Current Topics in
Environmental Health and Preventive Medicine

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Biological Effects of Fibrous and Particulate Substances

Springer

Chapter 4
Reproductive and Developmental Effects of Nanomaterials

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Abstract Reproductive and developmental toxicity are among the most important factors for evaluating the safety of chemical substances. Some critical organs for reproduction are protected by biological barriers: the fetus is protected by the blood–placental barrier and the testes by the blood–testis barrier. The small size of nanomaterials affords them unique biodistribution characteristics and thus biological effects that differ from those of larger materials. Their small size might allow nanoparticles to penetrate barriers and cause unexpected reproductive and developmental toxicity. In this chapter, the reproductive and developmental toxicity of nanomaterials, including biodistribution within and biological effects on reproductive tissues, fetuses, and offspring, are reviewed. Investigations show that nanomaterials can penetrate biological barriers and can be distributed to the ovaries, testes, and fetuses of rodents. Nanomaterials thus have the potential to affect both male and female reproductive functions. Maternal exposure to nanomaterials during gestation or lactation could also adversely affect the fetus

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T. Otsuki et al. (eds.), *Biological Effects of Fibrous and Particulate Substances*,
Current Topics in Environmental Health and Preventive Medicine,
DOI 10.1007/978-4-431-55732-6_4

or offspring. This review compiles current knowledge and highlights remaining open questions in evaluating the reproductive and developmental toxicity of nanomaterials.

Keywords Nanomaterials • Nanoparticles • Blood–placental barrier • Blood–testis barrier • Reproductive and developmental toxicity

4.1 Introduction

Fetuses and infants are more sensitive than adults to environmental toxins [1, 2]. Therefore, some chemicals transmitted through the placenta or through breast milk could adversely affect fetuses or infants, even if the dose is low enough not to induce adverse effects in mothers [2–4]. In addition, adverse effects that are induced in offspring during the fetal or infant period of development could affect the offspring's subsequent growth [5]. Ensuring the safety of these susceptible populations is one of the most important issues in chemical safety. In addition, when evaluating the safety of chemicals for progeny, effects on reproductive functions of parents should also be considered. Rates of male and female infertility continue to increase, and infertility has been a difficult problem to solve [6, 7]. Unlike toxicity-induced problems such as congenital abnormalities, infertility is less visibly apparent. Nevertheless, the damaging effect of infertility is severe when one considers the potential lives lost. Therefore, the reproductive and developmental toxicity of chemicals are very important points to be evaluated.

There is increasing concern regarding the safety of fine particles. For example, exposure to particulate matter less than 2.5 μm in diameter (PM2.5) is well known to increase cardiovascular or respiratory mortality [8]. In addition, effects of PM2.5 on fetuses, infants, or reproductive functions of parents have also been reported [9, 10]. Collection of more detailed information about reproductive and developmental toxicity of fine particles is still urgently needed. Importantly, recent epidemiological studies have shown that exposure to nanoparticles, rather than microparticles, highly relates to relate health risks caused by fine particles [11, 12]. In addition, there is an increasing use of engineered nanomaterials, including nanoparticles, nanofibers, and nanosheets, in various applications such as foods, cosmetics, and medicines [13–15]. Consequently, opportunities for humans to be exposed to nanoparticles are increasing rapidly. Some health risks of these engineered nanoparticles on humans have been reported [16–18]. Thus, further collection and understanding of safety information of nanomaterials should be regarded as an urgent need.

In this chapter, we summarize the current body of knowledge regarding the reproductive and developmental toxicity of nanomaterials, focusing mainly on *in vivo* and *ex vivo* studies of nanoparticle toxicity in mammals.

