Preconception and/or preimplantation exposure to a mixture of environmental contaminants altered fetoplacental development and placental function in a rabbit model

Jeanne Bozec, Delphine Rousseau-Ralliard, Luc Jouneau, Audrey Prézelin, Michèle Dahirel, Christophe Richard, Valérie Gelin, Natalie Fournier, Virginie Helies, Thierry Joly, et al.

Environmental Research 262 (2024) 119829

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Preconception and/or preimplantation exposure to a mixture of environmental contaminants altered fetoplacental development and placental function in a rabbit model

Jeanne Bozec [a,b], Delphine Rousseau-Ralliard [a,b], Luc Jouneau [a,b], Audrey Prézelin [a,b], Michèle Dahirel [a,b], Christophe Richard [a,b], Valérie Gelin [a,b], Natalie Fournier [c,d], Virginie Helies [e], Thierry Joly [f,g], Sara El Fouikar [h], Roger Léandri [h,i], Pascale Chavatte-Palmer [a,b], Anne Couturier-Tarrade [a,b,*]

[a] Université Paris-Saclay, UVSQ, INRAE, BREED, 78350, Jouy-en-Josas, France
[b] Ecole Nationale Vétérinaire d'Alfort, BREED, 94700, Maisons-Alfort, France
[c] Lip(Sys)2 - EA 7357, Athérosclérose et Macrophages: Impact des Phospholipides et des Fonctions Mitochondriales sur L'efflux du Cholestérol, Université Paris Saclay, UFR de Pharmacie, 91400, Orsay, France
[d] Laboratoire de Biochimie, AP-HP (Assistance Publique-Hôpitaux de Paris), Hôpital Européen Georges Pompidou, 75015, Paris, France
[e] GenPhySE, INRAE, Université de Toulouse, INPT, ENVT, Castanet Tolosan, France
[f] Université de Lyon, VetAgro Sup, UPSP Interaction Cellule Environnement, 69280, Marcy L'Etoile, France
[g] Université de Lyon, ISARA-Lyon, 69007, Lyon, France
[h] ToxAlim (Research Center in Food Toxicology), Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France
[i] Médecine de La Reproduction, Hôpital Paule de Viguier, Centre Hospitalier Universitaire de Toulouse, Toulouse, France

ARTICLE INFO

Keywords:
Placenta
DOHaD
Endocrine disruptors
Fetal programming
Mixture exposure
Rabbit model

ABSTRACT

Pregnant women are daily exposed to environmental contaminants, including endocrine disruptors that can impact the offspring's health. This study aimed to evaluate the effects of maternal oral exposure to a mixture of contaminants at a dose mimicking women's exposure, during folliculogenesis and/or preimplantation period (FED and ED groups, respectively) on the fetoplacental phenotype in a rabbit model. The mixture (DEHP, pp'DDE, β-HCH, HCB, BDE-47, BPS, PFOS, PFOA) was defined based on data from HELIX and INMA cohorts. FED and ED females or unexposed females (control) were inseminated, their embryos were collected and transferred to unexposed control recipient rabbits at 80 h post-insemination. The effects of maternal FED and ED exposure were evaluated on fetoplacental growth and development by ultrasound, fetoplacental biometry, fetal metabolism, placental structure and function. The results demonstrated that the mixture weakly affected ultrasound measurements, as only placental volume increased significantly in FED *vs* ED. Analysis of placental structure demonstrated that the volume fraction of the maternal blood space was increased in FED *vs* control. Pre- and/or periconception exposure did not affect biometric at the end of gestation, but affected FED fetal biochemistry. Plasma triglyceride concentration was reduced compared to control. However, total cholesterol, urea, ASAT and ALAT in fetal blood were affected in both exposed groups. Multiple factor analysis, including biometric, biochemical, and stereological datasets, indicated that the three groups were significantly different. Additionally, several placental genes were differentially expressed between groups, compared two by two, in a sex-specific manner, with more difference in females than in males. The differentially expressed genes were involved in lipid, cholesterol, and drug/xenobiotic metabolism in both sexes. These results indicate that maternal exposure to environmental contaminants during crucial developmental windows only mildly impaired fetoplacental development but disturbed fetal blood biochemistry and placental gene expression with potential long-term effects on offspring phenotype.

* Corresponding author. UMR 1198 Biologie de la Reproduction, Environnement, Epigénétique et Développement, INRAE Domaine de Vilvert, 78352, Jouy-en-Josas Cedex, France.
E-mail address: anne.couturier-tarrade@inrae.fr (A. Couturier-Tarrade).

https://doi.org/10.1016/j.envres.2024.119829
Received 3 April 2024; Received in revised form 19 August 2024; Accepted 20 August 2024
Available online 22 August 2024

Funding sources and approval of experiments

This study is part of the FEDEXPO project, " Folliculogenesis and Embryo Development EXPOsure" to a mixture of toxicants, supported by Agence Nationale de la Recherche, France [grant no. ANR-17-CE34-0015].

Animal experimentation was authorized by the French Ministry of Research under APAFIS no. 14787–201804201607003 v3). In addition, ARRIVE guidelines (Animal Research: Reporting of the In Vivo Experiments available at http://arriveguidelines.org/) were followed for this experiment.

1. Introduction

The general population is exposed daily by ingestion, inhalation, or skin contact with various chemical compounds that may have endocrine-disrupting activities (Nappi et al., 2016). These compounds include the families of organochlorine pesticides, bisphenols, perfluoroalkyl substances, phthalates, and flame retardants. Organochlorine pesticides include hexachlorobenzene (HCB) as well as dichloro-diphenyl-trichloro-ethane (DDT), which are two pesticides and persistent organic pollutants (POPs) currently found in the environment (Fernández-Rodríguez et al., 2015; Paíga et al., 2021). Bisphenols and phthalates are endocrine-disrupting plasticizers widely used in the manufacture of food packaging, toys, or cosmetics (Kirchnawy et al., 2020; Koniecki et al., 2011; Zlatnik, 2016). Bisphenol A (BPA) has been banned in many products (Alharbi et al., 2022), but has been replaced by chemically related compounds such as bisphenol S (BPS) (Fouyet et al., 2021). Perfluoroalkylated substances, common in textile manufacture and various household and food products, can also accumulate in the body (Abercrombie et al., 2019; Brusseau et al., 2020). Finally, many industrial and consumer products have used the polybrominated diphenyl ether (PBDE) family, which are brominated flame retardants (Alaee, 2003).

The developmental origins of health and disease (DOHaD) are based on the observation that the maternal environment influences offspring development and long-term phenotype (Barker et al., 1989). Maternal nutritional status during pregnancy was first demonstrated as a major environmental factor favouring the onset of non-communicable diseases in adulthood such as hypertension, diabetes, or coronary heart disease (Osmond and Barker, 2000; Fernandez-Twinn and Ozanne, 2010). This concept has then been extended to any deleterious maternal environment during the preconception period shown to negatively influence offspring's risk of developing non-communicable diseases in adulthood. Preconception period included folliculogenesis. During this period, the primordial follicles pool is established in the fetal ovary (Kerr et al., 2013). Then, this pool is continually depleted due to both initiation of the process of folliculogenesis and atresia (Kerr et al., 2013). From puberty to menopause, the folliculogenesis can lead to the ovulation of a mature and fertilizable oocyte. So, the oocyte quality is linked to the folliculogenesis period, which is sensitive to chemical environment (Johansson et al., 2017; Land et al., 2022). After fertilisation, the zygote and the preimplantation embryo are submitted to several molecular events leading to embryonic genome activation (Marcho et al., 2015). The preimplantation period seems to be a period of vulnerability, but in humans, this period is not easy to investigate and the potential impact of environmental contaminants during this short window has hardly been studied.

The placenta, located at the interface of mother and fetus, has endocrine, metabolic, and immune functions and ensures the control of fetal growth through the supply of nutrients. It is considered as the programming agent of fetal phenotype (Longtine and Nelson, 2011). In response to maternal environmental disturbances, the placenta adapts to ensure fetal survival, interfering with fetal metabolic programming (Maltepe and Fisher, 2015), and thus affecting the individual's future health (Gingrich et al., 2020; Vrooman et al., 2016). The placenta is an organ of dual origin: maternal and embryonic. On the embryonic side, it is the trophectoderm, an outer epithelium of the blastocyst, which differentiates to form the placental trophoblasts. In contrast, the other placental and extra-placental structures come from the embryo itself (Maltepe and Fisher, 2015). The embryonic origin of the placenta is derived from the functionality of the two parent gametes. An environmental disturbance during either spermatogenesis or folliculogenesis can have consequences for embryonic and fetal development, with long-term repercussions on the offspring's phenotype (Capobianco and Pirrone, 2023; Jorge et al., 2023). In addition, the preimplantation embryo is also sensitive to environmental perturbation (Reik et al., 2001; Velazquez et al., 2023).

The general population is not exposed to a single contaminant but to a multitude of compounds, contributing to the human exposome (Buck Louis et al., 2017). In mixtures, contaminants could interact synergistically or antagonistically (Martin et al., 2021), justifying the need for studies on the effect of exposure to mixtures that better reflect the reality of exposure. Indeed, the HOME human cohort demonstrated that high exposure to a combination of contaminants (POPs, phenols, and phthalates) during gestation affects fetal development and growth associated with decreased gestational length (Kalloo et al., 2020). This study is part of the FEDEXPO project ("Folliculogenesis and Embryo Development EXPOsure to a mixture of toxicants") that was designed to mimic in an animal model a complex exposure to environmental contaminants at levels described in women of childbearing age (El Fouikar et al., 2023).

The present study aims to evaluate, using a rabbit model, the fetoplacental effects of maternal exposure to a mixture of eight environmental contaminants, representative of the different families of endocrine disruptors mentioned above, during two critical windows in reproduction, namely folliculogenesis in females and preimplantation development. To ensure our study will focus on observing the effects of the mixture during these two developmental windows only, embryos from these exposed females were transferred into synchronized non-exposed females. The mixture of environmental contaminants was defined based on the characterization of exposomes from the HELIX (Human Early Life Exposome) consortium of mother-child cohorts (Maitre et al., 2018), including the INMA (Infancia y Medio Ambiente) (Guxens et al., 2012; Robinson et al., 2015) cohort, dedicated to the follow-up of growth and development of the progeny related to maternal contaminant exposure during pregnancy. Human exposure doses were adapted to the rabbit animal model and validated after carrying out a toxicokinetic study (Gayrard et al., 2021). The rabbit model was chosen as it offers several physiological advantages (Brewer, 2006), such as a discoid and haemochorial placentation closer to that of humans than that of rodents (Fischer et al., 2012), and for its use in toxicology studies (Foote and Carney, 2000; Theunissen et al., 2016). The objectives of the present study were to explore the effects of maternal exposure during the two windows of interest described above on middle and late gestation fetoplacental growth and development, fetal metabolism, placental structure, and function.

2. Materials and methods

2.1. Experimental protocol

The experiment was authorized by the French Ministry of Research (APAFIS number No. 14787–201804201607003 v3). The authors complied with the ARRIVE guidelines (Animal Research: Reporting of the In Vivo Experiments available at http://arriveguidelines.org/). Sixteen New Zealand white female rabbits (INRA1777 line) were exposed daily by oral route (feeding metal cannula) to a mixture of 8 environmental chemicals dissolved in corn oil (0.5 ml/kg of body weight per day). This cannula was attached to a syringe containing the volume of solution concerned, taking into account the dead volume of the cannula. It was not a force-feeding procedure, the syringe was gently pushed into the deep mouth so that the solution was swallowed. A first group has been exposed to the contaminant mixture during folliculogenesis (between 2

and 19 weeks of age) and during the pre-implantation period, corresponding to the group of folliculogenesis and early embryo development (FED, n = 6). A second group was exposed to corn oil during folliculogenesis and then exposed to the contaminant mixture during the pre-implantation period (from 19 weeks of age when artificial insemination takes place, and up to 80 h after insemination), corresponding to the group of early embryo development (ED, n = 6). Controls received corn oil daily during folliculogenesis and preimplantation period (0.5 ml/kg of body weight, non-exposed group (CON, n = 4), as described elsewhere (El Fouikar et al., 2023). Rabbits were reared in groups of 3 per cage until 10 weeks of age, then divided into individual cages after 10 weeks. Rabbits were fed *ad libitum* (Stabifibre commercial feed) and tap water was given *ad libitum* in a glass bottle. Levels of background exposure of rabbits via water and food were also measured (Table S1). Rabbits were housed and reared in such a way as to avoid unintentional contamination by potentially endocrine-disrupting substances (El Fouikar et al., 2023). Animals were bred in rooms isolated from other rabbit holdings *via* a dedicated airlock with independent systems of air-handling, heating and watering. To reduce the rabbits' exposure to potential other endocrine disruptors, they were housed in cages fully made of galvanized steel and water was supplied using glass bottles with a threaded stainless-steel pipette. The ambient temperature of the rabbit housing was maintained at around 18 °C throughout the protocol, and the lighting cycle was 12h night:12h daylight. The rabbits were handled daily to accustom them to humans and limit stress. According to the scoring grids available for this species, the procedures did not induce any pain, suffering, or distress throughout the experimental protocol.

To mimic the daily exposure of women, the exposure doses were adapted to the rabbit model (Gayrard et al., 2021) to approximate plasma concentrations observed in two mother-infant cohorts *HELIX* (Maitre et al., 2018) and *INMA* (Guxens et al., 2012; Robinson et al., 2015). The concentrations to be reached for BDE-47 (2,2′4,4′-tetrabromodiphenyl ether), pp'DDE, HCB, PFOS (perfluorooctanesulfonic acid), and PFOA (perfluorooctanoic acid) were established according to the Tamayo-Uria study (Tamayo-Uria et al., 2019). The target concentrations for BPS, β-HCH, and DEHP were based on results from Gerona (Gerona et al., 2018), Vrijheid (Vrijheid et al., 2012), and Assens (Assens et al., 2019), respectively. These eight contaminants were selected to include at least one substance from each family of pollutants detected in 90% of the pregnant women in the cohorts, and because of their associations with fetoplacental and neonatal health effects. In order to implement the project, Gayrard and colleagues have modelized and validated the exposure scheme in the female rabbit. This scheme encompasses a loading dose administered on the first day to rapidly reach serum equilibrium concentration. A daily maintenance doses were given in the subsequent days in order to maintain daily stability of the targeted serum concentrations (Gayrard et al., 2021). In the loading dose, BDE-47, pp'DDE, HCB, β-HCH, PFOA, PFOS, DEHP, and BPS were diluted in corn oil at the concentrations of 8.9, 1051, 180, 165, 9.9, 56.7, 262 and 32 μg/kg-d, respectively. For the maintenance dose, the concentrations were 1.1, 96, 5.6, 10, 9.8, 1.0, 770 and 122 μg/kg-d, respectively, to achieve serum concentrations 10 times higher than the 90-95th percentile concentrations observed in women (El Fouikar et al., 2024; Gayrard et al., 2021) (Table S2). This high dose mimics the highest or maximum exposures to these chemicals that have been observed in mother-child cohorts, internationally.

At 19 weeks of age, ovulation of rabbit donors (n = 16) was induced by intramuscular injection of a GnRH agonist (Fertagyl®, MSD Animal Health, Brussels) and rabbits were artificially inseminated with pool of 4 fresh semen from the same 4 unexposed adult males known for their fertile potential (n = 4). To target the two vulnerability windows of interest (i.e. folliculogenesis and/or pre-implantation), 11 recipient rabbits were synchronized with the donors for embryo transfer, as described elsewhere (Rousseau-Ralliard et al., 2019, 2021). Briefly, 80 h after insemination, female rabbits were euthanized, and pre-implantation embryos (stage 80h post-insemination) were collected from oviducts by flushing and transferred in uterine horn in anaesthetized recipient rabbits after laparotomy. The mean number of recovered embryos per female was 8, 9 and 6 in the FED, ED and CON groups, respectively. Before transfer, health of embryos was evaluated as the proportion of embryos at the blastocyst stage at the time of their recovery, which was not different among groups. Four recipients received embryos from the ED group, 4 from the FED group, and 3 recipients received embryos from the CON group. One recipient rabbit received embryos form on or two donors. The mean number of embryos transferred per recipient was 9, 10 and 8 for FED, ED and CON embryos, respectively. Recipient dams were never exposed to the mixture nor during gestation. At 28 dpi (days post-insemination), i.e., 3 days from term, recipient rabbits were sedated (intramuscular injection of Torbugesic, 20 min *prior* euthanasia) and then euthanized by electronarcosis followed by exsanguination and fetoplacental units were collected for biometric measurements and blood and tissue sampling for further analyses (Fig. 1). On average, 96% of embryos transferred resulted in a fetus at 28 dpi for the control group (CON), versus 81% for the ED group and 77% for the FED group.

2.2. Ultrasound and Doppler monitoring

During gestation, recipient dams were examined at 14 and 28 dpi by ultrasonography using a Voluson E8 (GE Medical Systems) and an 8–16 MHz linear volume probe (RSP6-16). B-mode was performed transabdominally on 3–4 fetoplacental units per dam, 2 conceptuses per uterine horn sufficiently spaced apart to ensure that the same ones are not measured twice (i.e., n = 24 FED, n = 23 ED, and n = 16 CON fetoplacental units). At 14 dpi, body length and width, head length, biparietal diameter, fetal diameter and circumference, placental volume, and fetal heart rate were measured. At 28 dpi, 2/3D Doppler scans were performed transabdominally on 60 fetoplacental units (i.e., n = 21 FED, n = 23 ED, and n = 16 CON fetoplacental units) to measure cerebral and umbilical blood flows, as described elsewhere (Valentino et al., 2015), including the following parameters: systolic velocity (SV), diastolic velocity (DV), systolic/diastolic velocity ratio (SDV), resistance index (RI), pulsatility index (PI), umbilical and cerebral pulse (UP and CP, respectively). Placental volume was measured after 3D reconstruction, and placental perfusion was evaluated according to three indices: i) the vascularization index (VI) representing the vascular density, ii) the flow index (FI) representing the average blood flow intensity, and iii) the vascularization flow index (VFI), a combination of the first two representing placental perfusion (Lecarpentier et al., 2012; Valentino et al., 2015).

2.3. Feto-placental biometry

At 28 dpi, fetoplacental units (i.e., n = 27 FED, n = 31 ED, and n = 22 CON fetoplacental units) were weighed. Head length and biparietal diameter of the head (BIP) were measured with an electronic calliper. Abdominal circumference was measured using a string that encircles the fetus at the umbilicus without compressing it. The string was held in place by a clamp, and the distance between the clamp and the end of the string was plotted on a double decimeter. To measure the length of the fetuses, photos were taken on graph paper and loaded into image J software to measure the crown-to-rump length.

Fetuses were sexed by visual examination of the gonads as previously described (Valentino et al., 2015). The fetal brain, lungs, heart, liver, and kidneys were collected and weighed. Whole placentas were weighed and dissected out to separate and individually weigh labyrinth and decidua.

2.4. Biochemistry assays

Classical biochemistry (glycaemia, triglycerides, cholesterol, urea, creatinine, ASAT (aspartate aminotransferase) and ALAT (alanine aminotransferase)) was assayed on fetal plasma (n = 27 FED, n = 31 ED

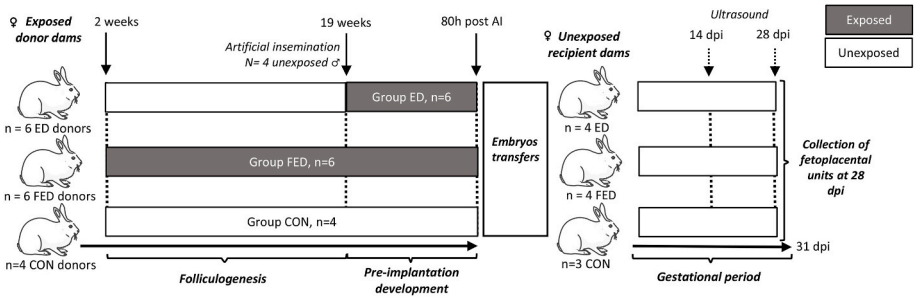


Fig. 1. Experimental protocol. Sixteen donor rabbits were exposed to the mixture of eight contaminants in corn oil: 6 donor does were exposed either during folliculogenesis and pre-implantation development (from 2 to 19 weeks of age when artificial insemination occurred, and up to 80 h post-insemination, FED group), 6 donor does exposed only during pre-implantation development (ED group) but exposed to corn oil only during folliculogenesis. Four donor controls (group of CON) were exposed to corn oil only from 2 weeks of age. Exactly 80 h after insemination, embryos were collected and transferred to synchronized recipient females. Each recipient received 6 embryos per horn, to obtain n = 4 FED, n = 4 ED, and n = 3 CON recipient groups. Ultrasound monitoring was performed at 14 and 28 days post-insemination (dpi). Pregnant females were euthanized at 28 dpi (3 days from term) to collect fetoplacental units.

and n = 22 CON fetuses) using a classical automated biochemistry analyzer (AU 5800 Beckman Coulter equipment). Plasma insulin was performed with chemiluminescent immunoassay using paramagnetic particles on an automaton analyzer (DXi Beckman Coulter equipment).

2.5. Placental immunohistochemistry and stereological analysis

The placental labyrinthine area of two males and two females per litter were randomly selected (n = 16 FED, n = 15 ED, and n = 12 CON placentas). Samples of the labyrinthine area were fixed and then embedded in paraffin. Vimentin immunodetection was performed on 7 μm sections to stain fetal vessels as previously described (Lecarpentier et al., 2012; Tarrade et al., 2014; Valentino et al., 2015) using a mouse monoclonal anti-vimentin antibody (IgG1, Clone V9, Sigma Aldrich, 1:200 in 2% BSA) and a biotinylated donkey anti-mouse secondary antibody (Jackson Immunoresearch, 1:500 in 2% BSA). Reaction amplification was performed using the ABC kit (Vector Laboratories, USA) containing avidin coupled to horse radish peroxidase (HRP). Staining of anti-vimentin sites was revealed with diaminobenzidine (DAB at 667 ng/ml) and 2% nickel ammonium, generating a black

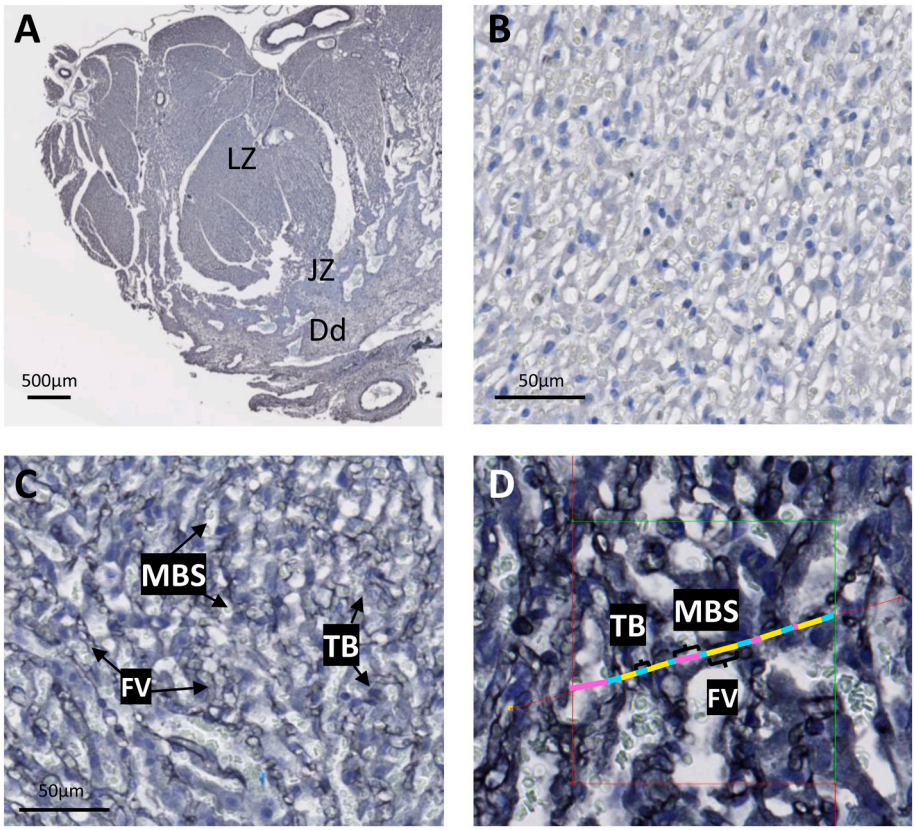


Fig. 2. Histology and immunohistochemistry of the rabbit placenta at 28 dpi. (A) Histological section of the placenta after immunodetection of vimentin. The rabbit placenta is composed of the labyrinthe zone (LZ) on the fetal side where exchanges take place, the decidua (Dd) on the maternal side, and the junctional zone (JZ) located between the other. Scale bar: 500 μm. **(B)** Negative control of vimentin immunodetection on a section (7 μm) of the labyrinthe zone of a rabbit placenta. Scale bar: 50 μm. **(C)** Immunodetection of a histological section of the labyrinthe zone of a rabbit placenta to stain fetal vessels (FV) and distinguish maternal blood space (MBS) and trophoblast (TB). Scale bar: 50 μm. **(D)** Stereological quantification of the three cellular structures that make up the labyrinthine zone: trophoblast (TB, blue bar), fetal vessels (FV, yellow bar) and maternal blood space (MBS, pink bar).

precipitate. The nuclei were stained with 0.1% toluidine blue. After scanning the slides (Nanozoomer, Hamamatsu™), the volume fraction (Vv) and the surface density (Sv) of the labyrinthine zone including trophoblast, maternal blood space, and fetal vessels (Fig. 2) were quantified using Mercator software and the One Stop Stereology tool as previously described (Favaron et al., 2013; Tarrade et al., 2014; Valentino et al., 2015).

2.6. RNA isolation and integrity

Total RNAs were extracted from 44 samples of labyrinthine area (i.e., n = 16 FED, n = 16 ED, n = 12 CON placentas; 2 males and 2 females per litter and per group) according to the method of Chomczynski and Sacchi (1987) using trizol (Fisher Scientific™). Total RNAs were collected and precipitated. RNA purification was then performed using the ISOLATE II RNA mini kit (Bioline, Meridian Bioscience).

2.7. Transcriptomic analysis

Transcriptomic analysis was performed using an Illumina NextSeq 500 sequencer (Illumina Inc.) with paired-end sequencing at an average depth of 45 million reads (n = 16 ED, n = 15 FED and n = 12 CON samples). The sequencing data were available in the NCBI BioProject database under accession number PRJNA1070544.

Descriptive analyses and analyses of differentially expressed genes according to group and sex were performed using the DESeq2 package (Love et al., 2014) of R software (version 4.1.2), resulting in 12 comparisons: (i) between each group (3 comparisons; n = 16 ED *vs.* n = 12 CON; n = 15 FED *vs.* n = 12 CON; n = 15 FED *vs.* n = 16 ED), (ii) between males and females within each group (3 comparisons); n = 6 CON females *vs.* n = 6 CON males; n = 8 ED females *vs.* n = 8 ED males; n = 7 FED females *vs.* n = 8 FED males) (iii) between treatment groups in males and females (2 comparisons); n = 8 FED males *vs.* n = 8 ED males; n = 7 FED females *vs.* n = 8 ED females), (iv) between each treatment group and controls, in males and females separately (4 comparisons); n = 8 ED males *vs.* n = 6 CON males; n = 8 ED females *vs.* n = 6 CON females; n = 8 FED males *vs.* n = 6 CON males; n = 7 FED females *vs.* n = 6 CON females). The rabbit genome was mapped using the OryCun2 assembly version, while gene definition and annotation were based on Ensembl version 105. Genes with an adjusted p-value <0.05 (Benjamini Hochberg method) and a Fold Change >1.5 or <1/1.5 were considered differentially expressed.

Gene Set Enrichment Analysis (GSEA) was carried out to identify enrichments in induced or repressed gene networks in response to exposure. For these analyses, five databases were used: i) GO BP (Gene Ontology - Biological Process), ii) GO CC (Gene Ontology - Cellular Component), iii) GO MF (Gene Ontology - Molecular Function), iv) KEGG (Kyoto Encyclopaedia of Gene and Genomes) and v) REACTOME. The normalized enrichment score (NES>|1.6|) obtained from the GSEA analysis, the False Discovery Rate (FDR<0.25), and the raw p-value (<0.05) were used to identify the most deregulated gene networks in the placenta in response to exposure to a mixture of eight environmental contaminants about the DEGs obtained.

2.8. Quantitative real-time polymerase chain reaction

One microgram RNA samples (N = 15 FED, N = 16 ED, and N = 11 CON placentas) were reverse transcribed into single-stranded cDNA in a 15 μL reaction mixture (Invitrogen). Real-time PCR was performed in a 15 μL reaction volume containing 10 μL of SYBR Green Mix (Applied Biosystems, USA), 5 μL template cDNA, and 300 nM primers using the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). Thermocycling conditions were, for 40 cycles, 95 °C for 15 s followed by 60 °C for 1min, and ended with a gradual rise to 95 °C. The results were analyzed using the StepOnePlus software (Applied Biosystems, USA). Melting curve analysis was performed to verify the amplification of a single product. The mRNA levels of each gene of interest were normalized and quantified using QbasPLUS software (Biogazelle). EIF4A2 (Eukaryotic translation initiation factor 4E family member 2), RPL18 (Ribosomal protein L18) and SDHA (Succinate dehydrogenase complex flavoprotein subunit A) mRNA levels were chosen as reference genes as their expression was not affected by treatment or sex. The primers used are listed in Table S3. They were synthesized by Eurogentec and standard curves were generated to calculate their efficiency.

2.9. Statistical analysis

Data are expressed as median [1st quartile; 3rd quartile]. Statistical analysis was performed using R software (version 4.1.2). Linear mixed-effects models were applied for biometric, biochemical, and stereological datasets using the nlme package (Pinheiro et al., 2022; Pinheiro and Bates, 2000), followed by type III ANOVA (Anova function in the car package (Weisberg and Fox, 2019)) with the recipient dam as a random effect, with treatment and fetal sex adjustment. For biometric, stereological, and biochemical data, the linear model was also applied with sex and litter size as fixed effects. If data distribution did not follow a normal distribution, a Permutation test was used (pgirmess package) (Giraudoux, 2022). When the group effect was statistically significant ($p<0.05$), a pairwise comparison (Tukey post-hoc test) of the three groups was performed using the lsmeans package (Lenth, 2016). Before applying the linear models, outliers were identified using the Rosner test (EnvStats package) (Millard, 2013).

Principal component analysis (PCA) and multiple factor analysis (MFA) of the different data sets (ultrasound, stereological, biometric, and biochemical) and their graphical representations were performed using the FactoMineR (Lê et al., 2008) and factoextra (Kassambara and Mundt, 2020) packages.

Because the normality of the RT-qPCR data was not validated, nonparametric statistical analyses were performed to compare the three exposed groups (i.e. FED, ED, and CON), taking into consideration fetal sex, using the Kruskal-Wallis test (stats package (R Core Team, 2022)), followed by pairwise comparison using Dunn's Kruskal-Wallis Multiple Comparisons test with Benjamini-Hochberg adjustment (FSA package (Ogle et al., 2023)) when the difference between groups was significant ($p<0.05$).

3. Results

3.1. Fetoplacental development and growth

Contaminant exposure during the FED and ED periods had no significant effect on the different ultrasound measurements at 14 dpi compared to CON group (Table S4).

At 28 dpi, maternal exposure to contaminants during the FED and ED periods had no significant effects on umbilical and cerebral blood flow compared to CON group (Table S5). Placental volume was not significantly different between the two exposed groups (FED and ED) compared to CON group. In contrast, a significant increase in placental volume (+14.63%, $p = 0.035$) was observed between groups of FED and ED, but placental perfusion indices were not statistically different between groups (Fig. 3).

3.2. Fetal and placental biometry

Exposure during folliculogenesis and/or pre-implantation had no significant effect on 28 dpi fetoplacental biometric data, nor on fetal morphometry or organ biometry, relative organ biometry, placental biometry, and placental efficiency defined as the gram of fetus produced per gram of placenta i.e. the fetal-to-placental weight ratio compared to CON group (Table S6).

Principal component analysis (PCA) of the fetoplacental biometric measurements demonstrated a difference between the ED and FED

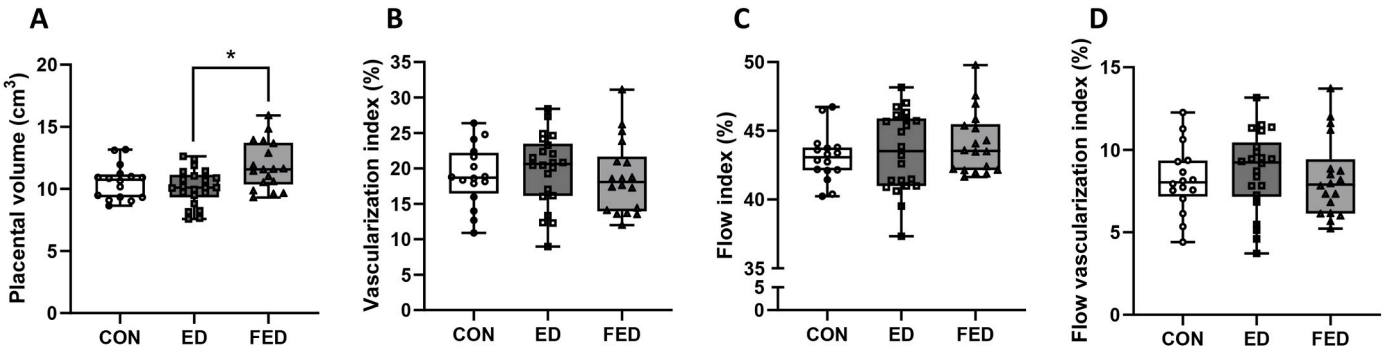


Fig. 3. Ultrasound monitoring of the rabbit placenta at 28 dpi. Quantitative 3D Doppler was performed to determine placental volume **(A)**, vascularization **(B)**, flow **(C)**, and flow vascularization index **(D)**. CON control group (n = 22); ED, exposed during preimplantation development (n = 31); FED, exposed during folliculogenesis and preimplantation development (n = 27). Significant differences between conditions are indicated by * (p-value <0.05).

groups and the CON group, with distinct confidence ellipses (Fig. S1A). The separation of the exposed groups (FED and ED) from the CON group is partly explained by the quantitative variable "fetal length", which was greater for the CON group. Additionally, the ED group was separated from the other groups by the brain weight, whereas the deciduae and labyrinthine weights separated the FED group from the others. When fetal sex was considered, however, confidence ellipses overlapped (Fig. S1B), erasing the small group effects.

3.3. Fetal biochemistry

Fetal biochemistry was largely affected by maternal exposure (Table 1). First, fetal plasma triglyceride concentrations ($p = 0.001$) significantly differed when comparing the three groups. Pairwise comparison showed that fetal FED concentrations were significantly lower (−15.09%, $p = 0.035$) than CON group (Fig. S2A). Both FED and ED exposure significantly affected total cholesterol, urea, ASAT, and ALAT in fetal plasma ($p = 0.027$, $p = 0.022$, $p = 0.015$, and $p = 0.032$, respectively) compared to CON group, but the pairwise comparison did not reach statistical significance between groups (Figs. S2B, D, E and F). Plasma HDL cholesterol concentrations were significantly lower (−17.28%, $p = 0.028$) in FED fetuses compared to ED fetuses (Fig. S2C). Exposure did not affect glycaemia, plasma insulin, or non-HDL cholesterol or plasma creatinine concentrations in fetuses. No sex effects were observed for the measured biochemical parameters.

PCA of fetal biochemical data (Fig. S3) showed that FED fetuses were different from the other two groups with distinct ellipses. The FED fetuses differed from the ED and CON fetuses on dimension 5, in terms of plasma creatinine, urea, and glucose concentrations. The ED and FED fetuses differed on dimensions 2 and 5, because a high glycaemia and plasma urea concentrations described the ED fetuses (Fig. S3A). When considering the sex of the fetuses (Fig. S3B), PCA showed that there was no sex-specific effect within the same group but that a sex-specific response to exposure occurred in females with the ellipses of the group of FED females that were clearly separated from those of the group of ED females. Thus, this sex-specific response occurred in females, according to the exposure period, in terms of plasma insulin and non-HDL cholesterol concentrations, as seen in dimension 5.

3.4. Placental morphometry

No effect of exposure was observed on fetal vessels and trophoblast volume fractions, nor on the surface density of the three cellular structures composing the labyrinth, whatever the group (Table S7). The maternal blood space volume fraction was significantly decreased in the FED labyrinthine compared to controls (−12.49%, $p = 0.036$).

3.5. MFA combining biometric, stereological, and biochemical datasets

The MFA combining biometric, biochemical, and stereological data sets from the same individuals at 28 dpi indicated significant differences between the three groups (i.e. FED, ED, and CON) as well as between males and females within the same group, with distinct confidence ellipses (Fig. 4A). The combination of the three data sets enabled the grouping of the fetoplacental units into 3 main clusters, specific to each exposure (Fig. 4B). These clusters could be explained by differences in biometric, biochemical, and stereological measurements characterizing each group (Fig. 4C). The effect of the exposure period was represented

Table 1
Effects of maternal exposure to a mixture of contaminants, during preimplantation (ED) or folliculogenesis and preimplantation period (FED) periods, compared to control rabbit (CON), on fetal biochemistry at 28 dpi, in rabbit.

Biochemical assay	Number of fetuses			Median [Q1; Q3]			p-value
	CON	ED	FED	CON	ED	FED	
Glycaemia (mmol/L)	22	31	26	3.68 [3.34; 3.91]	3.45 [2.89; 3.83]	3.49 [3.05; 3.79]	0.255
Insulin (mUI/L)	22	31	27	2.53 [1.95; 3.24]	2.24 [1.37; 3.10]	3.11 [1.99; 3.94]	0.433
Triglycerides (mmol/L)	22	31	27	1.06 [0.93; 1.32]	0.99 [0.90; 1.09]	0.90 [0.76; 1.02]a	0.001*
Cholesterol total (mmol/L)	22	31	27	2.52 [2.26; 2.84]	2.71 [2.46; 3.02]	2.54 [2.23; 2.73]	0.027*
HDL cholesterol (mmol/L)	22	31	26	0.74 [0.67; 0.84]	0.81 [0.71; 0.89]	0.67 [0.60; 0.79]b	0.001*
LDL cholesterol (mmol/L)	22	31	27	1.80 [1.60; 2.00]	1.84 [1.77; 2.07]	1.78 [1.60; 2.05]	0.556
Urea (mmol/L)	20	31	25	4.95 [4.62; 5.07]	5.15 [4.99; 5.40]	4.48 [3.88; 4.75]	0.022*
Creatinine (µmol/L)	22	31	27	66.20 [64.48; 69.58]	69.60 [64.70; 72.70]	70.80 [67.00; 76.20]	0.121
ASAT (UI/L)	22	31	25	159.5 [114.7; 198.3]	132.1 [115.4; 152]	158.2 [145.9; 190.1]	0.015*
ALAT (UI/L)	22	31	26	12.85 [8.60; 17.63]	9.50 [7.70; 11.60]	14.05 [9.73; 15.98]	0.032*

Data are expressed as: Median [1st quartile; 3rd quartile]. The statistical analysis of the data was performed by applying linear mixed effects models, followed by type III ANOVA. ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase. When the group effect was statistically significant (*p < 0,05), a pairwise comparison was performed by least-squares means test. a indicates a significant difference compared to the CON group. b indicates a significant difference compared to the ED group.

on dimension 1, with the two exposed groups (FED and ED), positively and negatively correlated, respectively to that dimension. FED fetuses differed from the CON fetuses by the decidua weight. The ED fetuses differed from those in the CON group by the fetal length and placental efficiency. In contrast, the ED group differed from FED group in terms of placental efficiency, relative brain weight, plasma lipids and urea. The graph of the variable groups of the MFA (Fig. 4D) highlighted that the data variabilities were mainly explained by the maternal effect, followed by the effect of exposure (i.e. FED, ED, or CON) and then by the effect of exposure according to the sex (i.e. FEDF, FEDM, EDF, EDM, CONF or CONM), which are the qualitative variables most correlated with the dimensions represented ($R^2 > 0.75$). Indeed, a sex-specific effect was observed on dimension 5 (data not shown, see Fig. 4E), with females and males positively and negatively correlated, respectively, whatever the exposure group.

3.6. Placental function

3.6.1. Overall PCA analysis

PCA of placental transcriptome from the CON, ED, and FED groups showed that there is a significant difference between the exposed placentas (ED and FED) compared to the CON placentas as well as between the two exposed groups (ED and FED). Indeed, the confidence ellipses of the three groups (CON, ED, and FED) are distinct in the plane delimited by the dimensions 2 and 4 (Fig. 5A). The expression of all placental genes therefore seems to be specific to each group. Furthermore, when groups are compared considering fetal sex, only males of each group CON, ED, and FED are different from each other, with well-separated ellipses (Fig. 5B). Thus, considering fetal sex, differential analysis showed that maternal exposure to contaminants dysregulated placental gene expression in a sex-specific manner whatever the comparison (Table 2).

3.6.2. Differential gene expression analysis

3.6.2.1. ED vs control. Comparison between the groups of ED and CON revealed 7 differentially expressed genes (DEGs, underexpressed in ED vs CON) in males (Table 2, Fig. 6A) and 39 in females (9 overexpressed genes and 30 underexpressed genes; Table 2, Fig. 6B).

In males, DEG were underexpressed and involved in lipids and atherosclerosis like *PLA2G4E* (Phospholipase A2 group IVE, underexpressed) and *TLR6* (Toll-like receptor 6, underexpressed).

In females, *TLR6* was also underexpressed, and *CYP1B1* (Cytochrome P450 family 1 subfamily B member 1), involved in AhR (Aryl hydrocarbon Receptor) signaling pathways was overexpressed.

TRL6 was underexpressed both in females and males in the ED group compared to those in the control group (CON F and CON M, respectively). However, this underexpression was similar in males and females of each group.

3.6.2.2. FED vs control. Fifty-nine DEGs (17 overexpressed and 42 underexpressed) were identified in FED compared to CON males (Table 2, Fig. 6C), whereas 219 genes were differentially expressed in females (166 overexpressed and 53 underexpressed) (Table 2, Fig. 6D).

In males, DEG were involved in drug/xenobiotic metabolism, such as *TK1* (Thymidine kinase 1, overexpressed).

In females, *HMGCS1* (3-hydroxy-3-methylglutaryl-CoA synthase 1), *MMP1 (matrix metallopeptidase 1)* and *IL1RN* (Interleukin 1 receptor antagonist) genes were overexpressed, as well as the *PLA1A* (Phospholipase A1 member A) gene that is involved in glycerophospholipid metabolism. In contrast, *CPT1B* (Carnitine palmitoyl transferase 1B) involved in the PPAR (Peroxisome proliferator-activated receptor) signaling pathway, was underexpressed. Other DEGs were involved in drug/xenobiotic metabolism, such as *FMO1* (Flavin containing dimethylaniline monooxygenase 1; underexpressed) and *RRM2* (Ribonucleotide reductase regulatory subunit M2; overexpressed).

3.6.2.3. FED vs ED. Regarding the comparison between groups of FED and ED males, 20 genes were differentially expressed (12 overexpressed and 8 under-expressed in FED compared to ED; Table 2, Fig. 6E) while 222 genes (184 overexpressed and 38 under-expressed) were differentially expressed between females (Table 2, Fig. 6F).

In males only, the *TK1* gene involved in drug and xenobiotic metabolism was overexpressed. *SLC27A2* (Solute carrier family 27 member 2), *MMP1,* and *IL1RN genes* were overexpressed in females, as well as *DGKG* (Diacylglycerol kinase gamma) and *PLA1A* genes involved in lipid metabolism and PPAR signaling pathway. Genes involved in drug and xenobiotic metabolism such as *GSTA5* (Glutathione S-transferase Yc), *RRM2* and *ALDH1A1* (Aldehyde dehydrogenase 1 family member A1) were overexpressed, as well as the *IL1B* gene involved in lipid and atherosclerosis. In contrast, the *CYP1B1* gene involved in the AhR signaling pathway was underexpressed.

3.6.3. RT-qPCR

The results of the transcriptomic analysis were confirmed by RT-qPCR for 12 genes of interest involved in the PPAR or AhR signaling pathways (*SLC27A2, HMGCS1, CPT1B*), in lipid metabolism (*DGKG, PLA1A),* in the inflammatory response. *(IL1B, TLR6*) or in drug and xenobiotic metabolism (*FMO1, GSTA5, TK1, RRM2, ALDH1A1*) (Fig. S4, Table S8).

3.6.4. Gene set enrichment analysis

The GSEA analysis was carried out on the entire groups, including males and females, and aimed to study the deregulation of specific gene networks during the different exposure windows.

3.6.4.1. ED vs control. For ED placentas, enrichment analysis revealed 112 modulated gene networks, across all databases, including 54 depleted and 57 enriched gene networks compared with the CON placentas.

Among the depleted gene networks, three ("NAD + nucleosidase activity", "antigen processing of cross-presentation" and the "regulation of TLR by endogenous ligands") included the under-expressed *TLR6* gene identified in the comparison of ED versus CON placental gene expression (Fig. 7A). The *SLC27A2* and *ALDH8A1* genes, which were not deregulated in this comparison but overexpressed in FED placentas, were also identified in 4 gene networks enriched here (“complement and coagulation cascades", “tryptophan metabolism”, “peroxisomal lipid metabolism”, and “integral component of endoplasmatic reticulum membrane”).

3.6.4.2. FED vs control. Across all databases, the enrichment analysis showed that 279 gene networks were altered in FED *vs* CON placentas. Of these gene networks, 208 were enriched and 71 were depleted.

Among the enriched gene networks (Fig. 7B), two include the over-expressed *HMGCS1* gene ("cholesterol biosynthesis" and PPAR signaling pathways"). This latter gene network also includes the *CPT1B* gene, that is underexpressed in this comparison (FED versus CON). This gene is also found in the "signaling by retinoic acid" enriched network. The "MMPs activation" enriched gene network obviously includes the *MMP9* gene previously identified as being over-expressed in the FED group compared with the control.

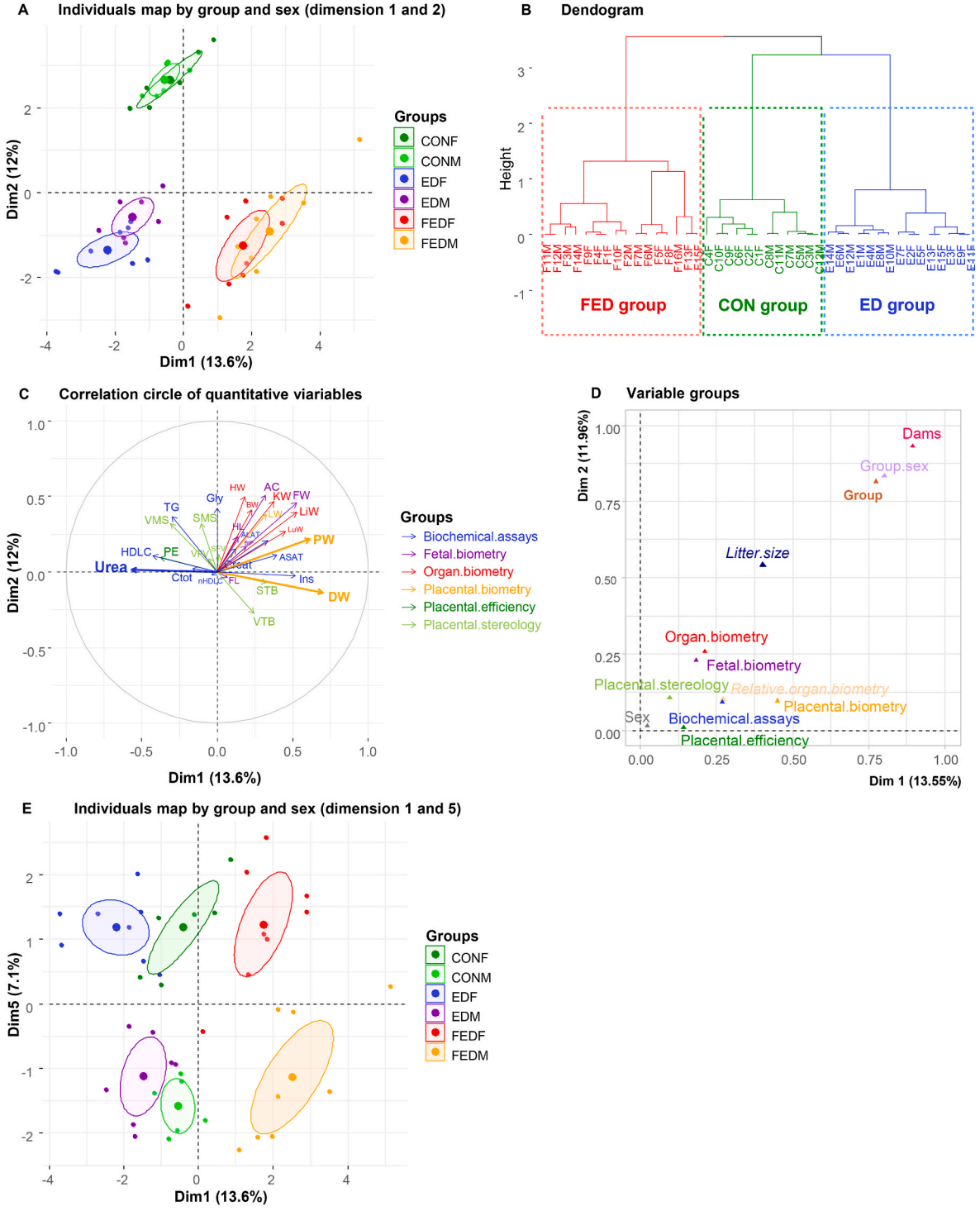
3.6.4.3. FED vs ED. Altogether, 419 gene networks were deregulated in the FED placentas compared to the ED placentas. Of these gene networks, 252 were enriched and 167 were depleted.

Enriched gene networks included the "PPAR signaling pathway" with the overexpressed MMP1 gene and the "lipid and atherosclerosis" network with the overexpressed IL1B and MMP1 genes. The DEGs globally involved in drug and xenobiotic metabolism, identified above

(*FMO1, RRM2, ALDH1A1, ALDH8A1, CYP1B1, GSTA5*), were also found in several enriched gene networks, such as: "phase I functionalization of compounds", "drug metabolism - other enzymes", glutathione metabolic process" and 'monooxygenase activity. Among the enriched gene networks identified in this comparison (Fig. 7C) were "retinol metabolism", "diseases of metabolism", "regulation of lipid metabolism by PPARα" and "metabolism of steroids" gene networks.

4. Discussion

Most studies relating maternal exposure to environmental contaminants and adverse effects in infants used maternal biomonitoring data during pregnancy. However, the exposure of women within human cohorts begins well before pregnancy and even before the parental desire to conceive the future child, because chemical pollution of our environment is omnipresent. Since the first publication of Barker's hypothesis (Barker et al., 1989), several publications have pointed out the importance of the periconception period in the process of DOHaD,



(caption on next page)

Fig. 4. Multiple factor analysis (MFA) of data sets including biometric, stereological, and biochemical measurements of fetoplacental units at 28 dpi from groups of ED, FED, and CON fetuses (on the plane defined by dimensions 1 and 2). **(A)** Multiple factor analysis (MFA) map of individuals. Fetoplacental units from control dams (CON; n = 22) are shown in dark green (females) and light green (males). Fetoplacental units from rabbits exposed during folliculogenesis and pre-implantation development (FED; n = 27) are shown in red (females) and orange (males) and those of rabbits exposed only during preimplantation development (ED; n = 31) are shown in blue (females) and purple (males). Dimensions 1 and 2, presented here, explain 25.6% of the variability in the data. Qualitative factor analysis shows that the groups of CON males and females are positively correlated with dimension 2 (v.test = |3,79| > 2 and v.test = |3.78| > 2, respectively), whereas the groups of ED et FED fetuses are negatively correlated to this dimension, reflecting an effect of exposure to the mixture. The groups of ED males and females are negatively correlated with dimension 1 (v.test = |-2.17| > 2 and v.test = |-3.51| > 2, respectively). In contrast, the groups of FED males and females are positively correlated with this dimension (v.test = |3.97| > 2 and v.test = |2,75| > 2, respectively), reflecting an effect of the window of exposure to the mixture. The ellipses for each group are distinct but the ellipses between males and females in each condition overlap. **(B)** Dendrogram of hierarchical cluster obtained using MFA. The MFA grouped the individuals into 3 clusters according to biometric, biochemical, and stereological parameters. The red cluster is composed of the group of FED fetuses and the green cluster is composed of the group of CON fetuses. The blue cluster is composed of the group of ED fetuses. **(C)** Correlation circle of quantitative variables from the MFA, with blue vectors for biochemical parameters, purple vectors for fetal biometric parameters, red vectors for fetal organ biometry, orange vectors for placental efficiency, dark green vectors for placental biometry and light green vectors for placental stereological parameters. The description of the dimensions shows that the weight of the decidua contributes strongly positively to dimension 1 ($R^2 = 0.70$, $p = 1.71^{e-07}$) as well as placental weight ($R^2 = 0.61$, $p = 1.15^{e-05}$) whereas fetal urea concentration contributes strongly and is negatively correlated to this dimension ($R^2 = -0.57$, $p = 7.31^{e-05}$). Biometric data of fetal organs contribute strongly and positively to dimension 2. **(D)** Plot of variable groups illustrates the correlation between variable groups and dimensions. Dimensions 1 and 2, presented here, explain 25.6% of the variability in the data. The "dams" qualitative variable is positively correlated with dimensions 1 and 2 ($R^2 = 0.89$, $p = 8.69^{e-13,}$ and $R^2 = 0.93$, $p = 7.72^{e-16}$, respectively), this reflects a very wide maternal effect on the fetus and placental datasets; followed by an effect of exposure, indeed the "group" variable (i.e. FED, ED or CON) which is positively correlated with dimension 1 ($R^2 = 0.77$, $p = 1.22^{e-13}$) and with dimension 2 ($R^2 = 0.82$, $p = 2^{e-15}$). The effect of exposure considering the sex of the fetuses (GroupSex) is also correlated with dimension 1 ($R^2 = 0.81$, $p = 5.22^{e-12}$) and dimension 2 ($R^2 = 0.83$, $p = 1.86^{e-13}$). **(E)** Multiple factor analysis (MFA) map of individuals on the plane defined by dimensions 1 and 5. Dimensions 1 and 5, presented here, explain 20.7% of the variability in the data. Qualitative factor analysis shows that individuals are separated by sex on dimension 5, since females are positively and males negatively correlated with this dimension (v.test = |5.60| > 2 and v.test = |-5.60| > 2, respectively). The description of the dimension 5 shows that the qualitative variable "Sex" and "GroupSex" were strongly and positively correlated to this dimension ($R^2 = 0.75$, $p = 8.58^{e-14}$ and $R^2 = 0.76$, $p = 1.95^{e-10}$, respectively). VFV, volume fraction of fetal vessels; VTB, volume fraction of trophoblast; VMS, volume fraction of maternal blood space; SFV, surface density of fetal vessels; STB, surface density of trophoblast; SMS, surface density of maternal blood space; FW, fetal weight; FL, fetal length; HL, head length; BIP, biparietal diameter; AC, abdominal circumference; BW, brain weight; LuW, lung weight; HW, heart weight; LiW, liver weight; KW, kidney weight; PW, placental weight; LW, labyrinth weight; DW, decidua weight; PE, placental efficiency; Gly, glycaemia; Ins, insulin; TG, Triglycerides; Ctot, cholesterol total; HDLC, HDL cholesterol; nHDLC, non-HDL cholesterol; Creat, creatinine; ASAL, aspartate aminotransferase; ALAT, alanine aminotransferase.

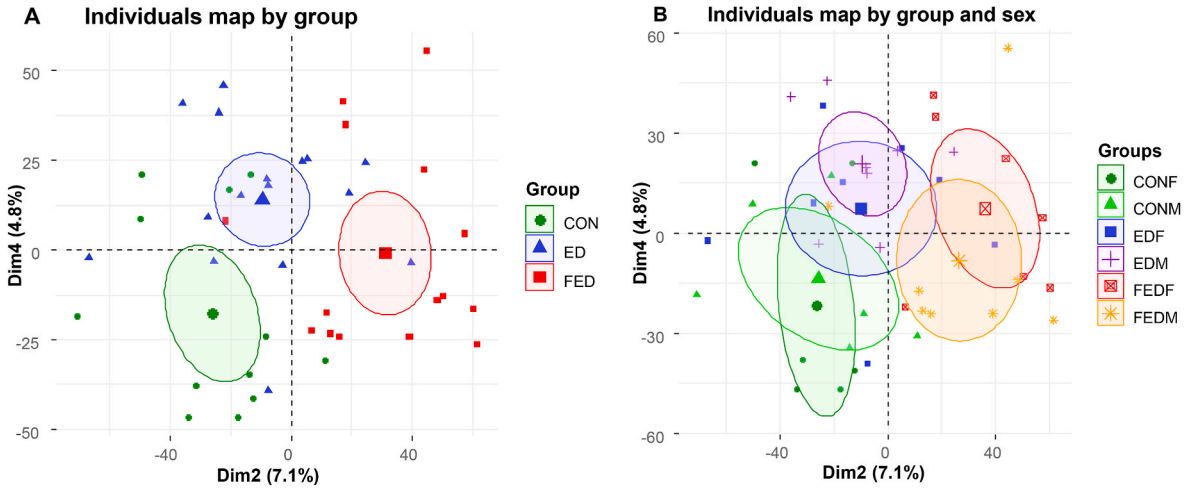


Fig. 5. Principal Component Analysis of placental gene expression at 28 dpi exposed or not to the mixture of eight environmental contaminants during folliculogenesis and/or pre-implantation development defined by dimensions 2 and 4. Dimensions 2 and 4 explain 11.8% of the variability between the 3 groups. **(A)** Individuals-PCA map considering the effects of FED and ED exposure compared to the control (CON). Control placentas (CON) are shown in green (n = 12), exposed ones during folliculogenesis and preimplantation development (FED) are shown in red (n = 16) and those exposed only during preimplantation development (ED) are shown in blue (n = 16). The well-separated confidence ellipses indicate that the gene expression is group-specific. Dimension 2 represents the difference between the groups of FED and CON placentas, and dimension 4 between the groups of ED and CON placentas. Indeed, the analysis of qualitative factors shows that the group of CON placenta is negatively correlated (v.test = |-3.03| > 2) while the group of FED placentas is positively correlated (v.test = |4.39| > 2) to dimension 2. The group of ED placentas is positively correlated with dimension 4 (v.test = |2.63| > 2). **(B)** Individuals-PCA map considering the effects of both exposure and the sex of the fetuses. Group of CON females are shown in dark green and males in light green. Groups of ED females and males are represented in blue and purple, respectively. The group of FED females is represented in red and those of FED males in orange. The analysis of qualitative factors shows that the groups of CON males and females are negatively correlated (v.test = |-2.01| > 2 and v.test = |-2.08| > 2, respectively) whereas the groups of FED males and females are positively correlated (v.test = |2.47| > 2 and v.test = |3,11| > 2, respectively) to dimension 2. The group of ED males is positively correlated with dimension 4 (v.test = |2,35| > 2).

particularly in contexts of obesity or maternal overnutrition, undernutrition or use of assisted reproductive technologies (Hadar et al., 2015; Velazquez et al., 2019). Although the effects of maternal exposure to chemical substances on the fetal and postnatal phenotype are increasingly being explored throughout gestation, little data is available to date to identify the windows of vulnerability most susceptible to endocrine disruption and their effects on offspring (Green et al., 2021). To better understand the effects of an identified but complex chemical exposome, it is necessary to rely on an animal model, in particular, to target specific windows of vulnerability, such as folliculogenesis and pre-implantation

Table 2
Number of differentially expressed genes (DEGs) in the placenta maternally exposed to a mixture of contaminants, during preimplantation (ED) or folliculogenesis and preimplantation period FED) periods, compared to the control (CON), according to the group and the sex of the fetuses.

Comparison	Number of DEGs (adj_p < 0.05, FC > 1.5 or <1/1.5)	Number of overexpressed gene	Number of underexpressed gene
ED vs CON	60	15	45
FED vs CON	268	130	138
FED vs ED	206	150	56
CON ♂ vs CON ♀	4	1	3
ED♂ vs ED♀	7	1	6
FED♂ vs FED ♀	4	2	2
ED♂ vs CON ♂	7	0	7
FED♂ vs CON ♂	59	17	42
FED♂ vs ED♂	20	12	8
ED♀ vs CON ♀	39	9	30
FED♀ vs CON ♀	219	166	53
FED♀ vs ED♀	222	184	38

♂, females; ♀, males; adj p, adjusted p-value; FC, fold change.

development, as explored in the present study.

To identify the most sensitive windows of vulnerability to environmental perturbations, a mixture of eight compounds, including PFOS, PFOA, pp'DDE, HCB, β-HCH, BDE-27, DEHP and BPS, was selected on the basis of the INMA-Sabadell cohort (INfancia y Medio Ambiente, 1997–2008) as described by Robinson et al. (2015). This cohort was selected among other European cohorts regrouped in the HELIX project ((Maitre et al., 2018), 1999–2010). In the present study, we chose to reproduce in female rabbit serum concentrations that equal ten times the 90-95th percentile values observed in European women. It is essential to acknowledge that the percentiles used in our study are based on assessments predating 2020. It is highly likely that some percentiles of women's exposure have changed since then. Moreover, these reference values are not only time-dependent but also geographically dependent. Although we chose to focus on a specific population (Western women), reference values can vary significantly depending on the chemicals, even among Western countries (Fillol et al., 2021) and according to recent reports from the French cohort Esteban https://www.santepubliquefrance.fr/). For example, our 0.156 ng/mL serum target for BDE-47 is inferior to the 75th percentile value in the US HOME cohort (Kalloo et al., 2020) and the 95th percentile value in the Canadian MIREC cohort (Fisher et al., 2016), as described by El Fouikar (El Fouikar et al., 2023). We, therefore, believe that our target concentrations are relevant for highly exposed women in developed countries and for common exposures in developing countries.

To characterize the effects of exposure to the selected mixture in the targeted windows of vulnerability, the present study was performed on a rabbit model. The rabbit was chosen for its numerous advantages: stage of embryo implantation, discoid and haemochorial placentation close to that of humans compared to rodents or sheep, monitoring of the feto-placental development at several stages without anaesthesia, relevant model to study toxicity (Fischer et al., 2012; Theunissen et al., 2016) and long-term health effects. Moreover, the rabbit is a more suitable animal model for toxicokinetic purposes since, in rabbits as in humans, urinary elimination predominates unlike other species (Toutain et al., 2010). The rabbit model is also relevant because gonadal steroid production during fetal life is similar to that of humans (androgens by testes, estrogens by ovaries) (George and Wilson, 1979; Jolivet et al., 2022). However, this model presents several disadvantages: herbivore diet, short gestational period (31 days), and polytocous species. The latter could be an advantage for studying the sex-specific response of the offspring since we always have at least one female and male fetus per litter. Indeed, following environmental stress, such as exposure to a high-fat diet, the rabbit model has already revealed sex-specific responses (Tarrade et al., 2013).

To evaluate the effects of preconception and/or preimplantation periods, embryo transfers were performed at 80 h post-insemination, as well as embryos from the control group. The CON group received corn oil daily during folliculogenesis and preimplantation period to compare FED or ED to CON group and FED and ED with each other. Feto-placental units were collected from 3 does in the control group and 4 does in the exposure groups. As described by El Fouikar (El Fouikar et al., 2023), hypothesizing a decrease of 33% in the embryo implantation rate in the exposed groups, i.e. 40% instead of 60% in the CON group, at least 48 embryos in the 2 exposed groups and 36 in the CON group have been transferred into, respectively, 4, 4 and 3 non-exposed recipient mothers, to reach 12 embryos per female. Consequently, the experimental protocol was designed to consider this estimate, even if the number of female recipients appears small. In addition, the data were analyzed by a robust and severe statistical method, namely a linear mixed-effects model with the recipient dam as a random effect, with treatment and fetal sex adjustment to analyse all the data.

At 28 dpi, neither the FED nor ED group was different from the control group in terms of fetal growth and development. This lack of biometric effect in the FED and ED groups probably results from the fact that the female recipients' of embryos, involved in carrying them throughout gestation, have never been exposed to the mixture of pollutants. The conceptuses were therefore not exposed from implantation until the end of gestation. In contrast, in human cohorts, pregnant women are exposed before and throughout their pregnancy, and contaminants such as brominated flame retardants, organochlorine pesticides (pp'DDE, HCB, and β-HCH) as well as PFAS (PFOS and PFOA) and BPS, were negatively associated to infant birth size (Lopez-Espinosa et al., 2015; Tanner et al., 2020; Hu et al., 2019; Marks et al., 2019). However, these effects are not consensual, as exposure to DEHP was significantly correlated with an increase in birth weight, but only in boys (Zhu et al., 2018). Regarding effects observed in animal models, preconception and gestational exposure to BPA in rats increased fetal birth weight (Benincasa et al., 2020). In mice, maternal exposure to the same contaminant, BPA, would have the same effect on birth weight (Alonso-Magdalena et al., 2010). However, depending on the dose of exposure to BPA, effects on birth weight have not always been observed in mice (Luo et al., 2016). Consistent with the majority of epidemiological studies, maternal exposure to DEHP in rats reduces birth weight (Wei et al., 2012). In rats, exposure to a mixture of 15 phthalates and pesticides at twice the NOAEL (no observable adverse effect level) was shown to reduce pup weight at PND2 (post-natal day 2), although this effect was not observed at PND9 (Conley et al., 2021). Also, in rats, maternal exposure to pp'DDE had no effect on female fetal weight (Makita, 2004, 2008).

Even without any visible macroscopic effect on fetal growth and development, maternal exposure to the mixture of contaminant during the FED period decreased plasma triglyceride concentrations compared to the control group, while HDL cholesterol level was lower in the FED fetuses compared to the ED fetuses. These data suggest that the exposure to the mixture during the preconception period is sufficient to disrupt lipid metabolism. In fact, several studies have described similar effects on biochemical parameters in offspring's blood samples following gestational exposure to chemicals included in the mixture tested here. Thus, prenatal exposure to PFOA and PFOS was associated with increased levels of triglycerides, in a cohort of Chinese infants (Sinisalu et al., 2021), as well as prenatal exposure to PBDE in girls in a Dutch cohort (Berghuis et al., 2022). In rats, prenatal exposure to low doses of DEHP increased hepatic and serum triglycerides and serum cholesterol in offspring (Su et al., 2024). Similar effects on triglycerides and total cholesterol were observed, again in rats, after maternal exposure to BPA (Yang et al., 2022). In mice, prenatal exposure to BPS or BDE-27 increased liver triglyceride in newborn offspring (Zhang et al., 2023), but BDE-27 exposure decreased blood triglycerides in 10 months old male offspring (Khalil et al., 2018). The effect of pollutants on fetal

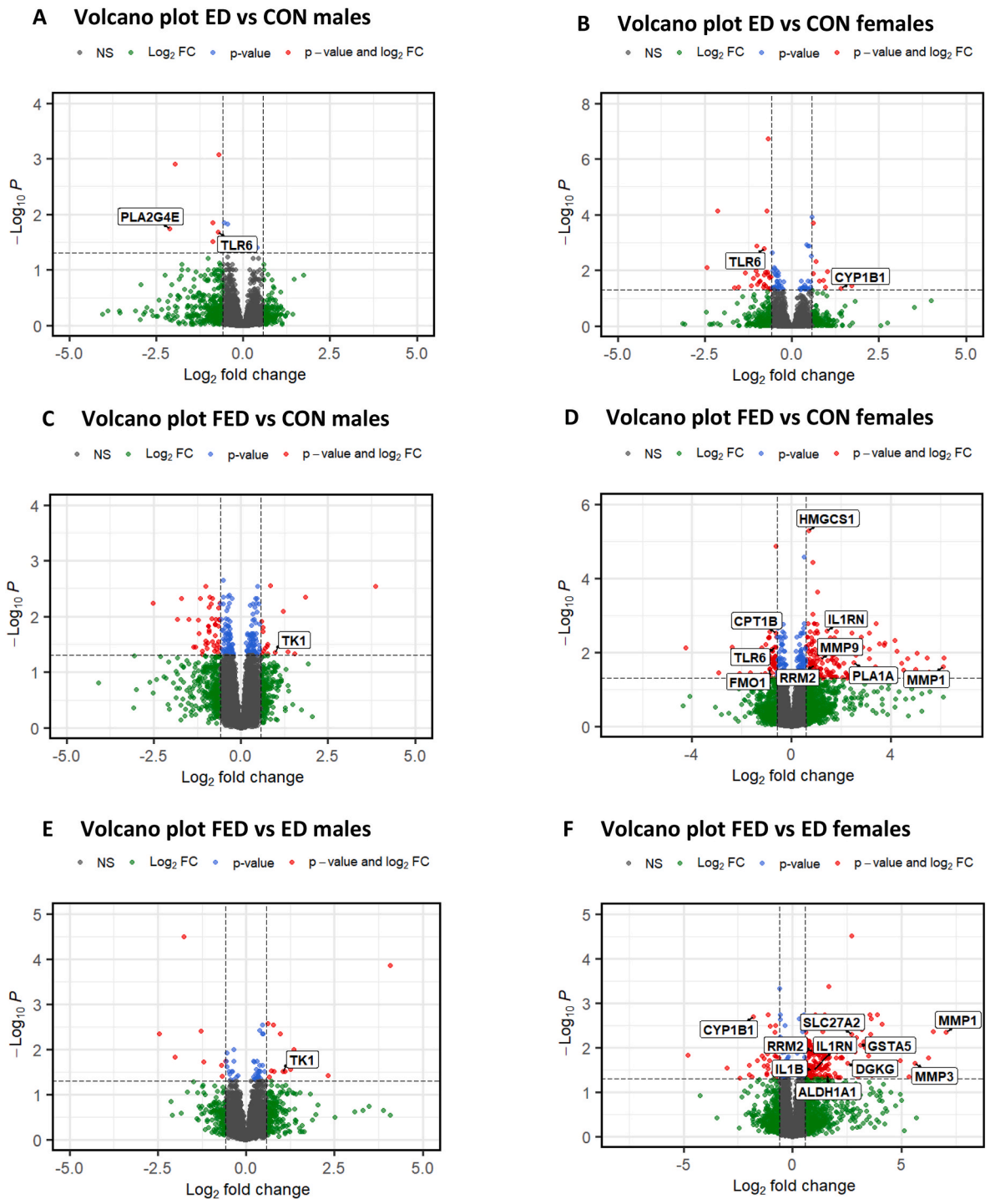


Figure 6. Volcano plot comparing pairwise placental gene expression of females and male fetuses from a maternal group exposed to a mixture of contaminants during preimplantation (ED) or during folliculogenesis and preimplantation (FED) or control group (CON), in pairs. Red dots represent significantly differentially expressed genes (DEGs; adjusted p-value <0.05 and Fold Change >1.5 or <1/1.5), blue dots represent DEGs with adjusted p-value <0.05, green dots represent DEGs with Fold Change >1.5 or <1/1.5 and grey dots represent non-differentially expressed genes. (A) Volcano plot representing placental gene expression of male fetuses from the groups of ED and CON. (B) Volcano plot comparing placental gene expression from the groups of ED and CON females. (C) Volcano plot pairwise representation of placental gene expression from the groups of FED and CON males. (D) Volcano plot comparing placental gene expression from the groups of FED and CON females. (E) Volcano plot representing placental gene expression from the groups of FED and ED males. (F) Volcano plot comparing placental gene expression from the groups of FED and ED females. Raw.pValue, raw p-value; and FC, Fold change describes the difference in gene expression between the first and the second condition.

metabolism could result from impaired fetal hepatic metabolism and/or placental transfer due to poor programming of hepatic and/or trophoblastic stem cells, respectively, at the embryonic stage. These questions will deserve to be addressed in the future.

Faced with this lack of clear effect (fetoplacental biometry) or these weak effects (fetal biochemistry and placental stereology (a weak decrease in the volume fraction of maternal blood space in the labyrinthine area in FED vs control placenta)), these three sets of data were

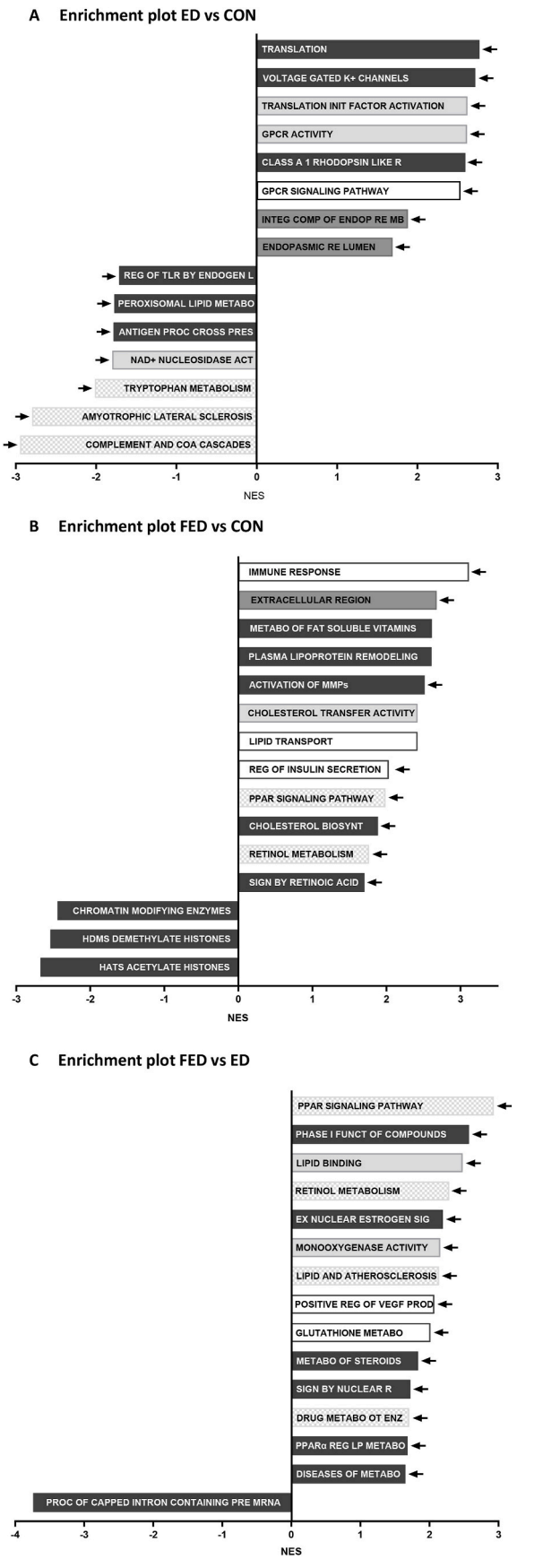


Figure 7. Gene set enrichment analysis (GSEA) in placentas at 28 dpi between groups compared two by two. Histogram representing the most depleted and enriched pathways in the placenta concerning the DEGs identified in the transcriptomic analysis. based on five databases: KEGG (white bar with grey check bar), REACTOME (black bar), GOBP (white bar), GOMF (light grey bar) and GOCC (dark grey bar) from (A) the group of ED placentas, exposed to contaminants during pre-implantation development compared with the group of control placentas (CON), (B) The group of FED placentas, exposed to contaminants during the folliculogenesis and the pre-implantation development, compared to those of the control placentas (CON) and (C) from the group of FED placentas compared to the group of ED placentas. Black arrows indicate the gene network in which DEGs are involved. K+, potassium; INIT, initiation; GPCR, G Protein-coupled receptor; R, receptor; INTEG, integral; COMP, component; ENDO, endoplasmic; RE, reticulum; MB, membrane; REG, regulation; ENDOGEN, endogenous; L, ligand; METABO, metabolism; PROC, processing; PRES, presentation; ACT, activity; COA, coagulation; MMPs, Matrix Metalloproteinases; BIOSYNT, biosynthesis; SIGN, signaling; FUNCT, functionalization; SIG, signaling; PROD, production; ENZ, enzymes; LP, lipid.

(caption on next column)

combined in MFA in order to better characterize the three groups of fetoplacental units (CON, ED and FED). As a result, this descriptive analysis showed a strong separation of the individuals into three clusters corresponding perfectly to FED, ED and CON group. Thus, the well-separated confidence ellipses indicated that the fetoplacental units were very different according to the exposure window. Moreover, a small sex-specific effect was observed but on the 5th dimension. This weak effect is probably due to the small number of fetuses. The variability of the data is explained, once maternal (or litter) effect is excluded, by an effect of the exposed group but only after stratification by the sex of the fetuses. Sexual dimorphism has also been shown elsewhere at the metabolic level, especially in response to a high-fat diet (Tarrade et al., 2013) but also in response to exposure to endocrine disruptors (Goel et al., 2008; Ruiz et al., 2019). The small size of the experimental groups remains a limitation of the study to highlight the effects observable in cohorts or other animal experiments.

As the placenta is described as a programming agent for offspring phenotype (Thornburg et al., 2016), quantitative 3D Doppler ultrasound has been performed to evaluate uteroplacental vascularization, during gestation, using three flow indices (VI, FI, and VFI) (Lecarpentier et al., 2012; Luria et al., 2012; Noguchi et al., 2009). Placental hypoperfusion has been associated with intrauterine growth retardation in rabbits using this approach (Lecarpentier et al., 2012; Luria et al., 2012; Noguchi et al., 2009). Here, placental perfusion indices at 28 dpi were similar between the three groups, corroborating the absence of fetal growth retardation. These data agree with the lack of difference of placental volume, placental weight or efficiency in FED or ED groups compared to control group. Moreover, the decrease (−12.5%) in the volume fraction of maternal blood space in the labyrinthine area in the FED *vs* control placentas was not strong enough to alter fetal growth, placental vascularization, efficiency or weight. In contrast, exposure to a low dose of BPA throughout gestation in mice decreased the placental maternal blood space area while exposure to high doses of BPA increased it (Tait et al., 2015). Gestational exposure to PFOA, BPA, or DEHP in mice affected the surface of the labyrinthine area (Jiang et al., 2020; Tachibana et al., 2007; Zong et al., 2015). In addition to these structural effects, another study has described placental effects following gestational exposure to chemicals included in the mixture tested here. Thus, in rabbits, gestational exposure to a mixture of 10 PFAS (including PFOA and PFOS) was shown to affect placental efficiency in a sex-specific manner (Crute et al., 2022). But such placental effects may require an exposure period that extends beyond 80 h post-insemination, which may explain the lack of placental visible effects in the present study.

Interestingly, the placental volume was increased in the FED fetoplacental units compared to the ED group. This difference suggests that the mixture could affect oocyte quality linked to the process of

folliculogenesis, a period which was exposed for 17 weeks in our study, independent of any effect on the early embryo. Folliculogenesis is known as a sensitive period to the chemical environment in particular to DEHP, BPS, PFAS family including PFOS and PFOA, flame retardants, and pesticides, which are included in the mixture (Johansson et al., 2017; Land et al., 2022). The embryonic microenvironment inside the uterine horn in which the embryo evolves can have an impact on its fate, affecting the progenitor trophectoderm, which gives rise to the placenta, at the blastocyst stage. However, this will not be discussed further here to avoid any speculation.

To our knowledge, no data relating to the exploration of the effects of periconceptional exposure to endocrine disruptors have been published in rabbits or rodents, with or without embryo transfer. In contrast, maternal diet, during oocyte development and maturation, was shown to impact embryo development and the future health of offspring in mice (Watkins et al., 2008), as well as in sheep (Edwards and McMillen, 2002). In rabbits, day-old embryo transfers from donors fed a high-fat, high-cholesterol diet to control recipient females did not affect feto-placental development but disrupted placental fatty acid profiling and expression of placental genes without any effect of embryo transfer technologies (Rousseau-Ralliard et al., 2021). All these data highlight the importance of a maternal exposure during the preconception period.

In response to an altered maternal environment, such as exposure to endocrine disruptors, placental function adapts to support development and fetal growth and also its survival by modulating gene expression (Lapehn and Paquette, 2022). As placental function can be affected, omics approaches provide a good tool to explore potential functional effects (Rosenfeld, 2021). Thus, in the present study, the placental function was explored through a transcriptomic approach that identified differentially expressed genes (DEGs) between the three groups. First, changes in placental gene expression were greater in females than in males (2–20-fold higher), suggesting different sex-specific adaptive strategies in response to adverse environment (Clifton, 2010). Sexual dimorphism is thought to have early placental origins (Myatt, 2006) and is believed to depend on the window of vulnerability at which the environmental disturbance occurs. Females would be more sensitive when the disturbance occurs early in gestation, as in the present study with exposure to early stages of development. On the other hand, males would be more sensitive to disturbances occurring in mid- or late-gestation (Kalisch-Smith et al., 2017). For example, in a rat model of periconceptional alcohol exposure, female-specific effects on trophoblast differentiation, embryo-uterine communication, and formation of the placental vascularization were affected (Kalisch-Smith et al., 2019).

Among DEGs in the FED *vs* CON females, *HMGCS1, PLA1A, MMP1* and *IL1*RN genes were overexpressed, whereas *CPTB1* gene was underexpressed. These genes were identified in the PPAR signaling pathway gene network, a nuclear receptor involved in lipid metabolism, as shown by GSEA. The mechanisms of action of BPA and phthalates, particularly DEHP and its metabolite MEHP, would involve PPARs and MMPs (Yang et al., 2019), as well as BDE-47, a flame retardant. As PFOA was recently described to impact the expression of genes related to lipid metabolism in the human placenta, this pollutant could also be involved in this pathway (Chen et al., 2023). Deregulation in the expression of placental genes involved in lipid metabolism and the PPAR signaling pathway in the FED group could potentially affect placental lipid transfers from the maternal to the fetal side, which could result in a susceptibility to developing metabolic disorders in adulthood, such as obesity or diabetes (Grün and Blumberg, 2006; Martínez-Razo et al., 2021; Szilagyi et al., 2020). In FED, *TK1* (Thymidine kinase 1, overexpressed) in males and *FMO1* (Flavin containing dimethylaniline monooxygenase 1; underexpressed) as well as *RRM2* (Ribonucleotide reductase regulatory subunit M2; overexpressed), in females, were deregulated. These genes are implicated in the drug/xenobiotic metabolism pathway. They could reflect a greater activation of placental mechanisms for metabolizing contaminants that were present in the exposure mixture to which their mothers were exposed, during early embryo development (pre- and periconception, corresponding to oocyte and embryo exposure) compared to control placentas.

