governed by the rules of quantum mechanics rather than classical physics. The most important feature of nanostructures that distinguishes them from chemical particles or ions is the defined surface of the nanomaterials. Atoms on the surface of a nanostructure are in a higher energy state than the core atoms. Furthermore, some atomic orbitals do not form bonds with neighboring atoms, which gives them unique physical and chemical properties (Jiang et al., 2009). Therefore, it can be said that the attributes of nanomaterials strongly depend on their size, shape, composition, dimensionality and morphology, which are the key factors determining their ultimate performance and applications (Ashby et al., 2009; Cao and Wang, 2011).

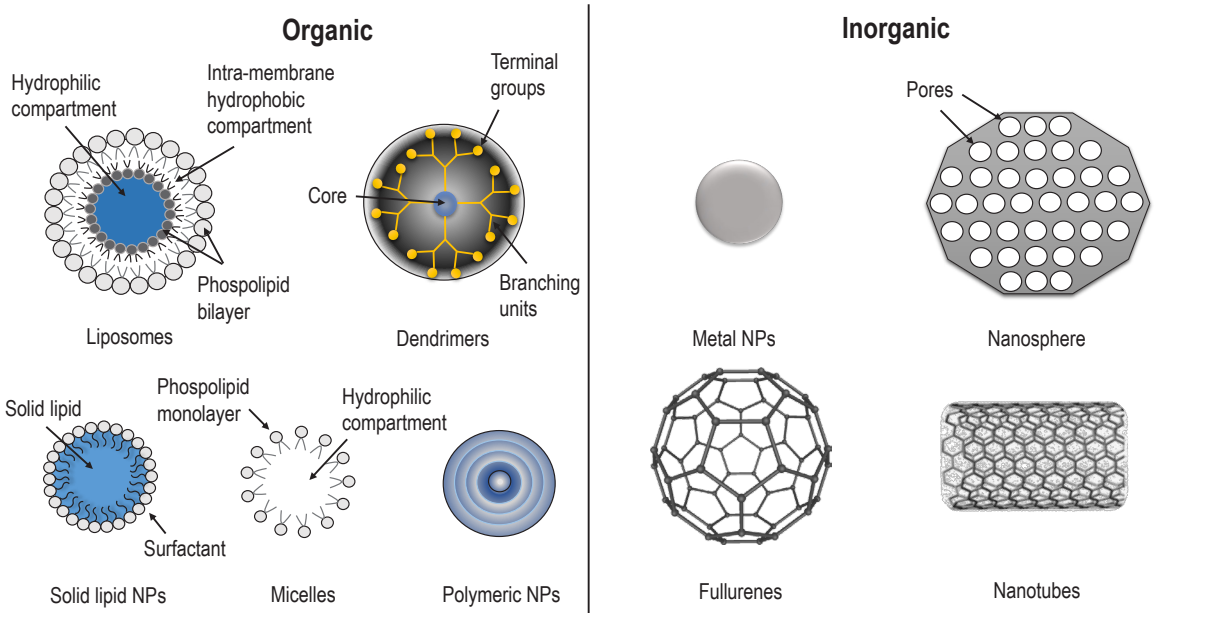

Figure 1. Biomedically-applied nanomaterials

The spatial structure of nanoobjects can be used to facilitate their classification. We distinguish zero-dimension (0D) structures with all dimensions within the nanometric size range. The nanoobjects in this category appear to be uniform nanoparticle (NPs) arrays: quantum dots, heterogeneous particle arrays, core–shell quantum dots, hollow spheres and nanolenses. The class of one-dimension (1D) materials comprises nanowires, nanorods, nanotubes, nanobelts, nanoribbons and hierarchical nanostructures. Those having two dimensional (2D) exceeding the nanometric size range belong to the 2D class that includes junctions – continuous islands, branched structures, nanoprisms, nanoplates, nanosheets, nanowalls and nanodisks. Three-dimensional (3D) constructions, such as nanoballs, dendritic structures, nanocoils, nanocones, nanopillers and nanoflowers, have recently become the subject of intensive study due to their highly active surface areas (Pokropivny and Skorokhod, 2007). Nanomaterials can also be defined according to a specific material. Organic nanomaterials are composed mainly of carbon and polymers such as chitosan (Figure 1). Inorganic ones consist of noble metals, oxides and semiconductors with optical properties, such as cadmium, selenium, tellurium (Barkalina et al., 2014a).

The expansion of nanomaterials has rapidly encompassed many areas of science and everyday life. Materials produced through nanotechnology or by classical technology, but having a size smaller than 100 nm, are used on a very large scale in practical applications, including energy supply, construction and transportation, food production, data storage and telecommunications, healthcare, the manufacturing of consumer products and biotechnology (Barkalina et al., 2014a).

Nanostructures in biological systems

Nanobiotechnology, which is described as the design and application of biomolecules on the nanometer scale, is a rapidly evolving interdisciplinary field at the crossroads of nanoscience, biology and engineering (Niemeyer and Mirkin, 2004). Advanced nanobiotechnology is multifunctional in nature and can benefit from the full potential of nanomaterials, transforming their optical, mechanical, magnetic and catalytic properties (Ashby et al., 2009; Cao and Wang, 2011). Over the last de-cade, nanomaterials have been used in biomedicine mainly as active agents, drug delivery systems and diagnostic molecules (Figure 2) to treat and prevent diseases at the systemic, cellular and molecular levels (Emerich, 2005).

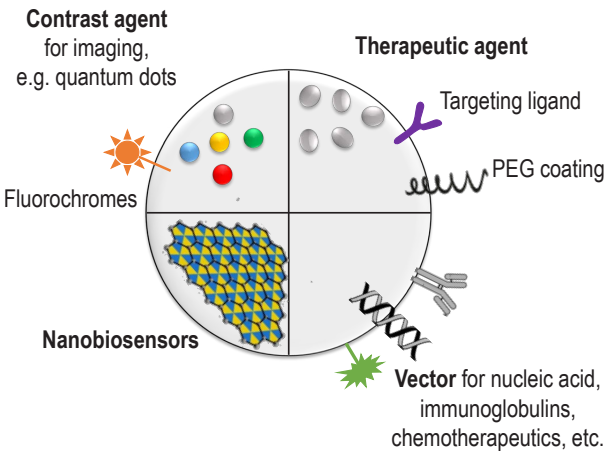

Figure 2. Direction of nanomaterials application

Active agents. The biocompatibility and toxicity of nanostructures define the limitations of their use. Although the number of studies examining the behaviour of nanostructures within living organisms has rapidly increased, many mechanisms are still not understood. Consequently, to use nanostructures as active agents, i.e. as new, alternative drugs, the interaction between them and the biostructures of the living body must be well known.

Biodistribution and clearance of nanostructures from the body are crucial aspects with regard to applicability in biomedical science; detailed research on them is being continued. Circulation of nanoparticles (NPs) is determined by their chemical and physical properties, including origin, size, surface charge, and also route of administration (Figure 3). Nanostructure distribution is also correlated with the physiological state of an organism. Inflammation, solid tumors and deliberate disruption of endothelial vessels contribute to increased leakiness that provides vascular contents greater access to extravascular targets (Faraji and Wipf, 2009). Nanostructures are efficiently taken up into cells, usually *via* the endosomal pathway through membrane fusion or receptor b-mediated endocytosis. Parab et al. (2009) observed a shape-dependency of NP uptake as a result of a smaller contact area with cell-membrane receptors. Cells exhibit increased uptake of spherical particles in comparison with rod- or cylinder-shaped ones. The optimal size of NPs for effective uptake depends on the cell that has been chosen. Each cell type can express varying levels of target receptors and can utilize different internalization pathways (Albanese et al., 2012). Biodistribution and accumulation of nanomaterials have been investigated for a number of applications. In the study by Dziendzikowska et al. (2012) translocation of single intravenous boluses of silver (Ag) NPs from the blood to organs was demonstrated. The concentration of Ag in tissues was size dependent and significantly higher in rats treated with 20 nm AgNPs as compared with 200 nm AgNPs. Circulation of Ag was also time dependent and the highest concentration of NPs was found in the liver after 24 h, in lungs and spleen after 7 days, and in the kidneys and brain after 28 days.

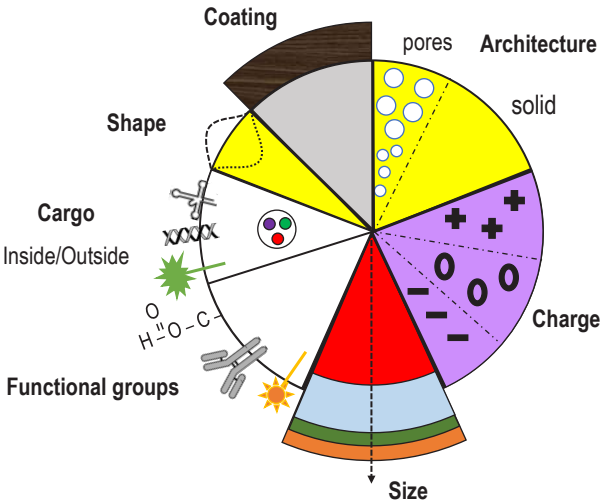

Figure 3. Properties of nanomaterials influenced on its biocompatibility (modified, according to Barkalina et al., 2014a)

One of the most frequent uses of nanostructures is related to their unique antimicrobial properties. Nanostructures are increasingly perceived as an alternative to antibiotics due to their vast spectrum of antibacterial actions, encompassing even those microbial strains that are antibiotic resistant, and their effect can be observed at low concentrations (Herman and Herman, 2014). The mechanism through which nanomaterials exert antibacterial effects strongly depends on the type of NP, its physical characteristics (size and shape) and preparation methods. There are two main categories of these antibacterial actions. The first is based on physical damage to the cell by increased membrane permeability (Padmavathy and Vijayaraghavan, 2008), accumulation in the bacterial membrane (Sondi and Salopek-Sondi, 2004), or immobilization of microorganisms while inducing membrane stress by direct contact with a sharp edge (Kurantowicz et al., 2015). The second type of actions relies on inhibition of a crucial physiological process or the cell cycle, resulting in suppression of the growth of pathogens. NPs induce oxidative stress (Kurantowicz et al., 2015), form free radicals (Rajeshkumar et al., 2012), inhibit the microorganism's respiratory system (Amin et al., 2012), or disturb replication by interacting with phosphorus-containing compounds like DNA (Sunkar and Nachiyar, 2012). Considering emerging and globally spreading new mechanisms of pathogen resistance that threaten the ability to treat common infectious diseases, nanostructures may prove to be the drug of choice.

