[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.

Table 4.3 Postnatal effects of intrauterine exposure to nanomaterials

Nanomaterials	Animals	Exposure protocol	Results	References
Titanium dioxide nanoparticles (anatase, 25–70 nm)	ICR mice	Subcutaneous injection on GD 3, 7, 10, and 14 at 0.1 mg/mouse	Reduced sperm production and increased apoptosis in olfactory bulb were observed in offspring	[27]
Titanium dioxide nanoparticles (2570 nm)	ICR mice	Subcutaneous injection on GD 6, 9, 12, and 15 at 0.1 mg/mouse	Changes in the expression of genes associated with apoptosis, brain development, response to oxidative stress, neurotransmitters, and psychiatric diseases were found in the brain of pups	[50]
Titanium dioxide nanoparticles (anatase, 25–70 nm)	ICR mice	Subcutaneous injection on GD 6, 9, 12, and 15 at 0.1 mg/mouse	Alteration of gene expression in the cerebral cortex, olfactory bulb, and regions related to dopamine systems of pups	[51]
Titanium dioxide nanoparticles (anatase, 25–70 nm)	ICR mice	Subcutaneous injection on GD 6, 9, 12, 15, and 18 at 0.1 mg/mouse	Dopamine and its metabolites were increased in the prefrontal cortex and the neostriatum of pups	[52]
Titanium dioxide nanoparticles (97 nm)	C57BL/6BomTac mice	Inhalation 1 h/day of 42 mg/m^3 aerosolized powder from GD 8 to 18	Offspring tended to avoid the central zone of the open field and female offspring displayed enhanced prepulse inhibition	[53]
Titanium dioxide nanoparticles (97 nm)	C57BL/6BomTac mice	Inhalation 1 h/day of 42 mg/m^3 aerosolized powder from GD8 to 18	Changes in gene expression related to the retinoic acid signaling pathway in the female pups	[54]
Titanium dioxide nanoparticles	BALB/c mice	Single intranasal administration on GD 14 at 50 μg/mouse	Increased asthma susceptibility in offspring	[55]
Carbon nanoparticles (14 nm)	ICR mice	Intratracheal administration on GD7 and 14 at 0.2 mg/mouse	Histological abnormalities in testes and reduced daily sperm production of pups	[56]

(continued)

Table 4.3 (continued)

Nanomaterials	Animals	Exposure protocol	Results	References
Carbon black nanoparticles (140 nm)	C57BL/6 J mice	Intratracheal administration on GD 7, 10, 15, and 18 at 67 μg/mouse	F2 offspring, whose fathers were prenatally exposed to carbon black nanoparticles, showed lowered sperm production	[57]
Carbon black nanoparticles (140 nm)	C57BL/6BomTac mice	Intratracheal administration on GD 7, 10, 15, and 18 with total doses of 11, 54, and 268 μg/mouse	Female offspring displayed altered habituation pattern during the open-field test	[58]
Carbon black nanoparticles (140 nm)	C57BL/6BomTac mice	Intratracheal administration on GD 7, 10, 15, and 18 with total doses of 11, 54, and 268 μg/mouse	Gene expression changes in pup's liver	[59]
Carbon black nanoparticles (140 nm)	C57BL/6BomTac mice	Inhalation of 42 mg/m^3 carbon black nanoparticles for 1 h/day from GD 8 to 18, or intratracheal administration on GD 7, 10, 15, and 18 with total doses of 11, 54, or 268 μg/mouse	Inhalation exposure induced DNA strand breaks in the liver of offspring, whereas intratracheal administration did not	[60]
Carbon black nanoparticles (14 nm)	ICR mice	Intranasal administration on GD 5 and 9 at 50 μg/mouse	Increased expression of the gene encoding collagen, type VIII, alpha 1 in the tubular cells in the kidney of 12-week-old offspring mice	[61]
Iron oxide nanoparticles coated with dimercaptosuccinic acid (3–9 nm)	BALB/c mice	Single intraperitoneal injection on GD8 at 50, 100, 200, or 300 mg/kg	Decreased growth of pups; decrease in spermatogonia, spermatocytes, spermatids, and mature sperm of pups	[30]
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 induced delayed neonatal growth	[41]

