HHS Public Access

Author manuscript

Adv Redox Res. Author manuscript; available in PMC 2024 April 01.

Published in final edited form as:

Adv Redox Res. 2024 April ; 10: . doi:10.1016/j.arres.2023.100090.

Maternal nano-titanium dioxide inhalation exposure alters placental cyclooxygenase and oxidant balance in a sexually dimorphic manner

Julie A. Griffith[a,b], **Rachel D. King**[c], **Allison C. Dunn**[a,b], **Sara E. Lewis**[a], **Brooke A. Maxwell**[a], **Timothy R. Nurkiewicz**[a,b], **William T. Goldsmith**[a,b], **Eric E. Kelley**[a,b], **Elizabeth C. Bowdridge**[a,b,*]

[a]Department of Physiology, Pharmacology and Toxicology, West Virginia University School of Medicine, Morgantown, WV, USA

[b]Center for Inhalation Toxicology, West Virginia University School of Medicine, Morgantown, WV, USA

[c]Department of Biochemistry, West Virginia University School of Medicine, Morgantown, WV, USA

Abstract

The placenta plays a critical role in nutrient-waste exchange between the maternal and fetal circulation, and thus impacts fetal growth and development. We have previously shown that nano-titanium dioxide (nano-TiO_2) inhalation exposure during gestation decreased fetal female pup and placenta mass [1], which persists in the following generation [2]. In utero exposed females, once mated, their offspring's placentas had increased capacity for H_2O_2 production. Generation of oxidants such as hydrogen peroxide (H_2O_2), have been shown to impact cyclooxygenase activity, specifically metabolites such as prostacyclin (PGI_2) or thromboxane (TXA_2). Therefore, we hypothesized that maternal nano-TiO_2 inhalation exposure during gestation results in alterations in placental production of prostacyclin and thromboxane mediated by enhanced H_2O_2 production in a sexually dimorphic manner. Pregnant Sprague-Dawley rats were exposed to nano-TiO_2 aerosols or filtered air (sham--control) from gestational day (GD) 10–19. Dams were euthanized on GD 20, and fetal serum and placental tissue were collected based on fetal sex. Fetal placental zones (junctional zone (JZ) and labyrinth zone (LZ)) were assessed for xanthine oxidoreductase

*Corresponding author at: Department of Physiology, Pharmacology, and Toxicology, University of West Virginia, School of Medicine, 3076 Health Sciences Center North, PO Box 9229, Morgantown, WV 26506-9229, USA. ebowdrid@hsc.wvu.edu (E.C. Bowdridge).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Julie A. Griffith: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Rachel D. King:** Data curation, Investigation, Writing – review & editing. **Allison C. Dunn:** Data curation, Investigation, Writing – review & editing. **Sara E. Lewis:** Data curation, Investigation, Writing – review & editing. **Brooke A. Maxwell:** Data curation, Investigation, Writing – review & editing. **Timothy R. Nurkiewicz:** Formal analysis, Funding acquisition, Writing – review & editing. **William T. Goldsmith:** Methodology, Writing – review & editing. **Eric E. Kelley:** Resources, Supervision, Writing – review & editing. **Elizabeth C. Bowdridge:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing.

(XOR) activity, H_2O_2, and catalase activity, as well as 6-keto-$PGF_1\alpha$ and TXB_2 levels. Nano-TiO_2 exposed fetal female LZ demonstrated significantly greater XOR activity compared to exposed males. Exposed fetal female LZ also demonstrated significantly diminished catalase activity compared to sham-control females. Exposed fetal female LZ had significantly increased abundance of 6-keto-$PGF_1\alpha$ compared to sham-control females and increased TXB_2 compared to exposed males. In the aggregate these data indicate that maternal nano-TiO_2 inhalation exposure has a greater impact on redox homeostasis and PGI_2/TXA_2 balance in the fetal female LZ. Future studies need to address if treatment with an XO inhibitor during gestation can prevent diminished fetal female growth during maternal nano-TiO_2 inhalation exposure.

Keywords

Titanium dioxide; Thromboxane; Prostacyclin; Sexual dimorphism; Oxidants; Xanthine oxidoreductase

Introduction

Historically, the placenta has been considered an asexual organ, however the fertilized egg gives rise to both fetus and placenta, thus the placenta and fetus are the same sex [1,3,4]. Sex differences in fetal development and growth have long been established, with males having accelerated growth rates relative to females, which can be observed as early as the blastocyst stage [5–7]. Additionally, growth of the placental unit is also sex dependent. In humans, male placentas exhibit greater placental growth (mass) compared to females, which facilitates the increased growth demands of the male fetus [8]. Blood flow to the placenta also differs between sexes with male fetuses from normotensive pregnancies having increased blood flow over time compared to female fetuses [9]. Sex also impacts placental nutrient transfer. Female placentas exhibit increased fatty acid oxidation, purine degradation, and uric acid levels, indicating that females may be more susceptible to oxidative stress [10]. Additional disease states [11,12] or environmental insults [13,14] can further alter and exacerbate oxidative stress within the placenta.

Oxidants are produced through aerobic metabolism [15] and can impair cellular function through damage to proteins, lipids, and DNA [16]. To combat oxidants, the body produces antioxidants to maintain a homeostatic balance of these redox compounds [15]. This balance is especially important in early gestation as redox signaling is required for placentation [15]. A study of hexavalent chromium (a by-product of industrial processes involving stainless steel or metals containing chromium [17]) exposure during gestation in humans found that male placentas had increased oxidative stress markers compared to females and exhibited a greater reduction in antioxidant activity of the enzymes, catalase and superoxide dismutase (SOD) [18]. Hydroxyl radicals ($HO^\bullet$ and hydroperoxyl, $HO_2^\bullet$) can result in lipid peroxidation and $HO^\bullet$ is thought to be formed *via* the interaction of H_2O_2 and free iron (Fe^{2+}) [19]. The hydroperoxyl radical, $HO_2^\bullet$, can yield H_2O_2 which can further react with metals, such as iron or copper, to further generate more $HO^\bullet$ [19]. To add to this complex system, COX, along with lipoxygenase, have been demonstrated to be minor endogenous sources of these oxidants [20–23]. The rat and human placenta have been shown to express

increasing cyclooxygenase (COX)-1 and COX-2, with COX-2 expression towards parturition [24]. In a mouse model, oxidative stress increased close to term and the authors proposed that this played a physiologic role by increasing prostaglandin synthesis, some of which helped to induce parturition [25]. Nano-TiO_2 (a white powder used for its optical properties in paint or photocatalyst [26]) inhalation exposure during gestation increased maternal liver oxidant load and placentas from nano-TiO_2 exposed dams had greater H_2O_2 production capacity [2]. This indicates that nano-TiO_2 exposure during gestation may increase oxidant balance.

Maternal nano-TiO_2 inhalation exposure during gestation increased inflammatory signals from the maternal lung, such as interleukin (IL)-4, -5, and -13 [27]. We also previously demonstrated increased prostacyclin synthase and decreased prostacyclin receptor mRNA expression in the exposed dam lungs [28]. Pulmonary inflammatory signals may enter the circulation due to the interdependence of the pulmonary and cardiovascular systems and therefore act systemically throughout the body. Nano-TiO_2 inhalation exposure decreased endothelium-dependent relaxation in thoracic and femoral arteries [26], uterine radial arterioles [29], and increased uterine radial arteriole vasoconstriction in response to TXA_2 mimetic, U46619 [28]. Additionally, maternal nano-TiO_2 inhalation exposure during gestation increased maternal hepatic mass and H_2O_2 production capacity [2], and decreased GSH: GSSH ratio. Fetal female placental outflow [1] was decreased in response to cyclooxygenase metabolites after maternal nano-TiO_2 inhalation exposure during gestation. Hepatic oxidant production is linked to vascular oxidant production and atherosclerosis [30]. Changes in redox balance during gestation may underlie the hindered fetal development and increased cyclooxygenase vasoreactivity. Whole, placental samples had increased mass [31] and H_2O_2 production capacity [2] from nano-TiO_2 exposed dams (highlighted in Fig. 1). Due to the inflammatory changes within the lungs, microcirculatory adaptations in COX metabolites, and liver oxidant signaling changes, we sought to investigate the changes that occurred within distinct placental zones and how fetal sex impacted these changes. This led to our hypothesis that maternal nano-TiO_2 inhalation during gestation increased placental production of PGI_2 and TXA_2 through H_2O_2 in a sexually dimorphic manner.

Materials and methods

Nanomaterial

Nano-TiO_2 powder was obtained from Evonik (P25 Aeroxide TiO_2, Parsippany, NJ) and is composed of a mixture of anatase (80 %) and rutile (20 %) TiO_2. Particle characteristics have previously been determined, including primary particle size (21 nm), specific surface area (48.08 m^2/g), and Zeta potential (−56.6 mV) [32].

Inhalation exposure and aerosol characterization

A high-pressure acoustical generator (HPAG, IEStechno, Morgantown, WV) created nano-TiO_2 aerosols from bulk TiO_2 dust. These aerosols were fed into a Venturi pump (JS-60M, Vaccon, Medway, MA) to further de-agglomerate the particles. The aerosols were passed into a stainless-steel whole-body exposure chamber (Cube 150, IEStechno, Morgantown, WV) where concentration levels were monitored with a light scattering device (pDR-1500;

Thermo Environmental Instruments Inc, Franklin, MA) and regulated with software driven feedback loops to maintain a stable mass aerosol concentration. Real-time aerosol size distributions were regulated in the exposure chamber at a target mass concentration of 12 mg/m^3 for 6 h each exposure day. Similar exposure chambers were utilized only for sham-control dams which were exposed to HEPA-filtered air only. In order to increase the likelihood of a viable pregnancy and pups to study, pregnant rats were exposed after implantation during mid to late gestation (gestational day [GD] 10–19) for a total of 6 days. The last exposure occurs on GD 19, 24 h prior tissue collection. The exposure paradigm and aerosol characterization utilized in this study have been previously described [1].