Notwithstanding the above, the most spectacular trend in nanobiotechnological implementations, one which has aroused great expectations in the scientific community, is cancer treatment. Cancer therapy is based on precise marking, augmenting, or suppressing endogenous functional activity in a selected target cell population. Nanooncology may make use of the specificity of the tumor microenvironment, which remarkably differs from the surrounding normal tissue (Thakor and Gambhir, 2013).

This attribute can be used to make oncotherapy safer and more efficient (Sultana et al., 2013). Nanostructures can attack tumor cells *per se*, as a therapeutic agent in photodynamic therapy. This treatment generates cytotoxic oxygen-based molecules, which in consequence cause damage to subcellular organelles and plasma membranes, resulting in cell death by apoptosis, necrosis or autophagy (Thakor and Gambhir, 2013).

Moreover, combating cancer requires a multitrack approach, therefore, anticancer treatment is also focusing on antiangiogenesis by synergic combination of NPs and drugs. Carbon nanostructures, including diamond, graphite, graphene and fullerenes, exhibit antiangiogenic properties (Wierzbicki et al., 2013). Meng et al. (2010) observed increased antiangiogenic properties of fullerenes conjugated with multiple hydroxyl groups, which are capable of downregulating more than 10 angiogenic factors.

Drug delivery systems. Robust nanoplatforms are a non-invasive transporter of all types of biological cargo, such as targeting ligands and biomarkers (Yu et al., 2012). A loaded drug can be adsorbed, dissolved, or dispersed throughout the NP complex or, alternatively, it can be covalently attached to its surface. The connection between carrier and ligand may result from the natural properties of individual nanostructures. An example of such properties is the strong affinity of gold (Au) NPs to thiol groups (Ghosh et al., 2008), where bio-complexes with organic compounds are created in the process of self-assembly (self-organization) driven by van der Waals forces (Pyrpassopoulos et al., 2007). In turn, the nanosurface can be modified in a forced manner by coating and/or charge changing, which allows manipulating the interaction with ligands and targets (Figure 3) (Petros and DeSimone, 2010; Albanese et al., 2012). Furthermore, binding of functional agents, like antibodies, proteins or nucleic acids, improves biodistribution and uptake by the target cell population (Faraji and Wipf, 2009; Ballarín-González and Howard, 2012; Wang et al., 2012). Moreover, the cargo delivered by NPs can also be formulated on a nanoscale. Implementation of this technique increased therapeutic efficiency in a living animal model due to greater bioavailability and a longer sustainable therapeutic time, while maintaining low toxicity (Thakor and Gambhir, 2013).

In this context, applicable nanocarriers should fulfill certain criteria, which include the ability to bind or contain ligands and maintain stability in the serum or cells. They should also exhibit affinity to target structures, be able to release a drug, and be created from a biological or biologically inert material with a limited lifespan to allow safe degradation. Appropriate components for these vectors are lipids, phospholipids, dextran and chitosan. Likewise, nonbiodegradable nanomaterials can be used as active carriers. Artificial nanostructures mimic naturally produced phospholipid vesicles, and thus they can act as powerful mediators of cell communication. In contrast to organic vehicles, inorganic ones are relatively stable over a wide temperature and pH range (Faraji and Wipf, 2009).

One of the varieties of a nanodelivery system used in nanooncology, is the application of nanostructures as a carrier for intracellular delivery (Liu and Zhang, 2011) *via* passive, active or magnetic pathways (Schleich et al., 2014). This treatment can reverse the resistance of tumors to chemotherapy and also can help reduce systemic toxicity. Beyond chemotherapeutics, nanocarriers can deliver genetically active agents in experimental gene therapy, which is currently being extensively examined. The most promising effects of gene therapy are observed when neutral nanoliposomes from biological material are used (Díaz and Vivas-Mejia, 2013). Also, NPs are used in gene silencing therapy based on siRNAs, short double-stranded RNA fragments with the ability to interfere with the translation of specific mRNAs complimentary to their nucleotide sequence (Fire et al., 1998). siRNA bind to the RNA-induced silencing complex (RISC) and activate its essential catalytic component – a multifunctional Argonaute protein, which, through its endonuclease activity, leads to silencing of gene expression. The process may simultaneously silence with high efficacy and specificity several genes that contribute to cancer progression. Using nanocarriers allows excluding several limitations of this method, like delivery problems or side effects due to off-target actions (Thakor and Gambhir, 2013). Therefore, nanobiotechnology creates a novel possibility for manipulation of cell function (Dragovic et al., 2011; Gercel-Taylor et al., 2012).

Diagnostics. The great versatility of nanostructures permits their use in theranostics, which refers to the simultaneous integration of therapy and diagnosis into a single, integrated system (Muthu et al., 2014). The implementation of nanostructures in this new area provides an individual approach to the patient and his disease. It enables increasing the effectiveness of therapy and reducing adverse effects by controlled and targeted co-delivery of therapeutic drugs and steady imaging, even during the treatment regimen (Xie et al., 2010). Nanoplatforms can also serve as quick and cost effective nanobiosensors for the detection of novel cancer biomarkers (Yuan et al., 2012).

Nanostructures in reproductive biology

Reproductive success is crucial for assurance of the continuation of species and is strictly dependent on the performance of the reproductive system. Disordered homeostasis of this system can lead to infertility. The problem of male and female subfertility has been recognized by the World Health Organization as a social disease. International studies have reported that reproductive dysfunction affects 12% to 20% of couples in the world, with most of them in developed or developing countries. This situation is probably related to the reproductive age shift and pollution. That is why medical support of fertility for couples with unexplained subfertility and unfavourable prognosis is a continuously expanding field of reproductive medicine. The role of assisted reproduction techniques (ART) is still growing, and increasing knowledge is probably leading to increasingly better efficiency. Conventional ART are mainly aimed at various forms of subfertility in both sexes, including severe or obstructive male infertility, bilateral tubal pathology, poor ovarian reserve, advanced maternal age, anovulation and endometriosis (Kamphuis et al., 2014). The main ART is still *in vitro* fertilization (IVF) and correlated procedures including selection and micromanipulation of gametes and embryos, *in vitro* maturation of oocytes, preimplantation genetic testing, cryopreservation of gametes and reproductive tissues. Novel and experimental techniques, like time-lapse imaging of embryo development, assisted egg activation and nuclear genome transfer, are also emerging (Lemmen et al., 2008; Fragouli and Wells, 2012). Extended use of IVF unfortunately increases the risk of damage associated with maternal and perinatal complications such as gestational diabetes, foetal growth restriction, pre-eclampsia and premature birth (Pinborg et al., 2013; Kamphuis et al., 2014). Therefore, new and improved coping strategies with infertility are still desired and nanobiotechnology has clear potential in this area. The use of nanotechnology for reproductive applications is currently in its earliest stages and scientists are still looking for possible implementations of nanomaterials (Figure 4).

One of the proven approaches is application of nanosolutions as a safer and less invasive alternative to standard surgical intervention. For example, a theranostic approach uses nanostructures to treat ectopic pregnancy and trophoblastic diseases (Kaitu'u-Lino et al., 2013), endometriosis (Lee et al., 2012), as well as uterine fibroids (Ali et al., 2013).

Nanosupport of assisted reproduction techniques. Nanobiotechnology is also a route for ART improvement. Nanomaterials as carriers of various functional molecules can find a broad spectrum of application. Nanovehicles are serving, among others, in hormone therapy, including hormone replacement therapy. Hormones with poor oral bioavailability, such as oestradiol, can be encapsulated into biodegradable polylactic-glycolic acid (PLGA) NPs and administered orally (Hariharan et al., 2006) or transdermally (Tomoda et al., 2012). In both studies, nanoparticulate formulations of the drug demonstrated greater permeability compared with its

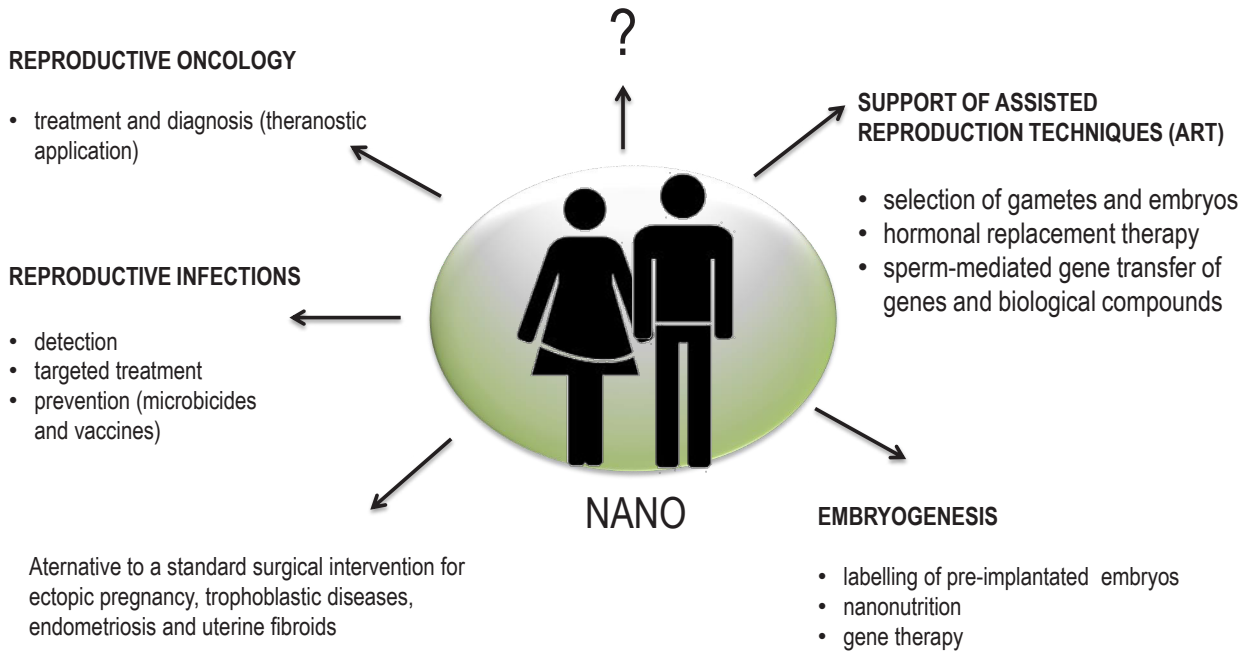

Figure 4. Current and experimental application of nanomaterials in reproductive medicine

standard form. In contradistinction to nonbiodegradable nanomaterials, those treatments did not cause an inflammatory response or undesired distribution in the body (Hariharan et al., 2006).

Moreover, NPs, as an alternative to traditional fluorophores, are used for spermatozoa sorting by flow cytometry and fluorescent-activated cell sorting (Chattopadhyay et al., 2010). Several publications report using AuNPs covalently bonded with membrane-penetration agents for sorting populations of gametes by recognizing and binding with specific DNA sequences at the Y-chromosome (Barchanski et al., 2011; Rath et al., 2013) without affecting fertilization potential (Feugang et al., 2012). Improvement of this technique will allow not only broader use of sex-sorted spermatozoa, mainly in breeding livestock (Rath et al., 2013), but also development of bioimaging systems in the future (Barchanski et al., 2011).

Another reproductive support technique in which nanosolutions can be deployed is sperm-mediated gene transfer (SMGT). This method is designed to breed transgenic animals for use as preclinical models of human diseases, bioreactors for pharmaceutical products and in xenotransplantation experiments (Canovas et al., 2010; Parrington et al., 2011). Traditional and forced transfection methods for SMGT are electroporation, lipofection, DNA/DMSO complexes, and restriction-enzyme-mediated integration. Nonetheless, the number of transgenic offspring obtained by this technique remains low (Spadafora, 2007). Actions of nanotransfectants, such as nanopolymers, are based on the sperm's inherent ability to spontaneously bind and incorporate exogenous DNA during co-incubation. Thereby they serve as natural vectors for transfection of the oocyte and, consequently, of a developing embryo *via* IVF (Campos et al., 2011b). This treatment did not affect sperm motility or viability, but both X- and Y-sorted sperm had decreased DNA uptake in comparison with unsorted sperm (Campos et al., 2011a). Constructs of NPs conjugated with exogenous DNA are successfully transferred *via* SMGT into fertilized oocytes, but expression of the assayed protein in morulae and blastocysts is not always detectible (Kim et al., 2010; Campos et al., 2011a).

From the research perspective, this approach could provide invaluable insight into the physiological pathways associated with fertilization and early embryo development. Another stage of proceeding with gametes within ART is cryopreservation of spermatozoa and oocytes. Nanomaterials have been evaluated as potential nanocryoprotectants improving the storage conditions for semen and the freeze/thaw cycle (Silva, 2014). Likewise, in relation to female gametes, NPs can hinder the recrystallization of vitrification solution during rewarming of oocytes (Zhou et al., 2015).

Antimicrobial treatment. Depending on the antibacterial and antiviral properties of nanostructures, they can be used for the detection and treatment of genital infections, including *Chlamydia trachomatis* (Tang et al., 2010), *Neisseria gonorrheae* (Singh et al., 2011), *Candida* spp. (Bansod et al., 2013) and *Herpes simplex virus* (Bernstein et al., 2003). In particular, studies on counteracting chlamydial infection, which has long-term consequences including male and female infertility, are well-advanced (Wyrick, 2010). The unique life cycle of this pathogen makes it remarkably antibiotic resistant. Anti-chlamydial nanotreatment can be conducted on three levels: drug delivery system (Toti et al., 2011), polyamidoamine dendrimer-based intravaginal knockdown of gene expression (Mishra et al., 2012) and effective anti-chlamydia immunization by biodegradable PLGA NPs as a vaccine subunit (Fairley et al., 2013).

Reproductive oncology. To date a number of nanomaterial-based reproductive applications have been investigated. The main focus of detection and targeted therapy is reproductive oncology. Traditional treatment with cytotoxic agents can expose patients to temporary or permanent loss of fertility through ovarian tissue damage and impaired follicle function (Levine et al., 2010). The ferto-toxicity of conventional drugs may influence treatment decisions for many premenopausal women suffering from cancer. Nanomaterial-mediated delivery of chemotherapeutics permits marked improvement in their efficiency and also helps to reduce systemic toxicity. Researchers have demonstrated the potential of biodegradable NPs, such as derivatives of PLGA (Le Broc-Ryckewaert et al., 2013) and bovine serum albumin (Zhao et al., 2010). Also non-biodegradable materials can be applied, including magnetic iron (Lee et al., 2013) and gold (de Oliveira et al., 2013). Binding ligands to nanocarriers facilitates the delivery of chemotherapeutic agents into target cancer cells and significantly potentiates their anti-tumor effects compared with the respective free molecules (Ahn et al., 2013).

The treatments referred to above are intended to ensure reproductive success. Currently, equally intensive research attempts to apply nanotheranostics in embryo development are being made (Kohli and Elezzabi, 2009).

Embryogenesis. During embryogenesis the intensive processes associated with the formation and proper development of all systems, such as the

nervous, respiratory, circulatory, digestive and excretory systems, affect the entire further life of the foetus. All transformations taking place in the embryo are relatively intensive and dynamic. The risk of affecting the foetus is higher in some critical periods of embryogenesis, such as implantation, placenta formation and organogenesis (Kulvietis et al., 2011).

Embryos can be exposed to nanostructures as a result of accidental contact in the maternal surroundings, which include air, food and water. Intentional action through diagnostics and treatment is also possible. There are ongoing studies concerning optimization of *in utero* treatment of embryo renal anomalies by gene therapy. Nanomaterial-mediated gene transfer into foetal tissues was demonstrated by Yang et al. (2011). The authors utilized intra-amniotic injections into mouse embryos of chitosan NPs conjugated with the enhanced green fluorescent protein (EGFP) gene and observed expression of the transgene in the alveolar epithelium of the lungs and the luminal intestinal epithelium. However, the effect was temporary and limited to the listed tissues. Nanomaterials also have been evaluated as potential labels for preimplantation embryos in IVF for identification purposes. Fynewever et al. (2007) co-incubated or microinjected polystyrene and polyacrylonitrile NPs into mouse 1-cell embryos for external and cytoplasmic tagging, respectively. Embryo development was similar for externally applied polystyrene nanoparticles and controls. Intracytoplasmic injections of the same NPs resulted in a reduced proportion of developing embryos compared with control groups. Regardless of the administration method, the negative effects of polyacrilonitrile NPs were more pronounced. Therefore, further research in both nanoapplications is required to optimize the techniques and timings of this procedure in order to achieve more stable outcomes.

Nanostructures *per se* or as vectors can be utilized as supports in nanonutrition. Nanonutrition refers to additional supporting of development in prenatal as well as postnatal life, when the content of nutrients is not sufficient to fully support embryogenesis (Foye et al., 2006). Nanonutrition comprises direct administration to the environment of the embryo such extra nutrients as carbohydrates, minerals, vitamins and other modulators that can assist foetal formation. Nanoapplication may have a positive effect on such fundamental processes as angiogenesis (Mroczek-Sosnowska et al., 2015), myogenesis (Zielinska et al., 2011) and osteogenesis (Sikorska et al., 2010). Angiogenesis is the process in which a new blood vessel is formed and developed, and is regulated by specific metabolic pathways. It is essential for proper growth of the embryo by providing appropriate supplies of oxygen and nutrients. Using an embryo chorioallantoic membrane model, Mroczek-Sosnowska et al. (2015) demonstrated that copper (Cu) NPs *per se* have pro-angiogenic properties at the systemic level, and also change the morphology of vessels and their thickness. Cu is a pleiotrophic agent that influences numerous mediators of angiogenesis (Xie and Kang, 2009). Nano-sized Cu induces more enhanced pro-angiogenic effects than copper in its salt ($CuSO_4$) and metal (Cu°) forms. Cerium oxide nanoparticles (CeNPs) are able to induce angiogenesis, too. CeNPs are a nontoxic redox-active agent (Celarado et al., 2011), which act by modulating the intracellular oxygen environment and stabilizing hypoxia. *In vitro* and *in vivo* research demonstrated robust induction of endothelial cell proliferation as well as vascular sprouting (Das et al., 2012).

Nanonutritional support also promotes progress of another crucial process, i.e. myogenesis. Muscle cells are created and developed from the undifferentiated mesodermal germ layer, and are nearly established just before birth. The muscle structure is mainly determined by the total number of muscle fibres and their thickness. The process of myogenesis depends on genetic and environmental factors, and is affected by the proliferation, differentiation and maturation of cells. Sawosz et al. (2013) and Grodzik et al. (2013) observed that AgNPs and diamond NPs (DNPs) used, respectively, as an active agent and as a protective nanocarrier for adenosine triphosphate (ATP) or amino acid L-glutamine, increased embryo muscle mass and upregulated expression of genes involved in myogenesis, such as fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF) and myogenic differentiation 1 (MyoD1). Furthermore, AuNPs with natural compounds of the extracellular matrix, such as taurine and heparan sulphate, increased the number of nuclei per cell, the number of satellite cells, fibre diameter, as well as muscle mass (Zielinska et al., 2011, 2012).

Nanostructures have also been evaluated as potential candidates for carriers into bones of micronutrients promoting osteogenesis (Sikorska et al., 2010). Reinforcement of bone tissue repair potential during embryogenesis may improve spontaneous regeneration in postnatal life (Gusić et al., 2014).

Risk of the use of nanomaterials

The benefits of using nanostructures must be, however, examined and discussed in parallel with potential toxic and/or negative effects of these materials on living organisms. One of the main problematic issues associated with nanoapplications

in various fields is the potential impact on reproduction. The praxis of nanobiotechnological methods in a range of clinical applications has undoubted advantages. The increasing use of nanomedicine brings with it the potential risk of systemic and local toxicity of engineered nanomaterials. Nanostructures are able to accumulate in organs, tissues and intracellular structures, so they are able to cause long-term metabolic, immune and carcinogenic effects (Barkalina et al., 2014a). Special concern arises about the uncontrolled circulation in the body of the nanomaterials currently widely used in both medical and nonmedical applications. Accumulation of nanomaterials in somatic cells can induce inflammatory responses and carcinogenesis (Taylor et al., 2012). However, in some respects accumulation of nanostructures in reproductive tissues and gametes is even more dangerous because it activates a range of changes in physiological processes associated with reproduction, which may lead to fertility disorders and/or consequential impairments in the offspring. This potential health hazard requires in-depth investigation.

It is very important to select nanomaterials in terms of safety and efficacy when considering exposure of sensitive reproductive organs and gametes to them, because many nanomaterials that are widely used for delivery into somatic cells can demonstrate toxicity towards gametes (Taylor et al., 2012; Tiedemann et al., 2014). This difficult task requires knowledge of the mechanisms governing interaction of nanomaterials with cells. These interactions depend on a combination of factors, including the physical and chemical properties of the nanocarrier (size, surface charge, coating and presence of functional groups) and morphological/physiological features of the particular target cell population. In nanocharacteristics studies there is no place for generalizations, because every nanostructure is unique and requires an individual approach. Consequently, the number of studies on potential toxicity is still growing, but the obtained data are inconsistent.

Reports concerning the influence of nanotreatment on male reproduction parameters are hard to interpret because these studies involved varied types of nanostructures, kinds of applications and treated species. Some researchers have demonstrated biocompatibility with mammalian sperm for magnetic iron NPs (Kim et al., 2010), mesoporous silica NPs (Barkalina et al., 2014b), halloysite clay nanotubes and commercial nanopolymer-based transfectants (Campos et al., 2011a). In turn, alternative data regarding the reproductive toxicity of most commonly used nanostructures, such as AuNPs, AgNPs and titanium dioxide (TiO_2) NPs, remain highly contradictory. Application of noble-metal NPs may impact sperm vitality parameters like motility, morphology and membrane integrity, resulting in impairment of male gametes, which become incapable of fertilization. These effects were observed after incubation of spermatozoa with extremely high concentrations of ligand-free AuNPs and AgNPs (Barchanski et al., 2011; Moretti et al., 2013). This cytotoxic consequence was manifested by failure of chromatin to decondense and in the nucleus structure (Zakhidov et al., 2010). It should also be remembered that that nanoparticles can pass through the blood-testis barrier (Gao et al., 2013). It has been shown that long-term administration by gavage of AgNPs to adult male rats led to depletion of germ cells, germinal cell necrosis, especially in spermatogonia, abnormal fibroblast-like appearance of Leydig cells and abnormal spaces between neighboring Sertoli cells (Thakur et al., 2014). Also, oral intake of AgNPs in the prepubertal period caused alterations in adult sperm parameters, such as reduction of the acrosome and plasma membrane integrities, decreased mitochondrial activity and increased abnormalities of the sperm, delay of puberty onset. However, in this case AgNPs did not influence the sex hormone profile (Sleiman et al., 2013; Mathias et al., 2015). In turn, Garcia et al. (2014) observed the effects of AgNPs on Leydig cell function after intravenous administration, which resulted in increased testicular and serum testosterone levels. Of particular importance are TiO_2NPs because they are frequently used for commercial purposes in a variety of products such as air and water filters, and also in sun screens and coatings of self-cleaning windows (Kale and Meena, 2012). Intraperitoneal injection of TiO_2NPs (Mohammadi Fartkhooni et al., 2013) as well as oral intake (Tassinari et al., 2014) or intragastric long-term administration (Gao et al., 2013) in male rodents resulted in disordered sex hormone profiles, testicular lesions and sperm malformations. Researchers also observed important changes in over 140 crucial genes involved in spermatogenesis and associated with steroid and hormone metabolism (Gao et al., 2013). The particular mechanisms of action are still unrecognized, but the suggested reasons for cellular damage include production of reactive oxygen species and interaction with DNA (Taylor et al., 2012).

Oogenesis, in turn, begins very early in foetal life and is continued in the primary ovarian reserve until the end of the reproductive period. Therefore, the effect of nanostructures on the female reproductive system can cause permanent damage and

subfertility. The few available studies focused on these areas have demonstrated that the spontaneous biokinetics of nanostructures of various origins allow their uncontrolled translocation, especially when unmodified nanomaterials are used. Gao et al. (2012) showed that after long-term intragastric administration to female mice, TiO_2NPs directly affected ovarian function. The NPs accumulated in the ovary either caused ovarian damage and decreased fertility, or affected the pregnancy rate through up- or down- regulation of over 200 genes, which implies an imbalance of sex hormone metabolism. NPs can also directly affect female gametes. In cultured preantral follicles obtained from rats receiving TiO_2NPs, Hou et al. (2009) observed morphological changes in the follicles and a reduced number of matured oocytes.

Another aspect of contact of females with nanomaterials pertains to the period of pregnancy and the maternal influence on embryogenesis. In the case of mammals, the nanoimpact on the foetus is dependent on the passage of nanostructures through the placental barrier. New reports show that many conditions, such as small size, appropriate hydrostatic pressure, coating, negative charge, hydrophilicity and chemical composition, can enable NPs to cross the trophoblast (Myllynen et al., 2008; Menjoge et al., 2010; Praetner et al., 2010). The mechanism of NP transition through the placenta is not yet known. Some authors believe that NPs can penetrate by active transcellular transport connected with specific membrane proteins or directly through an injured placental barrier. However, results are inconsistent. TiO_2NPs applied to time-mated mice through inhalation induced long-term lung inflammation in females, but no NPs were found in foetuses (Hougaard et al., 2010). In contrast, Yamashita et al. (2011) reported that fluorescently labeled TiO_2NPs and silica NPs smaller than 100 nm injected intravenously into pregnant mice were found in the placenta, foetal brain and foetal liver. Moreover, NPs larger than 300 nm were not observed in placenta or foetus after injection. These authors also observed that mice treated with NPs had smaller uteri and smaller foetuses compared with untreated females. Research indicates that nanoparticles can cause dose- and size-dependent embryo toxicity, resulting in growth inhibition, resorption of foetus, and placental dysfunction. Studies indicate that these adverse changes may be caused by pathological changes in placental structure that reduce blood flow (Yamashita et al., 2011). Other studies concerning subcutaneous injection of TiO_2NPs into pregnant mice, pointed to the possibility of effects on the expression of genes related to the development and function of the central nervous system (Shimizu et al., 2009). Another study showed that comparable exposure increased levels of dopamine in the brains of offspring (Takahashi et al., 2010). Gao et al. (2011) observed that oral administration of TiO_2NPs to rats during gestation decreased synaptic plasticity in the hippocampus of the foetus. However, not only the nervous system of foetuses was affected after maternal nanoexposure. Subcutaneous contact of pregnant female mice with TiO_2NPs caused irreversible changes in their male offspring. Takeda et al. (2009) observed various functional and pathologic disorders in 6-week-old male mice, such as reduced daily sperm production and histological disorders in testicular tissue. This negative effect of NPs can be prevented by coating the surface of nanoparticles with carboxyl or amino groups, thus producing safe nanomaterials (Yamashita et al., 2011).

Several studies have also investigated the effect of NPs on cultured early-stage embryos. The effects of cobalt–chromium NPs on human trophoblast choriocarcinoma cell lines and a layer of BeWo b30 cells were examined, and DNA damage in the fibroblasts was noted despite indirect exposure (Bhabra et al., 2009). In turn, Li et al. (2010) observed increased apoptosis, decreased cell numbers and decreased implantation success rates after AgNP treatment of mouse blastocysts.

Although the number of studies concerning mammalian embryogenesis is still small, extensive nanotoxicology research on chicken embryos and zebrafish embryos has been conducted. Exposition of zebrafish larvae to AgNPs induces cardiac and eye deformities, yolk sac oedema, finfold and tail abnormalities, skeletal flexure, head oedema, cardiac malformation and often abnormal tail (Lee et al., 2012). The frequent adverse effects of AgNPs can be explained by the action of Ag^+ ions released in an aqueous environment (Powers et al., 2011). Browning et al. (2009) observed concentration-dependent cardiac malformation and yolk sac oedema after exposure to AuNPs. In contrast to the above-cited studies, it seems that Ag does not influence chicken development as negatively as in zebrafish. *In ovo* injection of Ag/Cu or Ag/Pd NPs into chicken embryos did not influence embryonic development (Sawosz et al., 2009; Studnicka et al., 2009) or produce any negative effects on embryonic survival, growth, development, or morphology of chicken embryos.

Some kind of safety valve is natural potential for agglomeration of NPs. Aggregates can affect the total surface area and change the chemical properties

of NPs by increasing the total size. Aggregated, not sonicated NPs, significantly decreased mortality of fishes' embryos compared with exposure to particles that were just stirred (Laban et al., 2010).

Conclusions

Subfertility is becoming a matter of growing concern, therefore, the use of dedicated systems to deliver pharmaceutical products in targeted, non-invasive treatment of chronic reproductive pathologies instead of surgery can clearly optimize the chances for conception in future. The treatment based on the nanobiotechnological achievments is very promising in this discipline of medicine.

However, there are many factors that can disrupt the biological activity of nanoparticles and all of them have to be monitored during experiments to provide consistent conditions. Unfortunately, the available analytical methods are still unsuitable for study of the properties of nanostructures. Indirect methods such as visualization of particles bonded with fluorochromes are inadequate. The potential biotoxicity of nanomaterials is strongly dependent on their overall physicochemical properties, their origin and method of preparation. Understanding the relationship between the physical and chemical properties of nanostructures and their conduct *in vivo* will provide a basis for evaluating the response of organisms. Therefore, a very individual approach to each nanosolution, both in respect to the type of nanostructure, its dose, as well as target organisms must be adopted. The controversial nature of this discipline remains a matter of discussion. The delicate nature of reproductive tissues and gametes elicit ethical controversy surrounding experimental techniques in reproductive medicine. Although considerable experimental data related to nanostructure toxicity at the molecular, cellular and whole organism levels have been published, the results are often conflicting. In this young discipline there is a lack of studies defining effects of nanoexposure measured in years of postnatal life. Further investigations focusing more on the exact mechanism behind the observed effects are urgently needed.

In every aspect of life, even in nanobiotechnology, to balance pros and cons, the words of Paracelsus should be heeded: *'Omnia sunt venena, nihil est sine veneno. Sola dosis facit venenum.'*

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