4.2 Reproductive and Developmental Toxicity of Nanomaterials in Females

4.2.1 Effects of Nanomaterials on Female Reproductive Functions

Compared to the amount of safety information focused on gestational and lactational exposure (described in Sects. 4.2.2 and 4.2.3), relatively little information has been collected regarding the effects of nanomaterials on female reproductive functions. Gao G. et al. showed that, after oral administration for 90 days at 10 mg/kg in mice, titanium oxide nanoparticles could be distributed to the ovaries and could induce ovarian damage, altered gene expression in the ovaries, an imbalance of sex hormones, and decreased fertility [19]. Distribution to ovaries and an imbalance of sex hormones after oral administration of titanium dioxide nanoparticles (1 or 2 mg/kg for 5 days) were demonstrated also by Tassinari et al. [20]. At the moment, it is difficult to judge whether these results can be generalized to multiple types of nanomaterials or whether they are specific to titanium dioxide. Therefore, the effects of nanomaterials on female reproductive functions should be investigated more thoroughly through the use of various types and sizes of nanoparticles.

4.2.2 Safety of Intrauterine Exposure to Nanomaterials

4.2.2.1 Penetration of Blood–Placental Barrier by Nanomaterials

Many nanomaterials such as gold nanoparticles [21–23], carbon nanotubes [24], fullerenes [25, 26], titanium oxide nanoparticles [27, 28], silica nanoparticles [28], polystyrene nanoparticles [29], iron oxide nanoparticles [30], silver nanoparticles [31], and quantum dots [32] have been reported to penetrate the blood–placental barrier (BPB) and to be distributed to fetuses in rodent studies. However, in other studies, gold nanoparticles [33, 34] and quantum dots [35] have been reported to be unable to penetrate the BPB. These conflicting results may be due to differences in detection methods or detection limits. Studies that have demonstrated nanoparticles' penetration of the BPB have used high-sensitivity quantitative methods such as inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), and radioisotope measurements [21–23, 32]. In contrast, studies that have shown that nanoparticles do not penetrate the BPB have used relatively insensitive methods such as autometallography, spectroscopic examination, and microscopic examination [33, 35]. Although an *ex vivo* human placenta perfusion study that showed that gold nanoparticles do not penetrate the BPB [34] used a quantitative method (ICP-MS), the detection limit of gold in the fetal side of the placenta was only

0.13–0.2 % of the amount detectable in the maternal side. Considering the low transfer rate of gold nanoparticles through the BPB in late pregnancy period by rodent study (about 0.00005–0.07 % of the amount of gold administered to dams) [21–23], the sensitivity of the detection methods might have been insufficient to detect gold in the fetal side. In addition, penetration of the human BPB by polystyrene nanoparticles has been demonstrated by Wick et al. and Grafmüller et al. [36, 37]. Therefore, it is possible that nanomaterials could penetrate the BPB in humans and rodents, although the transfer rate would be low. These reports about transitivity of nanomaterials to fetuses by means of the BPB are summarized in Table 4.1. Some factors that determine the transitivity of nanomaterials through the BPB have been revealed. One of the factors is the size of the nanomaterials: smaller particles were reported to be more easily distributed to the fetus than were larger particles for gold [21, 22], silica [28], and polystyrene [29, 36, 37] nanoparticles as well as quantum dots [32]. The gestational day is also an important factor. Yang et al. showed that 13-nm gold nanoparticles were distributed more readily to fetal tissue before gestational day 9.5 than after gestational day 11.5 [23]. In addition, surface modification or coating with polyethylene glycol (PEG), acids, amino or carboxyl groups, proteins, or SiO_2 can change the nanoparticles' transitivity of the BPB [23, 29, 32]. Therefore, it could be possible to regulate the transitivity of nanomaterials to fetuses by designing appropriate particles or by using nanomaterials during times in the pregnancy term that are determined to be safe.