Animal model

Female Sprague-Dawley (SD) rats were purchased from Hilltop Laboratories (Scottdale, PA) and single-housed in an American Association for Accreditation of Laboratory Animal Care (AAALAC) approved facility at West Virginia University (WVU). Temperature (20–26 °C), relative humidity (30–70 %), and light-dark cycle (12:12 h) was maintained for housed rats. After 48–72 h for acclimation, rats were randomly assigned to either sham-control ($N = 13$) or nano-TiO_2 ($N = 14$) exposure groups. Rats had ad libitum access to standard chow (2918X; Envigo, Indianapolis, IN) and water throughout the acclimation and exposure periods. Rats were euthanized *via* thoracotomy and heart removal and then fetal distribution within the uterine horns and fetal sex were recorded. Fetal tissue was weighed and grouped according to fetal sex: placental junctional zone (endocrine production site), and placental labyrinth zone (nutrient-waste exchange), serum, liver, and lung were collected. Placental junctional zone (JZ) and labyrinth zone (LZ) were bluntly dissected [33], flash frozen in liquid nitrogen, and stored at −80 °C until use. All procedures were approved by the WVU Institutional Animal Care and Use Committee.

Briefly, once dams were euthanized and pup count was recorded (previously published [1]), the uterus was surgically excised and placed into a dissection dish containing physiological salt solution (PSS, in mmol/L: 129.8 NaCl, 5.4 KCl, 0.5 NaH_2PO_4, 0.83 $MgSO_4$, 19.0 $NaHCO_3$, 1.8 $CaCl_2$, 5.5 glucose). The uterus was incised longitudinally, and amnionic sacs were opened to allow for quick identification of fetal sex. Fetal sex and position within the horn was recorded, then the first male and female nearest the cervix were removed with the placenta still attached. This unit was utilized for placental hemodynamic assessment and has been previously published [1]. The next set of male and female units were collected for H&E and IHC assessment and has been previously published [1]. The remaining males and females in the litter were utilized for serum and tissue collection for this study.

Serum collection and protein isolation

Fetal blood samples (500 μL) collected by cardiac puncture were collected and pooled based on sex for each litter (BD SST Microtainer Tubes; Becton, Dickinson and Co, Franklin Lakes, NJ). Samples were allowed to clot at 20 °C for 30 min and centrifuged at 10,000 × g for 90 s. Serum was collected and flash frozen until use. JZ and LZ were individually homogenized with a bead mill homogenizer (1.6 mm stainless steel beads; Next Advance, Troy, New York) in phosphate buffered saline (PBS; Fisher Scientific, Waltham,

MA) containing a protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA). Homogenates were centrifuged at 4 °C and 14,000 × g for 15 min using a tabletop centrifuge (Hettich Lab Instrument AB, Stockholm, Sweden). Supernatant was collected, kept on ice, and total protein concentrations assessed according to the Bradford Method [34]. Isolated protein samples were stored at −80 °C until assayed.

Xanthine oxidoreductase activity

JZ and LZ samples were homogenized in ice cold RIPA containing protease inhibitor cocktail (Sigma) and were briefly spun down. Tissue samples were processed with a sample volume of 10 μL. Potential urate oxidase (uricase) activity was inhibited by the addition of oxonic acid (100 μM) to ensure UA levels were not altered and thus enzyme activity was not underestimated. Total UA production in 60 min at 37 °C in the presence of xanthine (75 μM) served as the basis for quantification of total xanthine oxidoreductase (XOR) activity. To ensure XO dependence on urate formation as well as establish base-line UA levels, allopurinol (100 μM) was used in parallel samples to inhibit XOR. Following incubation for 60 min at 37 °C, protein was precipitated with ice cold acetonitrile. The samples were centrifuged for 12 min at 13,200 × g, at 4 °C. Following centrifugation, the supernatant was removed, placed in borosilicate glass tubes, dried (60 min), and resuspended in isocratic mobile phase (300 μL) and filtered through 0.20 μm nylon membrane filter unit into 11 mm plastic snap top auto-sample vials. The UA content was measured by electrochemical detection (Vanquish UltiMate 3000 ECD-3000RS) coupled to reverse-phase HPLC using a C18 column (150 × 4.6 mm, 3 μm particle size, Luna Phenomenex) and isocratic mobile phase (50 mM sodium dihydrogen phosphate, 4 mM dodecyl-trimethylammonium chloride, 2.5 % methanol, pH 7.0). One unit of activity (U) was defined as 1 μmole/min urate formed at 37 °C and pH 7.4. Results were expressed as a concentration relative to total sample protein content [35]. Inter- and intra-assay variability were less than 10 %.

Hydrogen peroxide activity assay

Placental zone samples were assessed for hydrogen peroxide activity using the Amplex™ Red Hydrogen Peroxide/Peroxidase Assay Kit (A22188; Invitrogen, Waltham, MA) and performed according to manufacturer recommendations. Inter- and intra-assay variability were less than 10 %.

Catalase activity assay

Placental zone tissue and serum samples were assayed for catalase activity using the Catalase Activity Assay Kit (ab83464; Abcam, Cambridge, U.K.) and performed according to manufacturer recommendations. Inter- and intra-assay variability were less than 10 %.

Enzyme-linked immunosorbent assays (ELISA)

Fetal tissue extracts and serum were diluted with assay buffer and assays were performed to assess TXB_2 (501020; Thromboxane B2 Elisa Kit) and 6-keto-$PGF_1\alpha$ (515211, 6-keto Prostaglandin $F_1\alpha$ Elisa Kit) according to manufacturer recommendations (Cayman Chemical Company, Ann Arbor, MI). Inter- and intra-assay variability were less than 10 %.

mRNA analysis by real-time PCR

Total RNA was extracted from placental zones, fetal lungs, and resorption sites *via* the RNeasy Kit based on manufacturers recommendations (74104; Qiagen, Hilden, Germany). Total RNA was then transcribed to cDNA *via* the High-Capacity RNA-to-cDNA kit (4387406; Thermo Fisher Scientific, Waltham, MA).

Using real-time PCR, prostacyclin synthase (*Ptgis*), prostacyclin receptor (*Ptgir*), thromboxane receptor (*Tbxa2r*), superoxide dismutase (*Sod1*), catalase (*Cat*), estrogen receptor alpha (*Esr1*), and estrogen receptor beta (*Esr2*) were assessed. Primers were purchased from Integrated DNA Technologies (Coralville, IA); GenBank accession numbers, primer sequences, and references are listed in Table 1. Samples were analyzed in triplicate using iTAQ Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). Relative fold changes in expression of candidate genes were obtained using the $2^{-\Delta\Delta Ct}$ method [36]. The Ct values were used to calculate ΔCt values for genes of interest [Ct(test) – Ct(housekeeping)]. A housekeeping panel consisting of *beta actin, ribosomal small subunit 18*, and GAPDH was run on each tissue. Across tested tissue types, GAPDH was the most stable housekeeping gene and therefore tissues were normalized to GAPDH (*gapdh*). Statistical representation for each gene is based on fold change with respect to GAPDH.

Statistics

Values for xanthine oxidase, catalase, hydrogen peroxide, results from tissue ELISA's, and mRNA analyses were assessed *via* a two-way mixed-effects ANOVA. The two-way mixed-effects ANOVA was utilized to compare exposures and fetal sex in a two-by-two comparison. Litter size was nested within the two-way ANOVA to account for differences in pup number between dams. Exposure effects, sex effects, and an interaction of exposure and sex were assessed. If exposure, sex, or an interaction occurred, then individual group differences could be assessed with post-hoc tests. If statistical significance was noted, then Šídák›s post-hoc test was used. All statistical analysis were performed with Graph Pad Prism 9 (San Diego, CA). Significance was set at $p \leq 0.05$, with N representing the number of litters per group. All data is reported as the mean ± SEM, unless otherwise stated.

Results

Nano-TiO_2 exposed fetal females have increased heart mass and decreased liver mass compared to nano-TiO_2 exposed fetal males

An exposure effect was observed with fetal heart mass, with nano-TiO_2 exposed hearts being significantly heavier (Fig. 2A). A sex effect was seen in heart mass, of which fetal females had significantly heavier hearts. Additionally, there was an interaction between exposure and sex, whereby nano-TiO_2 fetal females had significantly increased heart mass compared to nano-TiO_2 fetal males and sham-control fetal females (Fig. 2A). This same pattern persists with normalized fetal heart mass normalized to total fetal mass. There was an exposure effect, sex effect, and an interaction of exposure and sex. Nano-TiO_2 fetal female normalized heart mass was significantly increased compared to nano-TiO_2 fetal males and sham-control fetal females (Fig. 2B).

No exposure effect, sex effect, or an interaction of sex and exposure was seen with fetal lung tissue (Fig. 2C). Normalized fetal lung mass to fetal mass had an exposure effect, which nano-TiO_2 had decreased normalized lung tissue compared to sham-control (Fig. 2D). There was no effect of sex or an interaction of exposure and sex.

Fetal liver mass did not have an exposure effect or sex effect but did have a trend (p = 0.054) towards an interaction of exposure and sex. Nano-TiO_2 fetal female livers trended (p = 0.051) towards significantly decreased in mass compared to nano-TiO_2 exposed fetal male liver mass (Fig. 2E). Normalized fetal liver mass to total fetal mass did not have an exposure effect or sex effect but trended (p = 0.054) towards an interaction of exposure and sex (Fig. 2F). Nano-TiO_2 fetal female normalized liver mass was significantly decreased compared to sham-control fetal female and nano-TiO_2 fetal male normalized liver masses.

XOR activity is decreased in nano-TiO_2 fetal female JZ versus sham-control female JZ

In the JZ, there was a significant effect of exposure on XOR activity, with decreased XOR activity in nano-TiO_2 JZ compared to sham-control JZ (Fig. 3A). There was not an effect of fetal sex, nor an interaction of exposure and fetal sex. In the LZ, there was not an effect of exposure on XOR activity, but there was a significant effect of fetal sex on XOR activity. Female LZ XOR activity was significantly increased compared to males. LZ XOR activity was increased in sham-control females compared to sham-control males and in exposed fetal females compared to exposed fetal males (Fig. 3B). There was no interaction of exposure and fetal sex.

Hydrogen peroxide levels are decreased in nano-TiO_2 fetal male JZ

In the JZ, there was a trend (p = 0.06) for an exposure effect, where nano-TiO_2 JZ's exhibited decreased H_2O_2 concentration compared to sham-control JZ's (Fig. 3C). There was no difference for sex effect or an interaction of exposure and sex. In the LZ, there were no differences among exposure, sex, or an interaction between groups for H_2O_2 concentration, Fig. 3D.

Catalase activity was decreased in nano-TiO_2 fetal female LZ versus sham-control fetal female

An exposure effect was not seen in fetal serum catalase activity. Catalase activity in fetal serum differed in a sex-dependent manner, which levels were greater in males than females. There was no interaction between exposure or sex. Fetal serum catalase activity was significantly less in sham-control females compared to sham-control males (Fig. 4A) and in nano-TiO_2 exposed pups.

In the JZ, exposed groups demonstrated significantly decreased levels of catalase activity than nonexposed groups (Fig. 4B), but there was no effect of sex or an interaction of sex and exposure. Catalase activity within the LZ was impacted by exposure as catalase activity was significantly diminished in exposed than sham-control groups. There was also a trend (p = 0.08) toward an effect of sex. Females had increased catalase activity compared to males. LZ catalase activity was decreased in nano-TiO_2 exposed fetal females compared to

sham-control fetal females (Fig. 4C). Nano-TiO_2 exposed fetal males catalase activity was not different compared to sham-control males.

Fetal female LZ TXB_2 levels were increased compared to sham-control females

There were significant effects of exposure, sex, and an exposure by sex interaction for circulating levels of fetal 6-keto-$PGF_1\alpha$. Nano-TiO_2 fetal serum had increased 6-keto-$PGF_1\alpha$ compared to sham-control fetal serum. Fetal females had increased 6-keto-$PGF_1\alpha$ compared to males. Due to an exposure by sex interaction, there was significantly increased levels of 6-keto-$PGF_1\alpha$ in exposed fetal females compared to exposed males and sham-control females (Fig. 5A). In contrast, no effect of exposure was observed in circulating levels of fetal TXB_2. However, TXB_2 demonstrated an effect of sex as levels were increased in fetal females compared to males. TXB_2 was increased for sham-control females compared to sham-control males (Fig. 5B). There was no interaction between exposure and sex.

Levels of 6-keto-$PGF_1\alpha$ in the JZ did not show an effect of exposure, sex, or interaction (Fig. 5C). However, concentrations of TXB_2 in JZ were not impacted by exposure but were significantly impacted by sex. TXB_2 JZ concentration was increased in females compared to males. JZ TXB_2 concentrations had a significant interaction of exposure and sex. JZ TXB_2 levels were significantly increased in exposed fetal females compared to exposed fetal males (Fig. 5D) and sham-control fetal females. There was not a difference between sham-control females and males.

There was a significant effect of exposure on levels of 6-keto-$PGF_1\alpha$ in LZs as values were increased in exposed groups. There was no effect of sex or an interaction of exposure by sex. LZ 6-keto-$PGF_1\alpha$ was increased in exposed fetal females compared to sham-control fetal females (Fig. 5E), the same pattern exists for fetal males. Conversely, LZ TXB_2 levels were significantly affected by exposure, sex, and their interaction. TXB_2 concentrations within the LZ were significantly increased in exposed LZs compared to sham-control. Fetal female LZ TXB_2 levels were increased compared to fetal male LZ's. TXB_2 concentrations was significantly increased in the exposed fetal female LZ compared to exposed males and sham-control female LZ's (Fig. 5F).

Exposed fetal female JZ had decreased catalase mRNA expression

JZ prostacyclin receptor gene expression was not impacted by exposure but was significantly affected by sex and the interaction of sex and exposure. Nano-TiO_2 exposed fetal JZ had significantly increased prostacyclin receptor mRNA expression compared to exposed fetal male JZs (Table 2) and sham-control females. JZ catalase expression was not different for exposure, but was significantly impacted by sex and the interaction of sex and exposure. Wherein catalase expression in exposed females was decreased compared to exposed males (Table 2). JZ mRNA expression was not different for thromboxane receptor, prostacyclin synthase, superoxide dismutase, ERα, or ERβ (Table 2). Statistical differences were not detected in LZ mRNA expression for any of the evaluated targets across groups.

Fetal lung and implantation sites, where the embryo embeds into the uterus and is the last maternal interface that attaches to the placenta, were assessed for cyclooxygenase metabolites, prostacyclin synthase, prostacyclin receptor, and thromboxane receptor. Fetal

lung thromboxane receptor mRNA expression expressed a trend for an effect of exposure ($p = 0.06$). Expression of thromboxane receptor mRNA was decreased in exposed fetal lung compared to sham-control, especially in male nano-TiO2 fetal lung (Table 3). There were no differences in fetal lung mRNA expression of prostacyclin receptor or prostacyclin synthase. Implantation site expression of prostacyclin synthase was not different for exposure but was impacted by sex and the interaction of sex and exposure. Nano-TiO2 exposed fetal female implantation sites had significantly increased prostacyclin synthase mRNA expression compared to exposed male counterparts (Table 3) and sham-control females. There were no significant effects of exposure or sex for implantation sites in mRNA expression of thromboxane receptor or prostacyclin receptor.

Discussion

This study aimed to identify the relationship between H_2O_2 and the cyclooxygenase metabolites, PGI2 and TXA2, in male and female feto-placental units after maternal nano-TiO_2 inhalation exposure during gestation. We have previously demonstrated that maternal nano-TiO_2 inhalation exposure during gestation reduces fetal female mass, female placenta mass and area, as well as placenta immune cell invasion [1]. Liver and placental tissues from dams directly exposed to nano-TiO_2 exhibit increased indices of oxidant stress and an increased H_2O_2 abundance, indicating that these tissues have increased oxidant load [2]. As adults, females exposed to nano-TiO_2 *in utero* exhibited decreased circulating estrogen levels during gestation, decreased litter size, and decreased pup mass [2]. This suggests that maternal nano-TiO_2 inhalation exposure during gestation not only impacts redox balance in the dam but also impacts female feto-placental growth and development during gestation and later in life. Modifications in redox balance could potentially contribute to the reduced cyclooxygenase metabolite vasoreactivity in dam uterine radial arterioles [28] and/or decreased placental outflow in the presence of U46619, the stable TXA_2 mimetic, in exposed fetal females [1] previously shown. In this study, placentas from female pups whose mothers were exposed to nano-TiO_2 during gestation had increased XOR activity and decreased catalase activity, which indicates an oxidative stress/antioxidant imbalance. Taken together, placentas from female pups exposed *in utero* demonstrate redox and cyclooxygenase imbalance, impacts placental nutrient-waste exchange, and ultimately fetal growth and development.

It is important to note that toxicant exposure consistently increases oxidant production [37], decreases catalase activity [38], and increases prostacyclin metabolite production [39], in HUVECs or guinea pig alveolar cells. This implies that toxicants, such as nano-TiO_2, can modify the balance between oxidants and antioxidants, thus disrupting downstream signaling by molecules such as prostacyclin or thromboxane. Additionally, placental growth occurs in a hypoxic environment, with oxygen tension residing at 2–3 % (25–38 μM dissolved O_2) in the first trimester and beginning of the second [40]. This becomes important when considering that the low O_2 tension diminishes superoxide generation by xanthine oxidase (XO), thus elevates H_2O_2 production to nearly 90 % [41]. Our current study found that nano-TiO_2 exposed females had increased XDH activity. This will lead to increased oxidants and uric acid production, which may increase placental inflammation and insufficiency, both of which we have previously demonstrated in exposed fetal females [1].

This implies that increased XOR in these exposed fetal females could contribute to their increased placental resistance and modified placental structure, thus impacting fetal female growth and development.

XOR can generate superoxide, H_2O_2, and NADH when transferring electrons from xanthine to oxygen and NAD^+ [42]. Due to the increased XOR activity found in exposed fetal female placentas, we examined H_2O_2 levels. No differences were observed in H_2O_2 levels across fetal sex or placental zones. This differs with previous results from our laboratory, which demonstrated increased placental capacity to produce H_2O_2 in collected tissue from nano-TiO_2 exposed dams [2]. In a mouse model of gestational exposure to hypoxia, placentas from hypoxic male fetuses were heavier than the hypoxia exposed fetal female placentas [43], and the hypoxic fetal female placentas demonstrated increased oxidative stress markers. This study highlights that oxidative stress is impacted by fetal sex. In homeostasis, redox balance is maintained *via* the action of small molecule antioxidants and enzymes such as catalase. Herein, we demonstrated that catalase activity in nano-TiO_2 females was significantly decreased within the LZ. Therefore, the decreased growth in female fetuses previously shown to occur due to maternal nano-TiO_2 inhalation exposure [1] may be explained, at least in part, by oxidative imbalance within the LZ, where nutrient-waste exchange occurs.

Previous results from our laboratory demonstrated significantly decreased circulating TXB_2 levels in nano-TiO_2 exposed dams [28]. Based on these results, both 6-keto-$PGF_1\alpha$ and TXB_2 are important vasoactive compounds, thus it was necessary to measure placental zone and fetal serum levels of 6-keto-$PGF_1\alpha$ and TXB_2. Increased circulating levels of 6-keto-$PGF_1\alpha$ were found in the fetal females from nano-TiO_2 exposed dams, which could be indicative of an elevated immune response. Complications during gestation, such as asthma and gestational diabetes, have been associated with increased placental expression of inflammatory immune markers such as, TNF-α, IL-1β, IL-6, and IL-8 [44,45]. Further, Hofbauer cells, which are fetal derived macrophages, have increased production of pro-inflammatory cytokines in gestational diabetes [45]. Maternal nano-TiO_2 exposure significantly increased female placenta Hofbauer cell invasion [1] and maternal circulating IL-1β and TNFα [27]. IL-1β is reported to induce COX-2 expression [46–48], which is the enzyme that catalyzes the production of PGI_2 [48], and could promote this increased 6-keto-$PGF_1\alpha$ levels in fetal females from nano-TiO_2 exposed dams. Placental tissue, specifically the LZ, exhibited significantly increased 6-keto-$PGF_1\alpha$ and TXB_2 levels from fetal females that were exposed *in utero*. Human pregnancies with intervillous blood flow below the normal mean (less than 130 mL/min) exhibit increased placental TXA_2 production [49]. In a preeclampsia mouse model that simulated modified blood flow to the fetoplacental unit, preeclampsia mice placentas had elevated TXA_2 synthase and plasma TXA_2, but not prostacyclin levels [50]. This indicates that the balance of TXA_2 and PGI_2 levels is critical in regulating blood flow to the feto-placental unit. A rat intrauterine growth restriction (IUGR) study found that IUGR male umbilical arteries had decreased maximal contraction in response to U46619, the TXA_2 mimetic, compared to IUGR females [51]. This highlights that changes in placental production of TXA_2, and PGI_2 to a lesser extent, impact blood flow and further impact fetal growth and development in a sex-based manner. It is possible that the redox imbalance, specifically the increased XOR-catalyzed peroxide production and

decreased catalase activity within the LZ, leads to the overall increase in PGI_2 and TXA_2 levels seen in the LZ of exposed fetal females. This would ultimately result in modified uteroplacental blood flow [1], as we have observed previously, and impair fetal growth and development due to modified redox homeostasis.

However, with all this information, there are a few study limitations that need to be addressed. The first limitation is that we do not know where the XO is coming from. A way to address this concern would be the incorporation of an XO-tissue specific knock-out mouse model. We would need to run a few small studies on wild-type mice first, to verify that the pregnant mice would respond to nano-TiO_2 inhalation exposure during gestation similarly to our current rat model. Further, we do not have any febuxostat data in this study, to verify that XO could be driving these changes seen in H_2O_2 and catalase activity after maternal nano-TiO_2 inhalation exposure during gestation. This is already an on-going study in our laboratory. Another limitation that should be addressed is the difference of H_2O_2 levels seen in this study and a previous one from our laboratory [2]. The differences are most likely caused by a few things. First, herein, the placentas were split into the two main zones, JZ (the endocrine production site) and the LZ (nutrient-waste exchange site), previously the whole placenta was assessed. Second, this study assessed the impacts of fetal sex on placental tissue, which fetal sex does matter in relation to placental and fetal tissue assessment. The sex of the fetus was not accounted for in the previous study. Lastly, herein, we utilized an entirely different assay, and measurement for H_2O_2. In this study, we utilized an Amplex Red H_2O_2 kit, which measured the amount of H_2O_2. Whereas the previous study used a Coumarin boronic acid (CBA) assay, which measures the production rate of H_2O_2. These limitations are important to note and are points that are planned to be addressed in future experiments in our laboratory.

In conclusion, this study sought to determine if maternal nano-TiO_2 inhalation exposure resulted in modified redox homeostasis that altered production of PGI_2/TXA_2 within the placental zones in a sexually dimorphic manner. Our exposure paradigm has provided evidence that maternal nano-TiO_2 inhalation exposure modified redox homeostasis within placental zones and impacted fetal female growth and mass, placental development, and hemodynamics [1]. These modifications likely impact COX metabolite production, ultimately modifying fetal growth in a sexually dimorphic manner, with the greatest growth impacts on exposed females (Fig. 6). These adaptations in the exposed fetal female likely ensures survival throughout gestation but could have lasting negative health impacts on females later in life. Future studies should focus on if treatment with an XO inhibitor, such as febuxostat, during gestation can prevent diminished fetal female growth and development during maternal nano-TiO_2 inhalation exposure. This will help elucidate if dysregulated redox homeostasis underlies the fetal and placental deficits observed after exposure and provide a possible treatment option to women who have imbalanced redox and COX metabolites.

Acknowledgments

The authors are grateful to Dr. Stan Hileman for his review comments on the manuscript.

Funding

This work was supported by the following sources: National Institutes of Health K01 10029010 (ECB), WV-CTSI U54 GM104942-05 (ECB), R01 ES015022 (TRN), T32 AG 52375 (JAG), T32 ES032920 (JAG), WV-INBRE P20 GM103434.

Data availability

Data will be made available on request.

Abbreviations:

COX	cyclooxygenase
GD	gestational day
H_2O_2	hydrogen peroxide
JZ	junctional zone
LZ	labyrinth zone
PGI_2	prostacyclin
SOD	superoxide dismutase
TXA_2	thromboxane
XDH	xanthine dehydrogenase
XO	xanthine oxidase
XOR	xanthine oxidoreductase

References

[1]. Griffith JA, Dunn A, DeVallance E, Schafner KJ, Engles KJ, Batchelor TP, Goldsmith WT, Wix K, Hussain S, Bowdridge EC, Nurkiewicz TR, Maternal nano-titanium dioxide inhalation alters fetoplacental outcomes in a sexually dimorphic manner, Front. Toxicol. 5 (2023), 1096173. [PubMed: 36950144]

[2]. Bowdridge EC, DeVallance E, Garner KL, Griffith JA, Schafner K, Seaman M, Engels KJ, Wix K, Batchelor TP, Goldsmith WT, Hussain S, Nurkiewicz TR, Nano-titanium dioxide inhalation exposure during gestation drives redox dysregulation and vascular dysfunction across generations, Part. Fibre Toxicol. 19 (2022) 18, 10.1186/s12989-022-00457-y. [PubMed: 35260159]

[3]. Gonzalez TL, Sun T, Koeppel AF, Lee B, Wang ET, Farber CR, Rich SS, Sundheimer LW, Buttle RA, Chen Y-DI, Rotter JI, Turner SD, Williams J, Goodarzi MO, Pisarska MD, Sex differences in the late first trimester human placenta transcriptome, Biol. Sex Differ. 9 (2018) 4, 10.1186/s13293-018-0165-y. [PubMed: 29335024]

[4]. Kalisch-Smith JI, Simmons DG, Dickinson H, Moritz KM, Review: sexual dimorphism in the formation, function and adaptation of the placenta, Placenta 54 (2017) 10–16, 10.1016/j.placenta.2016.12.008. [PubMed: 27979377]

[5]. Meakin AS, Cuffe JSM, Darby JRT, Morrison JL, Clifton VL, Let's talk about placental sex, baby: understanding mechanisms that drive female- and male-specific fetal growth and developmental outcomes, Int. J. Mol. Sci. 22 (2021) 6386, 10.3390/ijms22126386. [PubMed: 34203717]

[6]. Gabory A, Roseboom TJ, Moore T, Moore LG, Junien C, Placental contribution to the origins of sexual dimorphism in health and diseases: sex chromosomes and epigenetics, Biol. Sex Differ. 4 (2013) 5, 10.1186/2042-6410-4-5. [PubMed: 23514128]

[7]. Tarrade A, Panchenko P, Junien C, Gabory A, Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism, J. Exp. Biol. 218 (2015) 50–58, 10.1242/jeb.110320. [PubMed: 25568451]

[8]. Kalisch-Smith JI, Simmons DG, Pantaleon M, Moritz KM, Sex differences in rat placental development: from pre-implantation to late gestation, Biol. Sex Differ. 8 (2017) 17, 10.1186/s13293-017-0138-6. [PubMed: 28523122]

[9]. Stark MJ, Clifton VL, Wright IMR, Neonates born to mothers with preeclampsia exhibit sex-specific alterations in microvascular function, Pediatr. Res. 65 (2009) 291–295, 10.1203/PDR.0b013e318193edf1.

[10]. Saoi M, Kennedy KM, Gohir W, Sloboda DM, Britz-McKibbin P, Placental metabolomics for assessment of sex-specific differences in fetal development during normal gestation, Sci. Rep. 10 (2020) 9399, 10.1038/s41598-020-66222-3. [PubMed: 32523064]

[11]. Chiarello DI, Abad C, Rojas D, Toledo F, Vázquez CM, Mate A, Sobrevia L, Marín R, Oxidative stress: normal pregnancy versus preeclampsia, Biochim. Biophys. Acta BBA Mol. Basis Dis. 1866 (2020), 165354, 10.1016/j.bbadis.2018.12.005.

[12]. Saad MI, Abdelkhalek TM, Saleh MM, Haiba MM, Tawfik SH, Kamel MA, Maternal diabetes impairs oxidative and inflammatory response in murine placenta, Springerplus 5 (2016) 532, 10.1186/s40064-016-2180-y.

[13]. Aycicek A, Varma M, Ahmet K, Abdurrahim K, Erel O, Maternal active or passive smoking causes oxidative stress in placental tissue, Eur. J. Pediatr. 170 (2011) 645–651, 10.1007/s00431-010-1338-9. [PubMed: 20981440]

[14]. Weldy CS, Liu Y, Liggitt HD, Chin MT, In utero exposure to diesel exhaust air pollution promotes adverse intrauterine conditions, resulting in weight gain, altered blood pressure, and increased susceptibility to heart failure in adult mice, PLOS One 9 (2014) e88582, 10.1371/journal.pone.0088582. [PubMed: 24533117]

[15]. Rodríguez-Rodríguez P, Ramiro-Cortijo D, Reyes-Hernández CG, López de Pablo AL, González MC, Arribas SM, Implication of oxidative stress in fetal programming of cardiovascular disease, Front. Physiol. 9 (2018) 602. [PubMed: 29875698]

[16]. Silvestro S, Calcaterra V, Pelizzo G, Bramanti P, Mazzon E, Prenatal hypoxia and placental oxidative stress: insights from animal models to clinical evidences, Antioxidants 9 (2020) 414, 10.3390/antiox9050414. [PubMed: 32408702]

[17]. Remy LL, Byers V, Clay T, Reproductive outcomes after non-occupational exposure to hexavalent chromium, Willits California, 1983–2014, Environ. Health 16 (2017) 18, 10.1186/s12940-017-0222-8. [PubMed: 28264679]

[18]. Banu SK, Stanley JA, Taylor RJ, Sivakumar KK, Arosh JA, Zeng L, Pennathur S, Padmanabhan V, Sexually dimorphic impact of chromium accumulation on human placental oxidative stress and apoptosis, Toxicol. Sci. 161 (2018) 375–387, 10.1093/toxsci/kfx224. [PubMed: 29069462]

[19]. Ayala A, Muñoz MF, Argüelles S, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, Oxid. Med. Cell. Longev. 2014 (2014), e360438, 10.1155/2014/360438.

[20]. Biswas S, Das R, Banerjee ER, Biswas S, Das R, Banerjee ER, Role of free radicals in human inflammatory diseases, AIMS Biophys. 4 (2017) 596–614, 10.3934/biophy.2017.4.596.

[21]. Kontos HA, Wei EP, Ellis EF, Jenkins LW, Povlishock JT, Rowe GT, Hess ML, Appearance of superoxide anion radical in cerebral extracellular space during increased prostaglandin synthesis in cats, Circ. Res. 57 (1985) 142–151, 10.1161/01.RES.57.1.142. [PubMed: 2988819]

[22]. McIntyre M, Bohr DF, Dominiczak AF, Endothelial function in hypertension, Hypertension 34 (1999) 539–545, 10.1161/01.HYP.34.4.539. [PubMed: 10523323]

[23]. Roy P, Roy SK, Mitra A, Kulkarni AP, Superoxide generation by lipoxygenase in the presence of NADH and NADPH, Biochim. Biophys. Acta 1214 (1994) 171–179, 10.1016/0005-2760(94)90041-8. [PubMed: 7918597]

[24]. Xu Y, Knipp GT, Cook TJ, Expression of cyclooxygenase isoforms in developing rat placenta, human term placenta, and bewo human trophoblast model, ACS Publ. (2005), 10.1021/mp0500519.

[25]. Burdon C, Mann C, Cindrova-Davies T, Ferguson-Smith AC, Burton GJ, Oxidative stress and the induction of cyclooxygenase enzymes and apoptosis in the murine placenta, Placenta 28 (2007) 724–733, 10.1016/j.placenta.2006.12.001. [PubMed: 17222904]

[26]. Abukabda AB, Stapleton PA, McBride CR, Yi J, Nurkiewicz TR, Heterogeneous vascular bed responses to pulmonary titanium dioxide nanoparticle exposure, Front. Cardiovasc. Med. 4 (2017) 33, 10.3389/fcvm.2017.00033. [PubMed: 28596957]

[27]. Abukabda AB, McBride CR, Batchelor TP, Goldsmith WT, Bowdridge EC, Garner KL, Friend S, Nurkiewicz TR, Group II innate lymphoid cells and microvascular dysfunction from pulmonary titanium dioxide nanoparticle exposure, Part. Fibre Toxicol. 15 (2018) 43, 10.1186/s12989-018-0280-2. [PubMed: 30413212]

[28]. Griffith JA, Garner KL, Bowdridge EC, DeVallance E, Schafner KJ, Engles KJ, Batchelor TP, Goldsmith WT, Wix K, Hussain S, Nurkiewicz TR, Nanomaterial inhalation during pregnancy alters systemic vascular function in a cyclooxygenase-dependent manner, Toxicol. Sci. Off. J. Soc. Toxicol. (2022) kfac055, 10.1093/toxsci/kfac055.

[29]. Garner KL, Bowdridge EC, Griffith JA, DeVallance E, Seman MG, Engels KJ, Groth CP, Goldsmith WT, Wix K, Batchelor TP, Nurkiewicz TR, Maternal nanomaterial inhalation exposure: critical gestational period in the uterine microcirculation is angiotensin II dependent, Cardiovasc. Toxicol. 22 (2022) 167–180, 10.1007/s12012-021-09712-8. [PubMed: 35066857]

[30]. Madamanchi NR, Vendrov A, Runge MS, Oxidative stress and vascular disease, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 29–38, 10.1161/01.ATV.0000150649.39934.13. [PubMed: 15539615]

[31]. Bowdridge EC, Abukabda AB, Engles KJ, McBride CR, Batchelor TP, Goldsmith WT, Garner KL, Friend S, Nurkiewicz TR, Maternal engineered nanomaterial inhalation during gestation disrupts vascular kisspeptin reactivity, Toxicol. Sci. Off. J. Soc. Toxicol. 169 (2019) 524–533, 10.1093/toxsci/kfz064.

[32]. Yi J, Chen BT, Schwegler-Berry D, Frazer D, Castranova V, McBride C, Knuckles TL, Stapleton PA, Minarchick VC, Nurkiewicz TR, Whole-body nanoparticle aerosol inhalation exposures, J. Vis. Exp. JoVE (2013) e50263, 10.3791/50263. [PubMed: 23685643]

[33]. Qu D, McDonald A, Whiteley KJ, Bainbridge SA, Adamson SL, Croy BA, Yamada AT, DeMayo FJ, Adamson SL, 44 - layer-enriched tissue dissection of the mouse placenta in late gestation. The Guide to Investigation of Mouse Pregnancy, Academic Press, 2014, pp. 529–535, 10.1016/B978-0-12-394445-0.00044-8. Boston.

[34]. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254, 10.1016/0003-2697(76)90527-3. [PubMed: 942051]

[35]. Butts B, Brown JA, Denney TS, Ballinger S, Lloyd SG, Oparil S, Sanders P, Merriman TR, Gaffo A, Singh J, Kelley EE, Calhoun DA, Dell'Italia LJ, Racial differences in XO (xanthine oxidase) and mitochondrial DNA damage-associated molecular patterns in resistant hypertension, Hypertension 79 (2022) 775–784, 10.1161/HYPERTENSIONAHA.121.18298. [PubMed: 35164526]

[36]. Livak KJ, Schmittgen TD, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method, Methods San Diego Calif. 25 (2001) 402–408, 10.1006/meth.2001.1262. [PubMed: 11846609]

[37]. Lai T-C, Chen Y-C, Cheng H-H, Lee T-L, Tsai J-S, Lee I-T, Peng K-T, Lee C-W, Hsu L-F, Chen Y-L, Combined exposure to fine particulate matter and high glucose aggravates endothelial damage by increasing inflammation and mitophagy: the involvement of vitamin D, Part. Fibre Toxicol. 19 (2022) 25, 10.1186/s12989-022-00462-1. [PubMed: 35351169]

[38]. Mori Y, Murakami S, Sagae T, Hayashi H, Sakata M, Sagai M, Kumagai Y, Inhibition of catalase activity *in vitro* by diesel exhaust particles, J. Toxicol. Environ. Health. 47 (1996) 125–134, 10.1080/009841096161834. [PubMed: 8598569]

[39]. Bhavaraju L, Examination of diesel and biodiesel exhaust exposure induced disruption of arachidonic acid metabolism, (2015).

[40]. Tuuli MG, Longtine MS, Nelson DM, Review: oxygen and trophoblast biology — A source of controversy, Placenta 32 (2011) S109–S118, 10.1016/j.placenta.2010.12.013. [PubMed: 21216006]

[41]. Kelley EE, Khoo NKH, Hundley NJ, Malik UZ, Freeman BA, Tarpey MM, Hydrogen peroxide is the major oxidant product of xanthine oxidase, Free Radic. Biol. Med. 48 (2010) 493–498, 10.1016/j.freeradbiomed.2009.11.012. [PubMed: 19941951]

[42]. Berry CE, Hare JM, Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications, J. Physiol. 555 (2004) 589–606, 10.1113/jphysiol.2003.055913. [PubMed: 14694147]

[43]. Matheson H, Veerbeek JHW, Charnock-Jones DS, Burton GJ, Yung HW, Morphological and molecular changes in the murine placenta exposed to normobaric hypoxia throughout pregnancy, J. Physiol. 594 (2016) 1371–1388, 10.1113/JP271073. [PubMed: 26278110]

[44]. Scott NM, Hodyl NA, Murphy VE, Osei-Kumah A, Wyper H, Hodgson DM, Smith R, Clifton VL, Placental cytokine expression covaries with maternal asthma severity and fetal sex1, J. Immunol. 182 (2009) 1411–1420, 10.4049/jimmunol.182.3.1411. [PubMed: 19155488]

[45]. Schliefsteiner C, Peinhaupt M, Kopp S, Lögl J, Lang-Olip I, Hiden U, Heinemann A, Desoye G, Wadsack C, Human placental hofbauer cells maintain an anti-inflammatory M2 phenotype despite the presence of gestational diabetes mellitus, Front. Immunol. 8 (2017), 888. [PubMed: 28824621]

[46]. Said FA, Werts C, Elalamy I, Couetil J-P, Jacquemin C, Hatmi M, TNF-α, inefficient by itself, potentiates IL-1β-induced PGHS-2 expression in human pulmonary microvascular endothelial cells: requirement of NF-κB and p38 MAPK pathways, Br. J. Pharmacol. 136 (2002) 1005–1014, 10.1038/sj.bjp.0704811. [PubMed: 12145100]

[47]. El-Haroun H, Bradbury D, Clayton A, Knox AJ, Interleukin-1β, transforming growth factor-β1, and bradykinin attenuate cyclic AMP production by human pulmonary artery smooth muscle cells in response to prostacyclin analogues and prostaglandin E2 by cyclooxygenase-2 induction and downregulation of adenylyl cyclase isoforms 1, 2, and 4, Circ. Res. 94 (2004) 353–361, 10.1161/01.RES.0000111801.48626.F4. [PubMed: 14670842]

[48]. Beasley D, COX-2 and cytosolic PLA2mediate IL-1β-induced cAMP production in human vascular smooth muscle cells, Am. J. Physiol. Heart Circ. Physiol. 276 (1999) H1369–H1378, 10.1152/ajpheart.1999.276.4.H1369.

[49]. Um M, P J, P K, L V, O Y, Placental thromboxane and prostacyclin in the regulation of placental blood flow, Obstet. Gynecol. 68 (4) (1986) 537–540. [PubMed: 3528955]

[50]. Pai C-H, Yen C-T, Chen C-P, Yu I-S, Lin S-W, Lin S-R, Lack of thromboxane synthase prevents hypertension and fetal growth restriction after high salt treatment during pregnancy, PLOS One 11 (2016), e0151617, 10.1371/journal.pone.0151617. [PubMed: 26974824]

[51]. Sicotte B, Brochu M, Fetal sex and fetal environment interact to alter diameter, myogenic tone, and contractile response to thromboxane analog in rat umbilical cord vessels, Front. Physiol. 12 (2021), 620058, 10.3389/fphys.2021.620058. [PubMed: 34603067]

[52]. Chen J, Rider DA, Ruan R, Identification of valid housekeeping genes and antioxidant enzyme gene expression change in the aging rat liver, J. Gerontol. A. Biol. Sci. Med. Sci. 61 (2006) 20–27, 10.1093/gerona/61.1.20. [PubMed: 16456191]

[53]. Scudiero R, Verderame M, Gene expression profile of estrogen receptors alpha and beta in rat brain during aging and following high fat diet, C. R. Biol. 340 (2017) 372–378, 10.1016/j.crvi.2017.08.001. [PubMed: 28869142]

[54]. Jankowski M, Rachelska G, Donghao W, McCann SM, Gutkowska J, Estrogen receptors activate atrial natriuretic peptide in the rat heart, Proc. Natl. Acad. Sci. 98 (2001) 11765–11770, 10.1073/pnas.201394198. [PubMed: 11562484]

[55]. Zhang Y, Mi M, Xie Y-H, Wang S-W, Edvinsson L, Xu C-B, Downregulation of thromboxane A2 receptor occurs mainly via nuclear factor-kappab signaling pathway in rat renal artery, Adv. Pharmacol. Sci. 2017 (2017), e6507048, 10.1155/2017/6507048.

[56]. Numaguchi Y, Harada M, Osanai H, Hayashi K, Toki Y, Okumura K, Ito T, Hayakawa T, Altered gene expression of prostacyclin synthase and prostacyclin receptor in the thoracic

aorta of spontaneously hypertensive rats, Cardiovasc. Res. 41 (1999) 682–688, 10.1016/S0008-6363(98)00239-9. [PubMed: 10435040]

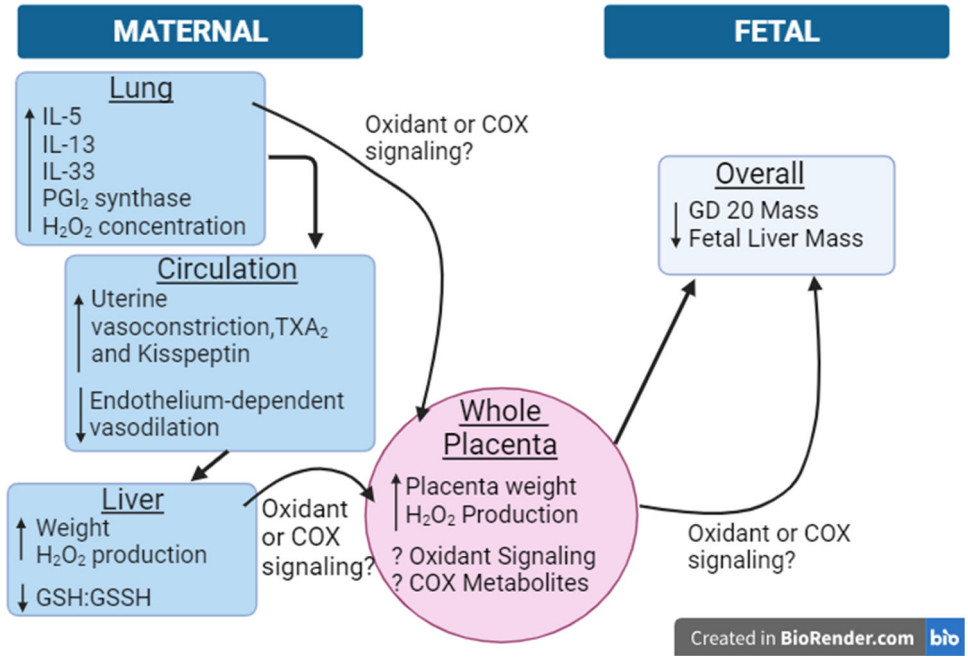

Fig. 1. Graphical representation of lung-liver-placenta axis interaction.

Maternal nano-TiO_2 inhalation exposure increases inflammatory signals within the maternal lung, which may spill over into the circulation. Maternal nano-TiO_2 inhalation exposure has been shown to decrease endothelium-dependent relaxation in many vascular beds. Further, maternal hepatic mass and H_2O_2 production capacity is increased in nano-TiO_2 exposed dams. Changes in redox balance during gestation may underlie hindered fetal female development and increased cyclooxygenase vasoreactivity in fetal female placental outflow.

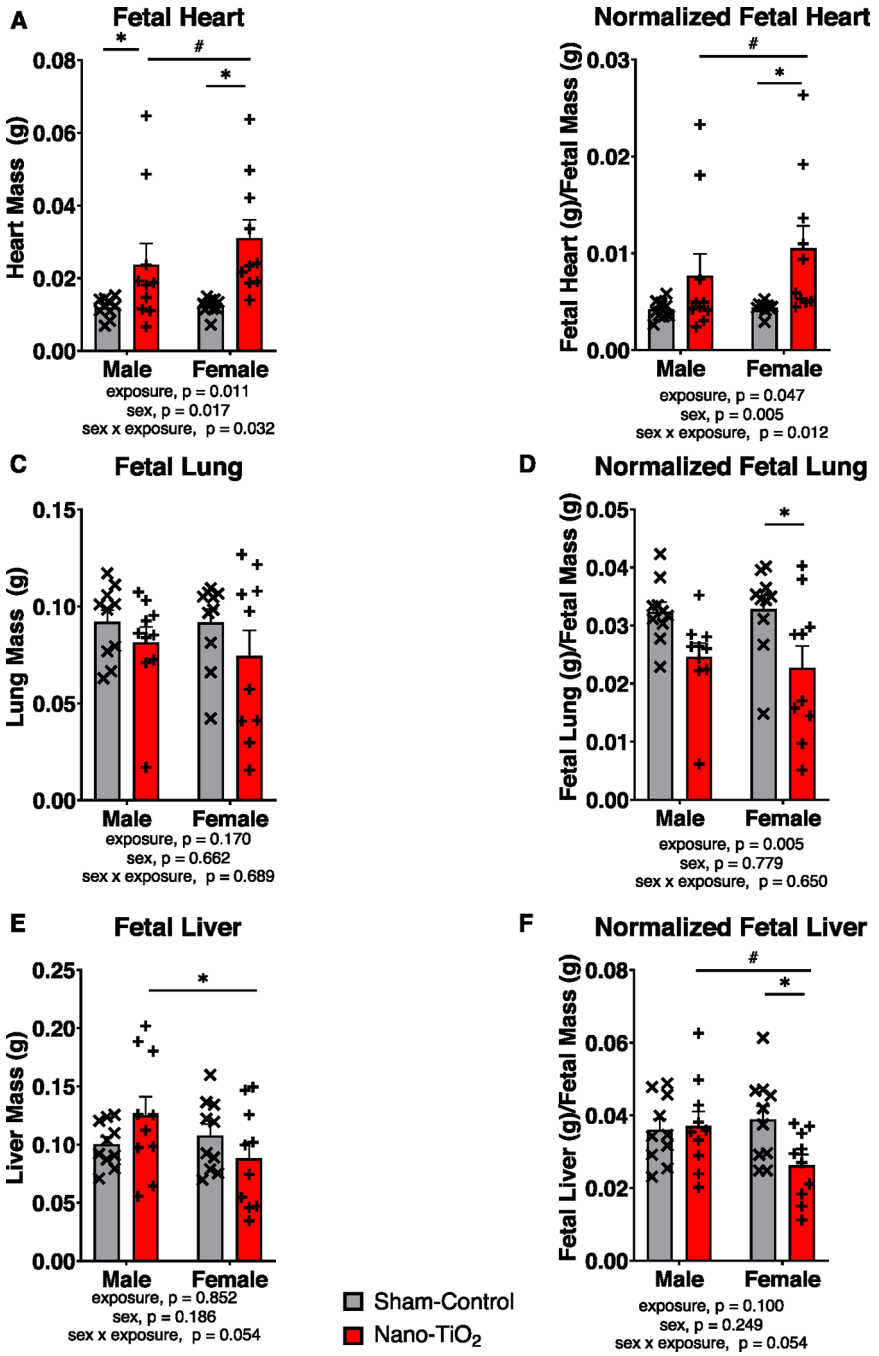

Fig. 2. Fetal tissue mass and normalized fetal tissue mass.

Fetal wet tissue mass was weighed and then normalized to fetal weight mass. (A) Fetal heart mass. (B) Normalized fetal heart to fetal mass. (C) Fetal lung mass. (D) Normalized fetal lung to fetal mass. (E) Fetal liver mass. (F) Normalized fetal liver. Sham-control male (N = 10), sham-control female (N = 10), nano-TiO_2 male (N = 10), and nano-TiO_2 female (N = 10). *, $p \leq 0.05$ exposure effect. #, $p \leq 0.05$ for sex effect.

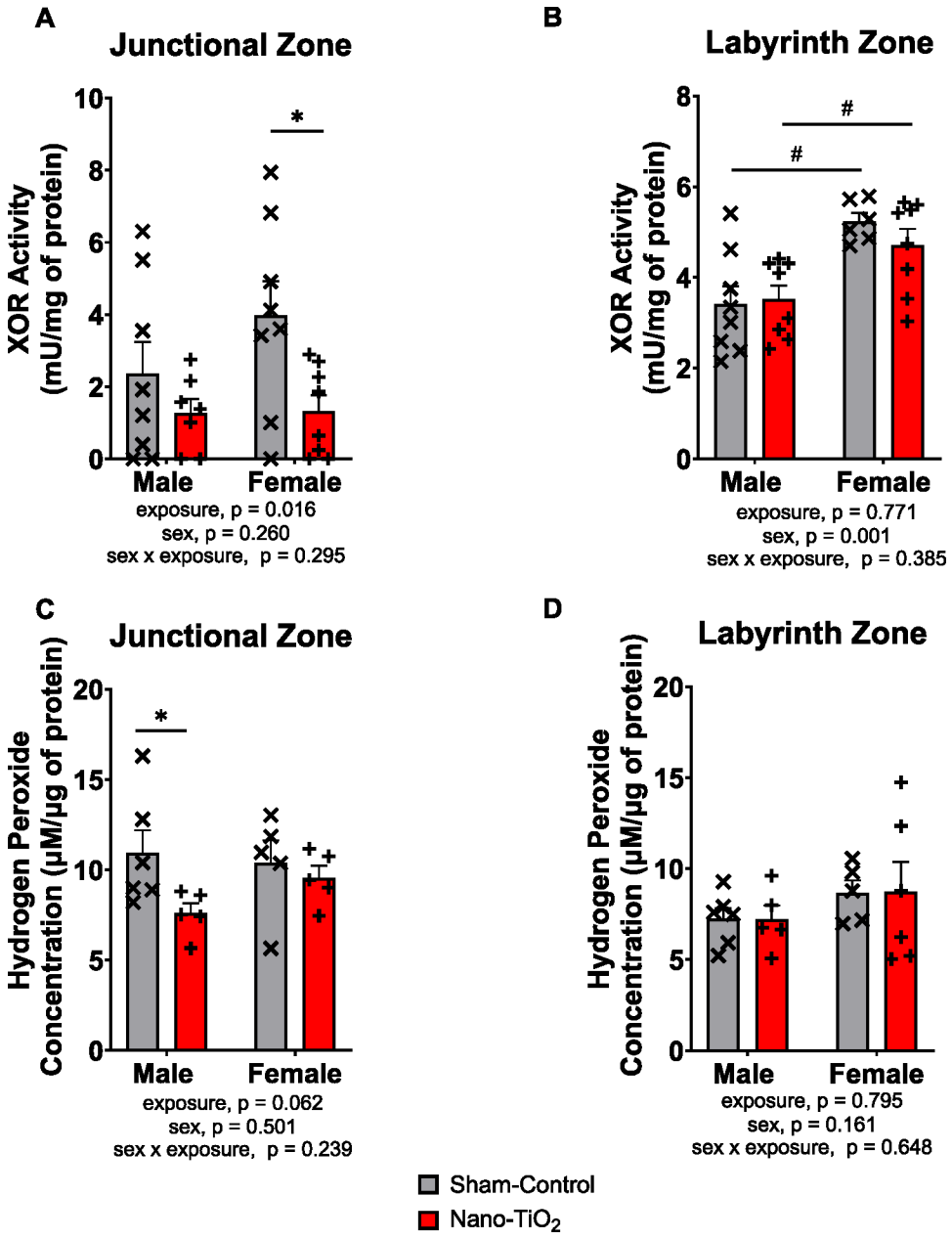

Fig. 3. Placental xanthine oxidoreductase activity and hydrogen peroxide concentration.

Placental activity of xanthine oxidoreductase (XOR) and hydrogen peroxide (H_2O_2) concentration were assessed in each placenta zone. (A) Junctional zone (JZ) XOR activity. (B) Labyrinth zone (LZ) XOR activity. (C) JZ H_2O_2 levels. (D) LZ H_2O_2 concentration. Sham-control male ($N = 8$), sham-control female ($N = 6–8$), nano-TiO_2 male ($N = 8$), and nano-TiO_2 female ($N = 8$). *, $p \leq 0.05$ exposure effect. #, $p \leq 0.05$ for sex effect.

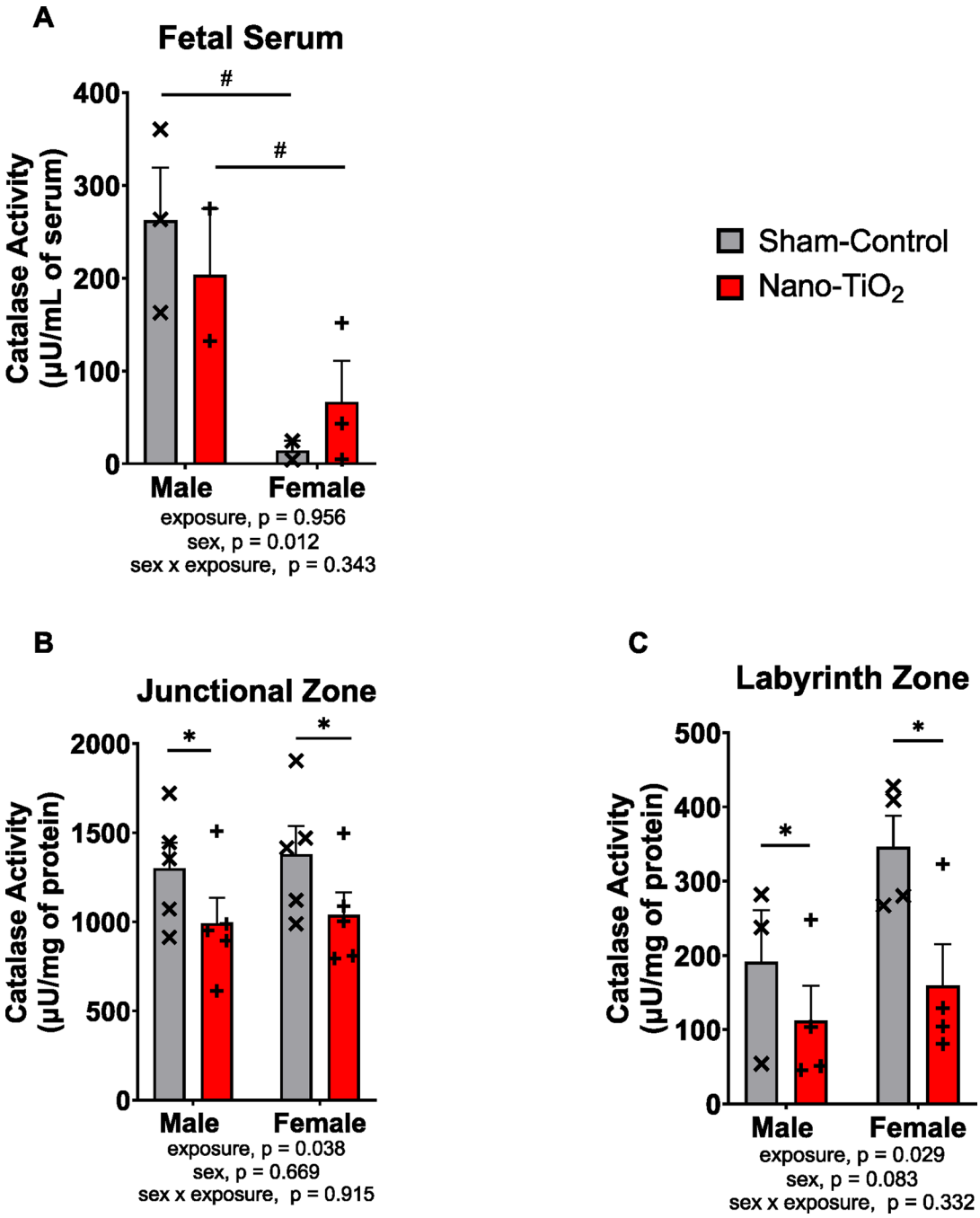

Fig. 4. Placental and fetal serum catalase activity.

Catalase activity was assessed in fetal serum and in each placenta zone. (A). Fetal serum catalase activity. (B) JZ catalase activity. (C) LZ catalase activity. Sham-control male (N = 3–4), sham-control female (N = 3–4), nano-TiO_2 male (N = 3–4), and nano-TiO_2 female (N = 3–4). *, $p \leq 0.05$ exposure effect. #, $p \leq 0.05$ for sex effect.

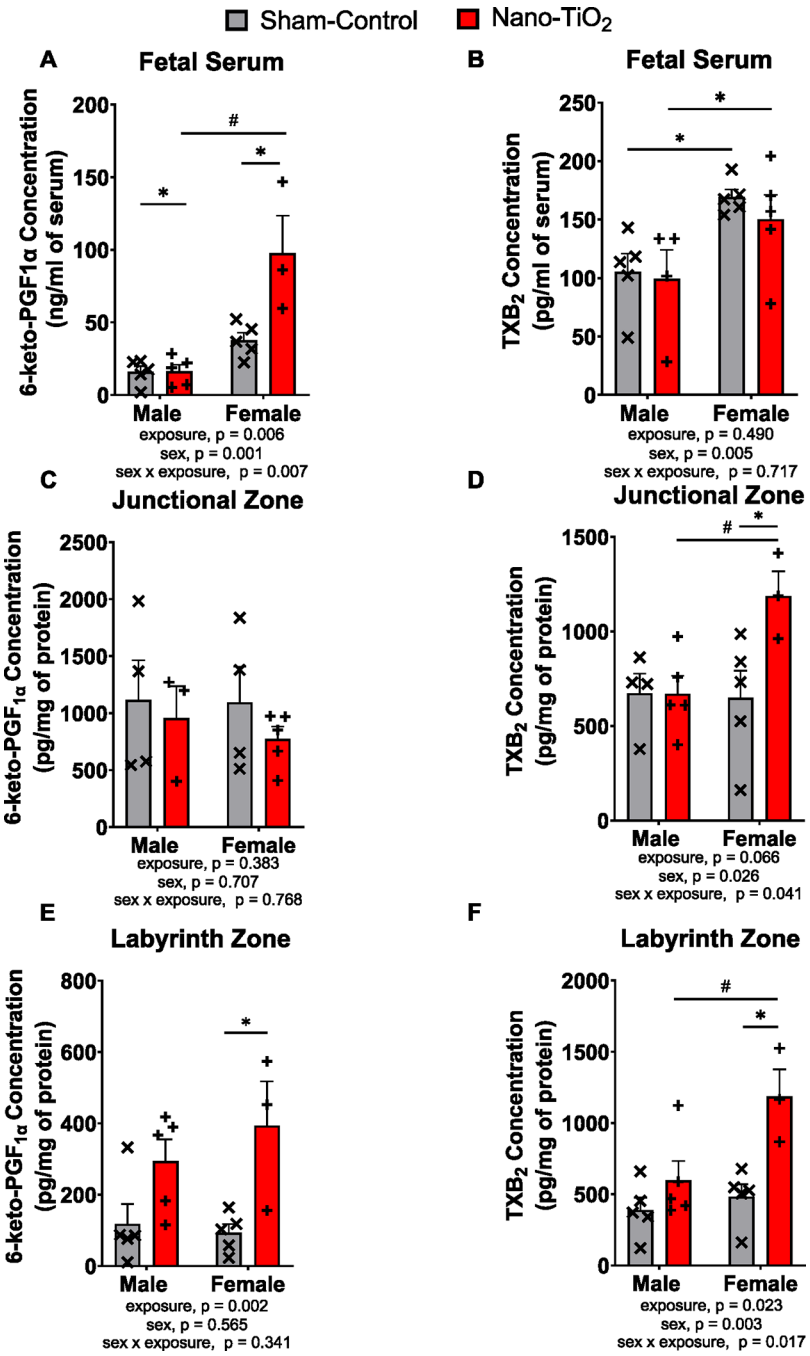

Fig. 5. Placental and fetal serum 6-keto-PGF$_{1\alpha}$ and TXB$_2$ production.

Cyclooxygenase metabolites (6-keto-PGF$_{1\alpha}$ and TXB$_2$) were assessed in fetal serum and each placenta zone. (A) Fetal serum levels of 6-keto-PGF$_{1\alpha}$. (B) Fetal serum TXB$_2$ levels. (C) JZ 6-keto-PGF$_{1\alpha}$ levels. (D) JZ production of TXB$_2$. (E) LZ levels of 6-keto-PGF$_{1\alpha}$. (F) LZ TXB$_2$ levels. Sham-control males (N = 5), sham-control females (N = 5), nano-TiO$_2$ males (N = 4–5), and nano-TiO$_2$ female (N = 3–5). *, $p \leq 0.05$ exposure effect. #, $p \leq 0.05$ for sex effect.

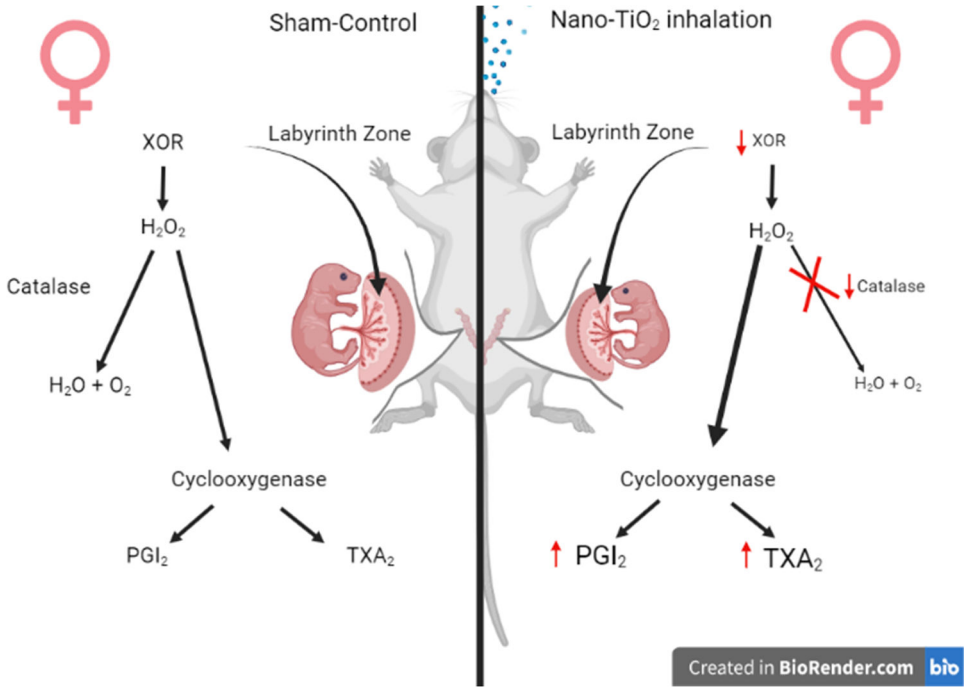

Fig. 6. Summary of the impacts of maternal nano-TiO_2 inhalation exposure during gestation on placental oxidant balance in a sex dependent manner.

Maternal nano-TiO_2 inhalation exposure during gestation results in sexually dimorphic modifications to redox balance and thus impacts cyclooxygenase metabolites in the labyrinth zone at GD 20. Females have demonstrated greater impact in their mass (JZ and LZ), area (JZ) compared to exposed males [1]. Females also have increased XDH activity (LZ), decreased catalase activity (LZ), and increased production of both, 6-keto-$PGF_{1\alpha}$ and TXB_2 (LZ). These resulting changes are likely responsible for the decreased fetal female weight at GD 20, of which progresses to female deficits into adulthood [2].

Table 1

Real-time PCR primers, their sequence, length, and references.

	Forward primer (5'–3')	Reverse primer (5'–3')	Length (bp)	Ref.
Gapdh NM_017008	AGGGCTGCCTTCTCTTGTGAC	TGGGTAGAATCATACTGGAACATGTAG	101	[52]
Esr1 NM_012689	GCACATTCCTTCCTTCCGTC	CTCGTTCCCTTGGATCTGGT	216	[53]
Esr2 NM_012754	TTCCCGGCAGCACCAGTAACC	TCCCTCTTTGCGTTTGGACTA	262	[54]
Cat NM_012520	ACAACTCCCAGAAGCCTAAGAATG	GCTTTTCCCTTGGCAGCTATG	76	[52]
Sod2 NM_017051	CGAGCATGGGTTCCATGTC	CTGGACCGCCATGTTTCTTAG	100	[52]
Tbxa2r NM_017054.1	ATCTCCCATCTTGCCATAGTCC	CCGATGATCCTTGAGCCTAAAG	1880	[55]
Ptgis NM_031557	TGGTGTGGGATCTGCGTACA	CCTCCACTCCATACAGGGTCA	567	[56]
Ptgir NM_001077644	TGCTGGAACATCACCTACGT	GTTTCGAGCATAGGCCACAA	422	[56]

Primers were used in placental junctional zone, labyrinth zone, fetal liver, fetal lung, implantation site, and resorption site tissue.

Table 2

mRNA Expression profiles for prostacyclin synthase (PGIS) and receptor (PGIR), thromboxane receptor (TXR), superoxide dismutase (SOD), catalase (CAT), and estrogen receptors (ERα or ERβ) or in placental tissue, JZ and LZ.

Tissue	Group	N	Prostacyclin Synthase	Prostacyclin Receptor	Thromboxane Receptor	Superoxide Dismutase	Catalase	ERα	ERβ
Junctional zone	Sham-control Male	3–5	1.00 ± 0.19	1.00 ± 0.32	1.00 ± 0.21	1.00 ± 0.17	1.00 ± 0.18	1.00 ± 0.73	1.00 ± 0.96
	Nano-TiO_2 Male	3–5	0.68 ± 0.22	0.80 ± 0.18	1.13 ± 0.31	0.89 ± 0.09	1.46 ± 0.12	0.59 ± 0.20	1.36 ± 1.10
	Sham-control Female	3–5	1.00 ± 0.33	1.00 ± 0.26	1.00 ± 0.29	1.00 ± 0.09	1.00 ± 0.21	1.00 ± 0.54	1.00 ± 0.15
	Nano-TiO_2 Female	3–5	0.58 ± 0.12	**2.32 ± 0.37**[ab]	1.54 ± 0.23	0.85 ± 0.03	**0.79 ± 0.09**[b]	1.21 ± 0.42	3.07 ± 0.08
Labyrinth zone	Sham-control Male	3	1.00 ± 1.40	1.00 ± 0.42	1.00 ± 0.83	1.00 ± 0.27	1.00 ± 0.21	1.00 ± 0.62	1.00 ± 8.56
	Nano-TiO_2 Male	3	0.64 ± 0.72	0.76 ± 0.18	1.46 ± 0.31	1.03 ± 0.27	1.16 ± 0.30	1.55 ± 1.02	0.22 ± 1.34
	Sham-control Female	3	1.00 ± 0.28	1.00 ± 0.08	1.00 ± 0.18	1.00 ± 0.13	1.00 ± 0.17	1.00 ± 0.29	1.00 ± 0.18
	Nano-TiO_2 Female	3	2.92 ± 1.74	1.27 ± 0.22	0.89 ± 0.04	1.55 ± 0.05	1.52 ± 0.19	1.55 ± 0.88	2.38 ± 1.34

N represents the number of litters per group. Data are fold-change and represent mean ± SEM. JZ TXR, PGIS, SOD, ERα, and ERβ do not have significance for exposure, sex, or an interaction effect ($p > 0.05$). JZ PGIR has a trend to an exposure effect ($p = 0.074$), a sex effect ($p = 0.019$), and an interaction ($p = 0.019$). JZ CAT does not have an exposure effect ($p > 0.05$), a trend for a sex effect ($p = 0.053$), and an interaction ($p = 0.053$). LZ SOD, TXR, PGIS, PGIR, CAT, ERα, or ERβ did not have an exposure, sex, or interaction effect ($p > 0.05$).

[a] $p \leq 0.05$ vs sham-control female.

[b] $p \leq 0.05$ vs nano-TiO2 male.

Table 3

mRNA Expression for prostacyclin synthase (PGIS) and receptor (PGIR) and thromboxane receptor (TXR).

Tissue	Exposure/Group	N	Prostacyclin Synthase	Prostacyclin Receptor	Thromboxane Receptor
Lung	Sham-control Male	5	1.00 ± 0.08	1.00 ± 0.23	1.00 ± 0.11
	Nano-TiO_2 Male	5	0.94 ± 0.33	0.73 ± 0.21	0.42 ± 0.09
	Sham-control Female	5	1.00 ± 0.26	1.00 ± 0.22	1.00 ± 0.29
	Nano-TiO_2 Female	5	0.99 ± 0.07	1.16 ± 0.26	0.87 ± 0.15
Implantation site	Sham-control Male	3	1.00 ± 0.15	1.00 ± 0.18	1.00 ± 0.17
	Nano-TiO_2 Male	3	1.85 ± 0.44	1.05 ± 0.35	1.25 ± 0.31
	Sham-control Female	3	1.00 ± 0.92	1.00 ± 0.05	1.00 ± 0.16
	Nano-TiO_2 Female	3	**5.78 ± 1.27**^{ab}	1.23 ± 0.40	1.48 ± 1.06

N represents the number of litters per group. Data are fold-change and represent mean ± SEM. Fetal lung PGIR and PGIS did not have significance for exposure, sex, or an interaction effect ($p > 0.05$). Fetal lung TXR had a trend toward significance ($p = 0.060$) for exposure effect but did not have significance for sex or an interaction effect ($p > 0.05$). Implantation sites TXR or PGIR did not have an exposure, sex, or interaction effects ($p > 0.05$). Implantation site PGIS had an exposure effect ($p = 0.009$), a sex effect ($p = 0.043$), and an interaction ($p = 0.043$) effect.

[a] $p \leq 0.05$ vs sham-control female.

[b] $p \leq 0.05$ vs nano-TiO_2 male.

[c] $p \leq 0.05$ vs sham-control male.