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Per- and polyfluoroalkyl substances (PFAS) in placental compartments: Histopathological and toxicological data integration in an Italian cohort

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are synthetic environmental contaminants with widespread industrial and consumer applications, characterized by strong chemical stability and environmental persistence. Recent studies have highlighted placental permeability to PFAS, though evidence of direct histopathological impairment remains limited. This study aimed to investigate potential associations between PFAS exposure and histopathological abnormalities in placental samples. A total of 23 at-term pregnant women were recruited from two hospitals in Italy as part of a multicenter study. Placental samples, divided into maternal (decidua) and fetal (villi) compartments, were analyzed for PFAS concentration and histopathological alterations. PFAS were detected in 95.7 % of samples. The most frequently detected PFAS were PFOS (88 %), followed by PFHxS (83 %), PFOA (83 %), PFBS (54 %) and PFHxA (54 %). Preliminary findings suggest variable PFAS concentrations among subjects, with histopathological examination revealing placental alterations of potential clinical relevance. The observed histopathological alterations, particularly in cases of malperfusion and angiogenesis changes, suggest that PFAS may contribute to placental dysfunction, potentially affecting pregnancy outcomes. In particular, it could be hypothesized that PFHxA could exert an adverse influence on placental angiogenesis, due to pre-placental hypoxia stimulating the angiogenesis and resulting in increased ramification and number of branches. While direct causative links remain to be fully elucidated, these results underscore the need for further investigations into PFAS-related placental effects and their implications for fetal development.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic environmental contaminants that pose significant health risks. These man-made chemicals, introduced in the mid-20th century, have been widely incorporated into industrial and consumer products due to their exceptional ability to repel both grease and water. They are characterized by a chain of carbon atoms bonded to fluorine atoms, creating one of the strongest chemical bonds in organic chemistry that resist degradation for extended periods of time under normal environment conditions. The same physical-chemical properties that make PFAS so useful in various applications also contribute to their persistence and bioaccumulation in the environment and throughout the food chain. In recent years, environmental and trophic global exposure to various PFAS congeners has been extensively documented through numerous studies worldwide and significant qualitative and quantitative variations have been observed in samples from different geographical regions, primarily associated with industrial activities and anthropogenic influences (Piva et al., 2021). Both acute and chronic toxicity of several PFAS compounds have been extensively investigated providing valuable insights into the potential health impacts of this huge group of environmental contaminants. For example, in vitro studies have elucidated potential mechanisms of PFAS toxicity, such as oxidative stress induction and endocrine disruption at cellular level; while animal models have provided crucial information on systemic effects, organ-specific toxicity and dose-response relationships for various PFAS compounds

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(Feng et al., 2023; Ríos-Bonilla et al., 2024; Fenton et al., 2021). In humans, PFAS health effects have been studied in occupational settings and/or in population exposed to environmental contamination associated to industrial activities (Piva et al., 2021). Epidemiological studies have provided significant insights into the long-term health risks associated with exposure to per- and polyfluoroalkyl substances (PFAS). These studies have highlighted the bioaccumulation and persistent exposure effects of PFAS on liver function, immune response, and reproductive health. Regarding the reproductive health, it has been suggested that PFAS exposure negatively affects reproductive health by disrupting hormone levels. For example, Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been linked to decreased levels of sex hormone-binding globulin (SHBG) in men and follicle-stimulating hormone (FSH) in women, which can impair spermatogenesis and menstrual cycles (Shi et al., 2024; Rickard et al., 2022). They can also affect parturition by mimicking estrogens, potentially leading to preterm birth (Qin et al., 2023). Reproductive impairment has also been deeply investigated in animal models such as *C. elegans* in concentration-dependent studies; Perfluorohexanoic acid (PFHxA) exposure resulted in prolonged generation time and significantly reduced brood size at higher concentrations. The authors suggested a gene dysregulation PFHxA-mediated of the genes playing a role in germ cell development and fertility (Sana et al., 2025). Researchers observed that reproductive effects in animals are primarily linked to adverse health consequences in the mother during pregnancy, which in turn impact the child's development. In fact, for example, maternal exposure to PFOA has been associated with multiple adverse health outcomes, including increased gestational weight gain (GWG), pregnancy-induced hypertension, preeclampsia and finally reduced birth weight in a dose-dependent manner (Blake et al., 2020). In this frame, regulatory agencies such as the European Food Safety Agency (EFSA) worked to define maximum levels of weekly intake for PFOS, PFOA, perfluorononanoic acid (PFNA) and perfluorohexanesulfonic acid (PFHxS) (EFSA, 2020a,b) and risk assessment evaluation (EFSA, 2020a,b). Moreover, in 2023, the International Agency for Research on Cancer (IARC) classified PFOA as a human carcinogen and for the first time, designated perfluorooctanesulfonic acid (PFOS) as a possible human carcinogen. (Zahm et al.). Another compound, PFHxA and its inorganic salts, is under evaluation by the European Chemical Agency (ECHA) to be classified in category 1B, clear evidence, for reproductive toxicity (ECHA, 2025). Several studies have focused on pregnancy as a time window of exposure to PFAS also in humans by assessing maternal and/or fetal blood or PFAS content in the placenta. As the transducer of the maternal to fetal environment during pregnancy, a healthy placenta is important for adequate transport of nutrients, removal of waste, secretion of hormones and fetal protection (Griffiths and Campbell, 2015). In fact, placental pathologies, such as maternal vascular malperfusion (MVM), histological chorioamnionitis (HCA) are associated with adverse preterm births (Budal et al., 2022). Studies have shown that the placenta accumulates PFAS, with concentrations more closely resembling fetal blood than maternal blood (Mamsen et al., 2019; Groisman et al., 2023). Another study identified distinct partitioning patterns of PFAS between the maternal and fetal sides of the placenta, suggesting a potential correlation with the physicochemical properties of these compounds (e.g., chain length, functional groups, and protein-binding affinity) (Pascali et al., 2023). While earlier research primarily focused on bioaccumulation patterns, newer investigations reveal structural and functional impairments linked to PFAS exposure. Mouse studies demonstrate placental labyrinth congestion (abnormal blood pooling) and atrophy following gestational exposure to PFOA and the ammonium salt of hexafluoropropylene oxide dimer acid (GenX) (EPA, 2023). These structural changes correlate with disrupted fetal-placental weight ratios and altered thyroxine levels (Khan et al., 2025). In vitro human trophoblast models show PFAS-induced reduced migratory capacity in the range of 57–79 % wound closure inhibition and mitochondrial dysfunction, suggesting impaired placental development. Notwithstanding the efforts in research, a lack of evidence or uncertainty exists, and more work is needed in the field.

The aim of the present study was to observe clinical or subclinical pathological condition associated with histopathological abnormalities in placenta samples in relation to PFAS presence, characterized by number of congeners, type and concentration. The women were recruited as part of a multicenter study conducted in two distinct regions of Italy, each characterized by varying degrees and types of human activities. These anthropogenic influences, including industrial operations, agricultural practices, urban development, and traffic emissions, have the potential to significantly affect environmental quality and, consequently, the levels of contaminants to which the population may be exposed. Placental samples were analyzed by separately examining the two main tissue components: the decidua, representing the maternal portion, and the chorionic villi, which constitute the fetal component. This distinction allowed for a more detailed assessment of the distribution and localization of contaminants across the maternal–fetal interface.

2. Materials and methods

2.1. Study population: design and data extraction

The study was approved by the Research Ethical Board of Area Vasta Emilia Nord (protocol number 859/2021/OSS/IRCCSRE9) and by the Ethical Committee of the University of Bologna (CE AVEC: 718/2023/Sper/AOUBo). At-term (gestational week >37) pregnant women were enrolled from the Hospital Arcispedale Santa Maria Nuova (ASMN) of Reggio Emilia and the University-Hospital Sant'Orsola of Bologna, Italy in the period December 2023–November 2024. Volunteers gave their written informed consent to participate in the research. Inclusion criteria were age above 18 years old and women from the general population living in regions with minimal environmental exposure to PFAS and not impacted by proximity to identified contaminated sites; women under no pharmacological treatment; absence of alcohol and/or illicit drug abuse, as assessed through anamnestic data collected before hospitalization.

Placental samples were collected fresh at the time of delivery and each placenta was divided into maternal (decidua) and fetal (villi) compartments for toxicological analysis. The placenta was sent for histopathological evaluation soon after. The amount of tissue submitted to toxicological analysis was in the order of 5–10 g. The maternal compartment (decidua) was accurately scraped through a scalpel. The fetal compartment (villi) was subsequently sampled with iron pliers and a scalpel to avoid cross-contamination between the maternal and fetal sides. Samples were stored in polypropylene screw vials at − 20 °C until analysis. After sampling for toxicological analysis, the remaining placenta and annexes were included in 10 % formalin and submitted to macroscopical and histopathological analyses. The placenta submitted to histopathological evaluation was sampled according to Amsterdam Consensus criteria (Khong et al., 2016). A thorough pathological assessment was conducted by a placental pathology expert. Gross evaluation included: placental weight, linear measurements, and thickness; cord length, number of vessels, diameter, coiling index, and insertion into the chorionic plate; membrane integrity, consistency, and color (yellowish for infection, greenish for meconium exposure). In the parenchyma, main lesions such as infarcts, hematomas, thrombo-hematomas, and their extension were recorded. Histologically, membrane meconium exposure, lesions of chorionic vessels and their branches, abnormalities in parenchymal morphology, and maturation were evaluated. These histopathological alterations are considered clinically relevant, as they are commonly associated with adverse pregnancy outcomes such as preeclampsia, intrauterine growth restriction (IUGR), stillbirth, and placental abruption, all of which can significantly compromise both maternal and fetal health. A total of 23 subjects were included in the study.

2.2. PFAS materials, sample preparation and analysis

2.2.1. Materials

All PFAS analytes (>98 % purity) and PFAS mass labelled standards used as surrogate and injection standards were from Wellington Laboratories (Guelph, Ontario, Canada). Target perfluoroalkyl analytes were prepared at serial dilution of 0.05–0.1–0.25-1-2.5–5 ng/ml in water/methanol (90/10, v/v) for calibration; surrogate mass-labelled compounds were used at the concentration of 500 ng/ml; injection standards were used at the concentration of 1000 ng/ml. Methanol, acetonitrile and formic acid 98–100 % (all LC–MS grade) were supplied by Merck (Darmstadt, Germany). Water for mobile phase was produced by Sartorius Arium mini apparatus (Sartorius, Goettingen, Germany). Ammonium acetate was provided by Sigma-Aldrich (S.Louis, MO, USA). WAX polymer (150 mg, 6 ml) cartridges were from Agilent Technologies (Santa Clara, US).

2.2.2. Sample preparation and PFAS analysis

A previously validated method for the determination of 23 PFAS in placenta was applied to assess the PFAS content (Pascali et al., 2023), namely PFBA, PFPeA, PFMBA, PFBS, PFEESA, PFHxA, 4:2 FTS, 6:2 FTS, 8:2 FTS, PFPeS, PFHxS, PFHpA, ADONA, PFOA, PFHpS, PFOS, HFPO-DA, 9Cl-PF3ONS, 11Cl-PF3OUdS, PFNA, PFDA, PFUnA, PFDoA (details in supplemental material). Briefly, 3 g ± 0.5 g of sample was added of 2.5 μl of mass labelled standard solution and of 10 ml of purified water, followed by homogenation (model S, VWR International, Radnor, USA) until dissolution. The homogenate was transferred into a polypropylene 50 ml vial and added of 10 ml of acetonitrile. Samples were vortexed for 30″ (IKA, Staufen, Germany), and debris removed by centrifugation at 4500 rpm for 10 min. Supernatant was collected and purified by weak anion exchange SPE. Column was conditioned with 5 ml of methanol and 5 ml of 2 % formic acid in water. Column washing was carried out with 5 ml of 2 % formic acid in water and 5 ml of methanol. Elution consisted of two steps, each composed of 2.5 ml of methanol/NH4OH (90/10, v/v). Extracts were evaporated to dryness under a stream of nitrogen at 40 °C, then reconstituted in 500 μl of water/methanol (90/10, v/v) to which 2.5 μl of injection internal standard solution was added. Analyses were carried out in a 1290 Infinity II LC coupled to 6546 quadrupole-time-of-flight mass spectrometer (Q-TOF) (Agilent Technologies, Santa Clara, USA). Chromatographic separation was performed in a Poroshell EC-C18 column (2.1 × 100 mm, 1.9 μm) (Agilent Technologies) at 40 °C. An LC C18 column (EclipsePlus -C18, 3.0 × 50 mm 1.8 μm) was placed after pump exit to delay any perfluorinated interferents possibly originating from fluidic system. Analyses were carried out in 20 mM ammonium acetate in water (mobile phase A) and methanol (mobile phase B), both added of 0.1 % formic acid (v/v) under gradient. The flow rate was 0.250 ml/min. The injected volume was 5 μl. The Q-TOF instrument was set in negative acquisition mode and source parameters were as follow: capillary 3500 V, gas temperature 320 °C, sheat gas temperature 350 °C, drying gas 8 l/min, nebulizer 35 psi, sheat gas flow 12 l/min. All source parameters were optimized under LC conditions. Mass scan was in the range 100–1000 m/z at a rate of 3 spectra/sec. Method performance parameters are summarized in the supplementary material.

2.3. Histopathological assessment

For each participant, the following demographic and clinical data was extracted from the clinical records: maternal age and clinical history; data on fetuses/newborns, including newborn sex, birth weight, relevant fetal conditions such as intrauterine growth restriction (IUGR). The results of histopathological analysis were categorized based on:

- presence/absence of any abnormality in the histopathology of decidua;
- presence/absence of any abnormality in the histopathology of villi.

Focal changes were not deemed as pathological, and, for statistical analyses, samples with focal modifications were considered normal. