Review

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Impacts and potential mechanisms of fine particulate matter ($PM_{2.5}$) on male testosterone biosynthesis disruption

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Abstract: Exposure to $PM_{2.5}$ is the most significant air pollutant for health risk. The testosterone level in male is vulnerable to environmental toxicants. In the past, researchers focused more attention on the impacts of $PM_{2.5}$ on respiratory system, cardiovascular system, and nervous system, and few researchers focused attention on the reproductive system. Recent studies have reported that $PM_{2.5}$ involved in male testosterone biosynthesis disruption, which is closely associated with male reproductive health. However, the underlying mechanisms by which $PM_{2.5}$ causes testosterone biosynthesis disruption are still not clear. To better understand its potential mechanisms, we based on the existing scientific publications to critically and comprehensively reviewed the role and potential mechanisms of $PM_{2.5}$ that are participated in testosterone biosynthesis in male. In this review, we summarized the potential mechanisms of $PM_{2.5}$ triggering the change of testosterone level in male, which involve in oxidative stress, inflammatory response, ferroptosis, pyroptosis, autophagy and mitophagy, microRNAs (miRNAs), endoplasmic reticulum (ER) stress, and N6-methyladenosine (m6A) modification. It will provide new suggestions and ideas for prevention and treatment of testosterone biosynthesis disruption caused by $PM_{2.5}$ for future research.

Keywords: $PM_{2.5}$; testosterone biosynthesis; toxic mechanism; male

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Introduction

The rapid urbanization and industrialization have accompanied with a growing number of air pollution. Ambient fine particulate matter (aero dynamic diameter ≤2.5 μm, $PM_{2.5}$) is believed to be the most hazardous air pollution and has become a major public health problem, attracting accumulating attention in bioscience research [1]. $PM_{2.5}$ is a complex mixture of suspending particles originated from traffic exhaust emission, coal combustion and open heating sources [2]. The main chemical composition of $PM_{2.5}$ includes metal elements, inorganic ions, polycyclic aromatic hydrocarbons (PAHs) and endocrine disrupting chemicals [3]. However, the constituents of $PM_{2.5}$ varies with location, season, and source, which indicated that $PM_{2.5}$ adverse effects on health are complex and extensive. Due to the particles with small size, large surface area and complicated toxic substance, $PM_{2.5}$ is very easily to enter and accumulate in human various organs and has a marked adverse health on the human [4, 5]. For decades, the relationship between $PM_{2.5}$ exposure and reproductive dysfunctions in male has attracted an increasing public attention. The epidemiological and experimental evidence have suggested that $PM_{2.5}$ exposure can impair sperm quality and the level of sex hormones via testicular damage [6, 7].

Over the past decades, there has been a worldwide decline on male sperm quality and an increase on the incidence of infertility [8]. Testosterone is the main male androgen, which is essential for secondary sexual development, metabolism, and spermatogenesis. In the absence of testosterone, males are infertile because of spermatogenesis dysfunction [9]. Testosterone contributes to the maintain of the blood-testis barrier (BTB) [10, 11], which provides nutrients and safe space for the development, survival, and maturation of germ cells [12]. The BTB is one of the tightest blood-tissue barriers in the mammalian animals, constituted by tight junction, gap junction, ectoplasmic specialization, and desmosomes [13]. The tight structure of the BTB can prevent the environmental toxicants from entering. Toxicological studies have shown that the decreasing of testosterone impairs the BTB components in mice [14, 15],

which means that testosterone biosynthesis disruption may accelerate $PM_{2.5}$ accumulation in testis via reducing the integrity of the BTB to impair Leydig cells which is the center of testosterone synthesis and secretion in male. When the level of testosterone reduced, the developing spermatids detached from Sertoli cells in the seminiferous epithelium, which demonstrated that testosterone is important for keeping the BTB function [16]. In addition, $PM_{2.5}$ can also accumulate in testis and directly damage the BTB through reducing the BTB-related proteins [17, 18]. Thus, $PM_{2.5}$ may directly or indirectly disrupt the complete structure of the BTB to accelerate Leydig cell damage via multiple toxic mechanisms. The absence of testosterone results in abnormal attachment and release of germ cells via caused the dysfunction of Sertoli cells, which suggested the occurrence of infertility.

