Table 3
External and internal examinations of fetuses of rats given dinoseb by gavage or in the diet.

Dose	Gavage dose			Feeding dose		
	Control	8.0 mg/kg	10 mg/kg	Control	120 ppm	200 ppm
External examination						
Total no. of fetuses (litters) examined	174 (12)	171 (12)	147 (10)	172 (12)	165 (12)	153 (12)
No. of fetuses (litters) with external malformations	0	1 (1)	18 (4)*	0	0	0
Microphthalmia	0	0	17 (4)*	0	0	0
Cleft palate	0	0	1 (1)	0	0	0
Anotia	0	0	2 (1)	0	0	0
Brachygnathia	0	1 (1)	0	0	0	0
Brachymelia	0	0	2 (1)	0	0	0
Ectrodactyly	0	0	2 (1)	0	0	0
Filamentous tail	0	0	1 (1)	0	0	0
No. of runt fetuses (litters)	0	1 (1)	2 (2)	0	0	0
Internal examination						
Total no. of fetuses (litters) examined	83 (12)	84 (12)	72 (10)	83 (12)	80 (12)	75 (12)
No. of fetuses (litters) with malformations	1 (1)	1 (1)	2 (1)	0	0	0
Small cerebrum/small inner ear	0	0	2 (1)	0	0	0
Dilatation of lateral ventricle	0	1 (1)	0	0	0	0
Situs inversus totalis	1 (1)	0	0	0	0	0
Small intermediate lobe of lung	1 (1)	0	0	0	0	0
No. of fetuses (litters) with variations	7 (5)	6 (3)	7 (6)	7 (5)	3 (3)	9 (7)
Thymic remnant in neck (partially undescended horn of thymus)	5 (4)	5 (2)	5 (4)	5 (3)	0	8 (6)
Dilatation of renal pelvis	1 (1)	1 (1)	2 (2)	2 (2)	1 (1)	1 (1)
Left-sided umbilical artery	1 (1)	0	0	1 (1)	2 (2)	0

* Significantly different from the control ($p < 0.05$).

of fetuses with skeletal malformations. At 10 mg/kg bw/day, there were between one and five fetuses with split thoracic centrum, thoracic hemivertebra, fusion of cervical/thoracic vertebral arches, absence or fusion of ribs, fusion of clavicle and scapula, short humerus and absence of radius, absence of forelimb phalanges or short/absent metacarpals. These anomalies were not observed in the control data of 12 studies in the laboratory that performed this study for past 7 years. The incidences of fetuses with skeletal variations were significantly increased in all dinoseb-treated groups. A significantly increased incidence of fetuses with supernumerary ribs was noted in all dinoseb-treated groups. The incidences of fetuses with unossified thoracic centrum, 27 presacral vertebrae and lumbarization of sacral vertebra were also significantly higher at 10 mg/kg bw/day. Significantly delayed ossification was noted as evidenced by the numbers of cervical centrum and metacarpi at 8.0 and 10 mg/kg bw/day and of cervical centrum at 200 ppm.

Table 4
Skeletal examinations of fetuses of rats given dinoseb by gavage or in the diet.

Dose	Gavage dose			Feeding dose		
	Control	8.0 mg/kg	10 mg/kg	Control	120 ppm	200 ppm
Total no. of fetuses (litters) examined	91 (12)	87 (12)	75 (10)	89 (12)	85 (12)	78 (12)
No. of fetuses (litters) with malformations	3 (3)	1 (1)	6 (2)	3 (3)	0	1 (1)
Splitting of cervical centrum	1 (1)	1 (1)	0	0	0	1 (1)
Splittting of thoracic centrum	2 (2)	0	5 (1)	2 (2)	0	0
Fusion of cervical centrum	0	0	0	1 (1)	0	0
Thoracic hemivertebra	0	0	4 (2)	0	0	0
Fusion of cervical/thoracic vertebral arches	0	0	2 (1)	0	0	0
Absence of ribs	0	0	4 (2)	0	0	0
Fusion of ribs	0	0	1 (1)	0	0	0
Fusion of clavicle and scapula	0	0	1 (1)	0	0	0
Short humerus and absence of radius	0	0	1 (1)	0	0	0
Absence of forelimb phalanges	0	0	3 (1)	0	0	0
Short/absent metacarpals	0	0	2 (1)	0	0	0
No. of fetuses (litters) with variations	12 (6)	38 (10)**	69 (10)**	14 (6)	30 (10)*	29 (10)*
Bipartite ossification of thoracic centrum	0	1 (1)	3 (2)	2 (1)	1 (1)	3 (2)
Dumbbell ossification of thoracic centrum	0	0	1 (1)	5 (2)	1 (1)	1 (1)
Unossified thoracic centrum	0	3 (2)	10 (5)**	0	0	1 (1)
25 presacral vertebrae	0	0	0	1 (1)	0	0
27 presacral vertebrae	0	3 (2)	19 (5)**	0	1 (1)	1 (1)
Short supernumerary ribs	12 (6)	37 (10)**	66 (10)**	9 (6)	29 (10)*	24 (10)*
Lumbarization of sacral vertebra	0	2 (2)	9 (5)**	0	0	0
Bipartite ossification of sternebra	0	0	0	0	0	1 (1)
Misaligned ossification of sternebra	0	0	0	0	0	1 (1)
Degree of ossification						
Number of cervical centrum	0.55 ± 0.51[a]	0.26 ± 0.54*	0.04 ± 0.05**	0.88 ± 0.62	0.40 ± 0.58	0.23 ± 0.22*
Number of metacarpi	6.80 ± 0.52	6.33 ± 0.49*	6.02 ± 0.08**	7.18 ± 0.64	6.90 ± 0.55	6.64 ± 0.76

[a] Values are given as the mean ± SD (the litter is the unit evaluated).
* Significantly different from the control ($p < 0.05$).
** Significantly different from the control ($p < 0.01$).

Lower number of cervical centrum was also observed at 120 ppm, but it was within the historical control range (0.35–0.87) of the laboratory that performed this study.

4. Discussion

In this study, the effect of dinoseb on the morphological development of embryos was determined by administering relatively high doses of dinoseb by gavage or in the diet to pregnant rats during organogenesis. As expected, maternal toxicity was observed in all the dinoseb-treated groups. Dinoseb induced dose-dependent decreases in body weight gain and food consumption during pregnancy in the dinoseb-treated groups. The decrease in food consumption was greater in the feeding dose groups than the gavage dose groups; therefore, the decreased food consumption may be related to a reduced palatability of the diet in the feeding groups.

Although there was no increased incidence of intrauterine deaths in any dinoseb-treated groups, significantly decreased weights of fetuses were observed in all the dinoseb-treated groups, except for the group fed dinoseb at 120 ppm. A decrease in the gravid uterine weight, reflecting the decreases in the fetal weights, was also found in the treatment groups, and a significant decrease at 200 ppm seemed partly related to the incidentally low number of corpora lutea. Skeletal examinations of fetuses revealed an increased incidence of fetuses with skeletal variations in all dinoseb-treated groups and delayed ossification at 8.0 and 10 mg/kg bw/day and at 200 ppm. These findings indicate that dinoseb is developmentally toxic at 8.0 and 10 mg/kg bw/day by gavage and 120 and 200 ppm by feeding when administered during organogenesis.

An increased incidence of fetuses with external malformations was observed at 10 mg/kg bw/day, but there was no increased incidence of fetuses with external, internal or skeletal malformations in the groups given dinoseb at 8.0 mg/kg bw/day by gavage or 120 or 200 ppm by feeding. The results of morphological examinations of fetuses revealed that dinoseb is teratogenic at the maternally toxic dose of 10 mg/kg bw/day when administered by gavage during organogenesis.

A recent study analyzing 125 developmental toxicity bioassays indicated that reduced maternal body weight gain was associated with fetal development [22]. To further evaluate dinoseb-induced developmental toxicity, maternal toxicity in the 10 mg/kg bw/day group was compared between litters with malformations and litters without malformations. A remarkable reduction in maternal body weight gain over days 6–16 was observed in the litters with malformations (19.0 ± 6.7 g vs. 30.0 ± 6.1 g; with vs. without malformations). In addition, placental weight was reduced in the litters with malformations (0.415 ± 0.024 g) compared to the litters without malformations (0.448 ± 0.054 g). These findings indicated that dinoseb was teratogenic at maternally toxic doses, but seems unrelated to maternal dietary deficiency.

Although the feeding dose of dinoseb at 200 ppm (15 mg/kg bw/day) was previously reported to be teratogenic in rats [4], the feeding dose of dinoseb up to 200 ppm (8.5 mg/kg bw/day) did not induce teratogenicity in the present study. Dose levels of dinoseb in the current study might not have been sufficiently high to induce teratogenicity; however, pregnant rats did not consume sufficiently high amounts of dinoseb to produce fetal malformations because food consumption was reduced in the feeding groups. It seems unlikely that a feeding study is appropriate to evaluate the toxicity of dinoseb.

Microphthalmia, which was found in rats after exposure to dinoseb by gavage or feeding [4,19] and in rabbits by gavage [23] or dermal application [7], was predominantly observed after administration of dinoseb at 10 mg/kg bw/day by gavage. As a rule, the administration of a suitable dosage of a teratogen generally results in the production of some normal offspring, some malformed offspring, and some dead or resorbed offspring [24]. In the present study, the increased incidence of malformed fetuses was not accompanied by an increased incidence of intrauterine deaths of offspring after the administration of dinoseb. This phenomenon was also observed in the previous studies of Giavini et al. [4,19]. One possible explanation for this is that microphthalmia itself is not lethal *in utero* as well as probably postnatally.

Giavini et al. showed that teratogenic potential in rats was influenced by the mode of administration or even the dietary composition [4,19]; however, conditions under which malformations occurred were not clearly described in these papers. The diets used in these studies did not meet the nutrient requirement of rats for fat (more than 5%) [25,26] while the diet used in our study is a standard rat diet; however, fat concentration seems unrelated to dinoseb-induced teratogenicity. Teratogenic effects were not observed after the gavage dose of dinoseb at 8.0 mg/kg bw/day. Because maternal death was observed after the gavage dose of dinoseb at 10 mg/kg bw/day, the exposure range of dinoseb where malformations are observed seems to be narrow. The findings of the present study confirmed the experimental condition that could induce malformation in rats fed a standard diet.