In ED *vs* control group, some of the DEGs were involved in lipid and atherosclerosis, such as *PLAGEG4E* and *TLR6* in males. In females, *CYP1B1* implicated in the AhR signaling pathway (Aromatic hydrocarbon Receptor) (Chen et al., 2023; Wakx et al., 2018), a nuclear receptor involved a crucial role in the metabolism of drugs and xenobiotics (Larigot et al., 2018), was overexpressed. In males, *TLR6* is involved in the immune and inflammatory response, notably in the secretion of interleukins 6 and 8, and is thought to play a role in placental inflammation during the first trimester of pregnancy in women (Tangerås et al., 2014) and at the start of gestation in ewes (Kaya et al., 2017). The inflammatory and innate immune response would, therefore, be negatively impacted when exposed to environmental contaminants in the mixture during pre-implantation development in addition to presenting sex-specific responses (Baines and West, 2023). Moreover, bisphenol included in the mixture could contribute to increase placental inflammation (Arita et al., 2019), whereas PFOA could play a role in the innate immune response (Chen et al., 2023).

Among DEG in FED compared to the ED group, the *TK1* gene, in FED males, involved in drug and xenobiotic metabolism was deregulated. While in FED females, deregulated genes were involved in lipid metabolism and PPAR signaling pathway (*SLC27A2, MMP1, IL1RN, DGKG, and PLA1A*), in lipid and atherosclerosis (*IL1B*), in drug and xenobiotic metabolism (*GSTA5, RRM2, ALDH1A1, IL1B*), as well as the AhR signaling pathway (*CYP1B1*), which is sensitive to pp'DDE (Wójtowicz et al., 2011). These data indicate multiple adaptations in females placental compared to males to cope with the same adverse maternal environment as suggested by Clifton (2010), and as demonstrated by specific gene expression signatures in a sex-specific manner. Moreover, as observed in the FED fetal biochemistry or placental volume, the preconception period seems to have a more significant impact on placental function.

Concerning AhR signaling, AhR binds many endocrine disruptors such as bisphenol and can regulate CYP1B1 detectable in the human placenta during the first trimester of pregnancy and at term (Hakkola, 1997; Li et al., 2017). Although these detoxification and metabolism mechanisms are mainly described in the liver, they could also be active in the placenta (Datta et al., 1994), notably through Glutathione S-transferases activity, involving enzymes of the cytochrome P450 family and other enzymes such as aldehyde dehydrogenase (Hakkola et al., 1998; Myllynen et al., 2005; Syme et al., 2004). Furthermore, between the exposed and control groups and between the two exposed groups, genes and pathways of the endocrine system were slightly deregulated. GSEA demonstrated that the thyroid hormone signalling pathway was also deregulated, with a negative enrichment score in FED and ED males compared to control males. The deregulation of this pathway could be due to DEHP but also to bisphenol, both included in the mixture since this compound disturbed the placental thyroid receptor (Yu et al., 2018; Wu et al., 2022). However, no genes involved in this pathway were identified as deregulated in these comparisons. Between the FED and CON females, the GSEA revealed enrichments in various pathways related to hormone activity such as binding to estrogen receptor. In ED females compared to CON, the estrogen signaling pathway was also positively enriched. The GSEA analysis made it possible to show, independently of the DEGs, that exposure to the mixture of pollutants did indeed have an effect on endocrine signaling, which could be linked to bisphenols (Cao et al., 2022), DEHP (Zhang et al., 2020), or PFOS (Lee et al., 2015; Zhang et al., 2015) included in the mixture.

Most studies in the literature have explored, on animal models, the effect of a single contaminant, even if mixture studies are increasingly emerging, based on the identification of the chemical exposome of human cohorts. If the study of the effects of exposure of animal models to a mixture of pollutants brings us closer to the reality of the exposome of women, it will always be difficult to identify the specific action of

each molecule in the mixture. It might be necessary to resort to *in vitro* approaches to characterize the mechanistic mode of action of pollutants individually, by couple or by family, then in combination to explain the global effects observed.

5. Conclusion

Oral exposure to a mixture of eight environmental contaminants at realistic dose during the preconception and preimplantation periods has a slight effect on fetoplacental development at the macroscopic level, but altered fetal metabolism and placental function at the molecular level, mainly affecting metabolic pathways, to a greater extent in females than in males. Although the mixture used in our study is far from exhaustively reflecting the real human exposome, these molecules were chosen from the complex exposome of pregnant women because of their associations with perinatal health events, and used at doses realistic. The study of the placenta, i.e. its development and function, is essential because this transient organ, easily accessible at birth, is the memory of all the events that occurred during the *in utero* life of an individual, and it is described as the programming agent of the phenotype able to predict long-term effects (Thornburg et al., 2010).

Despite the drawbacks of the animal model, we were able to show that these two windows were sensitive to a mixture of pollutants and highlight the importance of these two exposure windows. These data raise questions about the exposure to endocrine disruptors in women of childbearing age and the contribution of chemical exposure during these two periods on the future offspring health. These data also raise questions about applying a precautionary principle during these vulnerable periods.

CRediT authorship contribution statement

Jeanne Bozec: Writing – original draft, Methodology, Formal analysis, Data curation. **Delphine Rousseau-Ralliard:** Writing – original draft, Visualization, Supervision, Resources, Project administration, Investigation, Data curation, Conceptualization. **Luc Jouneau:** Formal analysis. **Audrey Prézelin:** Investigation, Formal analysis. **Michèle Dahirel:** Investigation, Formal analysis. **Christophe Richard:** Investigation, Formal analysis. **Valérie Gelin:** Investigation, Formal analysis. **Natalie Fournier:** Investigation. **Virginie Helies:** Resources. **Thierry Joly:** Resources, Methodology. **Sara El Fouikar:** Investigation. **Roger Léandri:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Pascale Chavatte-Palmer:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Anne Couturier-Tarrade:** Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

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.

Data availability

Data will be made available on request.

Acknowledgments

The authors are grateful to the staff of the GenPhySE experimental unit for all the help given in animal care and animal experimental procedures. Moreover, the authors thank the MIMA2 Imaging Core Facility, *Microscopie et Imagerie des Microorganismes, Animaux et Aliments, INRAE, Jouy-en-Josas*, doi.org/10.15454/1.5572348210007727E12), particularly the CIMA (*Chirurgie et Imagerie Médicale chez l'Animal*) team for the help they provided in carrying out the ultrasounds and providing access to medical imaging equipment. The authors thank all persons who participated in this project from the BREED unit or elsewhere.

This work was supported by Agence Nationale de la Recherche [grant no. ANR-17-CE34-0015].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.119829.

References

Abercrombie, S.A., de Perre, C., Choi, Y.J., Tornabene, B.J., Sepúlveda, M.S., Lee, L.S., Hoverman, J.T., 2019. Larval amphibians rapidly bioaccumulate poly- and perfluoroalkyl substances. Ecotoxicol. Environ. Saf. 178, 137–145. https://doi.org/10.1016/j.ecoenv.2019.04.022.

Alaee, M., 2003. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environ. Int. 29, 683–689. https://doi.org/10.1016/S0160-4120(03)00121-1.

Alharbi, H.F., Algonaiman, R., Alduwayghiri, R., Aljutaily, T., Algheshairy, R.M., Almutairi, A.S., Alharbi, R.M., Alfurayh, L.A., Alshahwan, A.A., Alsadun, A.F., Barakat, H., 2022. Exposure to bisphenol A substitutes, bisphenol S and bisphenol F, and its association with developing obesity and diabetes mellitus: a narrative review. IJERPH 19, 15918. https://doi.org/10.3390/ijerph192315918.

Alonso-Magdalena, P., Vieira, E., Soriano, S., Menes, L., Burks, D., Quesada, I., Nadal, A., 2010. Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ. Health Perspect. 118, 1243–1250. https://doi.org/10.1289/ehp.1001993.

Arita, Y., Park, H.J., Cantillon, A., Getahun, D., Menon, R., Peltier, M.R., 2019. Effect of bisphenol-A (BPA) on placental biomarkers for inflammation, neurodevelopment and oxidative stress. J. Perinat. Med. 47, 741–749. https://doi.org/10.1515/jpm-2019-0045.

Assens, M., Frederiksen, H., Petersen, J.H., Larsen, T., Skakkebæk, N.E., Juul, A., Andersson, A.-M., Main, K.M., 2019. Variations in repeated serum concentrations of UV filters, phthalates, phenols and parabens during pregnancy. Environ. Int. 123, 318–324. https://doi.org/10.1016/j.envint.2018.11.047.

Baines, K.J., West, R.C., 2023. Sex differences in innate and adaptive immunity impact fetal, placental, and maternal health. Biol. Reprod. 109, 256–270. https://doi.org/10.1093/biolre/ioad072.

Barker, D.J., Osmond, C., Golding, J., Kuh, D., Wadsworth, M.E., 1989. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ 298, 564–567. https://doi.org/10.1136/bmj.298.6673.564.

Benincasa, L., Mandalà, M., Paulesu, L., Barberio, L., Ietta, F., 2020. Prenatal nutrition containing bisphenol A affects placenta glucose transfer: evidence in rats and human trophoblast. Nutrients 12, 1375. https://doi.org/10.3390/nu12051375.

Berghuis, S.A., Bos, A.F., Sauer, P.J.J., Bocca, G., 2022. Prenatal environmental exposure to persistent organic pollutants and indices of overweight and cardiovascular risk in Dutch adolescents. Nutrients 14, 2269. https://doi.org/10.3390/nu14112269.

Brewer, N.R., 2006. Biology of the rabbit. J Am Assoc Lab Anim Sci 45, 8–24.

Brusseau, M.L., Anderson, R.H., Guo, B., 2020. PFAS concentrations in soils: background levels versus contaminated sites. Sci. Total Environ. 740, 140017 https://doi.org/10.1016/j.scitotenv.2020.140017.

Buck Louis, G.M., Smarr, M.M., Patel, C.J., 2017. The exposome research paradigm: an opportunity to understand the environmental basis for human health and disease. Curr Environ Health Rep 4, 89–98. https://doi.org/10.1007/s40572-017-0126-3.

Cao, Y., Chen, Z., Zhang, M., Shi, L., Qin, S., Lv, D., Li, D., Ma, L., Zhang, Y., 2022. Maternal exposure to bisphenol A induces fetal growth restriction via upregulating the expression of estrogen receptors. Chemosphere 287, 132244. https://doi.org/10.1016/j.chemosphere.2021.132244.

Capobianco, E., Pirrone, I., 2023. Paternal programming of fetoplacental and offspring metabolic disorders. Placenta 141, 71–77. https://doi.org/10.1016/j.placenta.2023.06.009.

Chen, H., Kapidzic, M., Gantar, D., Aksel, S., Levan, J., Abrahamsson, D.P., Jigmeddagva, U., Basrai, S., San, A., Gaw, S.L., Woodruff, T.J., Fisher, S.J., Robinson, J.F., 2023. Perfluorooctanoic acid induces transcriptomic alterations in second trimester human cytotrophoblasts. Toxicol. Sci. 196, 187–199. https://doi.org/10.1093/toxsci/kfad097.

Chen, Y., Lu, Z., Li, B., Wang, H., Jiang, T., Xuan, M., Yang, H., Chen, J., Liu, X., Liang, H., Liu, Y., Tang, H., 2023. Human CYP1B1 enzyme-mediated, AhR enhanced activation of aflatoxin B1 for its genotoxicity in human cells. Toxicol. Lett. 373, 132–140. https://doi.org/10.1016/j.toxlet.2022.11.014.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159. https://doi.org/10.1006/abio.1987.9999.

Clifton, V.L., 2010. Review: sex and the human placenta: mediating differential strategies of fetal growth and survival. Placenta 31, S33–S39. https://doi.org/10.1016/j.placenta.2009.11.010.

Conley, J.M., Lambright, C.S., Evans, N., Cardon, M., Medlock-Kakaley, E., Wilson, V.S., Gray, L.E., 2021. A mixture of 15 phthalates and pesticides below individual

chemical no observed adverse effect levels (NOAELs) produces reproductive tract malformations in the male rat. Environ. Int. 156, 106615 https://doi.org/10.1016/j.envint.2021.106615.

Datta, K., Roy, S.K., Mitra, A.K., Kulkarni, A.P., 1994. Glutathione S-transferase mediated detoxification and bioactivation of xenobiotics during early human pregnancy. Early Hum. Dev. 37, 167–174. https://doi.org/10.1016/0378-3782(94)90075-2.

Edwards, L.J., McMillen, I.C., 2002. Impact of maternal undernutrition during the periconceptional period, fetal number, and fetal sex on the development of the hypothalamo-pituitary adrenal axis in sheep during late gestation. Biol. Reprod. 66, 1562–1569. https://doi.org/10.1095/biolreprod66.5.1562.

El Fouikar, S., Duranthon, V., Helies, V., Jammes, H., Couturier-Tarrade, A., Gayrard, V., Van Acker, N., Frenois, F.-X., Archilla, C., Rousseau-Ralliard, D., Gatimel, N., Léandri, R., 2023. Multigenerational effects of a complex human-relevant exposure during folliculogenesis and preimplantation embryo development: the FEDEXPO study. Toxics 11, 425. https://doi.org/10.3390/toxics11050425.

El Fouikar, S., Van Acker, N., Héliès, V., Frenois, F.-X., Giton, F., Gayrard, V., Dauwe, Y., Mselli-Lakhal, L., Rousseau-Ralliard, D., Fournier, N., Léandri, R., Gatimel, N., 2024. Folliculogenesis and steroidogenesis alterations after chronic exposure to a human-relevant mixture of environmental toxicants spare the ovarian reserve in the rabbit model. J. Ovarian Res. 17, 134. https://doi.org/10.1186/s13048-024-01457-6.

Favaron, P.O., Mess, A.M., de Oliveira, M.F., Gabory, A., Miglino, M.A., Chavatte-Palmer, P., Tarrade, A., 2013. Morphometric analysis of the placenta in the New World mouse Necromys lasiurus (Rodentia, Cricetidae): a comparison of placental development in cricetids and murids. Reprod. Biol. Endocrinol. 11, 10. https://doi.org/10.1186/1477-7827-11-10.

Fernández-Rodríguez, M., Arrebola, J.P., Artacho-Cordón, F., Amaya, E., Aragones, N., Llorca, J., Perez-Gomez, B., Ardanaz, E., Kogevinas, M., Castano-Vinyals, G., Pollan, M., Olea, N., 2015. Levels and predictors of persistent organic pollutants in an adult population from four Spanish regions. Sci. Total Environ. 538, 152–161. https://doi.org/10.1016/j.scitotenv.2015.07.162.

Fernandez-Twinn, D.S., Ozanne, S.E., 2010. Early life nutrition and metabolic programming: early life programming. Ann. N. Y. Acad. Sci. 1212, 78–96. https://doi.org/10.1111/j.1749-6632.2010.05798.x.

Fillol, C., Oleko, A., Saoudi, A., Zeghnoun, A., Balicco, A., Gane, J., Rambaud, L., Leblanc, A., Gaudreau, É., Marchand, P., Le Bizec, B., Bouchart, V., Le Gléau, F., Durand, G., Denys, S., 2021. Exposure of the French population to bisphenols, phthalates, parabens, glycol ethers, brominated flame retardants, and perfluorinated compounds in 2014-2016: results from the Esteban study. Environ. Int. 147, 106340 https://doi.org/10.1016/j.envint.2020.106340.

Fischer, B., Chavatte-Palmer, P., Viebahn, C., Navarrete Santos, A., Duranthon, V., 2012. Rabbit as a reproductive model for human health. Reproduction 144, 1–10. https://doi.org/10.1530/REP-12-0091.

Fisher, M., Arbuckle, T.E., Liang, C.L., LeBlanc, A., Gaudreau, E., Foster, W.G., Haines, D., Davis, K., Fraser, W.D., 2016. Concentrations of persistent organic pollutants in maternal and cord blood from the maternal-infant research on environmental chemicals (MIREC) cohort study. Environ. Health 15, 59. https://doi.org/10.1186/s12940-016-0143-y.

Foote, R.H., Carney, E.W., 2000. The rabbit as a model for reproductive and developmental toxicity studies. Reprod. Toxicol. 14, 477–493. https://doi.org/10.1016/S0890-6238(00)00101-5.

Fouyet, S., Olivier, E., Leproux, P., Dutot, M., Rat, P., 2021. Bisphenol A, bisphenol F, and bisphenol S: the bad and the ugly. Where is the good? Life 11, 314. https://doi.org/10.3390/life11040314.

Gayrard, V., Moreau, J., Picard-Hagen, N., Helies, V., Marchand, P., Antignac, J.-P., Toutain, P.-L., Leandri, R., 2021. Use of mixture dosing and nonlinear mixed effect modeling of eight environmental contaminants in rabbits to improve extrapolation value of toxicokinetic data. Environ. Health Perspect. 129, 117006 https://doi.org/10.1289/EHP8957.

George, F.W., Wilson, J.D., 1979. The regulation of androgen and estrogen formation in fetal gonads. Annales de biologie animale. biochimie, biophysique 19 (4B), 1297–1306.

Gerona, R.R., Schwartz, J.M., Pan, J., Friesen, M.M., Lin, T., Woodruff, T.J., 2018. Suspect screening of maternal serum to identify new environmental chemical biomonitoring targets using liquid chromatography–quadrupole time-of-flight mass spectrometry. J. Expo. Sci. Environ. Epidemiol. 28, 101–108. https://doi.org/10.1038/jes.2017.28.

Gingrich, J., Ticiani, E., Veiga-Lopez, A., 2020. Placenta disrupted: endocrine disrupting chemicals and pregnancy. Trends Endocrinol. Metabol. 31, 508–524. https://doi.org/10.1016/j.tem.2020.03.003.

Giraudoux, P., 2022. Spatial analysis and data mining for field ecologists [R package version 2.0.0] [WWW Document]. URL. https://CRAN.R-project.org/package=pgirmess.

Goel, A., Thor, D., Anderson, L., Rahimian, R., 2008. Sexual dimorphism in rabbit aortic endothelial function under acute hyperglycemic conditions and gender-specific responses to acute 17β-estradiol. Am. J. Physiol. Heart Circ. Physiol. 294, H2411–H2420. https://doi.org/10.1152/ajpheart.01217.2007.

Green, M.P., Harvey, A.J., Finger, B.J., Tarulli, G.A., 2021. Endocrine disrupting chemicals: impacts on human fertility and fecundity during the peri-conception period. Environ. Res. 194, 110694 https://doi.org/10.1016/j.envres.2020.110694.

Grün, F., Blumberg, B., 2006. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. Endocrinology 147, s50–s55. https://doi.org/10.1210/en.2005-1129.

Guxens, M., Ballester, F., Espada, M., Fernández, M.F., Grimalt, J.O., Ibarluzea, J., Olea, N., Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheid, M., Sunyer, J., 2012. Cohort profile: the INMA—INfancia y Medio ambiente—(environment and childhood) project. Int. J. Epidemiol. 41, 930–940. https://doi.org/10.1093/ije/dyr054.

Hadar, E., Ashwal, E., Hod, M., 2015. The preconceptional period as an opportunity for prediction and prevention of noncommunicable disease. Best Pract. Res. Clin. Obstet. Gynaecol. 29, 54–62. https://doi.org/10.1016/j.bpobgyn.2014.05.011.

Hakkola, J., 1997. Expression of CYP1B1 in human adult and fetal tissues and differential inducibility of CYP1B1 and CYP1A1 by Ah receptor ligands in human placenta and cultured cells. Carcinogenesis 18, 391–397. https://doi.org/10.1093/carcin/18.2.391.

Hakkola, J., Pelkonen, O., Pasanen, M., Raunio, H., 1998. Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto-placental unit: role in intrauterine toxicity. Crit. Rev. Toxicol. 28, 35–72. https://doi.org/10.1080/10408449891344173.

Hu, J., Zhao, H., Braun, J.M., Zheng, T., Zhang, B., Xia, W., Zhang, W., Li, Jiufeng, Zhou, Y., Li, H., Li, Jun, Zhou, A., Zhang, Y., Buka, S.L., Liu, S., Peng, Y., Wu, C., Jiang, M., Huo, W., Zhu, Y., Shi, K., Li, Y., Cai, Z., Xu, S., 2019. Associations of trimester-specific exposure to bisphenols with size at birth: a Chinese prenatal cohort study. Environ. Health Perspect. 127, 107001 https://doi.org/10.1289/EHP4664.

Johansson, H.K.L., Svingen, T., Fowler, P.A., Vinggaard, A.M., Boberg, J., 2017. Environmental influences on ovarian dysgenesis - developmental windows sensitive to chemical exposures. Nat. Rev. Endocrinol. 13, 400–414. https://doi.org/10.1038/nrendo.2017.36.

Jolivet, G., Daniel-Carlier, N., Harscoët, E., Airaud, E., Dewaele, A., Pierson, C., Giton, F., Boulanger, L., Daniel, N., Mandon-Pépin, B., Pannetier, M., Pailhoux, E., 2022. Fetal estrogens are not involved in sex determination but critical for early ovarian differentiation in rabbits. Endocrinology 163, bqab210. https://doi.org/10.1210/endocr/bqab210.

Jorge, B.C., Stein, J., Reis, A.C.C., Bueno, J.N., Paschoalini, B.R., Moreira, S. da S., Manoel, B. de M., Arena, A.C., 2023. Paternal low-dose benzo(a)pyrene exposure in rats impairs sexual development and fertility of the paternal lineage in F2 generation: a transgenerational study. Toxicology 494, 153585. https://doi.org/10.1016/j.tox.2023.153585.

Kalisch-Smith, J.I., Simmons, D.G., Pantaleon, M., Moritz, K.M., 2017. Sex differences in rat placental development: from pre-implantation to late gestation. Biol. Sex Differ. 8, 17. https://doi.org/10.1186/s13293-017-0138-6.

Kalisch-Smith, J.I., Steane, S.E., Simmons, D.G., Pantaleon, M., Anderson, S.T., Akison, L. K., Wlodek, M.E., Moritz, K.M., 2019. Periconceptional alcohol exposure causes female-specific perturbations to trophoblast differentiation and placental formation in the rat. Development 146, dev172205. https://doi.org/10.1242/dev.172205.

Kalloo, G., Wellenius, G.A., McCandless, L., Calafat, A.M., Sjodin, A., Romano, M.E., Karagas, M.R., Chen, A., Yolton, K., Lanphear, B.P., Braun, J.M., 2020. Exposures to chemical mixtures during pregnancy and neonatal outcomes: the HOME study. Environ. Int. 134, 105219 https://doi.org/10.1016/j.envint.2019.105219.

Kassambara, A., Mundt, F., 2020. Extract and visualize the results of multivariate data analyses [R package version 1.0.7] [WWW Document]. URL. https://CRAN.R-project.org/package=factoextra.

Kaya, M.S., Kose, M., Guzeloglu, A., Kıyma, Z., Atli, M.O., 2017. Early pregnancy-related changes in toll-like receptors expression in ovine trophoblasts and peripheral blood leukocytes. Theriogenology 93, 40–45. https://doi.org/10.1016/j.theriogenology.2017.01.031.

Kerr, J.B., Myers, M., Anderson, R.A., 2013. The dynamics of the primordial follicle reserve. Reproduction 146, R205–R215. https://doi.org/10.1530/REP-13-0181.

Khalil, A., Cevik, S.E., Hung, S., Kolla, S., Roy, M.A., Suvorov, A., 2018. Developmental exposure to 2,2′,4,4′-tetrabromodiphenyl ether permanently alters blood-liver balance of lipids in male mice. Front. Endocrinol. 9, 548. https://doi.org/10.3389/fendo.2018.00548.

Kirchnawy, C., Hager, F., Osorio Piniella, V., Jeschko, M., Washüttl, M., Mertl, J., Mathieu-Huart, A., Rousselle, C., 2020. Potential endocrine disrupting properties of toys for babies and infants. PLoS One 15, e0231171. https://doi.org/10.1371/journal.pone.0231171.

Koniecki, D., Wang, R., Moody, R.P., Zhu, J., 2011. Phthalates in cosmetic and personal care products: concentrations and possible dermal exposure. Environ. Res. 111, 329–336. https://doi.org/10.1016/j.envres.2011.01.013.

Land, K.L., Miller, F.G., Fugate, A.C., Hannon, P.R., 2022. The effects of endocrine-disrupting chemicals on ovarian- and ovulation-related fertility outcomes. Mol. Reprod. Dev. 89, 608–631. https://doi.org/10.1002/mrd.23652.

Lapehn, S., Paquette, A.G., 2022. The placental epigenome as a molecular link between prenatal exposures and fetal health outcomes through the DOHaD hypothesis. Curr Envir Health Rpt 9, 490–501. https://doi.org/10.1007/s40572-022-00354-8.

Larigot, L., Juricek, L., Dairou, J., Coumoul, X., 2018. AhR signaling pathways and regulatory functions. Biochimie Open 7, 1–9. https://doi.org/10.1016/j.biopen.2018.05.001.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR : an *R* package for multivariate analysis. J. Stat. Software 25. https://doi.org/10.18637/jss.v025.i01.

Lecarpentier, E., Morel, O., Tarrade, A., Dahirel, M., Bonneau, M., Gayat, E., Evain-brion, D., Chavatte-palmer, P., Tsatsaris, V., 2012. Quantification of utero-placental vascularization in a rabbit model of IUGR with three-dimensional power Doppler angiography. Placenta 33, 769–775. https://doi.org/10.1016/j.placenta.2012.06.013.

Lee, C.K., Kang, S.G., Lee, J.T., Lee, S.-W., Kim, J.H., Kim, D.H., Son, B.C., Kim, K.H., Suh, C.H., Kim, S.Y., Park, Y.B., 2015. Effects of perfluorooctane sulfuric acid on placental PRL-family hormone production and fetal growth retardation in mice. Mol. Cell. Endocrinol. 401, 165–172. https://doi.org/10.1016/j.mce.2014.10.026.

Lenth, R.V., 2016. Least-squares means: the *R* package **lsmeans**. J. Stat. Software 69. https://doi.org/10.18637/jss.v069.i01.

Li, F., Zhu, W., Gonzalez, F.J., 2017. Potential role of CYP1B1 in the development and treatment of metabolic diseases. Pharmacology & Therapeutics 178, 18–30. https://doi.org/10.1016/j.pharmthera.2017.03.007.
Longtine, M., Nelson, D.M., 2011. Placental dysfunction and fetal programming: the importance of placental size, shape, histopathology, and molecular composition. Semin. Reprod. Med. 29, 187–196. https://doi.org/10.1055/s-0031-1275515.
Lopez-Espinosa, M.-J., Costa, O., Vizcaino, E., Murcia, M., Fernandez-Somoano, A., Iñiguez, C., Llop, S., Grimalt, J.O., Ballester, F., Tardon, A., 2015. Prenatal exposure to polybrominated flame retardants and fetal growth in the INMA cohort (Spain). Environ. Sci. Technol. 49, 10108–10116. https://doi.org/10.1021/acs.est.5b01793.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550. https://doi.org/10.1186/s13059-014-0550-8.
Luo, S., Li, Yun, Li, Yingpei, Zhu, Q., Jiang, J., Wu, C., Shen, T., 2016. Gestational and lactational exposure to low-dose bisphenol A increases Th17 cells in mice offspring. Environ. Toxicol. Pharmacol. 47, 149–158. https://doi.org/10.1016/j.etap.2016.09.017.
Luria, O., Barnea, O., Shalev, J., Barkat, J., Kovo, M., Golan, A., Bar, J., 2012. Two-dimensional and three-dimensional Doppler assessment of fetal growth restriction with different severity and onset: three-dimensional Doppler assessment of fetal growth restriction. Prenat. Diagn. 32, 1174–1180. https://doi.org/10.1002/pd.3980.
Maitre, L., de Bont, J., Casas, M., Robinson, O., Aasvang, G.M., Agier, L., Andrušaitytė, S., Ballester, F., Basagaña, X., Borràs, E., Brochot, C., Bustamante, M., Carracedo, A., de Castro, M., Dedele, A., Donaire-Gonzalez, D., Estivill, X., Evandt, J., Fossati, S., Giorgis-Allemand, L., R Gonzalez, J., Granum, B., Grazuleviciene, R., Bjerve Gützkow, K., Småstuen Haug, L., Hernandez-Ferrer, C., Heude, B., Ibarluzea, J., Julvez, J., Karachaliou, M., Keun, H.C., Hjertager Krog, N., Lau, C.-H.E., Leventakou, V., Lyon-Caen, S., Manzano, C., Mason, D., McEachan, R., Meltzer, H. M., Petraviciene, I., Quentin, J., Roumeliotaki, T., Sabido, E., Saulnier, P.-J., Siskos, A.P., Siroux, V., Sunyer, J., Tamayo, I., Urquiza, J., Vafeiadi, M., van Gent, D., Vives-Usano, M., Waiblinger, D., Warembourg, C., Chatzi, L., Coen, M., van den Hazel, P., Nieuwenhuijsen, M.J., Slama, R., Thomsen, C., Wright, J., Vrijheid, M., 2018. Human Early Life Exposome (HELIX) study: a European population-based exposome cohort. BMJ Open 8, e021311. https://doi.org/10.1136/bmjopen-2017-021311.
Makita, Y., 2008. Effects of perinatal combined exposure to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) and tributyltin (TBT) on rat female reproductive system. Environ. Toxicol. Pharmacol. 25, 380–385. https://doi.org/10.1016/j.etap.2007.12.003.
Makita, Y., 2004. Effects of perinatal combined exposure to 1,4-dichlorobenzene and 1,1-dichloro-2, 2-bis (p-chlorophenyl) ethylene (p,p'-DDE) on rat female offspring. Basic Clin. Pharmacol. Toxicol. 95, 139–143. https://doi.org/10.1111/j.1742-7843.2004.950306.x.
Maltepe, E., Fisher, S.J., 2015. Placenta: the forgotten organ. Annu. Rev. Cell Dev. Biol. 31, 523–552. https://doi.org/10.1146/annurev-cellbio-100814-125620.
Marcho, C., Cui, W., Mager, J., 2015. Epigenetic dynamics during preimplantation development. Reproduction 150, R109–R120. https://doi.org/10.1530/REP-15-0180.
Marks, K.J., Cutler, A.J., Jeddy, Z., Northstone, K., Kato, K., Hartman, T.J., 2019. Maternal serum concentrations of perfluoroalkyl substances and birth size in British boys. Int. J. Hyg Environ. Health 222, 889–895. https://doi.org/10.1016/j.ijheh.2019.03.008.
Martin, O., Scholze, M., Ermler, S., McPhie, J., Bopp, S.K., Kienzler, A., Parissis, N., Kortenkamp, A., 2021. Ten years of research on synergisms and antagonisms in chemical mixtures: a systematic review and quantitative reappraisal of mixture studies. Environ. Int. 146, 106206 https://doi.org/10.1016/j.envint.2020.106206.
Martínez-Razo, L.D., Martínez-Ibarra, A., Vázquez-Martínez, E.R., Cerbón, M., 2021. The impact of Di-(2-ethylhexyl) Phthalate and Mono(2-ethylhexyl) Phthalate in placental development, function, and pathophysiology. Environ. Int. 146, 106228 https://doi.org/10.1016/j.envint.2020.106228.
Millard, S.P., 2013. EnvStats: an R Package for Environmental Statistics, 2. Springer, New York, NY.
Myatt, L., 2006. Placental adaptive responses and fetal programming: placenta and fetal programming. J. Physiol. 572, 25–30. https://doi.org/10.1113/jphysiol.2006.104968.
Myllynen, P., Pasanen, M., Pelkonen, O., 2005. Human placenta: a human organ for developmental toxicology research and biomonitoring. Placenta 26, 361–371. https://doi.org/10.1016/j.placenta.2004.09.006.
Nappi, F., Barrea, L., Di Somma, C., Savanelli, M., Muscogiuri, G., Orio, F., Savastano, S., 2016. Endocrine aspects of environmental "obesogen" pollutants. IJERPH 13, 765. https://doi.org/10.3390/ijerph13080765.
Noguchi, J., Hata, K., Tanaka, H., Hata, T., 2009. Placental vascular sonobiopsy using three-dimensional power Doppler ultrasound in normal and growth restricted fetuses. Placenta 30, 391–397. https://doi.org/10.1016/j.placenta.2009.02.010.
Ogle, D.H., Doll, J.C., Wheeler, A.P., Dinno, A., 2023. FSA: simple fisheries stock assessment methods [R package version 0.9.4] [WWW Document]. URL. https://CRAN.R-project.org/package=FSA.
Osmond, C., Barker, D.J., 2000. Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. Environ. Health Perspect. 108 (Suppl. 3), 545–553. https://doi.org/10.1289/ehp.00108s3545.
Paíga, P., Sousa, S., Vera, J., Bitencourt, L., Vieira, J., Jorge, S., Silva, J.G., Correia, M., Domingues, V.F., Delerue-Matos, C., 2021. Multi-residue analysis of fifty pesticides in river waters and in wastewaters. Environ. Sci. Pollut. Res. 28, 66787–66803. https://doi.org/10.1007/s11356-021-15134-4.
Pinheiro, J., Bates, D., 2000. Mixed-Effects Models in S and S-PLUS, Statistics and Computing. Springer-Verlag, New York. https://doi.org/10.1007/b98882.
Pinheiro, J., Bates, D., R Core Team, 2022. Linear and Nonlinear Mixed Effects Models [R package nlme version 3.1-163] [WWW Document]. URL. https://CRAN.R-project.org/package=nlme.
R Core Team, 2022. R: A Language and Environment for Statistical Computing [WWW Document]. R Foundation for Statistical Computing. URL. https://www.R-project.org/.
Reik, W., Dean, W., Walter, J., 2001. Epigenetic reprogramming in mammalian development. Science 293, 1089–1093. https://doi.org/10.1126/science.1063443.
Robinson, O., Basagaña, X., Agier, L., de Castro, M., Hernandez-Ferrer, C., Gonzalez, J.R., Grimalt, J.O., Nieuwenhuijsen, M., Sunyer, J., Slama, R., Vrijheid, M., 2015. The pregnancy exposome: multiple environmental exposures in the INMA-sabadell birth cohort. Environ. Sci. Technol. 49, 10632–10641. https://doi.org/10.1021/acs.est.5b01782.
Rosenfeld, C.S., 2021. Transcriptomics and other omics approaches to investigate effects of xenobiotics on the placenta. Front. Cell Dev. Biol. 9, 723656 https://doi.org/10.3389/fcell.2021.723656.
Rousseau-Ralliard, D., Aubrière, M.-C., Daniel, N., Dahirel, M., Morin, G., Prézelin, A., Bertrand, J., Rey, C., Chavatte-Palmer, P., Couturier-Tarrade, A., 2021. Importance of windows of exposure to maternal high-fat diet and feto-placental effects: discrimination between pre-conception and gestational periods in a rabbit model. Front. Physiol. 12, 784268 https://doi.org/10.3389/fphys.2021.784268.
Rousseau-Ralliard, D., Couturier-Tarrade, A., Thieme, R., Brat, R., Rolland, A., Boileau, P., Aubrière, M.-C., Daniel, N., Dahirel, M., Derisoud, E., Fournier, N., Schindler, M., Duranthon, V., Fischer, B., Santos, A.N., Chavatte-Palmer, P., 2019. A short periconceptional exposure to maternal type-1 diabetes is sufficient to disrupt the feto-placental phenotype in a rabbit model. Mol. Cell. Endocrinol. 480, 42–53. https://doi.org/10.1016/j.mce.2018.10.010.
Ruiz, D., Regnier, S.M., Kirkley, A.G., Hara, M., Haro, F., Aldirawi, H., Dybala, M.P., Sargis, R.M., 2019. Developmental exposure to the endocrine disruptor tolylfluanid induces sex-specific later-life metabolic dysfunction. Reprod. Toxicol. 89, 74–82. https://doi.org/10.1016/j.reprotox.2019.06.010.
Sinisalu, L., Yeung, L.W.Y., Wang, J., Pan, Y., Dai, J., Hyötyläinen, T., 2021. Prenatal exposure to poly-/per-fluoroalkyl substances is associated with alteration of lipid profiles in cord-blood. Metabolomics 17, 103. https://doi.org/10.1007/s11306-021-01853-9.
Su, H.-Y., Lai, C.-S., Lee, K.-H., Chiang, Y.-W., Chen, C.-C., Hsu, P.-C., 2024. Prenatal exposure to low-dose di-(2-ethylhexyl) phthalate (DEHP) induces potentially hepatic lipid accumulation and fibrotic changes in rat offspring. Ecotoxicol. Environ. Saf. 269, 115776 https://doi.org/10.1016/j.ecoenv.2023.115776.
Syme, M.R., Paxton, J.W., Keelan, J.A., 2004. Drug transfer and metabolism by the human placenta. Clin. Pharmacokinet. 43, 487–514. https://doi.org/10.2165/00003088-200443080-00001.
Szilagyi, J.T., Avula, V., Fry, R.C., 2020. Perfluoroalkyl substances (PFAS) and their effects on the placenta, pregnancy, and child development: a potential mechanistic role for placental peroxisome proliferator–activated receptors (PPARs). Curr Envir Health Rpt 7, 222–230. https://doi.org/10.1007/s40572-020-00279-0.
Tachibana, T., Wakimoto, Y., Nakamuta, N., Phichitraslip, T., Wakitani, S., Kusakabe, K., Hondo, E., Kiso, Y., 2007. Effects of bisphenol A (BPA) on placentation and survival of the neonates in mice. J. Reprod. Dev. 53, 509–514. https://doi.org/10.1262/jrd.18171.
Tait, S., Tassinari, R., Maranghi, F., Mantovani, A., 2015. Bisphenol A affects placental layers morphology and angiogenesis during early pregnancy phase in mice: BPA exposure affects placenta angiogenesis. J. Appl. Toxicol. 35, 1278–1291. https://doi.org/10.1002/jat.3176.
Tamayo-Uria, I., Maitre, L., Thomsen, C., Nieuwenhuijsen, M.J., Chatzi, L., Siroux, V., Aasvang, G.M., Agier, L., Andrusaityte, S., Casas, M., de Castro, M., Dedele, A., Haug, L.S., Heude, B., Grazuleviciene, R., Gutzkow, K.B., Krog, N.H., Mason, D., McEachan, R.R.C., Meltzer, H.M., Petraviciene, I., Robinson, O., Roumeliotaki, T., Sakhi, A.K., Urquiza, J., Vafeiadi, M., Waiblinger, D., Warembourg, C., Wright, J., Slama, R., Vrijheid, M., Basagaña, X., 2019. The early-life exposome: description and patterns in six European countries. Environ. Int. 123, 189–200. https://doi.org/10.1016/j.envint.2018.11.067.
Tangerås, L.H., Stødle, G.S., Olsen, G.D., Leknes, A.-H., Gundersen, A.S., Skei, B., Vikdal, A.J., Ryan, L., Steinkjer, B., Myklebost, M.F., Langaas, M., Austgulen, R., Iversen, A.-C., 2014. Functional Toll-like receptors in primary first-trimester trophoblasts. J. Reprod. Immunol. 106, 89–99. https://doi.org/10.1016/j.jri.2014.04.004.
Tanner, E.M., Bornehag, C.-G., Gennings, C., 2020. Dynamic growth metrics for examining prenatal exposure impacts on child growth trajectories: application to perfluorooctanoic acid (PFOA) and postnatal weight gain. Environ. Res. 182, 109044 https://doi.org/10.1016/j.envres.2019.109044.
Tarrade, A., Lecarpentier, E., Gil, S., Morel, O., Zahr, N., Dahirel, M., Tsatsaris, V., Chavatte-Palmer, P., 2014. Analysis of placental vascularization in a pharmacological rabbit model of IUGR induced by l-NAME, a nitric oxide synthase inhibitor. Placenta 35, 254–259. https://doi.org/10.1016/j.placenta.2014.01.013.
Tarrade, A., Rousseau-Ralliard, D., Aubrière, M.-C., Peynot, N., Dahirel, M., Bertrand-Michel, J., Aguirre-Lavin, T., Morel, O., Beaujean, N., Duranthon, V., Chavatte-Palmer, P., 2013. Sexual dimorphism of the feto-placental phenotype in response to a high fat and control maternal diets in a rabbit model. PLoS One 8, e83458. https://doi.org/10.1371/journal.pone.0083458.
Theunissen, P.T., Beken, S., Beyer, B.K., Breslin, W.J., Cappon, G.D., Chen, C.L., Chmielewski, G., De Schaepdrijver, L., Enright, B., Foreman, J.E., Harrouk, W., Hew, K.-W., Hoberman, A.M., Hui, J.Y., Knudsen, T.B., Laffan, S.B., Makris, S.L., Martin, M., McNerney, M.E., Siezen, C.L., Stanislaus, D.J., Stewart, J., Thompson, K.

E., Tornesi, B., Van der Laan, J.W., Weinbauer, G.F., Wood, S., Piersma, A.H., 2016. Comparison of rat and rabbit embryo–fetal developmental toxicity data for 379 pharmaceuticals: on the nature and severity of developmental effects. Crit. Rev. Toxicol. 46, 900–910. https://doi.org/10.1080/10408444.2016.1224807.

Thornburg, K.L., Kolahi, K., Pierce, M., Valent, A., Drake, R., Louey, S., 2016. Biological features of placental programming. Placenta 48, S47–S53. https://doi.org/10.1016/j.placenta.2016.10.012.

Thornburg, K.L., O'Tierney, P.F., Louey, S., 2010. Review: the placenta is a programming agent for cardiovascular disease. Placenta 31, S54–S59. https://doi.org/10.1016/j.placenta.2010.01.002.

Toutain, P.-L., Ferran, A., Bousquet-Mélou, A., 2010. Species differences in pharmacokinetics and pharmacodynamics. Handb. Exp. Pharmacol. 19–48. https://doi.org/10.1007/978-3-642-10324-7_2.

Valentino, S.A., Tarrade, A., Aioun, J., Mourier, E., Richard, C., Dahirel, M., Rousseau-Ralliard, D., Fournier, N., Aubrière, M.-C., Lallemand, M.-S., Camous, S., Guinot, M., Charlier, M., Aujean, E., Al Adhami, H., Fokkens, P.H., Agier, L., Boere, J.A., Cassee, F.R., Slama, R., Chavatte-Palmer, P., 2015. Maternal exposure to diluted diesel engine exhaust alters placental function and induces intergenerational effects in rabbits. Part. Fibre Toxicol. 13, 39. https://doi.org/10.1186/s12989-016-0151-7.

Velazquez, M.A., Fleming, T.P., Watkins, A.J., 2019. Periconceptional environment and the developmental origins of disease. J. Endocrinol. 242, T33–T49. https://doi.org/10.1530/JOE-18-0676.

Velazquez, M.A., Idriss, A., Chavatte-Palmer, P., Fleming, T.P., 2023. The mammalian preimplantation embryo: its role in the environmental programming of postnatal health and performance. Anim. Reprod. Sci. 256, 107321 https://doi.org/10.1016/j.anireprosci.2023.107321.

Vrijheid, M., Martinez, D., Aguilera, I., Ballester, F., Basterrechea, M., Esplugues, A., Guxens, M., Larrañaga, M., Lertxundi, A., Mendez, M., Murcia, M., Marina, L.S., Villanueva, C.M., Sunyer, J., 2012. Socioeconomic status and exposure to multiple environmental pollutants during pregnancy: evidence for environmental inequity? J. Epidemiol. Community Health 66, 106–113. https://doi.org/10.1136/jech.2010.117408.

Vrooman, L.A., Xin, F., Bartolomei, M.S., 2016. Morphologic and molecular changes in the placenta: what we can learn from environmental exposures. Fertil. Steril. 106, 930–940. https://doi.org/10.1016/j.fertnstert.2016.08.016.

Wakx, A., Nedder, M., Tomkiewicz-Raulet, C., Dalmasso, J., Chissey, A., Boland, S., Vibert, F., Degrelle, S., Fournier, T., Coumoul, X., Gil, S., Ferecatu, I., 2018. Expression, localization, and activity of the aryl hydrocarbon receptor in the human placenta. Indian J. Manag. Sci. 19, 3762. https://doi.org/10.3390/ijms19123762.

Watkins, A.J., Wilkins, A., Cunningham, C., Perry, V.H., Seet, M.J., Osmond, C., Eckert, J. J., Torrens, C., Cagampang, F.R.A., Cleal, J., Gray, W.P., Hanson, M.A., Fleming, T. P., 2008. Low protein diet fed exclusively during mouse oocyte maturation leads to behavioural and cardiovascular abnormalities in offspring. J Physiol 586, 2231–2244. https://doi.org/10.1113/jphysiol.2007.149229.

Wei, Z., Song, L., Wei, J., Chen, T., Chen, J., Lin, Y., Xia, W., Xu, B., Li, X., Chen, X., Li, Y., Xu, S., 2012. Maternal exposure to di-(2-ethylhexyl)phthalate alters kidney development through the renin–angiotensin system in offspring. Toxicol. Lett. 212, 212–221. https://doi.org/10.1016/j.toxlet.2012.05.023.

Weisberg, S., Fox, J., 2019. An {R} Companion to Applied Regression, Third. ed. Sage, Thousand Oaks CA.

Wójtowicz, A.K., Honkisz, E., Zięba-Przybylska, D., Milewicz, T., Kajta, M., 2011. Effects of two isomers of DDT and their metabolite DDE on CYP1A1 and AhR function in human placental cells. Pharmacol. Rep. 63, 1460–1468. https://doi.org/10.1016/s1734-1140(11)70710-1.

Wu, X., Yang, X., Geng, X., Ji, X., Zhang, X., Yue, H., Li, G., Sang, N., 2022. Bisphenol A analogs induce cellular dysfunction in human trophoblast cells in a thyroid hormone receptor-dependent manner: in silico and in vitro analyses. Environ. Sci. Technol. 56, 8384–8394. https://doi.org/10.1021/acs.est.1c08161.

Yang, C., Song, G., Lim, W., 2019. A mechanism for the effect of endocrine disrupting chemicals on placentation. Chemosphere 231, 326–336. https://doi.org/10.1016/j.chemosphere.2019.05.133.

Yang, Q., Mao, Y., Wang, J., Yu, H., Zhang, X., Pei, X., Duan, Z., Xiao, C., Ma, M., 2022. Gestational bisphenol A exposure impairs hepatic lipid metabolism by altering mTOR/CRTC2/SREBP1 in male rat offspring. Hum. Exp. Toxicol. 41, 096032712211298 https://doi.org/10.1177/09603271221129852.

Yu, Z., Han, Y., Shen, R., Huang, K., Xu, Y., Wang, Q., Zhou, S., Xu, D., Tao, F., 2018. Gestational di-(2-ethylhexyl) phthalate exposure causes fetal intrauterine growth restriction through disturbing placental thyroid hormone receptor signaling. Toxicol. Lett. 294, 1–10. https://doi.org/10.1016/j.toxlet.2018.05.013.

Zhang, N., Wang, W.S., Li, W.J., Liu, C., Wang, Y., Sun, K., 2015. Reduction of progesterone, estradiol and hCG secretion by perfluorooctane sulfonate via induction of apoptosis in human placental syncytiotrophoblasts. Placenta 36, 575–580. https://doi.org/10.1016/j.placenta.2015.02.008.

Zhang, R., Guo, J., Wang, Y., Sun, R., Dong, G., Wang, X., Du, G., 2023. Prenatal bisphenol S exposure induces hepatic lipid deposition in male mice offspring through downregulation of adipose-derived exosomal miR-29a-3p. J. Hazard Mater. 453, 131410 https://doi.org/10.1016/j.jhazmat.2023.131410.

Zhang, S., Sun, C., Zhao, S., Wang, B., Wang, H., Zhang, J., Wang, Y., Cheng, H., Zhu, L., Shen, R., Sun, M., Xu, T., Zhao, L., 2020. Exposure to DEHP or its metabolite MEHP promotes progesterone secretion and inhibits proliferation in mouse placenta or JEG-3 cells. Environ. Pollut. 257, 113593 https://doi.org/10.1016/j.envpol.2019.113593.

Zhu, Y., Wan, Y., Zhang, B., Zhou, A., Huo, W., Wu, C., Liu, H., Jiang, Y., Chen, Z., Jiang, M., Peng, Y., Xu, S., Xia, W., Li, Y., 2018. Relationship between maternal phthalate exposure and offspring size at birth. Sci. Total Environ. 612, 1072–1078. https://doi.org/10.1016/j.scitotenv.2017.08.207.

Zlatnik, M.G., 2016. Endocrine-disrupting chemicals and reproductive health. J. Midwifery Wom. Health 61, 442–455. https://doi.org/10.1111/jmwh.12500.

Zong, T., Lai, L., Hu, J., Guo, M., Li, M., Zhang, L., Zhong, C., Yang, B., Wu, L., Zhang, D., Tang, M., Kuang, H., 2015. Maternal exposure to di-(2-ethylhexyl) phthalate disrupts placental growth and development in pregnant mice. J. Hazard Mater. 297, 25–33. https://doi.org/10.1016/j.jhazmat.2015.04.065.