Testosterone biosynthesis is vulnerable to exposure to various toxic substances. Recently, several studies have demonstrated that $PM_{2.5}$ exposure is inversely associated with testosterone biosynthesis [14, 19, 20]. Leydig cells are the major site of testosterone biosynthesis and secretion in male. Testosterone production depends on the function of Leydig cells, which is tightly regulated via complex testosterone biosynthesis pathways (Figure 1) [21]. Testosterone is biosynthesized by various enzymes from cholesterol. First, cholesterol is transported to the inner mitochondrial membrane by StAR and then is converted to pregnenolone by CYP11A1. Pregnenolone is converted to testosterone through two pathways. On the one hand, pregnenolone is converted to androstenediol catalyzed by CYP17A1, AKR1C3 and CYP17B1/2. On the other hand, pregnenolone is converted to androstenedione catalyzed by HSD3B1/2 and CYP17A1. Thereafter, both androstenediol and androstenedione are converted to testosterone via HSD3B1/2

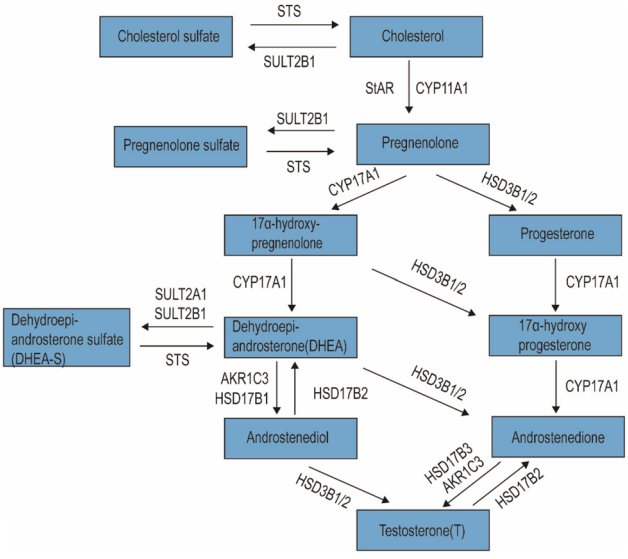

Figure 1: Testosterone biosynthesis pathways are shown.

and HSD17B2/3, respectively. Thus, $PM_{2.5}$ may impair testosterone biosynthesis via targeting Leydig cell. However, there is less reports on whether $PM_{2.5}$ exposure impairs Leydig cells function and its underlying mechanisms.

A growing number of evidence has shown that $PM_{2.5}$ impairs male testosterone biosynthesis (Table 1). After the human body inhales small particles matter (such as PM_{10}, $PM_{2.5}$ and $PM_{0.1}$), it will enter the systemic circulation and organs of the body, and even reach the testis [18]. We summarized epidemiological and experimental studies and found that most studies have shown that $PM_{2.5}$ decreased the testosterone level and is accompanied with reproductive impairment such as infertility, the decrease of sperm production and the increase of sperm abnormalities, although several articles have proposed opposite results on the change of testosterone level. Nevertheless, its potential molecular mechanism has not been fully clear. Further studies are needed for further exploring the potential mechanisms of $PM_{2.5}$-induced decline of testosterone level. In the review, we focus on reviewing the existing epidemiological and experimental studies and summarize potential molecular mechanisms. We propose a hypothesis that the process of $PM_{2.5}$ exposure impairing male testosterone biosynthesis involved in multiple mechanisms.

Search strategy

A careful literature search was carried out on April 2023 for eligible articles using the PubMed database according to the PRISMA criteria. Only studies in English were included. This review includes studies which used healthy participants, patients, animals *in vivo*, and cell cultures *in vitro*. We search the following terms for the search using the Advanced Search Builder: (relevant mechanism [Title/Abstract]) AND ((PM2.5 [Title/Abstract]) OR (particulate matter [Title/Abstract]) OR (PM [Title/Abstract])) and (relevant mechanisms [Title/Abstract]) AND (testosterone [Title/Abstract]). The search terms of relevant mechanisms include endoplasmic reticulum stress, ER stress, autophagy, mitophagy, ferroptosis, inflammatory response, inflammation, miR, N6-methyladenosine (m6A) RNA modification, m6A, pyroptosis, ROS and oxidative stress.

Potential mechanisms of $PM_{2.5}$-induced testosterone biosynthesis disruption

$PM_{2.5}$ exposure can cause testosterone biosynthesis disruption, while the alteration of testosterone level is associated

Table 1: Summary of epidemiological and experimental studies on the relationship between PM2.5 exposure and testosterone biosynthesis.

Subject	Location	$PM_{2.5}$ exposure	Effects on testosterone biosynthesis	Reference
Fischer male rat (beginning at birth)	Tokyo, Japan	Inhalation, 5.63 mg/m^3, 6 h/day, 5 days/week for 90 days (diesel exhaust particle)	Testosterone↑, impairs sperm quality and production	[22]
BALB/c male mice	Tokyo, Japan	Dorsal subcutaneous injection, 24.7, 74.0 or 220 μg/mouse, 10 times for 5 weeks (diesel exhaust particle)	Testosterone↑, decreases sperm productions and viabilities, increases sperm abnormalities	[23]
Men	Poland	Inhalation, arithmetic mean for a period of 90 days before semen collection	Testosterone↓, increases sperm abnormalities	[7]
Sprague-Dawley (SD) male rats	Beijing, China	Intratracheal instillation, 1.8, 5.4, 16.2 mg/kg.bw, 10 times for 30 days	Testosterone↓, impairs sperm quality	[14]
C57Bl/6J male mice	Baltimore, USA	Inhalation, 12.8 μg/m^3, 6 h/day, 5 days/week for 4 months	Testosterone↓, decreases sperm count	[24]
Male college students	Chongqing, China	Inhalation, 54.8 μg/m^3, from January 1, 2013, to December 31, 2015	Testosterone↑, decreases sperm quality, sperm count and sperm normal morphology	[25]
Male C57BL/6 mice	Shanghai, China	Inhalation, 153.05 μg/m^3, 8 h/day, 7 days/week for 125 days	Testosterone↓, decreases sperm concentration and motility	[26]
Male C57BL/6 mice	Shijiazhuang, China	Inhalation, 671.87 μg/m^3, 6 h/day, 7 days/week for 16 weeks	Testosterone↓, decreases sperm density and motility	[6]
Sprague-Dawley (SD) male rats	Zhengzhou, China	Intratracheal instillation, 1.5 mg/kg.bw, 5 days/week for 4 weeks	Testosterone↓, decreases sperm quality	[1]
Offspring male C57BL/6 mice (sacrificed at 8 weeks old)	Beijing, China	Intratracheal instillation, 4.8, 43.2 mg/kg.bw, every three days, 6 times in all (maternal exposure)	Testosterone↓	[27, 28]
Male adults	Beijing, China	Inhalation, 63.6 mg/m^3, from February 2014 to December 2019	Testosterone↓	[20]
Male Wistar-Kyoto (WKY) rats	North Carolina, USA	Intratracheal instillation, 5 mg/kg.bw, 1 time	Testosterone↓	[29]

with various reproductive system toxicity in male. The mechanisms in $PM_{2.5}$-induced testosterone biosynthesis disruption are various and complex. Therefore, we discuss the potential mechanisms from the following mechanisms: oxidative stress, inflammatory response, ferroptosis, pyroptosis, autophagy and mitophagy, microRNAs (miRNAs), endoplasmic reticulum (ER) stress, and N6-methyladenosine (m6A) modification (Figure 2). Above mechanisms are supported by existing epidemiological and experimental studies.

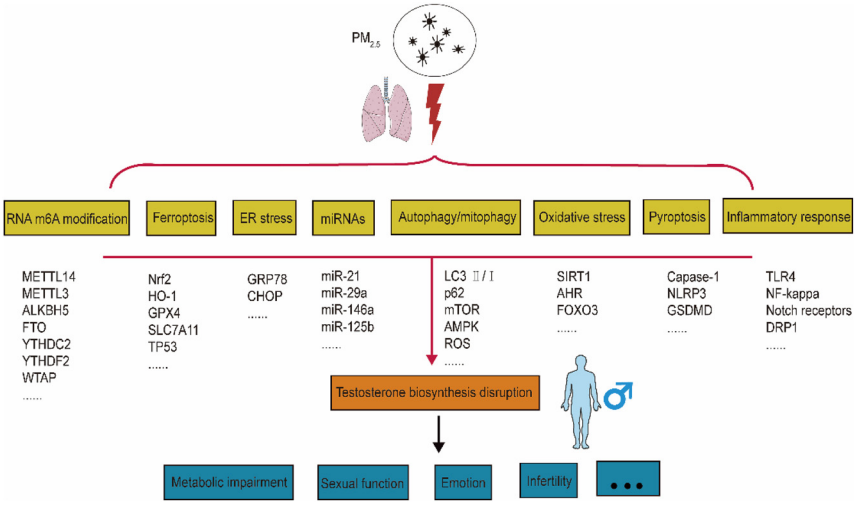

Figure 2: Potential mechanisms and its adverse outcomes of PM2.5-induced testosterone biosynthesis disruption on male.

Oxidative stress

Numerous studies have reported that oxidative stress mediated $PM_{2.5}$-induced toxicity and testosterone biosynthesis disruption. Oxidative stress is the crucial mediator of $PM_{2.5}$-related toxicity. Oxidative stress can activate other mechanisms, such as inflammation, autophagy, ferroptosis, and pyroptosis [30–32], which suggested that it is the central mediator of $PM_{2.5}$-induced toxicity. Thus, oxidative may play an upstream role to trigger other mechanisms or directly target Leydig cells and their testosterone biosynthesis pathway. It has been reported that $PM_{2.5}$ exposure promoted oxidative stress through inhibiting the expression of sirtuin1(SIRT1), a type III protein deacetylase [33]. SIRT1 is necessary for testosterone biosynthesis via regulating multiple molecules in Leydig cells. The knockout of SIRT1 resulted in a sharp decrease in testosterone in $SIRT1^{-/-}$ mice compared to control mice [34]. The SIRT1-mediated deacetylation of molecules such as Nrf2, p53, NF-kappa B and FOXO3, which performed important roles on driving oxidative stress [35]. All the above studies have suggested that SIRT1 may act as a critical molecular to regulate oxidative stress. The aromatic hydrocarbon receptor (AHR), a ligand-activated transcription factor, mediates the excessive reactive oxygen species (ROS) after exposure to $PM_{2.5}$ [36]. Recently, it has been evidenced that the long-term exposure to triclosan decreases the testosterone level through the activation of AHR in mouse neocortical neurons [37]. AHR also plays an important role on inhibiting testosterone secretion induced by polychlorinated naphthalene mixture in porcine ovaries [38].

Inflammatory response

Some studies have demonstrated that inflammatory response may mediate the testosterone biosynthesis disruption [6, 39, 40]. Toll-like receptor 4 (TLR4) is a type-Itransmembrane receptor protein. The extracellular domain recognizes pathogens while the cytoplasmic domain like IL-1 receptor family. NF-kappa B, which is the downstream target of TLR4, involves in initiating inflammatory response [41]. Prior evidence demonstrated that TLR4/NF-kappa B signaling pathway mediates inflammatory response caused by $PM_{2.5}$ *in vitro* and *in vivo* [42–44]. Interestingly, related research discovered that TLR4 could activate NF-kappa B and lead to the reduction of testosterone concentration via decreasing the expression of testosterone synthesis genes, including StAR, CYP11A1, 3β-HSD, CYP17A1 and 17β-HSD, in male piglets and pig Leydig cells [45]. Notch signaling pathway is highly conserved in animals, which includes Notch receptors (Notch 1–4) and their ligands (Delta1-3 and Jagged1-2) [46]. Studies have shown that $PM_{2.5}$ modulates airway inflammation by Notch signaling pathway [47, 48]. The knockdown of Notch receptors has shown that the expression of testosterone biosynthesis-related genes and proteins such as CYP11A1, StAR and HSD3B were lower in Leydig cells [49]. It has been demonstrated that NLRP3 inflammasome modulates the initiation of inflammation induced by $PM_{2.5}$ [50–52]. A recent publication has noted that NLRP3 inflammasome involves in testicular damage and decrease testosterone level [53]. COX-2/PGE2 signaling pathway is another inflammatory mediator, which is associated with inflammation caused by $PM_{2.5}$ exposure [54]. The increase of COX-2 and PGE2 level have been described during reproductive damage in male rats [55]. Mitochondria are highly dynamic organelles via fission and fusion to modulate their function [56]. DRP1 can promote fission during mitochondria dynamics. After treatment with urban particulate matter (PM), DRP1 highly expressed and mediated inflammatory response in EA. hy926 cells [57]. Previous studies have found that CREB activated DRP1 and led to decrease in StAR in Leydig cells [58], while DRP1 could increase inflammation [59]. $PM_{2.5}$ not only targets Leydig cells, but also targets hypothalamic-pituitary-gonadal (HPG) axis [29]. $PM_{2.5}$ is attributed to suppress HPG axis by inducing hypothalamic inflammation and result the downregulation of testosterone level in a mouse model [24].

Ferroptosis

Ferroptosis is an iron-dependent programmed cell death, characterized by the imbalance of the redox state in cell [60]. Iron plays a critical role in the initiation of ferroptosis. Interestingly, as one of the major mental components of $PM_{2.5}$, iron can overload and trigger ferroptosis in cells and animals [61, 62]. Ferroptosis contributed to targeting CYP11A1 and caused the deficiency of testosterone biosynthesis after treatment with cadmium in Leydig cells [63]. Thus, we hypothesize that ferroptosis may involve in the disruption of testosterone level by $PM_{2.5}$ exposure. Accumulating studies have unveiled that the inhibition of Nrf2 is related with $PM_{2.5}$ exposure-induced ferroptosis [64]. The mechanism of most classic ferroptosis inducers is the inhibition of the antioxidant system. Nrf2 can bind to the antioxidant response element of genes and mediate antioxidant responses in tissues and cells [65]. Nrf2 has been reported to alleviate ferroptosis [66]. Compared with wild-type mice/Leydig cells, the concentration of testosterone was reduced in knockout of Nrf2 mice/Leydig cells, accompanied with reduced antioxidant capacity and the expression of CYP11A1

and StAR [67]. The molecular mechanisms of ferroptosis are also associated with the system Xc−/GSH/GPX4 signaling pathway, PUFA-phospholipid peroxidation, TF/TFR signaling pathway, and p53 signaling pathway [68]. Currently, research on $PM_{2.5}$-induced ferroptosis is limited.

Pyroptosis

Pyroptosis also is a pro-inflammatory programmed cell death associated with caspases and cytokines. The characteristics of pyroptosis include DNA damage, chromatin, swelling, bubble-like protrusions, and membrane blebbing [69]. In canonical pathway of pyroptosis, cytosolic pattern recognition receptors (PRRs) assemble inflammasomes, which can cleave pro-caspase-1 to caspase-1 [70]. On the one hand, activated caspase-1 can cleaves GSDMD to form N-GSDMD [71, 72]. On the other hand, caspase-1 also cleaves pro-IL-1β and pro-IL-18 to activate IL-1β and IL-18 [73, 74]. Active IL-1β and IL-18 are released from the cell membrane pores, which are formed by N-GSDMD, and result in pyroptosis [75, 76]. In the non-canonical pathway, intracellular lipopolysaccharide (LPS) activates caspase-4/5/11, which can also cleave GSDMD into N-GSDMD [71]. Active caspase-4/5/11 and N-GSDMD can mediate the cleavage and secretion of IL-1β and IL-18 via NLRP3 inflammasome/caspase-1 signaling pathway [77, 78]. The activation of NLRP3 inflammasome is one of the mechanisms to mediate $PM_{2.5}$-induced toxicity [50, 52]. Emerging data have suggested that $PM_{2.5}$ causes pyroptosis via NLRP3 inflammasome/caspase-1 in various tissues and cells [79–81]. The activation of NLRP3 inflammasome has also been associated with testicular impairment and the decrease of testosterone level via reducing the expression of testosterone biosynthesis genes such as CYP11A1, CYP17A1, HSD3B, HSD17B and StAR [82–84]. The activation of pyroptosis not only involves in NLRP3 inflammasome, but also link with other inflammasomes, which suggests that they maybe regulate pyroptosis caused by $PM_{2.5}$. At present, studies on the activation of pyroptosis are mostly focused on the NLRP3 inflammasome [74], while studies on the other inflammasomes are rare. In addition, the non-canonical pathway of pyroptosis in testosterone biosynthesis disruption caused by $PM_{2.5}$ is still unclear.

Autophagy and mitophagy

Autophagy is a complex self-degradative process, which has been considered as an important mechanism of $PM_{2.5}$-induced toxicity. However, the functional role and underlying mechanisms of autophagy in testosterone biosynthesis are still unclear. There are three types of autophagy, including macro-autophagy, micro-autophagy, and chaperone-mediated autophagy, while macro-autophagy is the best studied [85]. Macro-autophagy process involves key steps: phagophore formation; completion of autophagosome; fusion of autophagosome with lysosome to form autolysosome, which mediates degradation of protein aggregates, organelles, and ribosomes [86]. Several studies have demonstrated that $PM_{2.5}$-mediated oxidative stress is responsible for autophagy triggered by $PM_{2.5}$ *in vivo* and *in vitro* [87–89]. Autophagy provides a protective effect to inhibit $PM_{2.5}$-induced apoptosis, necrosis, and cytotoxicity [90, 91]. mTOR, AMPK, PI3K-AKT, MAPK, and cAMP signaling pathways have been demonstrated to regulate autophagy [85, 86]. Interestingly, the inhibition of autophagy can improve conversely oxidative stress and inflammation caused by $PM_{2.5}$ [92, 93]. $PM_{2.5}$ cause the increase of LC3B-II/I(means the formation of autophagosomes) and the upregulation of P62 (means the block of autophagosomes), which suggests that the importance of autophagic flux in $PM_{2.5}$-induced toxicity [94, 95]. Recently, a study has shown that $PM_{2.5}$-induced male reproductive injury is accompanied by the downregulation of serum testosterone and the activation of autophagy [1]. When the body is invaded by external substance, autophagy is a double-edged sword. It can resist the damage of external obstacles to the body, but excessive autophagy may have adverse effects and promote cell death. An increasing body of studies has illustrated that activation of autophagy enhances testosterone secretion and increases StAR, HSD3B2, CYP17A1 and CYP11A1 in Leydig cells and testicular tissue [96–98], which have demonstrated that autophagy may resist and reduce the toxicants damage on testosterone biosynthesis. Based on the above findings, we can propose a hypothesis that autophagy may play a protective effect to inhibit $PM_{2.5}$-induced testosterone biosynthesis disruption. However, there is no research about the association between testosterone biosynthesis and autophagy caused by $PM_{2.5}$. Mitophagy is a selective autophagy, given its significance for $PM_{2.5}$-mediated toxicity [99, 100], which has also been observed in the exploration of testosterone biosynthesis [101].

MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are sensitive to environmentally hazardous substances and involve in negatively regulating of the expression of target genes. Importantly, numerous studies have reported that miRNAs are altered and participate in $PM_{2.5}$-induced toxicity in diverse human diseases [102–105]. However, there are limit microRNAs to

be identified as $PM_{2.5}$-sensitive. Potential miRNAs, which are verified to involve in pathological processes in different tissues and cells caused by $PM_{2.5}$ and may be associated with testosterone biosynthesis, are illustrated in Table 2. miR-21 previously found to be positively associated with serum levels of testosterone in patients with breast cancer or polycystic ovary syndrome (PCOS) [106]. However, there is less study to demonstrate the relationship between miR-21 and testosterone level in male. Recent studies have verified that $PM_{2.5}$ exposure induced miR-21 alteration that could trigger vascular endothelial and bronchial epithelial dysfunction [107, 108]. Interestingly, it has demonstrated that testicular vascular damage plays an important role in regulating testosterone such as testicular

Table 2: Potential miRNAs involve in PM2.5-induced testosterone biosynthesis disruption *in vivo* and *in vitro* model.

Model	Dose/duration	Trend of miRNAs which may be associated with testosterone biosynthesis	Effects	Reference
HBE cells	3 μg/cm^2, 24 h	**Up:** miR-375	Inflammation	[114]
Elderly men	3.83 μg/m^3, 7-day	**Down:** miR-1, miR-126, miR-146a, miR-155, miR-21, miR-222, miR-9	Cardiovascular disease	[115]
BEAS-2B cells	3, 12 μg/m^3, 24,48,72 h	**Up:** miR-21	Genotoxicity in lung cells	[116]
BEAS-2B cells	10 μg/cm^3, 24 h	**Up:** miR-1246,	Lung injury	[117]
Human	Time windows (1 day, 1 week, 1 month, 3 months, 6 months, and 1 year)	**Up:** miR-126-3p, miR-19b, miR-93, miR-223, miR-142, miR-23a, miR-150, miR-15a, Let-7a	Cardiovascular disease	[118]
SD rats	0.25, 2.5, and 25 mg per 3 days	**Up:** Let-7b, miR-466b **Down:** Let-7e	Neural diseases	[119]
SD rats, HUVEC cells	4 mg/kg.bw in SD rats per 3 days for 4 weeks, 80 μg/mL in HUVEC cells for 24 h	Up: miR-21	Vascular endothelial dysfunction	[107]
C57BL/6 mice	1 and 5 mg/kg.bw every other day for 4 weeks	**Down:** miR-574	Neural diseases	[120]
A549 cells	5, 50 μg/mL for 24 h	**Down:** Let-7a, miR-34a	Lung cancer	[121]
BALB/c mice	2.5, 10 and 20 mg/kg.bw for 1, 7, 14 days	**Up:** miR-139, miR-146	Lung inflammation	[122]
College students	53.1 μg/m^3	**Down:** miR-21, miR-146a, miR-1, miR-119a	Cardiovascular disease	[123]
Students	21.31 μg/m^3	**Up:** miR-29a, miR-92a	Health risks	[124]
Elderly men	11.67 μg/m^3	**Up:** miR-199b, miR-223	Blood pressure	[105]
HBE and BEAS-2B cells	10, 20, 30 μg/mL for 24 h	**Down:** miR-204	Carcinogenesis	[125]
EA.hy926 cells	2.5, 10 μg/cm^2 for 24 h	**Down:** miR-128, miR-28	Cardiovascular disease	[126]
C57BL/6 mice	900.21 μg/m^3 for 8 weeks, 671.87 μg/m^3 for 16 weeks	**Up:** miR-96, miR-182, miR-183	Testicular damage	[6]
HBECs, MLE-12, RAW264.7 cells	300 μg/cm^3 for 24 h	**Up:** miR-29b	Inflammatory responses	[127]
Wistar rats	1 mL of 1,2,2 mg/mL at day 0, 3, 7	**Down:** miR-125b, miR-21	Inflammatory responses	[128]
SD rats, AC16 cells	1.8, 5.4 and 16.2 mg/kg.bw every 3 days for 1 month in rat, 25 50 100 μg/mL in cells for 24 h	**Down:** miR-205	Cardiovascular diseases	[129]
NCI-H23 and Bet1A cells	5 μg/ml for 15 or 28 days	**Down:** miR-125a	Lung cancer	[130]
BALB/c mice, 16HBE cells	2.5, 10, 20 mg/kg.bw in mice, 25, 50, 100 μg/ml for 24–48 h in cells	**Down:** miR-139	Lung cancer	[131]
HBE cells	50 μg/ml for 24 h	**Down:** miR-145	Lung injury	[132]
HBE cells	50, 100 μg/ml for 24 h	**Down:** miR-222	Lung injury	[133]
Balb/c mice, A549 cells	20 mg/kg.bw in mice, 100 μg/ml for 24 h in cells	**Down:** miR-193b **Up:** miR-100, miR-125b	Lung cancer	[134]
C57BL/6 mice	87 μg/m^3 for 8 weeks	**Up:** miR-10b, miR-466b	Alzheimer's disease	[135]
ApoE$^{-/-}$ mice	157 μg/m^3 for 8 weeks	**Up:** miR-326	Atherosclerosis	[136]
C57BL/6J mice	0.6 mg/mouse once a week for 2 or 3 months	**Up:** miR-149	Pulmonary fibrosis	[137]

blood flow, vascular permeability, and endothelial surface [109], which has suggested that testicular vessels may be another mechanism of the impact of $PM_{2.5}$ on testosterone level. The increased expression of miR-29a is relevant with the decreased androgen production by downregulating the expression of HSD3B1 in Leydig cells [110]. Microarray analysis and real-time PCR analysis revealed that miR-29a positively expressed in associated with $PM_{2.5}$ exposure in human [111]. MiR-146a has recently been shown to be negatively associated with serum testosterone and its related pathways including Toll-like receptor signaling pathway, apoptosis, cell adhesion molecules and NF-kappa B signaling pathway [112]. MiR-146a is found to play an important role in pathological processes caused by $PM_{2.5}$ exposure [113]. Above results confirm the possible contribution of aberrant alteration of miRNA expression to $PM_{2.5}$-induced testosterone biosynthesis disruption in male.

Endoplasmic reticulum stress

As the organelles with the largest surface area in cells, the endoplasmic reticulum (ER) plays a key role in the synthesis, folding, and modifications of secreted proteins, which are synthesized in ER membrane-bound ribosomes and then injected into the ER lumen for next processes [138]. Although the protein-folding capacity of ER is exquisitely regulated, diverse external factors can disrupt this process and lead to ER stress due to the accumulation of unfolded/misfolded proteins in ER lumen [139]. The activation of ER stress initiates the unfolded protein response (UPR) to restore ER homeostasis through an adaptive mechanism [140]. In mammalian cells, inositol-requiring enzyme 1α (IRE1α), activating transcription factor 6 (ATF6) and PRKR-like ER kinase (PERK) are three ER transmembrane proteins which operate as ER stress sensors and initiate the adaptive mechanism [141]. Under conditions of inactivation of ER stress, the molecular chaperone glucose-regulated protein 78 (GRP78, alias BIP, HSPA5) binds with these sensors and persists them in an inactivated state. Once the event of the activation of ER stress occurs, GRP78 has a higher affinity to bind misfolded/unfolded proteins while dissociates from the sensors [142]. The discrete sensors enable induction of UPR. The moderate ER stress restores ER homeostasis, and thus causes cells to adapt to stress and survival. However, excessive, and unresolved ER stress can accelerate cell death. Previous studies have reported that $PM_{2.5}$ could induce ER stress and thus break the balance of ER homeostasis to result in function disorder in various systems [143–145]. Although numerous studies have demonstrated that ER stress also closely involved in testosterone biosynthesis disruption under adverse conditions in Leydig cells [146, 147], it is unclear whether $PM_{2.5}$ cause testosterone disorder through the relevant mechanism of ER stress. Thus, it is of interest to evaluate the involvement of ER stress and explore its related UPR pathway during the period of $PM_{2.5}$-mediated testosterone biosynthesis disruption (Figure 3).

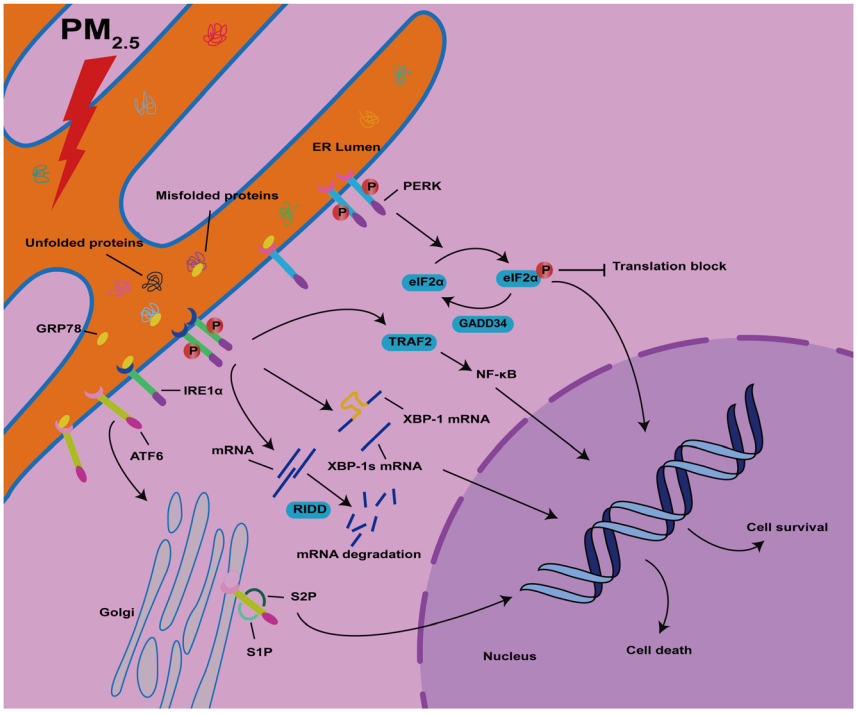

Figure 3: Related toxicity mechanisms involved in the UPR pathway activated by PM2.5-induced ER stress in mammals.

N6-methyladenosine (m6A) RNA modification

m6A RNA modification refers to the addition of a methylation at position N6 of adenosine in almost type of eukaryotes RNAs [148, 149]. m6A modification is the most abundant and common of RNA modifications and an average of 1–2 m6A modification in each 1000 nucleotides [150]. m6A modification has been identified to involved in regulating RNA translation, stability, splicing and translocation, folding and export [151, 152]. m6A modification frequently occurs around the 3′ untranslated regions (UTRs) and near stop codons in mRNAs [153]. In addition, most m6A modifications are enriched in a conserved motif RRACH (R=A/G, H=A/C/U) [154]. The m6A modification is catalyzed by methyltransferase complex (also called "writers", including METTL3/14/16, RBM15, VIRMA, ZC3H13 and WTAP), removed by demethylases (also called "erasers", including ALKBH5, and FTO), and recognized by a group of binding proteins (also called "readers", including YTHDC1, HNRNPA2B1, HNRNPC, and YTHDF1/2/3) [155]. Several studies have suggested that m6A modification plays a significant role in testicular injury and the decrease of testosterone concentration caused by exogenous toxicants via regulating the expression of testosterone biosynthesis-related enzymes *in vivo* and *in vitro* models [156, 157]. $PM_{2.5}$ can alter m6A modification level and the expression of RNA modulator gene [158]. Currently, only m6A writer METTL3 and METTL16 have been identified to participate in $PM_{2.5}$-induced injury [159, 160], while it is lack of research on m6A erasers, readers, and other writers.

Conclusions

$PM_{2.5}$ can lead to many adverse effects on male reproductive system, especially in developing countries which face to the challenge of air pollution. Testosterone plays an important role in maintaining male reproductive health, especially for fertility. Although $PM_{2.5}$ has a negative impact on male testosterone biosynthesis, few studies have focused on the potential mechanisms. In this review, we aim to explore the potential mechanisms of $PM_{2.5}$ toxic effects on testosterone level carefully and accurately according to scientific studies. We summarized relevant mechanisms, including oxidative stress, inflammatory response, ferroptosis, pyroptosis, autophagy and mitophagy, miRNAs, ER stress, and m6A modification, which might provide the basis for prevention and treatment of male testosterone level disruption caused by $PM_{2.5}$. In the future, researchers should pay more attention on the toxic mechanisms of $PM_{2.5}$ to testosterone biosynthesis. It is wish that under the theoretical framework of this review, researchers can find more exact mechanisms of these effects.

Highlights

$PM_{2.5}$ causes male testosterone biosynthesis disruption according to existing epidemiological and experimental studies.

Testosterone biosynthesis is closely regulated via complex pathways involving multiple catalytic enzymes.

$PM_{2.5}$-induced testosterone biosynthesis disruption may lead to various adverse outcome on male health.

Potential toxic mechanisms of $PM_{2.5}$ are oxidative stress, inflammatory response, ferroptosis, pyroptosis, autophagy and mitophagy, miRNAs, ER stress, and m6A modification.

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