Dinitro-o-cresol, a structural and mechanical analogue of dinoseb, also induced external or internal malformations in 29 out of 64 fetuses when pregnant rabbits were administered it by gavage from day 6 to day 18 of gestation at 25 mg/kg bw/day [27]. The most frequent malformations were microphthalmia/anophthalmia and hydrocephaly/microcephaly. These results were quite similar to the findings of a gavage dose study of dinoseb in rabbits [23]. Further teratology studies of other uncoupling agents may be needed to clarify that uncoupling agents can produce malformations with the same mode of action.

It is considered that the basic mechanism of toxicity of dinoseb is stimulation of oxidative metabolism in cell mitochondria by the uncoupling of oxidative phosphorylation, and the energy is released as heat [28,29]. However, there is no clear understanding of the fundamental mechanism of developmental toxicity of dinoseb, although an energy-deficient intrauterine environment due to uncoupling of cellular oxidative phosphorylation may explain dinoseb-induced developmental toxicity. A decreased placental weight was observed in the gavage dose group at 10 mg/kg bw/day, which may suggest intrauterine energy deficiency. A prenatal dose of thiabendazole, an ATP-synthesis inhibitor, induced a deformity involving reduced limb size in mice fetuses [30], and ATP levels in fore and hind limb buds of fetuses were related to the incidence of this deformity [31]. Dinoseb-induced teratogenicity may be related to the degree of reduction in ATP expression influenced by variable factors such as the mode of administration used in experiments. Recent studies have investigated the role that mitochondria play in mediating apoptotic signals [32–34]. Programmed cell death (PCD) is an essential component of normal physiological processes such as embryogenesis and normal tissue development [35]. Altering normal patterns of PCD could be teratogenic because areas of the body with a high incidence of malformations coincide with areas where PCD occurs [36,37]. Some studies showed a positive correlation between mitochondrial uncoupling activity and PCD [38,39], and 2,4-dinitrophenol, an uncoupling agent, enhanced the Fas apoptotic signal in Jurkat Bcl-2 cells [33]. These findings imply that the enhanced uncoupling of oxidative phosphorylation in mitochondria may alter normal patterns of PCD. However, the link between malformations and mitochondrial uncoupling activity are still poorly understood. Further mechanistic studies are necessary to clarify the teratogenicity of dinoseb.

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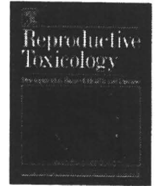

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Review

Reproductive and developmental toxicity studies of manufactured nanomaterials

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ABSTRACT

This paper reviews studies *in vivo* and *in vitro* on the reproductive and developmental toxicity of manufactured nanomaterials including metallic and metal oxide-based particles, fullerenes (C_{60}), carbon black (CB), and luminescent particles. Studies *in vivo* showed increased allergic susceptibility in offspring of mouse dams intranasally insufflated with respirable-size titanium dioxide (TiO_2), adverse effects on spermatogenesis and histopathological changes in the testes and changes in gene expression in the brain of mouse offspring after maternal subcutaneous injection of TiO_2 nanoparticles, transfer to rat fetuses of radiolabeled gold nanoparticles and C_{60} after maternal intravenous injection, death and morphological abnormalities in mouse embryos after maternal intraperitoneal injection of C_{60}, and adverse effects on spermatogenesis in mouse offspring after maternal intratracheal instillation of CB nanoparticles. Studies *in vitro* revealed that TiO_2 and CB nanoparticles affected the viability of mouse Leydig cells, that gold nanoparticles reduced the motility of human sperm, that silver, aluminum, and molybdenum trioxide were toxic to mouse spermatogonia stem cells, that silica nanoparticles and C_{60} inhibited the differentiation of mouse embryonic stem cells and midbrain cells, respectively, and that cadmium selenium-core quantum dots inhibited pre- and postimplantation development of mouse embryos. Although this paper provides initial information on the potential reproductive and developmental toxicity of manufactured nanomaterials, further studies, especially *in vivo*, using characterized nanoparticles, relevant routes of administration, and doses closely reflecting expected levels of exposure are needed.

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Contents

1. Introduction 344
2. Reproductive and developmental toxicity of manufactured nanomaterials 344
 2.1. Metallic and metal oxide-based particles 344
  2.1.1. Titanium dioxide (TiO_2) 344
  2.1.2. Gold 345
  2.1.3. Silver, aluminum, and molybdenum trioxide (MoO_3) 347
  2.1.4. Magnetic iron oxide (Fe_3O_4) 347
  2.1.5. Cobalt-chromium (CoCr) nanoparticles 348
  2.1.6. Silica (SiO_2) 348
 2.2. Fullerenes (C_{60}) 348
  2.2.1. *In vivo* study of fullerenes (C_{60}) 348
  2.2.2. *In vitro* study of fullerenes (C_{60}) 348
 2.3. Carbon black (CB) 348
  2.3.1. *In vivo* study of carbon black (CB) 350
  2.3.2. *In vitro* study of carbon black (CB) 350
 2.4. Luminescent particles 350
  2.4.1. Cadmium selenium-core quantum dots (CdSeQDs) 350
  2.4.2. Polystyrene-based fluorescent particles 350

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3. Discussion and conclusions 350
Conflict of interest 352
Acknowledgements 352
References 352

1. Introduction

Nanomaterials are defined as materials having a physicochemical structure on a scale greater than typical atomic/molecular dimensions but less than 100 nm (nanostructure), which exhibit physical, chemical and/or biological characteristics associated with a nanostructure [1]. Nanoparticles are defined as particles with at least one dimension smaller than 100 nm and include manufactured nanoparticles, ambient ultrafine particles and biological nanoparticles [1,2]. Humans have been exposed to airborne nanoparticles throughout evolution, but exposure has increased dramatically because of anthropogenic factors including combustion engines, power plants, and other sources of thermodegradation [2]. The rapidly developing field of nanotechnology, which is creating materials with size-dependent properties, is likely to become another source of exposure to nanomaterials. The surface and interface of particles are particularly important components of nanoparticles. Nanomaterials have an increased surface area: mass ratio thereby greatly enhancing their chemical/catalytic reactivity compared to normal-sized forms of the same substance. Surface coatings can be utilized to alter surface properties of nanoparticles to prevent aggregation or agglomeration with different particle-types, and/or serve to passivate the particle-type to migrate the effects of ultraviolet radiation-induced reactive oxidants [1]. The distinctive and often unique properties of nanomaterials offer the promise of broad advances for a wide range of technologies. Nanomaterials are used in a variety of areas including advanced materials, electronics, magnetics and optoelectronics, biomedicine, pharmaceuticals, cosmetics, energy, and catalytic and environmental detection and monitoring [3,4]. At present, there are relatively few environments where exposures are known to occur. However, if the commercialization of products using nanomaterials develops as anticipated, the potential for exposure is likely to increase notably over the coming decade [1]. Despite growing concern over the possible risk that nanomaterials pose, there is a lack of information on their potential toxicity. At this moment, there is a knowledge gap between the increasing development and use of nanomaterials and the prediction of possible health risks.

In recent years, reproductive and developmental toxicity has increasingly become recognized as an important part of overall toxicology. In fact, adverse effects of environmental chemicals on the reproductive success of wildlife populations have been noted [5]. It is reported that nanoparticles can pass through biological membranes [6,7]; raising fears that they can affect the physiology of any cell in the body. The possibility of chemicals entering biological systems is of great concern to the public with regard to possible reproductive and developmental toxicity. In this paper, we review studies on the reproductive and developmental toxicity of nanomaterials, published in openly available scientific literature.

2. Reproductive and developmental toxicity of manufactured nanomaterials

The literature on manufactured nanomaterials was searched using TOXNET/TOXLINE for studies *in vivo* and *in vitro* of reproductive and developmental toxicity, excluding abstracts. Although no information was available on the reproductive and developmental toxicity of single- or multi-wall carbon nanotubes, articles on metallic and metal oxide-based particles, fullerenes (C_{60}), and carbon black (CB) and luminescent particles were found. In this paper, we review studies using mammalian animals and cells on the reproductive and developmental effects of nanomaterials. The final search of the literature was conducted in March, 2010.

2.1. Metallic and metal oxide-based particles

In vivo and *in vitro* studies of titanium dioxide (TiO_2) nanoparticles, and *in vitro* studies of silver, aluminum, molybdenum trioxide (MoO_3), gold, magnetic iron oxide (Fe_3O_4), cobalt–chromium (CoCr) and silica nanoparticles have been published.

2.1.1. Titanium dioxide (TiO_2)

TiO_2 is widely used as a white pigment in paints, plastics, inks, paper, creams, cosmetics, drugs and foods. TiO_2 was previously classified as biologically inert in animals and humans [8–10] and has been used as a negative control particle in a variety of toxicological studies. Recently, concern has been raised on possible adverse effects of TiO_2 on human health because exposure to high concentrations of ultrafine TiO_2 was involved in the induction of lung inflammatory responses [11] and tumors [12]. Very recently, the International Agency for Research on Cancer (IARC) Monograph Working Group classified TiO_2 as possibly carcinogenic to humans (i.e., group 2B) based on results from studies in which the inhalation and intratracheal instillation of TiO_2 provided sufficient evidence in animals for carcinogenicity [13]. As for genotoxicity, the results of studies on TiO_2 nanoparticles are inconclusive [14,15]. *In vivo* and *in vitro* studies of TiO_2 are summarized in Table 1.

2.1.1.1. In vivo study of titanium dioxide (TiO_2). Pregnant BALB/c mice on gestational day (GD) 14 or nonpregnant control mice were administered respirable-size TiO_2 [16], that is less than 10 μm in particle size [17], suspended in phosphate-buffered saline (PBS) at 50 μg/mouse by a single intranasal insufflation. Pups obtained by spontaneous delivery received a single intraperitoneal injection of ovalbumin (OVA) with alum on postnatal day (PND) 4. These pups were exposed to aerosolized OVA on PNDs 12–14, and subjected to an examination of pulmonary function and a pathological analysis. Airway responsiveness to increasing concentrations of aerosolized methacholine was measured using whole body plethysmography. Bronchoalveolar lavage (BAL) differential cell counts and histopathological examinations of the lung were also performed. Lung inflammatory responses were determined 48 h postadministration in nonpregnant and pregnant mice ($n > 9$/group). TiO_2-treated nonpregnant mice exhibited minimal increases in BAL polymorphonuclear leukocyte counts, whereas pregnant mice showed acute neutrophilic inflammation. Pregnant mice exposed to TiO_2 had higher serum levels of cytokines, including interleukin-1β, tumor necrosis factor-α, interleukin-6 and chemokine, 48 h after exposure compared with nonpregnant mice ($n = 9$/group). Offspring of dams exposed to TiO_2 showed increased airway hyperresponsiveness, increased percentage of eosinophils, and pulmonary inflammation ($n = 17$–21/group). These findings indicate that TiO_2 caused acute cellular inflammation in pregnant mice and increased allergic susceptibility in their pups.

A TiO_2 nanopowder (25–70 nm in particle size, 20–25 m^2/g in surface area, anatase, Sigma-Aldrich Japan, Inc.) suspended in saline with 0.05% Tween 80 was subcutaneously injected into pregnant Slc:ICR mice ($n = 15$) on GDs 6, 9, 12 and 15 at

Table 1
In vivo and *in vitro* reproductive and developmental toxicity studies of titanium dioxide (TiO_2) particles.

In vivo/in vitro	Materials/characteristics	Animals/cells	Exposure			Findings	References
			Route/method	Duration/time	Dose/concentration		
In vivo	Respirable-size	BALB/c mice	Intranasal insufflation	Single on GD 14	50 μg/mouse	↑ Acute cellular inflammation in pregnant mice ↑ Susceptibility to allergy in pups	[16]
In vivo	25–70 nm in particle size, 20–25 m^2/g in surface area, anatase	Slc:ICR mice	Subcutaneous injection	GDs 6, 9, 12, and 15	100 μg/mouse/day (14–15 mice/group)	Changes in gene expression related to development and function of central nervous system in male pups	[18]
In vivo	25–70 nm in particle size, 20–25 m^2/g in surface area, anatase	Slc:ICR mice	Subcutaneous injection	GDs 3, 7, 10, and 14	100 μg/mouse/day (6 mice/group)	↓ Body weight of pups ↓ DSP of pups ↓ Epididymal sperm motility in pups ↓ Number of Sertoli cells in pups Histopathological changes in testis of pups	[19]
In vitro	25–70 nm in particle size	Mouse testis Leydig cell line TM3	Incubation	16, 24, or 48 h	1–1000 μg/mL	↓ Viability of TM3 at 100 μg/mL ↓ Proliferation of TM3 cells at 100 μg/mL No changes in HO-1 or StAR mRNA expression at up to 100 μg/mL	[20]

100 μg/mouse/day as the exposure group, and 100 μl of vehicle alone was injected into pregnant mice (n = 14) as the control group [18]. Brain tissue was obtained from male offspring on embryonic day 16 (n = 8/group) or on PND 2 (n = 10/group), PND 7 (n = 10/group), or PND 21 (n = 9/group), total RNA was extracted from whole brain, and gene expression was analyzed. Maternal exposure to TiO_2 caused changes in the expression of genes associated with brain development, cell death, response to oxidative stress, and mitochondria in the brain during the prenatal period, and genes associated with inflammation and neurotransmitters in the later stages. However, this study did not investigate how maternal behavior toward the pups changed and how this in turn altered gene expression. It is difficult to evaluate the change in gene expression using the toxicogenomic data of this study, because not enough microarray data was provided in the paper.

Slc:ICR mice (n = 6/group) were subcutaneously injected with TiO_2 nanoparticles (25–70 nm in particle size, 20–25 m^2/g in surface area, anatase, Sigma-Aldrich) suspended in saline with 0.05% Tween 80 at 100 μg/mouse/day on GDs 3, 7, 10 and 14 [19]. Male offspring were autopsied on PND 4 or postnatal week (PNW) 6. Lower body weights were found among offspring of dams exposed to TiO_2. Aggregates of TiO_2 nanoparticles (100–200 nm) were detected in Leydig cells, Sertoli cells, and spermatids in the testes of pups on PND 4 and PNW 6. Disorganized and disrupted seminiferous tubules, tubule lumens with few mature sperm, and decreases in daily sperm production (DSP), epididymal sperm motility, and numbers of Sertoli cells were observed at PNW 6 in pups of the TiO_2-treated group (n = 8/group). TiO_2 particles were detected in cells of the olfactory bulb and cerebral cortex of pups at PNW 6. There were many cells positive for caspase-3, an enzymatic marker of apoptosis, in the olfactory bulb of pups on PNW 6 in the TiO_2-exposed group. Although the possibility of adverse effects of TiO_2 nanoparticles on brain development is noted, the behavioral effects of nanoparticles were not investigated. There was a lack of description on the maternal findings in this report.

2.1.1.2. In vitro study of titanium dioxide (TiO_2). The direct effects of TiO_2 (25–70 nm in particle size, Aldrich) on testis-constituent cells was determined using the mouse Leydig cell line TM3, testosterone-producing cells of the testis [20]. TiO_2 was suspended in a balanced salt solution [0.05% Tween 80–0.25% DMSO in PBS (−)], and sonicated for 10 min immediately prior to use in the assay. TiO_2 was added to the culture system for 16, 24, or 48 h. The uptake of TiO_2 nanoparticles by Leydig cells was detected after incubation of cells with TiO_2 at 30 μg/mL for 48 h. Following incubation of cells with TiO_2 at 10 or 100 μg/mL, a remarkable inhibition of viability and transient reduction in proliferation of TM3 cells were observed at 100 μg/mL after 24 h. No effect of TiO_2 was found on the expression of heme oxygenase-1 (HO-1), a sensitive marker of oxidative stress, or steroidogenic acute regulatory (StAR) mRNA in TM3 cells treated for 16 h at up to 100 μg/mL or for 48 h at up to 30 μg/mL. These findings suggest that TiO_2 nanoparticles have no direct effect on the induction of oxidative stress or synthesis of testosterone in Leydig cells.

2.1.2. Gold

Colloidal gold has been used in medical applications and gold nanoparticles are used commercially in a wide array of catalytic applications and optical and electrical applications as components of various probes, sensors, and optical devices [21]. *In vivo* and *in vitro* studies of gold particles are shown in Table 2.

2.1.2.1. In vivo study of gold. The distribution of ^{198}Au-colloidal particles (4–200 nm) was determined after a single injection into the iliac artery of pregnant SD rats on GDs 16–18 [22]. Although more than 90% of the radiocolloid was found in the maternal liver

Table 2
In vivo and *in vitro* reproductive and developmental toxicity studies of gold particles.

In vivo/*in vitro*	Materials/characteristics	Animals/Cells	Exposure			Findings	References
			Route/method	Duration/time	Dose/concentration		
In vivo	^{198}Au-colloidal particles (4–200 nm in diameter)	SD rats	Intraarterial injection	Single during GDs 16–18	200 μL/rat	No detection of radioactivity in amniotic fluid, fetal membranes, or fetus	[22]
In vivo	^{198}Au-colloidal nanoparticles (5 or 30 nm in diameter)	Wistar rats	Intravenous injection	Single on GD 19	0.5 mL/rat of solution contained 20 μg of gold (7–10 rats/group)	Transfer rate to fetus: 0.018% for 5 nm particles Transfer rate to fetus: 0.005% for 30 nm particles	[23]
In vivo	Colloidal gold nanoparticles (2 or 40 nm in diameter)	C57BL/6 mice	Intravenous injection	On GDs 16–18	1 mL/rat of solution contained gold particle (5 mice/group)	No transfer to fetus	[24]
In vitro	Gold nanoparticles (10, 15, or 30 nm in diameter) coated with polyethylene glycol	Human placenta	Open perfusion	5 min	7.9×10^{11} for 15 nm particles and 7.8×10^{10} for 30 nm particles	Detection of high levels of nanoparticles soon after perfusion in maternal outflow No detection of nanoparticles in fetal outflow	[25]
In vitro	Gold nanoparticles (10, 15, or 30 nm in diameter) coated with polyethylene glycol	Human placenta	Recirculating perfusion	6 h	9.1×10^{9} for 10 nm particles and 2.0×10^{9} for 15 nm particles	No transplacental transfer of nanoparticles	[25]
In vitro	Gold nanoparticles (9 nm in size)	Human sperm	Mixed with semen		44 ppm	↓ Sperm motility	[26]

at 15 min after injection, no radioactivity was detected in the amniotic fluid, fetal membranes, or fetus. These findings indicate the impermeability of the rat placenta to colloidal gold. Detailed experimental conditions including concentrations of gold particles and numbers of rats used were not described in this report.

Pregnant Wistar rats (n = 7–10/group) were injected intravenously with ^{198}Au-colloidal particles (5 and 30 nm in diameter, Daichi Radio Isotope Co., Ltd. and Hoext Japan Co., Ltd., respectively) into the tail vein on GD 19 (vaginal plug = GD 1) and sacrificed 1 or 24 h later [23]. The 0.5 mL of solution injected contained 20 μg of gold. The clearance of ^{198}Au-colloid from blood was faster in dams injected with the 30 nm particles than in dams injected with the 5 nm particles, and, therefore, the radioactivity remaining in maternal blood was greater in the 5 nm-group. Fetal radioactivity was detected in pregnant rats sacrificed at 1 and 24 h after the injection of 5 nm particles and at 24 h after the injection of 30 nm particles. The transfer rate to the fetus was very small, being approximately 0.018 and 0.005% for the 5 and 30 nm particles, respectively. The levels of radioactivity in the fetal membrane and placenta were greater in the 5 nm-group that in 30 nm-group, and 100–300 times greater than the levels in the fetus for either group. The authors described that the transfer or deposition of ^{198}Au-colloid was directly affected not by particle size, but by the average concentration in maternal blood.

Pregnant C57BL/6 mice were intravenously injected into the tail vein with 1 mL of a solution containing 2 or 40 nm colloidal gold nanoparticles (n = 5/goroup) or 1 mL of saline (n = 3 as controls) on GDs 16–18 and killed 24 h after the last injection [24]. The 2 and 40 nm gold nanoparticles (Fitzgerald Industry Inc.) contained 15×10^{13} particles (12.13 μg) and 9×10^{10} particles (58.21 μg), respectively. The gold nanoparticles had a negative surface charge and were monodispers and spherical in shape. No particles were detected in the fetuses and placentae. These findings suggest that gold nanoparticles do not penetrate the placental barrier.

2.1.2.2. In vitro study of gold. The transplacental transfer of monodispersed gold particles (10, 15, and 30 nm in diameter before coating) coated with polyethylene glycol (PEG) was examined using placentae from healthy, nonsmoking mothers [25]. In the open perfusion as a "once-through" perfusion, nanoparticles (7.9×10^{11} for 15 nm particles and 7.8×10^{10} for 30 nm particles) were suspended in 5 mL of physiological saline and injected into the maternal artery within 5 min, and the maternal and fetal outflow were collected at 3-min intervals for 18 min. In the maternal outflow, the nanoparticles of 15 and 30 nm were detected at 570 and 678 ppb within 3–6 min of injection, and only 9.3 and 18.0 ppb, respectively, at the end of perfusion. No nanoparticles were detected in the fetal outflow. Recirculating perfusion was performed with 10 and 15 nm nanoparticles only. Both the maternal and fetal sides were recirculated. The nanoparticles (9.1×10^{9} for 10 nm particles and 2.0×10^{9} for 15 nm particles) were added to the maternal reservoir and the perfusion was continued for 6 h. Samples were taken from the maternal and fetal reservoirs every 30 min for the first 2 h, and once per hour thereafter. Nanoparticles did not cross the placenta regardless of particle size. At the end of the perfusion, concentrations of nanoparticles in maternal perfusate samples decreased 41 and 64% giving final concentrations of 24.2 and 22.2 ppb for the 10 and 15 nm nanoparticles, respectively. The gold aggregates were located in syncytiotrophoblasts and trophoblasts, but no gold particles were detected in the fetal capillary endothelium in perfused tissue. These findings indicate that PEGylayed gold nanoparticles do not cross the human placenta from the maternal to fetal circulation.

The effect of gold nanoparticles (9 nm) at a concentration of 44 ppm on human sperm was determined using a single, fresh, donor semen sample from a healthy male [26]. In a mixture of 500 μL of the gold nanoparticle solution and semen, 25% of sperm

were not motile. The rate of motility among the control sperm was 95%. The penetration of sperm heads and tails by gold nanoparticles, and fragmentation of sperm were found in the mixture. Toxicity parameters, except for motility, were not investigated in this study.

2.1.3. Silver, aluminum, and molybdenum trioxide (MoO_3)

Nanoscaled silver powder is used in biocides, transparent conductive inks and pastes, and various consumer and industrial products that need enhanced antimicrobial properties [21]. Nanoscaled aluminum powder is used in various electronic circuits and as a scratch-resistant coating for plastic lenses, antimicrobial agents, and new tissue-biopsy tools [21]. MoO_3 nanoparticles have electrochromic, photochromic, and gas-sensing properties [27]. *In vitro* studies of silver, aluminum, and MoO_3 particles are listed in Table 3.

2.1.3.1. In vitro study of silver, aluminum, and molybdenum trioxide (MoO_3). *In vitro* studies of silver (15 nm in diameter), aluminum (30 nm in diameter), and MoO_3 (30 nm in diameter) nanoparticles were performed using the C18-4 cell line, which was established from type A spermatogonia isolated from 6-day-old mouse testes [28]. The cells were immortalized and exhibited phenotypic characteristics of germline stem cells *in vivo*, were adherent, and responded to the growth factor glial cell line-derived neurotrophic factor. Nanoparticles were dispersed in PBS at final concentrations of 5, 10, 25, 50, and 100 μg/mL culture medium, and the C18-4 cells were incubated with nanoparticles for 48 h. Silver nanoparticles caused necrosis and apoptosis at 10 μg/mL and above. Aluminum nanoparticles did not induce shrinkage, necrosis, or apoptosis below 10 μg/mL. No distinct changes in cell morphology were observed at any concentration of MoO_3 nanoparticles. Reduced mitochondrial function and cell viability were noted after incubation with silver nanoparticles at 10 μg/mL, and the EC_{50} was calculated at 7.75 μg/mL. The effects of aluminum nanoparticles on mitochondrial function could not be determined because the particles accumulated in the cells and formed cytoplasmic aggregates at low concentrations. MoO_3 nanoparticles reduced mitochondrial function at 50 μg/mL and above, and the EC_{50} was 90 μg/mL. Silver nanoparticles slightly increased lactase dehydrogenase (LDH) leakage at 5 μg/mL, and the EC_{50} was 2.5 μg/mL. The leakage of LDH was increased by aluminum nanoparticles at 5 μg/mL and above, values reaching a plateau at around 25 μg/mL and the EC_{50} being 4.7 μg/mL. An increase in LDH leakage was observed with MoO_3 nanoparticles at 5 μg/mL and above, and the value reached a plateau at 10 μg/mL. The EC_{50} was 5 μg/mL. An increased number of apoptotic C18-4 cells were found after incubation with silver nanoparticles at 5 μg/mL, aluminum nanoparticles at 5 and 10 μg/mL, and MoO_3 nanoparticles at 50 μg/mL. These results indicate that silver nanoparticles are most toxic and MoO_3 nanoparticles are least toxic to this cell line. The authors noted that this cell line provides a valuable model to assess the cytotoxicity of nanoparticles in the germ line *in vitro*.

2.1.4. Magnetic iron oxide (Fe_3O_4)

The magnetic properties of magnetic iron oxide nanoparticles may lead to a range of new biomedical and diagnostic applications including cellular therapy by cell labeling and targeting, tissue repair, drug delivery, magnetic resonance imaging, and magnetofection [29]. An *in vivo* study of magnetic Fe_3O_4 particles is presented in Table 3.

2.1.4.1. In vitro study of magnetic iron oxide (Fe_3O_4). The effect of Fe_3O_4 on sperm was determined after incubation of bovine sperm in glucose-free modified Tyrode solution with an aqueous colloid solution of Fe_3O_4 nanoparticles coated with poly(vinyl alcohol) for 2 h at 37 °C [29]. The final concentration of Fe ions was 7.35 mM. In

Table 3
In vitro reproductive and developmental toxicity studies of silver, aluminum, molybdenum trioxide (MoO_3), magnetic iron oxide (Fe_3O_4), cobalt–chromium (CoCr) and silica (SiO_2) particles.

Materials/characteristics	Cells	Exposure			Findings	References
		Route/method	Duration/time	Dose/concentration		
Silver nanoparticles (15 nm in diameter)	Mouse spermatogonia stem cell line C18-4	Incubation	48 h	5–100 μg/mL	↑ Necrosis and apoptosis at 10 μg/mL and above ↓ Mitochondrial function and cell viability at 10 μg/mL ↑ LDH leakage at 5 μg/mL ↑ Apoptotic cells at 5 μg/mL	[28]
Aluminum nanoparticles (30 nm in diameter)	Mouse spermatogonia stem cell line C18-4	Incubation	48 h	5–100 μg/mL	No shrinkage, necrosis, or apoptosis of cells at below 10 μg/mL ↑ LDH leakage at 5 μg/mL ↑ Apoptotic cells at 5 and 10 μg/mL	[28]
MoO_3 nanoparticles (30 nm in diameter)	Mouse spermatogonia stem cell line C18-4	Incubation	48 h	5–100 μg/mL	No distinct change in cell morphology ↓ Mitochondrial function at 50 μg/mL and above ↑ LDH leakage at 5 μg/mL ↑ Apoptotic cells at 50 μg/mL	[28]
Magnetic Fe_3O_4 nanoparticles coated with poly(vinyl alcohol)	Bovine sperm	Incubation	6 h	7.35 mM (as Fe ions)	No adverse effect on sperm motility or acrosome reaction	[29]
CoCr nanoparticles (29.5 nm in diameter)	Human trophoblast choriocarcinoma cell line and Layer of BeWo b30 cells	Direct and indirect exposure	24 h	0.036 mg/cm^2	↑ DNA damage of fibroblasts by indirect exposure	[30]
Spherical amorphous silica nanoparticles (10, 30, 80, or 400 nm in average primary particle size)	D3 murine embryonic stem cell line	Incubation	10 days	1–100 μg/mL	↓ Differentiation of embryonic stem cells after exposure to 10 and 30 nm, but not 80 and 400 nm, particles	[32]

the first 20 min of incubation, 23% of the particles were taken up by sperm cells. Later on, about 60% of these particles were released from the cells and a further linear uptake was observed for an additional 1.5 h of incubation. Particles were bound to the acrosome in the head of the sperm, and to mitochondria in the tail of the sperm. The sperm was further incubated for 4 h. Motility and the ability to undergo an acrosome reaction, i.e. the ability to fertilize an egg, were not affected by the presence of the magnetic nanoparticles.

2.1.5. Cobalt–chromium (CoCr) nanoparticles

Internal exposure to CoCr nanoparticles can occur by wear mechanism associated with metal-on-metal (CoCr) orthopaedic joint replacements [30]. An *in vitro* study of CoCr particles is presented in Table 3.

2.1.5.1. In vitro study of cobalt–chromium (CoCr) nanoparticles. The cellular toxicity of CoCr nanoparticles (29.5 ± 6.3 nm in diameter, Osprey Metals) when located on the other side of a fully confluent cellular barrier was assessed using BeWo b30 cells, a human trophoblast choriocarcinoma-derived cell line, which were grown as a multi-layered (3–4 cells thick) barrier to simulate tight barriers in the body like the placental barrier [30]. Human fibroblast cells were placed on one side of this layer of cells, and CoCr particles on the other. The fibroblasts were checked for DNA damage using the alkaline comet assay after introduction of the particles. Indirect exposure to CoCr nanoparticles caused DNA damage. Indirect exposure to micrometer-sized CoCr (2.9 ± 1.1 μm in diameter) also damaged DNA. More than 95% of the nanoparticles were located within the cells of the superficial layer after 24 h of exposure, indicating that nanoparticles were internalized by the BeWo cells and did not pass through the barrier. The authors of this paper noted that the DNA damage was mediated by a novel mechanism involving pannexin and connexin hemichannels and gap junctions and purinergic signaling. These findings suggest that there is some possibility of placental transfer of particles.

2.1.6. Silica (SiO_2)

Industrial silica products are widely used in the electronics industry and as a food additive, and nanosized amorphous silica is used in a wide variety of applications including catalytic supports, photonic crystals, gene delivery, photodynamic therapy, and biomedical imaging [31]. An *in vitro* study of silica particles is presented in Table 3.

2.1.6.1. In vitro study of silica (SiO_2). The embryonic stem (ES) cell test using the D3 murine ES cell line was performed to determine the potential of spherical amorphous silica nanoparticles (10, 30, 80 and 400 nm in average primary particle size, Glantreo Ltd.) to inhibit the differentiation of ES cells into spontaneously contracting cardiomyocytes [32]. Silica nanoparticles were dialyzed against pure MilliQ water and diluted in distilled water, and the ES cells were exposed at 1–100 μg/mL throughout the entire 10-day test period. Transmission electron microscopy revealed that the dried silica particles were spherical and showed no substantial aggregation, except for the 10 nm particles, and measured diameters of the particles specified as 10, 30, 80, and 400 nm by the manufacturer were 11, 34, 34, and 248 nm, respectively. Silica particles of 30, 80 and 400 nm were observed in cells of the embryonic body. A concentration-dependent inhibition of the differentiation of ES cells into contracting cardiomyocytes was observed after exposure to 10 and 30 nm particles while the 80 and 400 nm particles did not inhibit the differentiation at up to 100 μg/mL. The inhibitory effect of the 30 nm particles was greater than that of the 10 nm particles as evidenced by the estimated ID_{50} values, 29 and 59 μg/mL, respectively. Inhibition of the differentiation of ES cells occurred below cytotoxic concentrations, suggesting a specific effect of the 10 and 30 nm particles on the differentiation of the ES cells.

2.2. Fullerenes (C_{60})

A fullerene is any molecule entirely in the form of a hollow sphere, ellipsoid, or tube. The first fullerene to be discovered is known as buckminsterfullerene C_{60}. Fullerenes have unique physicochemical properties that have been exploited for use in cosmetics, lubricants, dietary supplements, building materials, clothing treatment, electronics, and fuel cells [33]. *In vivo* and *in vitro* studies on fullerenes are listed in Table 4.

2.2.1. In vivo study of fullerenes (C_{60})

[60]Fullerene (C_{60}, purity > 99.9%, Terms Co.) was solubilized with poly(vinylpyrrolidone) (PVP). Pregnant Slc mice ($n = 2$/group) were intraperitoneally injected with C_{60} at 25, 50, or 137 mg/kg, PVP, or distilled water on DG 10, and their embryos were examined 18 h after injection [34]. No effects were observed in embryos of dams injected with PVP or distilled water. After the injection of C_{60}, all embryos died at 137 mg/kg. At 50 mg/kg, C_{60} was clearly distributed into the yolk sac and embryos and 50% of embryos were abnormal in shape predominantly in the head and tail regions. At 25 mg/kg, one pregnant mouse had all normal embryos and the other had only one abnormal embryo. The authors of this study speculated that C_{60} was incorporated into the concepts and the severely disrupted the function of the yolk sac and embryonic morphogenesis.

The distribution of $[^{14}C]_{60}$ was determined in rat dams and their pre- and postnatal offspring [35]. C_{60}, with an average particle size of less than 10 nm and estimated at 2 nm, was suspended in PVP. SD rats were given an intravenous injection of a suspension of approximately 0.3 mg $[^{14}C]_{60}$/kg into the tail vein on GD 15 or lactational day (LD) 8, and tissues of dams were collected 24 h ($n = 4$) and 48 h ($n = 3$) later. In pregnant dams at 24 h after injection, radioactivity was found in the liver (43% of the injected radioactivity), spleen (4%), reproductive tract (3%), and placenta (2%). Radioactivity was also detected in the digest of fetuses (0.87%). In lactating dams, radioactivity was detected in the liver (35%), spleen (4%), reproductive tract (0.10–0.42%), mammary tissue (0.48–0.94%), and milk at 24 h after injection. Radioactivity transferred to pups via lactation was found in the gastrointestinal tract (0.28%) in pups sacrificed at 24 h after injection, with an increase in distribution to the gastrointestinal tract of pups (0.43%) by 48 h after injection. The authors of this study noted that C_{60} distributed to the placenta and fetuses of exposed pregnant dams and to the milk and pups of exposed lactating dams.

2.2.2. In vitro study of fullerenes (C_{60})

Midbrain tissue samples of embryos of pregnant Slc-ICR mice on GD 11 were dissociated into individual cells, cell suspensions were prepared in culture medium, and a midbrain micromass culture was performed to evaluate the toxicity of C_{60} solubilized with PVP [34]. The C_{60} solution in the medium was incorporated into the midbrain culture plates, and further cultured for 6 days. The IC_{50} values of C_{60} for cell differentiation and proliferation were 0.43 and 0.47 mg/mL, respectively. Differentiation was inhibited as cytotoxicity increased. C_{60} was assumed to decrease cell proliferation via active oxygen species, because cell proliferation inhibited by C_{60} was partly restored by the addition of antioxidative enzymes.

2.3. Carbon black (CB)

CB is a low solubility particle produced industrially from incomplete thermal decomposition of hydrocarbons, a process controlled

Table 4
In vivo and *in vitro* reproductive and developmental toxicity studies of fullerenes (C_{60}), carbon black (CB), cadmium selenium-core quantum dots (CdSeQDs) and polystyrene-based fluorescent particles.

In vivo/in vitro	Materials/characteristics	Animals/cells	Exposure			Findings	References
			Route/method	Duration/time	Concentration		
In vivo	C_{60} (purity > 99.9%)	Slc mice	Intraperitoneal injection	Single on GD 10	25–137 mg/kg	Deaths of all embryos at 138 mg/kg Abnormalities in 50% of embryos at 50 mg/kg	[34]
In vivo	[^{14}C]C_{60} (>10 nm, estimated 2 nm in particle size)	SD rats	Intravenous injection	Single on DG 15 or LD 8	Approx. 0.3 mg/kg (3–4 rats/group)	Distribution of C_{60} to placentae and fetuses of exposed pregnant dams Distribution of C_{60} to milk and offspring of exposed lactating dams	[35]
In vitro	C_{60} (purity > 99.9%)	Midbrain cells of Slc:ICR mouse embryos at GD 11	Incubation	6 days	10–1000 μg/mL	IC_{50} for cell differentiation = 430 μg/mL IC_{50} for cell proliferation = 470 μg/mL	[34]
In vivo	CB Printex 90 (14 nm in particle size, 300 m^2/g in surface area) Printex 25 (56 nm in particle size, 45 m^2/g in surface area) Flammruss 101 (95 nm in particle size, 20 m^2/g in surface area)	ICR mice	Intratracheal instillation	10 times at weekly intervals	0.1 mg/mouse	No effect of 14, 56 or 95 nm particles on body weight or reproductive organs ↑ Serum testosterone levels after instillation of 14 and 56 nm particles ↓ DSP after instillation of 14, 56, and 90 nm particles	[37]
In vitro	CB (14 nm in particle size, Printex 90)	Mouse testis Leydig cell line TM3	Incubation	16, 24, or 48 h	1–1000 μg/mL	↓ Viability of TM3 at 1000 μg/ml No effect on proliferation of TM3 cells No changes in HO-1 mRNA expression at up to 100 μg/mL ↑ StAR mRNA expression at 30 μg/mL for 48 h-incubation	[20]
In vitro	CdSeQDs (approx. 3.5 nm in diameter) ZnS coating CdSeQDs	ICR mouse morulas and blastocysts	Incubation	24 h	125, 250, or 500 nmol/L	↓ Development of morulas into blastocysts at 250 and 500 nmol/L ↑ Number of apoptotic cells of blastocysts at 250 and 500 nmol/L ↓ Cell proliferation of blastocysts at 250 and 500 nmol/L ↓ Blastocyst development at 125 nmol/L and higher No cytotoxicity of ZnS coating CdSeQDs	[38]
In vitro	CdSeQDs (approx. 3.5 nm in diameter)	Female ICR and male C57BL/6J mice	Blastocysts were preincubated with CdSeQDs and transfered to pseudopregnat mice	Preincubation of blastocysts for 24 h	500 nmol/L	↓ Implantation rate ↑ Resorptions ↑ Embryos with abnormal development ↓ Fetal weight	[38]
In vitro	Polystyrene-based fluorescent nanoparticles (microspheres 40 to over 120 nm in size, Molecular Probes Inc.)	Two-cell stage mouse embryos	Incubation	4 days for 2-cell embryos 48 h for blastocysts	11.0 million/mL	No effect on development of 2-cell embryos No effect on hatching, implantation, or degeneration after exposure up to the blastocyst stage	[41]

to achieve pre-defined and reproducible particle sizes and properties suitable for a diverse range of industrial applications [36]. The CB particles so formed are complex, with a degenerated graphitic crystallite structure and high-power electron micrographs clearly show irregular layered graphitic plates. The most common use of CB is as a pigment and reinforcing phase in automobile tires. CB helps conduct heat away from the tread and belt area of the tire, reducing thermal damage and increasing tire life. CB is also employed in some radar-absorbent materials and in photocopiers and laser printer toner. *In vivo* and *in vitro* studies of CB are listed in Table 4.

2.3.1. In vivo study of carbon black (CB)

The effect of CB nanoparticles with a primary size of 14 nm (300 m^2/g in surface area, Printex 90, Degussa), 56 nm (45 m^2/g in surface area, Printex 25, Degussa), and 95 nm (20 m^2/g in surface area, Flammruss 101, Degussa) on the male reproductive system was determined [37]. Six-week-old male ICR mice (n = 15–16/group) were intratracheally instilled with CB particles suspended in normal saline containing 0.05% Tween 80 at 0.1 mg/mouse for the 14, 56, and 95 nm CB particles and 1.56 μg/mouse for the 14 nm CB (particle number concentration of 14 nm CB is the same as that of 56 nm CB). Mice received 10 weekly instillations and were killed on day after the last instillation. No effect of the 14, 56, or 96 nm particles was observed on body weight or male reproductive organ weights. Vacuolation of the seminiferous tubules and decreased DSP were found in mice instilled with all three sizes of CB particles. Levels of serum testosterone were increased after instillation of all three particles. The group exposed to the 14 nm particles, with approximately the same number of particles per unit volume as the 56 nm particles, showed fewer effects than did the group exposed to the 56 nm particles. The authors noted that CB nanoparticles impaired the function of Leydig cells, and the consequent fluctuation of sperm testosterone levels caused a reduction of DSP. These findings suggest that CB nanoparticles adversely affect mouse spermatogenesis and the effect depends on particle mass rather than particle number.

2.3.2. In vitro study of carbon black (CB)

The direct effects of CB (14 nm in particle size, Printex 90, Degussa) on testis-constituent cells was determined using the mouse Leydig cell line TM3 [20]. The test was performed using the procedure described above in the TiO_2 section. The uptake of CB nanoparticles by Leydig cells was detected after 48 h. Cell viability was markedly inhibited at 1000 μg/mL, but CB did not affect the proliferation of TM3 cells. No effect of CB was found on the expression of HO-1 mRNA in TM3 cells at up to 100 μg/mL. StAR mRNA expression was increased at 30 μg/mL after incubation for 48 h. These findings suggest that CB nanoparticles have no direct effect on the induction of oxidative stress but affect the production of steroid hormones in Leydig cells.

2.4. Luminescent particles

In vitro studies of cadmium selenium-core quatum dots (CdSeQDs) and polystyrene-based fluorescent particle have been published.

2.4.1. Cadmium selenium-core quantum dots (CdSeQDs)

Quantum dots are colloidal nanocrystalline semiconductors that have unique light-emitting properties and can be used as a novel luminescent material [38]. CdSeQDs are useful as an alternative to fluorescent dyes for use in biological imaging, due to their bright fluorescence, narrow emission, broad UV excitation, and high photostability [39]. An *in vitro* study of CdSeQDs is shown in Table 4.

2.4.1.1. In vitro study of cadmium selenium-core quantum dots (CdSeQDs). The developmental effect of CdSeQDs (approximately 3.5 nm in diameter) was determined using mouse embryos [38]. For water solubilization, the CdSeQDs were surface coupled with mercaptoacetic acid and suspended in PBS. Morulas and blastocysts were obtained from superovulating ICR female mice, which were mated with fertile males of the same strain, by flushing the fallopian tubes on GD 3 and flushing the uterine horns on GD 4, respectively. After incubation of morulas or blastocysts with CdSeQDs for 24 h, an inhibition of the preimplantation development of morulas into blastocysts, increased number of apoptotic cells in the inner cell mass (ICM) of blastocysts (n = 200/group) and inhibition of cell proliferation, primarily in the ICM, of blastocysts (n = 180/group) at 250 nmol/L and above, and inhibition of the postimplantation development of blastocysts at 125 nmol/L and above were observed. To examine the effect of CdSeQDs on the piostimplantation development of blastocysts, blastocysts (n = 200/group) exposed to 0 or 500 nmol/L for 24 h were transferred to recipient ICR mice (n = 25/group), which were mated with vasectomized C57BL/6J male mice, on pseudopregnant day (PD) 4 and killed on PD 18. A decreased implantation rate and fetal weight, and increased numbers of embryos with abnormal development and resorptions were observed in the CdSeQDs-treated group. CdSeQDs coated with ZnS had no significant cytotoxic effect on blastocyst development. These findings indicate that CdSeQDs affect adversely pre- and postimplantation embryonic survival and development and the ZnS coating alters the CdSeQD-induced toxicity.

2.4.2. Polystyrene-based fluorescent particles

Fluorescent nanoparticles are promising tools for optical data storage and other technical applications in biochemical, bioanalytical, and medical areas, and were successfully used for immunoassays [40]. An *in vitro* study of fluorescent nanoparticles is shown in Table 4.

2.4.2.1. In vitro study of polystyrene-based fluorescent particles. The effect of ultrafine polystyrene-based fluorescent particles (Molecular Probes Inc.), ranging from 40 nm to over 120 nm in size with different fluorescence colors corresponding to particle size, on mouse embryos was examined [41]. Two-cell stage embryos were incubated with fluorescent nanoparticles at 11.0 million/mL for 4 days, and development was assessed. Untreated embryos incubated for 4 days were further incubated with fluorescent nanoparticles at 11.0 million/mL for 48 h, and the developmental stages of the blastocysts were assessed. No effect of nanoparticles was found on the development of 2-cell stage embryos to the blastocyst stage. There was no effect of nanoparticles on hatching, implantation on the culture dish, or degeneration after additional exposure until the blastocyst stage. Although nanoparticles were internalized, the development of embryos was not affected. Nanoparticles were predominantly found in the trophoblast cells with a few located in the inner cell mass in hatched blastocysts. These findings show that fluorescent nanoparticles did not affect the development of mouse early embryos and suggest that internalized nanoparticles did not affect cellular processes or the expression of factors needed for development.

3. Discussion and conclusions

This paper reviewed the *in vivo* and *in vitro* studies on the reproductive and developmental toxicity of nanomaterials. Although it provides initial information on the potential toxicity of nanomaterials, it should be followed up by relevant hazard studies of nanomaterials.

In vivo studies have showed increased allergic susceptibility in offspring of mouse dams intranasally insufflated with respirable-

size TiO_2, adverse effects on spermatogenesis and histopathological changes in the testes, and changes in gene expression in the brain in mouse offspring after maternal subcutaneous injections of TiO_2 nanoparticles, transfer to rat fetuses of radiolabeled gold nanoparticles and C_{60} after maternal intravenous injection, death and morphological abnormalities in mouse embryos after maternal intraperitoneal injections of C_{60}, and adverse effects on spermatogenesis in mouse offspring after maternal intratracheal instillations of CB nanoparticles. However, these studies were performed with 1–10 administrations of a large bolus and/or a route of exposure not relevant to humans using relatively small numbers of animals. *In vivo* studies should be performed that include doses that closely reflect expected exposure levels. Major routes of exposure to nanoparticles are the respiratory tract, skin, eyes, and gastrointestinal tract. Studies using relevant routes of exposure are needed to clarify the toxicity of nanoparticles. The number of animals per group should be sufficient to allow meaningful interpretation of the data for reproductive and developmental toxicity studies, and a dose–response analysis is also needed to allow more realistic comparisons with actual human exposure. In the studies presented in this review paper, there was a lack of information regarding maternal toxicity. The investigation of maternal toxicity is essential for reproductive and developmental toxicity studies, because the toxicity to offspring may be modified or influenced by toxicity to the mother, and toxicity to offspring often occurs in conjunction with maternal toxicity in animal studies.

Radioactivity was detected in rat fetuses of dams intravenously injected with gold nanoparticles or C_{60}, but unlabeled gold nanoparticles were not detected in mouse fetuses of dams injected intravenously or in the fetal outflow of human placenta. *In vitro* study also revealed some possibility of placental transfer of CoCr particles mediated by a novel mechanism. In terms of developmental toxicity, information on the placental transfer of nanomaterials to offspring of dams given during gestation and lactation is of great interest in interpretation of the data. Measurements of the placental transfer of nanoparticles are an important source of information on the mechanism of action and the risk of nanoparticles, and may help to clarify the reproductive and developmental toxicity of nanoparticles.

As for the effect of nanoparticles on embryonic development, maternally administered C_{60} impaired embryonic development and the results of micromass culture suggest a dysmorphogenic effect of C_{60}. The C_{60} was clearly distributed into the yolk sac. These findings resemble those of developmental toxicity studies of trypan blue, which was teratogenic in mice, rats, hamsters, and guinea pigs [42]. It is generally accepted that teratogenic action of trypan blue in rats is due to its accumulation and interference in the function of the yolk sac, an organ of histotrophic nutrition that provides the principal source of nutrients before the initiation of functional chorio-allantoic placentae. Mice and rats have a yolk sac placenta, which plays a significant role during early in organogenesis. This is not the case for humans and monkeys in which the yolk sac placenta is of insignificant importance. Trypan blue produces malformations in rats and mice due to its accumulation in the yolk sac. This is not possible in humans and monkeys [43].

It is noted that test conducted and reported according to international accepted test guidelines and in compliance with the principles of Good Laboratory Practice (GLP) should have the highest grade of reliability and data for hazard identification must be evaluated considering their quality and adequacy for risk assessment [44]. At present, however, such studies are not available for reproductive and developmental toxicity of nanomaterials. Oberdörster et al. [1] described that studies to assess reproductive effects following pulmonary exposure to nanomaterials should follow protocols similar to OECD guideline 422 for the Testing of Chemicals (Combined Repeated Dose of Toxicity Study with the Reproduction/Developmental Toxicity Screening Test). The OECD guideline 421 for Testing of Chemicals (Reproduction/Developmental Toxicity Screening Test) is also useful to obtain initial information on possible effects on reproduction and development. In these tests, test materials are given to male rats for a minimum of 4 weeks beginning before mating and to females beginning before mating to shortly after parturition of pups. These screening tests are performed using relatively small numbers of animals in the dose groups and do not provide complete information on all aspects of reproduction and development due to the limitation of the exposure period and selectivity of endpoints. The two-generation study, which covers the whole reproductive cycles of at least one generation, may be adequate to evaluate the reproductive and developmental toxicity of nanomaterials. However, the concentrations, populations, and duration of exposure to nanomaterials are different from one another. It is required to modify the exposure period and the endpoints correlated with the exposure period. To further evaluate the reproductive and developmental toxicity of nanomaterials, a more specific test should be designed on a case-by-case basis according to the characterization of human exposure.

In vitro studies revealed high concentrations of TiO_2 nanoparticles to affect the viability and proliferation of mouse Leydig cells, but not the gene expression associated with spermatogenesis. Gold nanoparticles decreased the motility of human sperm, silver, aluminum, and MoO_3 were toxic to mouse spermatogonia stem cells, CoCr nanoparticles damaged DNA of human fibroblast cells, silica nanoparticles inhibited the differentiation of mouse ES cells, C_{60} inhibited the differentiation of mouse midbrain cells, CB decreased the viability of mouse Leydig cells, and CdSeQDs inhibited the pre- and postimplantation development of mouse embryos. In these studies, the concentrations of nanoparticles were very high and unlikely to occur in animal studies. The mechanistic pathways that operate at low realistic concentrations are likely to be different from those operating at very high concentrations when the cell's or organism's defenses are overwhelmed [2]. The findings of these *in vitro* studies are difficult to evaluate because of differences in the chemical composition and sizes of particles, target cells, duration of exposure, endpoints, and exposure concentrations among experiments. *In vivo* studies correlated with results obtained from *in vitro* studies should be performed.

Oxidative stress as a common mechanism for cell damage induced by nanoparticles is well known and a wide range of nanomaterial species have been shown to create reactive oxygen species (ROS) both *in vivo* and *in vitro*. It is suggested that a free radical-induced mechanism or another form of oxidative stress played a role in the developmental toxicity of C_{60} in zebrafish, in which C_{60} caused decreases in the embryonic survival rate, the hatching rate, heartbeat and pericardial edema, and the toxicity was effectively attenuated by adding glutathione, an antioxidant [45]. In mammals, TiO_2 nanoparticles in Leydig cells, Sertili cells, spermatids, and cells of the olfactory bulb and cerebral cortex of pups, and C_{60} in embryos and yolk sac were noted after a maternal administration. In *in vitro* studies, TiO_2 and CB nanoparticles in Leydig cells, Fe_3O_4 and gold nanoparticles in sperm cells, silica nanoparticles in cells of the embryonic body, CoCr nanoparticles in BeWo cells, and fluorescent nanoparticles in trophoblast cells were observed. Determination of the oxidative stress in these cells may help us to understand the reproductive and developmental toxicity of nanoparticles.

The contradicting results obtained from the studies presented in this review may be attributed to the use of different nanomaterials and experimental models, the exposure during different stages of offspring development, and evaluations with different endpoints. It is likely that the size, shapes, chemistry, crystallinity, surface properties, concentration, agglomeration, and dose of nanoparticles are all involved in detecting biological activity. The characterization of

administered materials in toxicity studies is fundamental, and characterizing delivered nanomaterials after administration in a test system or model provides the best quality data on dose and material properties that are related to observed responses, but this is limited by current methodological capabilities [2]. Further studies, especially *in vivo*, using different types of characterized materials, relevant routes of administration, and doses closely reflecting expected levels of exposure are needed to adequately evaluate the reproductive and developmental toxicity of nanomaterials.

Conflict of interest

None.

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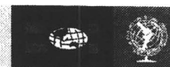

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CASE REPORT

High-dose unfractionated heparin therapy in a pregnant patient with antiphospolipid syndrome: a case report

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Abstract

A case of a 37-year-old pregnant patient with antiphospholipid syndrome (APS), who has a medical history of both thrombosis and recurrent fetal loss, is presented. She was treated with predonisolone and fixed-dose unfractionated heparin (UFH) infusion, followed by plasmaphereses and fixed-dose low-molecular-weight heparin infusion during her fourth pregnancy. Unfortunately, this treatment did not have beneficial effects, resulting in intrauterine growth restriction and finally neonatal death. Continuous intravenous UFH infusion and low-dose aspirin were administrated under the monitoring of the activated partial thromboplastin time to achieve a target level of 120 s during her fifth pregnancy. A healthy baby weighing 1818 g at birth was delivered by Cesarean section at the 34th week of pregnancy. High-dose UFH infusion may be considered to be one of the preferable options to manage pregnant patients with refractory APS.

Key words: activated partial thromboplastin time, anticoagulation, antiphospholipid syndrome, heparin, high intensity, pregnancy.

INTRODUCTION

Antiphospholipid syndrome (APS) is clinically characterized by arterial and/or venous thrombosis and obstetric complication(s) in association with the repeated positivity of antiphospholipid antibody (APLA).[1] The obstetric complications include fetal death, fetal growth restriction, premature birth, preeclampsia, and maternal thrombosis. A combination of unfractionated heparin (UFH) and low-dose aspirin (LDA) has generally been used to treat pregnant APS patients with a medical history of vascular thrombosis and/or obstetric complication(s). The combination therapy of low-molecular-weight heparin (LMWH) and LDA has also been widely accepted.[2] However, the dosage regimen has not been well established, including proper heparin dosage for pregnant APS patients at high risk of thrombosis and obstetric complication(s) and the frequency of heparin dosage adjustment. We report a case of a pregnant APS patient, who was treated with LDA and continuous high-dose intravenous UFH under monitoring of the activated partial thromboplastin time (APTT) during her fifth pregnancy.

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CASE REPORT

In November 2005, a 37-year-old pregnant woman was referred to our hospital for the management of her fifth pregnancy.

She became pregnant for the first time in 1996 and aborted a fetus spontaneously at the ninth week of pregnancy. She developed right cerebellar infarction 4 days after endometrial ablation. The examination detected the factors of APS; thrombocytopenia, prolonged APTT value (55.2 s; control value 32.6 s), lupus anticoagulant (LAC) by APTT and kaolin clotting time, anti-β2-glycoprotein I antibody (anti-β2GPI) (5.0 U/mL), antinuclear antibody (1 : 640), and IgG anti-double-stranded DNA antibody (108.7 U/mL). In the light of the examination results, she was suspected of having APS. However, her symptoms and clinical data did not satisfy the American College of Rheumatology (ACR) criteria for the classification of systemic lupus erythematosus (SLE).[3,4] Accordingly, warfarin and ticlopidine was continuously administered to her until her second pregnancy in 1997.

In terms of anti-β2GPI (6.8 U/mL) detected by the examination, she was diagnosed as APS according to Harris's criteria for APS[5] in this second pregnancy. Her symptoms and clinical data still did not satisfy ACR criteria for SLE, so her APS was considered to be primary.

The patient was treated with aspirin (162 mg/day); however, the fetus developed intrauterine growth restriction and its heart beat was lost at the 22nd week of pregnancy. The infarction was observed in 20–30% of the placenta.

Her third pregnancy was managed with predonisolone (5 mg/day) and aspirin (162 mg/day) in 2000. The fetal heart beat was suddenly lost at the 23rd week of pregnancy. Multiple infracted lesions were found in the placenta. She had developed left renal infarction in the period between the third and the fourth pregnancies. Warfarin was administered again.

She became pregnant for the fourth time in 2002. Warfarin was discontinued. Predonisolone (10 mg/day), LDA (81 mg/day), and intravenous UFH infusion at a fixed dose (10 000 units/day) were continuously administered starting at the fifth week of pregnancy. She developed cerebral infarction at the ninth week of pregnancy. Intravenous UFH infusion (10 000 units/day) was considered ineffective in treating this APS patient and this was changed to intravenous LMWH infusion at a fixed dose (5000 units/day). The dosage of predonisolone was increased to 30 mg/day. In addition, plasmapheresis was repeated 12 times in the period between the 9th and 24th weeks of pregnancy. Nevertheless, intrauterine growth retardation was observed at the 18th week of pregnancy. Reversed end-diastolic flow velocity was detected by ultrasonography and the emergency Cesarean section procedure was performed at the 24th week of pregnancy. The neonate died 7 days after birth. The administration of warfarin was resumed after diagnosis of her fourth pregnancy.

She had become pregnant for the fifth time in November 2004. When she was diagnosed at the fifth week of pregnancy, warfarin was changed to LDA (81 mg/day) and continuous intravenous UFH infusion (15 000 U/day) was started under hospitalization. She was referred to our hospital at the eighth week of pregnancy. Her data on body weight and APTT were 54.8 kg and 189.6 s, respectively, on the day of hospitalization. We decided to administer her LDA and high-dose UFH adjusted by APTT value. Her APTT value had been kept at around 60 s during the period of non-pregnancy. The target of her APTT value was set to 120 s, which was about 3.5 times the control value (32.6 s) and far higher than that in standard use of UFH for anticoagulation (1.5–2.0 times the control value or the upper limit of the normal APTT range). The APTT test was repeatedly performed at the intervals of about twice a week to adjust the daily dose of UFH. The required dose of UFH was 10 000 U/day at the eighth week of pregnancy, increased gradually up to 23 000 U/day at the 25th week and finally decreased (Fig. 1). To avoid unexpected loss of the fetus during the remainder of the pregnancy period

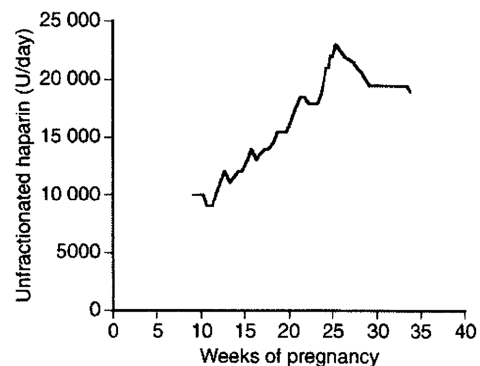

Figure 1 Daily dose of unfractionated heparin to maintain activated partial thromboplastin time at 120 seconds in the patient with antiphospholipid syndrome during her fifth pregnancy.

and to finish the labor in a short period of time, the Cesarean section procedure was performed at the 34th week of pregnancy, assuming that at this point, the fetus had matured enough to be in sound health after the delivery. The birth weight of the baby was 1818 g, which fell within the reference range of fetus weight at the 34th week of pregnancy. No infarction was observed in the placenta. No major complications developed throughout the course of pregnancy in this case. Both the APS patient and her baby are in good health at the present time 2 years after the delivery.

DISCUSSION

Pregnancy loss is one of the two major clinical components of APS. The obstetric complications induced by APLA were thought to be secondary to placental thrombosis,[6,7] possibly leading to placental dysfunction. Heparin and LDA were used as anticoagulant and antiplatelet agents, respectively; the combination therapy of heparin and LDA contributed to improved fetal outcomes.[8,9] LMWH, which have better bioavailability and lower risk of heparin-induced osteoporosis and thrombocytopenia, appeared to be superior to UFH despite both treatments having similar levels of efficacy.

The bioavailability of UFH deteriorated as the pregnancy progressed[10] due to such factors as: increased heparin-binding proteins and volume of maternal plasma; accelerated renal clearance; and degradated heparin by the placenta. The same applies to LMWH. Higher doses of LMWH may be needed for anticoagulation in pregnant patients[11] due to increased volume of maternal plasma and glomerular filtration; however, LMWH does not bind to many plasma proteins. Dose adjustment of UFH or LMWH plus LDA is recommended in pregnant patients with APLA and a medical history of thrombosis, who have higher risks of recurrence.[2] This article does not refer to the necessity of dose adjustment for pregnant APS patients with recurrent fetal loss. However, generally, prudence would seem to monitor the intensity of anticoagulation therapy using UFH or LMWH and adjust the dose of the drug in the pregnant APS patients, whose previous pregnancy resulted in fetal loss despite of the adoption of the fixed dose regimen.

It is unknown how often the dose of heparin should be adjusted in pregnant APS patients. The intensity of heparin therapy was measured one time or less in each trimester in some reports which showed the dose of heparin for stable anticoagulation was increased as pregnancy progressed.[11,12] The report in which the intensity of UFH therapy was measured more frequently showed that the dose of heparin for stable anticoagulation started to decrease in the third trimester,[13] as shown in this case report. An increase in heparin dose during pregnancy may not be proportional to the gestational age or body weight of the pregnant patient. Repeated measurement of the intensity of UFH therapy may be favorable to avoid overuse of the drug in the third trimester.

The inexpensive and simplified APTT test is suitable to monitor the intensity of UFH therapy; however, some patients with LAC have prolonged APTT value. It may be difficult to evaluate the intensity of UFH therapy for these patients by the APTT test. The factor Xa inhibition test is recommended to monitor anticoagulation levels in these patient populations;[14,15] however, this test is more expensive and takes longer for results to be obtained. For this reason, the factor Xa inhibition test is not suitable to frequent and prompt dose adjustment in UFH therapy. In reality, it is possible to monitor the intensity of UFH therapy by the APTT test if UFH is administered at high enough dose to allow the measured APTT values to far exceed the intrinsic prolonged APTT values of APS patients. Thus, the APTT test can be used to monitor high-dose UFH therapy in pregnant APS patients with prolonged APTT value.

The different mechanisms of heparin contributing to APS treatment have been recently revealed. UFH[16] and LMWH[17] inhibited APLA binding *in vitro*. It has also been reported that heparin inhibited the binding of β2-glycoprotein I to phospholipids.[18] Furthermore, previous studies have reported that complement activation was critical to obstetric complications in pregnant APS patients[19,20] and TNF-alpha was induced by C5 activation associated with fetal resorption in mouse models.[21] The deposition of complement components was observed in the placenta of an APS patient.[22] Heparin prevented antiphospholipid-induced fetal loss by inhibiting complement activation in mouse models.[23] These studies indicated that heparin has another action than as an anticoagulant; however, the mechanism of this action on pregnant APS patients in the early stage has not been well elucidated. Based on these findings, it seemed to be sensible to start heparin administration as soon as APS patients are proved to be pregnant. How to use heparin in pregnant APS patients may need to be reconsidered from an aspect different from the conventional one, that is, when used only as the anticoagulant.

The combination therapy of heparin and LDA contributed to improved fetal outcomes in pregnant APS patients, as mentioned earlier. The treatment of LDA alone was reported to have no significant effects compared with supportive care.[24] Heparin appears to be a great contributor to successful prevention of pregnant APS patients losing their fetuses, compared with LDA. High-dose UFH monotheraphy might be used in pregnant APS patients only after the effects and safety of LDA-combined high-dose UFH therapy have been thoroughly verified.

Thus, LDA-combined high-dose UFH therapy may be effective in managing pregnant APS patients with recurrent fetal loss. However, further studies must need to be conducted before this therapy can be used as an alternative to the conventional treatment of pregnant APS patients.

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日本における医薬品添付文書の記載要領と問題点

妊婦・授乳婦に対する医薬品の情報源として，医薬品添付文書は唯一薬事法に法的根拠を持ち，日本では最も重要な資料ということができる．医薬品に添付されている以外に，現在はインターネットを通じて，医療関係者のみならず一般の方々であっても，最新の医薬品添付文書を簡単に入手することができる[1]．医薬品には，使用者がドラッグストアなどで購入して使用する一般用医薬品と医療機関で処方される医療用医薬品があるが，そのいずれの添付文書も入手可能であり，医師や薬剤師などの医療従事者はもちろん，患者自身が利用できる点でも重要性が高い．

本項ではこれらの医薬品添付文書に関して，「妊娠・授乳と薬」の観点から，その記載要領と指摘されている問題点について，その問題点解決の方向性を含めて概説する．

医薬品添付文書の記載要領

医薬品添付文書は製薬企業がそれぞれ記載するものである．ただし，医薬品によって，あるいは製薬企業によってその様式が異なることがないよう，また必要事項の記載漏れがないよう，その記載要領が定められている．

このうち一般用医薬品については，平成11年8月12日，厚生省医薬安全局長が各都道府県知事あてに「一般用医薬品の添付文書記載要領について」という通知(医薬発第984号)を行った．本通知には「一般用医薬品の添付文書記載要領」が添えられており，これに加えて同日厚生省医薬安全局安全対策課長が各都道府県衛生主管部(局)長あてに通知した「一般用医薬品の添付文書記載要領の留意事項について」(医薬安第96号)などに基づいて，現在の一般用医薬品添付文書は記載されている．一方，医療用医薬品については，平成9年4月25日，厚生省薬務局長が各都道府県知事あてに行った「医療用医薬品添付文書の記載要領について」という通知(薬発第606号)に添えられた「医療用医薬品添付文書の記載要領」(**表2-9**)に従って，現在記載が行われている．

これらの医薬品添付文書においては，当然「妊娠・授乳と薬」に関連する記載もなされており，その記載要領もそれぞれ規定されている．

一般用医薬品については「使用上の注意」の第2項目として「相談すること」という項目が定められており，その記載要領「一般用医薬品の使用上の注意記載要領」(平成11年8月12日医薬発第983号別添)には，「疾病の種類，症状，合併症，既往歴，家族歴，体質，妊娠の可能性の有無，授乳の有無，年齢，性別等からみて，副作用による危険性が高い場合若しくは医師又は歯科医師の治療を受けている人であって，一般使用者の判断のみで

表2-9 医療用医薬品添付文書の記載要領

第一 「添付文書記載」の原則

医療用医薬品の添付文書は，薬事法第52条第1号の規定に基づき医薬品の適用を受ける患者の安全を確保し適正使用を図るために，医師，歯科医師及び薬剤師に対して必要な情報を提供する目的で当該医薬品の製造業者又は輸入販売業者が作成するものであること．
添付文書に記載すべき内容は，原則として当該医薬品が承認された範囲で用いられる場合に必要とされる事項とすること．ただし，その場合以外であっても重要で特に必要と認められる情報については評価して記載すること．
記載順序は，原則として「記載項目及び記載順序」に掲げるものに従うこと．
既に記載している事項の削除又は変更は，十分な根拠に基づいて行うこと．

第二 記載項目及び記載順序

作成又は改訂年月，日本標準商品分類番号等，薬効分類名，規制区分，名称，警告，禁忌，組成・性状，効能又は効果，用法及び用量，使用上の注意，薬物動態，臨床成績，薬効薬理，有効成分に関する理化学的知見，取扱い上の注意，承認条件，包装，主要文献及び文献請求先，製造業者又は輸入販売業者の氏名又は名称及び住所．

第三 記載要領

項目	記載要領
作成又は改訂年月	改訂した版数も記載すること．
日本標準商品分類番号等	日本標準商品分類番号，承認番号，薬価基準収載年月，販売開始年月，再審査結果の公表年月，再評価結果の公表年月，効能又は効果の追加承認年月，貯法等について記載すること．
薬効分類名	当該医薬品の薬効又は性格を正しく表すことのできる場合には記載することとし，使用者に誤解を招くおそれのある表現は避けること．
規制区分	毒薬，劇薬，麻薬，向精神薬，覚せい剤，覚せい剤原料，習慣性医薬品，指定医薬品及び要指示医薬品の区分を記載すること．
名　称	日本薬局方外医薬品にあっては，承認を受けた販売名を記載すること．なお，薬事法第42条第1項の規定に基づく基準(以下「法定の基準」という．)により記載が義務付けられている医薬品にあっては，基準名を併せて記載すること．またそれ以外の医薬品であって，一般的名称がある場合には，その一般的名称を併せて記載すること． 日本薬局方に収められている医薬品にあっては，日本薬局方で定められた名称を記載し，販売名がある場合は併記して差し支えないこと．
警　告	本文冒頭に記載すること． 平成9年4月25日薬発第607号「医療用医薬品の使用上の注意記載要領について」により記載すること．
禁　忌	原則として，警告に続けて記載することとし，警告がない場合は本文冒頭に記載すること． 平成9年4月25日薬発第607号「医療用医薬品の使用上の注意記載要領について」により記載すること．
組成・性状	組成： 有効成分の名称(一般的名称があるものにあっては，その一般的名称)及びその分量(有効成分が不明なものにあっては，その本質及び製造方法の要旨)及びその分量を記載すること． 医薬品添加物については，昭和63年10月1日薬発第853号薬務局長通知「医療用医薬品添加物の記載について」により記載すること． 日本薬局方に収められている医薬品又は法定の基準が定められている医薬品にあっては，(ii)に規定するものの他，日本薬局方又は法定の基準で添付文書への記載が義務付けられている医薬品の添加物について記載すること． 製剤の性状： 識別上に必要な色，味，におい，形状(散剤，顆粒剤等の別)，識別コードなどを記載すること．また，水性注射液にあっては，pH及び浸透圧比を，無菌製剤(注射剤を除く)にあっては，その旨を記載すること．

効能又は効果	承認を受けた効能又は効果を記載すること.
用法及び用量	承認を受けた用法及び用量を記載すること. なお, 効能又は効果に応じて用法及び用量が定められているものはこれを書き分けること.
使用上の注意	平成9年4月25日薬発第607号薬務局長通知「医療用医薬品の使用上の注意記載要領について」により記載すること. 「使用上の注意」で効能又は効果に関連する事項は, 効能又は効果の項目に続けて承認内容と明確に区別して記載すること. 「使用上の注意」で用法及び用量に関連する事項は, 用法及び用量の項目に続けて承認内容と明確に区別して記載すること. 「使用上の注意」のうちで, 警告, 禁忌, (二)及び(三)に該当する事項は, 原則としてこの項目に重複して記載する必要はないこと.
薬物動態	ヒトでの吸収, 分布, 代謝及び排泄に関するデータを記載すること. ヒトでの吸収, 分布, 代謝及び排泄に関するデータが得られないものについては, これを補足するために本項に動物実験の結果を記載すること. データの根拠がある場合には, 腎機能, 肝機能等の程度に応じた投与量, 投与間隔の解説を記載し, 慎重投与等の対象患者の記載の後に「薬物動態の項参照」と記載すること.
臨床成績	精密かつ客観的に行われた臨床試験の結果について, 投与量, 投与期間, 症例数, 有効率等を承認を受けた用法及び用量に従って記載すること. 他剤との比較を記載する場合には, その対照が繁用医薬品であり, 精密かつ客観的に行われた比較試験の成績がある場合にのみ記載することができること.
薬効薬理	効能又は効果を裏付ける薬理作用及び作用機序を記載すること. 動物実験の結果を用いる場合には動物種を, また*in vitro*試験の結果を用いる場合にはその旨をそれぞれ記載すること.
有効成分に関する理化学的知見	一般的名称, 化学名, 分子式, 化学構造式, 核物理学的特性(放射性物質に限る.)等必要に応じて記載すること.
取扱い上の注意	日本薬局方に収められている医薬品, 法定の基準が定められている医薬品又は承認を受けた医薬品であって, それぞれ日本薬局方, 基準又は承認の中で取扱い上の注意事項が定められているものにあっては, 少なくともそれぞれの当該注意事項を記載すること. その他の医薬品にあっては, 取扱い上の注意事項があればそれを記載すること.
承認条件	承認に当たって試験の実施等の条件を付された場合には, その内容を記載すること.
包装	
主要文献及び文献請求先	文献請求先にあっては, その氏名又は名称及び住所を記載すること.
製造業者又は輸入販売業者の氏名又は名称及び住所	

(医療用医薬品添付文書の記載要領について〔平成9年4月25日 薬発606号〕)

使用することが不適当な場合」については,「次の人は使用前に医師, 歯科医師又は薬剤師に相談すること(歯科医師については, 歯科医師が関係する場合にのみ記載すること)」としてこれらを記載することを求めている. そのため, 実際に販売されている一般用医薬品のほとんどで,「妊婦又は妊娠していると思われる人や授乳中の人は服用前に医師又は薬剤師に相談して下さい」といった内容の添付文書が添えられる結果となっている.