4.2.3 Safety Information Regarding Lactational Exposure to Nanomaterials

4.2.3.1 Distribution of Nanomaterials to Breast Milk

The possibility that nanomaterials could be distributed to breast milk has been demonstrated in a few studies. Titanium oxide nanoparticles [67] and silver nanoparticles [31] were detected in neonates after oral administration to lactating dams. However, these results are thought to be insufficient to verify the transitivity of nanomaterials to breast milk, because neonates could be exposed to nanomaterials not only through breast milk but also from contact with the dam's excretion or the dam herself. Among the very few studies that have directly examined nanomaterials' transitivity to breast milk, Sumner et al. showed that fullerenes could be distributed to breast milk after intravenous injection to lactating rats [26] and Hougaard et al. showed that titanium dioxide nanoparticles are not detected in breast milk after inhalation by pregnant mice [53]. Information about the transitivity of other nanoparticles to breast milk is limited. Therefore, the transitivity of nanomaterials to breast milk should be investigated more thoroughly, using various species and sizes of nanoparticles.

4.2.3.2 Biological Effects on Neonates by Lactational Exposure to Nanomaterials

Very little information is available regarding the hazards of neonatal exposure to nanomaterials via lactation, compared to the information available for in utero exposure. Gao X. et al. orally treated lactating rats with 100 mg/kg of titanium oxide nanoparticles from postnatal days 2–21, and they revealed that the offspring's synaptic plasticity, namely, input/output functions, paired pulse reaction, and long-term potentiation in the hippocampal dentate gyrus area, all were attenuated [67]. In addition, they showed that if dams were exposed to titanium oxide nanoparticles during pregnancy, rather than while lactating, only the paired pulse reaction was attenuated in the offspring. These results suggest that the susceptibility of neonates or infants to the nanomaterials varied among pregnancy and lactation periods. Therefore, more investigations focused on lactational exposure to nanomaterials would help to reveal more hazards to neonates, including the identification of target tissues, in addition to the hazards described in Sect. 4.2.2.3.

4.3 Effects of Nanomaterials on Male Reproductive Functions

4.3.1 Penetration of BTB by Nanomaterials

Many nanomaterials such as gold nanoparticles [68–70], carbon nanotubes [71], titanium oxide particles [72, 73], silica nanoparticles [74], iron oxide nanoparticles [75], silver nanoparticles [76–79], ceria nanoparticles [80], magnetic nanoparticles [81, 82], cobalt–chromium nanoparticles [83], and polymethyl methacrylate nanoparticles [84] have been reported to be distributed to the testes (Table 4.4). In addition, smaller nanomaterials were more easily distributed to the testes than were larger nanomaterials [68, 74, 77]. The BTB is the barrier between blood vessels and seminiferous tubules that is formed by tight junctions of Sertoli cells. Distribution to the testes itself does not mean that the BTB has been penetrated, because some interstitial testis cells exist on the external side of the BTB. However, gold nanoparticles [70], titanium oxide particles [72, 73], silica nanoparticles [74], and magnetic nanoparticles [81] have been detected inside seminiferous tubules or Sertoli cells raising the possibility that nanomaterials can penetrate the BTB. Furthermore, gold nanoparticles [70] and silica nanoparticles [74] have been shown to be distributed to male germ cells. Testicular distribution of nanomaterials has some interesting characteristics. Nanomaterials distributed to the testes are retained for a long time compared to those retained in other tissues [78, 79]. Accumulation of gold nanoparticles in testes has been reported to occur from 1 month postinjection [69]. Considering these findings of long retention and late accumulation, further studies on nanomaterials in testes should focus on long-term analysis.

4.3.2 Biological Effects of Nanomaterials on Male Reproductive Functions

Some nanomaterials have been reported to cause hazardous effects on the male reproductive functions of rodents *in vivo* (Table 4.5). Carbon nanotubes [71], carbon black nanoparticles [85], titanium oxide nanoparticles [72, 73, 86], silica nanoparticles [87], silver nanoparticles [88], and cobalt–chromium nanoparticles [83] can cause oxidative stress or tissue damage in the testes. Gold nanoparticles [70], carbon black nanoparticles [85], titanium oxide nanoparticles [20, 72], and nanoparticle-rich diesel exhaust [89, 90] have been shown to disrupt the endocrine activity of the male reproductive system. Carbon black nanoparticles [85], titanium oxide nanoparticles [72, 73, 86, 91], silver nanoparticles (especially smaller silver particles) [88], and cobalt–chromium nanoparticles [83] can affect sperm production or injure sperm (or germ cells). Although such occurrences are thought to be infrequent, male germ cells have been shown to be directly exposed to nanomaterials in some cases [70, 74]. In this regard, hazard information for male

Table 4.4 Distribution of nanomaterials to testes

Nanomaterials	Animals	Exposure protocol	Results	References
Gold nanoparticles (10, 50, 100, 250 nm)	Wistar rats	Single intravenous injection at 77, 96, 89, or 108 μg/rat (for 10-, 50-, 100-, and 250-nm particles, respectively)	Gold nanoparticles were detected in testes; 10-nm nanoparticles were the most easily distributed to testes	[68]
Gold nanoparticles (20 nm)	Wistar rats	Single intravenous injection at 3.02 μg/rat	Gold nanoparticles were detected in testes; significant accumulation of Au in testes took place only after 1–2 months postinjection	[69]
PEG-NH_2_modified or ω-methoxy and ω-aminoethyl poly (ethylene glycol)–modified gold nanoparticles (14 nm)	ICR mice	Single intravenous injection at 45 mg/kg	Gold nanoparticles were detected in testes; PEG-NH_2_modified gold nanoparticles accumulate more easily in testis than do ω-methoxy and ω-aminoethyl poly (ethylene glycol)–modified gold nanoparticles	[70]
Carboxylate-functionalized multi-walled carbon nanotubes	BALB/c mice	Single intravenous injection at 5 mg/kg	Carbon nanotubes were detected in testes	[71]
Titanium dioxide nanoparticles (anatase, 294 nm)	CD-1 mice	Intragastric administration for 90 days at 2.5, 5, or 10 mg/kg	Titanium dioxide nanoparticles were detected inside of the seminiferous tubules	[72]
Titanium dioxide nanoparticles (310 nm)	CD-1 mice	Intragastric administration for 90 days at 2.5, 5, or 10 mg/kg	Titanium dioxide nanoparticles were detected in Sertoli cells	[73]
Silica nanoparticles (70, 300 nm)	BALB/c mice	Intravenous injection on two consecutive days at 0.8 mg/mouse	70-nm silica nanoparticles were detected within Sertoli cells and spermatocytes, while 300-nm silica particles were not	[74]
Iron oxide nanoparticles (144 nm)	Sprague–Dawley rats	Single intratracheal administration at 4 mg/rat	Iron oxide nanoparticles were detected in testes	[75]
Silver nanoparticles (56 nm)	F344 rats	Oral administration for 90 days at 30, 125, or 500 mg/kg	Silver nanoparticles were detected in testes	[76]

(continued)

Table 4.4 (continued)

Nanomaterials	Animals	Exposure protocol	Results	References
Silver nanoparticles (22, 42, 71, 323 nm)	ICR mice	Oral administration for 14 days at 1 mg/kg	Smaller silver nanoparticles (22 nm and 42 nm) were detected in testes, while larger silver nanoparticles (71 and 323 nm) were not	[77]
<20-nm noncoated, or <15-nm polyvinylpyrrolidone-coated silver nanoparticles	Sprague–Dawley rats	Oral administration for 28 days at 90 mg/kg	Silver nanoparticles were detected in testes; silver nanoparticles were not cleared from the testes after 8 weeks post-dosing	[78]
Silver nanoparticles (10, 25 nm)	Sprague–Dawley rats	Oral administration for 28 days at 100 or 500 mg/kg	Silver nanoparticles were detected in testes; silver concentrations in the testes did not clear well after the 4-month recovery period	[79]
Ceria nanoparticles (30 nm)	Sprague–Dawley rats	Single intravenous administration at −100 mg/kg	Ceria nanoparticles were detected in testes	[80]
Silica-overcoated magnetic nanoparticles (50 nm)	ICR mice	Intraperitoneal administration for 4 weeks at 25, 50, or 100 mg/kg	Magnetic nanoparticles were detected inside of the seminiferous tubules	[81]
Magnetic nanoparticles (50 nm)	ICR mice	By nose-only exposure chamber system with a total particle number of 4.89×10^5 /cm^3 (low concentration) and 9.34×10^5 / cm^3 (high concentration) for 4 weeks (4 h/d, 5 d/wk)	Magnetic nanoparticles were detected in testes	[82]
Cobalt–chromium nanoparticles (55 nm)	Sprague–Dawley rats	Intra-articular administration once a week at 20, 100, or 500 μg/kg for 10 consecutive weeks	Cobalt–chromium nanoparticles were detected in testes	[83]
Polymethyl methacrylate nanoparticles (130 nm)	Wistar rats	Single oral administration	Polymethyl methacrylate nanoparticles were detected in testes	[84]

Table 4.5 Biological effects of nanomaterials on male reproductive functions

Nanomaterials	Animals	Exposure protocol	Results	References
Carboxylate- and amine-functionalized multi-walled carbon nanotubes	BALB/c mice	Intravenous injection every 3 days for 5 times at 5 mg/kg	Nanotubes generated oxidative stress and decreased the thickness of the seminiferous epithelium in the testes at 15 days after the first dose, but the damage was repaired at 60 and 90 days after the first dose	[71]
Carbon black nanoparticles (14, 56, 95 nm)	ICR mice	Intratracheal administration for 10 times every week at 0.1 mg/mouse	Carbon black nanoparticles induced increased serum testosterone, partial vacuolation of the seminiferous tubules, and reduced daily sperm production	[85]
Titanium dioxide nanoparticles (anatase, 294 nm)	CD-1 mice	Intragastric administration for 90 days at 2.5, 5, or 10 mg/kg	Titanium dioxide nanoparticles induced testicular lesions, sperm malformations, alterations in serum sex hormone levels, and altered gene expression in testes	[72]
Titanium dioxide nanoparticles (310 nm)	CD-1 mice	Intragastric administration for 90 days at 2.5, 5, or 10 mg/kg	Titanium dioxide nanoparticles induced testicular oxidative damage and/or apoptosis, altered gene expression in testes, and increased abnormal sperm	[73]
Titanium dioxide nanoparticles (33, 160 nm)	CBAB6F1 mice	Oral administration for 7 days at 40, 200, or 1000 mg/kg	Particles induced increased frequency of spermatids with two and more nuclei (33, 160 nm), apoptosis in testes (only 33 nm)	[86]

(continued)

Table 4.5 (continued)

Nanomaterials	Animals	Exposure protocol	Results	References
Silica nanoparticles (10–15 nm)	Wistar mice	Single oral administration at 333.33 mg/kg	Silica nanoparticles induced testicular lesions	[87]
Silver nanoparticles (20, 200 nm)	Wistar rats	Single intravenous injection at 5 (20 and 200 nm) or 10 (only 20 nm) mg/kg	Silver nanoparticles induced decrease of the epididymal sperm count, DNA damage in germ cells, and change in the testes seminiferous tubule morphometry	[88]
Cobalt–chromium nanoparticles (55 nm)	Sprague–Dawley rats	Intra-articular administration once a week at 20, 100, or 500 μg/kg for 10 consecutive weeks	Cobalt–chromium nanoparticles reduced epididymal sperm motility, viability, and concentration, increased abnormal sperm rate, and induced testicular damage and pathological changes via oxidative stress	[83]
PEG-NH_2–modified or ω-methoxy and ω-aminoethyl poly (ethylene glycol)–modified Gold nanoparticles (14 nm)	ICR mice	Three intravenous injection each other day at 45 or 225 (only for ω-methoxy and ω-aminoethyl poly (ethylene glycol)–modified gold nanoparticles) mg/kg	PEG-NH_2–modified gold nanoparticles increased plasma testosterone levels	[70]
Titanium dioxide nanoparticles	Sprague–Dawley rats	Oral administration for 5 days at 1 or 2 mg/kg	Titanium dioxide nanoparticles increased plasma testosterone levels	[20]
Nanoparticle-rich diesel exhaust	F344 rats	Inhalation for 4, 8, or 12 weeks (5 h/day, 5 days/week) at 15.37, 36.35, or 168.84 μg/m^3	Increased plasma testosterone, plasma inhibin, and testicular testosterone concentration	[89]
Nanoparticle-rich diesel exhaust	F344 rats	Inhalation for 4, 8, or 12 weeks (5 h/day, 5 days/week) at 15.37, 36.35, or 168.84 μg/m^3	Increased plasma testosterone concentration	[90]
Titanium dioxide nanoparticles	ICR mice	Intraperitoneal injection every other day for 5 times at 200 or 500 mg/kg	The high-dose group showed reduced sperm density and motility, increased sperm abnormality, and germ cell apoptosis	[91]

germ cells directly exposed to nanomaterials have been collected by some *in vitro* studies. In these studies, gold nanoparticles [92], magnetic nanoparticles [93], and silver nanoparticles [94] could penetrate into the sperm or the spermatogonial stem cell. Silver nanoparticles were shown to decrease motility and viability of sperm [95], and gold nanoparticles were shown to decrease motility of sperm, increase fragmentation of sperm, and disturb nuclear chromatin decondensation in sperm [92, 96]. In view of these reported hazards, opportunities of nanomaterial exposure to sperm, such as distribution of nanomaterials to seminal vesicle fluid, should be investigated in greater detail. In addition, transgenerational effects through fathers have been reported [97–100]. Therefore, biological effects on neonates from fathers exposed to nanomaterials should also be evaluated.

Some nanomaterials have been reported to have beneficial effects rather than hazardous effects on male reproductive functions *in vivo*. Hydrated fullerenes have been reported to restore decreased weight in reproductive tissues, blood testosterone, sperm motility, and sperm concentration in the epididymis and to mitigate testicular injury in streptozotocin-induced diabetic male rats [101]. In addition, fullerenol can prevent testicular oxidative stress induced by doxorubicin [102]. In humans, rates of male infertility continue to increase and male infertility has been an arduous problem [7]. Therefore, beneficial nanomaterials should be utilized to improve male fertility with consideration of the balance between risk and benefit.

4.4 Conclusion

In this chapter, the biodistribution of nanomaterials to reproductive tissues, fetuses, and infants and the potential effects of nanomaterials on these susceptible tissues and populations have been reviewed. When female rodents are exposed to nanomaterials, the nanomaterials might be distributed to the ovaries and affect sex hormone secretion and fertility. Exposure to nanomaterials during pregnancy results in accumulation of nanomaterials in the fetus and has the potential to cause miscarriage, fetal death, fetal resorption, fetal growth restriction, and fetal malformation. In utero exposure of nanomaterials also presents a risk of causing malfunctions in offspring, including hepatotoxicity, nephrotoxicity, reproductive toxicity, neurotoxicity, and immunotoxicity. Although more detailed studies of lactational exposure effects are needed, nanomaterials might be distributed to breast milk and cause neurotoxicity of breast-fed offspring mice. When male rodents were exposed to nanomaterials, the nanomaterials could be distributed to the testes or male germ cells and could affect sex hormone secretion and sperm production. Smaller nanomaterials are more easily distributed to the fetus or testes through the BPB or BTB. In addition, some hazardous effects are more severe for smaller particles than for larger particles. Therefore, special attention should be paid to nanoparticles, more so than larger particles, when considering reproductive and developmental toxicity. On the other hand, similarly sized particles can induce different biological effects when the surface coating or modification of the particles