4.2.2.2 Biological Effects of Nanomaterials on Embryos and Fetuses

Some nanomaterials have been reported to cause hazardous effects on fetuses in rodent studies (Table 4.2). Carbon nanotubes [24, 38–40], fullerenes [25], titanium oxide nanoparticles [28], silica nanoparticles [28], cadmium oxide nanoparticles [41], and quantum dots [32] present hazards leading to miscarriage, fetal death, fetal resorption, fetal growth restriction, or fetal malformation when exposed to dams. In addition, treatment of blastocysts or oocytes by silver nanoparticles [42] or quantum dots [43, 44] has been reported to cause increased resorption of post-implantation embryos and decreased fetal weights. Similar to the case of the transitivity through the BPB, the size and surface modification of the nanomaterials, as well as the pregnancy term, determine the hazard on the fetus. Smaller silica nanoparticles [28] and quantum dots [32] had a greater hazard of causing fetal death, fetal resorption, or fetal growth restriction than did larger particles. Surface modification or coating by PEG, 3-mercaptopropionic acid, amino groups, carboxyl groups, SiO_2, or ZnS could change the nanomaterials' hazard to the fetus [28, 32, 43, 44]. The degree of oxidation of carbon nanotubes was found to contribute to their toxicity [39]. The period of pregnancy during exposure also seems to determine what effects are observed on the fetus. Many studies focused on exposure during the organogenic period have revealed teratogenicity of nanomaterials [25, 38, 39]. On the other hand, exposure to nanomaterials late in pregnancy induced fetal death/resorption or fetal growth restriction [28, 32]. These results

Table 4.1 Distribution of nanomaterials to fetuses

Nanomaterials	Animals	Exposure protocol	Results	References
Gold nanoparticles (5, 30 nm)	Wistar rats	Single intravenous injection on gestational day (GD) 19 at 0.02 mg/rat	Gold nanoparticles were detected in fetus; fetal accumulation was greater for the smaller nanoparticles	[21]
Gold nanoparticles (1.4, 18 nm)	WKY rats	Single intravenous injection in third trimester	Gold nanoparticles were detected in fetus; fetal accumulation was greater for the smaller nanoparticles	[22]
13-nm gold nanoparticles with surface modifications (ferritin, PEG, or citrate)	CD-1 mice	Single intravenous injection at GD 5.5, 7.5, 9.5, 11.5, 13.5, or 15.5 (7.2 μg/g)	Gold nanoparticles were detected in fetal tissue; fetal gold levels declined dramatically post-E11.5; fetal accumulation of ferritin- or PEG-modified nanoparticles was considerably greater than that of citrate-capped nanoparticles	[23]
Oxidized multi-walled carbon nanotubes	Kunming mice	Single intravenous injection on GD 17 at 20 mg/kg	Carbon nanotubes were detected in fetus	[24]
Fullerene (C60)	SLC mice	Single intraperitoneal injection on GD 10 at 25–137 mg/kg	Fullerene was detected in embryos	[25]
Fullerene (C60)	Sprague–Dawley rats	Single intravenous injection on GD 15 at 0.3 mg/kg	Fullerene was detected in fetus	[26]
Titanium dioxide nanoparticles (anatase, 25–70 nm)	ICR mice	Subcutaneous injection on GD 3, 7, 10, and 14 at 0.1 mg/mouse	Titanium dioxide nanoparticles were detected in testes and brain of male offspring	[27]
Titanium dioxide nanoparticles (217 nm), silica nanoparticles (70, 300, 1000 nm), carboxyl-modified silica nanoparticles (70 nm), amine-modified silica nanoparticles (70 nm)	BALB/c mice	Intravenous injection on GD 16 and 17 at 0.8 mg/mouse	Titanium dioxide nanoparticles and silica nanoparticles with diameters of less than 70 nm were detected in fetal liver and brain; larger silica particles (300, 1000 nm) were not detected in fetus	[28]

(continued)

Table 4.1 (continued)

Nanomaterials	Animals	Exposure protocol	Results	References
Carboxyl-modified polystyrene nanoparticles (20, 100, 500 nm), amine-modified polystyrene nanoparticles (200 nm)	Mouse placenta (*ex vivo*)	Injection via extra-embryonic tissue on GD 7.5	20- and 200-nm polystyrene nanoparticles were detected in embryo; 100- and 500-nm polystyrene nanoparticles were not detected	[29]
Iron oxide nanoparticles coated with dimercapto-succinic acid (3–9 nm)	BALB/c mice	Single intraperitoneal injection on GD 8 at 50, 100, 200, and 300 mg/kg	Iron oxide nanoparticles were detected in fetal liver	[30]
Silver nanoparticles (35 nm)	Wistar rats	Single intragastrical administration on GD 20 at 1.69–2.21 mg/kg	Silver nanoparticles were detected in fetus	[31]
CdTe/CdS quantum dots (with various sizes and cappings)	Kunming mice	Single intravenous injection on 20–22 days after female mice were housed with male mice at 20, 50, 86, or 125 μg Cd/mouse	Smaller quantum dots were more easily transferred to fetus than larger ones; capping with an inorganic silica shell or organic polyethylene glycol reduced the transfer of quantum dots to fetus	[32]
Gold nanoparticles (2, 40 nm)	C57BL/6 mice	Single intravenous injection on GD 16 to 18 at 12.13 μg (2-nm gold nanoparticles) or 58.21 μg (40-nm gold nanoparticles)	Gold nanoparticles were not detected in fetal liver	[33]
PEGylated gold nanoparticles (10, 15, 30 nm)	Human placenta (*ex vivo*)	Once-through perfusions (15, 30 nm) or recirculating perfusions (15, 30 nm)	Gold nanoparticles were not detected in fetal outflow	[34]
CdSe/ZnS quantum dots coated with PEG	Wistar rats	Intraperitoneal injection on GD 13, at 0.4 nmol/rat	Quantum dots were not detected in fetus	[35]
Polystyrene nanoparticles (50, 80, 240, 500 nm)	Human placenta (*ex vivo*)	Dual recirculating perfusion	Polystyrene particles with diameter up to 240 nm were able to cross the placental barrier	[36]
Polystyrene nanoparticles (80, 500 nm)	Human placenta (*ex vivo*)	Dual recirculating perfusion	The 80-nm particles were able to cross the placental barrier while the 500-nm particles were not	[37]

Table 4.2 Biological effects of nanomaterials on fetuses

Nanomaterials	Animals	Exposure protocol	Results	References
Oxidized multi-walled carbon nanotubes	Kunming mice	Single intravenous injection on GD 17 at 20 mg/kg	Increased abortion rate	[24]
Single-walled carbon nanotubes functionalized with a hydroxyl group	CD-1 mice	Single oral administration on GD 9 at 10 or 100 mg/kg	Carbon nanotubes administration (10 mg/kg) increased the number of resorptions and resulted in fetal morphological and skeletal abnormalities	[38]
Pristine, oxidized, or ultraoxidized single-walled carbon nanotubes	CD-1 mice	Single intravenous injection on GD 5.5 at 0.01, 0.1, 0.3, 3, or 30 μg/mouse	A high percentage of early miscarriages and fetal malformations were observed in females exposed to single-walled carbon nanotubes, while lower percentages were found in animals exposed to the pristine material; the lowest effective dose was 0.1 μg/mouse	[39]
Multi-walled carbon nanotubes	ICR mice	Single intraperitoneal or intratracheal administration on GD 9 at 2, 3, 4, or 5 mg/kg (intraperitoneal) or 3, 4, or 5 mg/kg (intratracheal)	In the intraperitoneal study, various types of malformation were observed in all carbon nanotube-treated groups. Such malformations were observed in groups given 4 or 5 mg/kg body weight, but not in those treated with 3 mg/kg in the intratracheal study	[40]
Fullerene (C60)	SLC mice	Single intraperitoneal injection on GD 10 at 25–137 mg/kg	Increased fetal death (137 mg/kg) and fetal abnormalities (25, 50, and 137 mg/kg)	[25]

(continued)

Table 4.2 (continued)

Nanomaterials	Animals	Exposure protocol	Results	References
Titanium dioxide nanoparticles (217 nm), silica nanoparticles (70, 300, 1000 nm), carboxyl-modified silica nanoparticles (70 nm), amine-modified silica nanoparticles (70 nm)	BALB/c mice	Intravenous injection on GD 16 and 17 at 0.8 mg/mouse	Titanium dioxide nanoparticles and silica nanoparticles with diameters of less than 70 nm induced reduction of fetal weight and increased resorption rate while other materials did not	[28]
Cadmium oxide nanoparticles (10–15 nm)	CD-1 mice	Inhalation every other day of 100 μg of particles or daily inhalation of 230 μg particles from PND4.5 to 16.5	Daily inhalation of 230 μg particles decreased the incidence of pregnancy, delayed maternal weight gain, altered placental weight, and decreased fetal length	[41]
CdTe/CdS quantum dots (with various sizes and cappings)	Kun Ming mice	Single intravenous injection on 20–22 days after female mice were housed with male mice at 20, 50, 86, or 125 μg Cd/mouse	Smaller quantum dots produced dead pups; PEG or SiO_2 coating could enhance the survival of the pups	[32]

seem reasonable, because teratogenic effects arise mainly following exposure during the organogenic period. It remains to be clarified which period of pregnancy is most susceptible to exposure of nanomaterials. Hazardous effects, such as hepatotoxicity and nephrotoxicity, might be induced in fetuses at doses that do not induce adverse effects on mothers [28]. Since the "no observed adverse effects level" (NOAEL) is lower for fetuses than for mothers, particular attention should be paid to the fetal toxicity of nanomaterials. Unfortunately, the mechanism of these hazardous effects is not well understood and needs to be evaluated further. Our research revealed a partial mechanism of silica nanoparticle–induced fetal growth restriction in mice [28]. In our research, heparin treatment prevented decreased fetal weight caused by silica nanoparticles. Heparin mainly works as anticoagulant. However, heparin is also known to have an anticomplement activation effect [45] and a role as a placental growth factor [46–48]. Therefore, silica nanoparticle–induced fetal growth restriction might involve coagulation, complement activation, or placental dysfunctions. Extensive vascular lesions and increased production of reactive oxygen species (ROS) were also observed in placentas of malformed fetuses by carbon nanotube administration to dams [39]. Oxidative stress in the placenta can cause placental dysfunction and induce pregnancy complications

[49]. Therefore, ROS induction in the placenta and placental dysfunction is a possible mechanism of fetal toxicity by nanomaterials.

4.2.2.3 Postnatal Effects of Intrauterine Exposure to Nanomaterials

In utero exposure to nanomaterials can also induce postnatal effects in rodents (Table 4.3). Hazard information has been collected mainly for titanium oxide nanoparticles and carbon black nanoparticles. After intrauterine exposure, titanium oxide nanoparticles have been observed to distribute to the testes or brain of pups [27]. In addition, they have been shown to induce testicular injury and reduce sperm production [27], as well as brain dysfunctions: altered gene expression [50, 51], altered neurotransmitters [52], increased apoptosis in the olfactory bulb [27], and neurobehavioral alterations [53] of pups. Titanium oxide nanoparticles have also been reported to alter hepatic gene expression of pups [54] and to increase neonatal asthma susceptibility [55]. Carbon black nanoparticles presented hazards similar to those of titanium oxide nanoparticles upon intrauterine exposure, including the risk of causing testicular damage and reduced sperm production [56] (reduced sperm production was even observed in F2 pups [57]), neurobehavioral alterations [58], altered hepatic gene expression [59], hepatic gene damage [60], and renal abnormalities in pups [61]. As for other nanomaterials, decreased growth and abnormal spermatogenesis of pups caused by iron oxide nanoparticles [30] and delayed neonatal growth caused by cadmium oxide nanoparticles [41] have been reported. Taken together, the findings indicate that prenatal exposure to nanomaterials could affect the growth, liver, kidney, brain, and testes of neonates. Although the broad range of tissues and organs affected by the nanomaterials suggests that the toxicity of nanomaterials is not tissue-specific, it is interesting to note that many hazards were observed for the brain and testes of pups. These tissues might have been affected owing to the immaturity of the blood–brain barrier and blood–testis barrier (BTB) during the fetal and juvenile periods [62–65]. Substances that would rarely pass through these barriers in adults, such as nanomaterials or proinflammatory cytokines produced by exposure to nanomaterials, might be more easily distributed to the brain or testes during the fetal and juvenile periods, resulting in increased toxicity for these susceptible tissues. Proinflammatory cytokines, including maternal proinflammatory cytokines, have been shown to affect fetal brain development [66]. In view of these possibilities, more detailed mechanistic analysis of nanomaterials' postnatal effects should be conducted. In addition, for postnatal effects of nanomaterials, information about the relationship between toxicity and physical properties of nanomaterials such as size and surface modification is deficient and should be investigated in future studies.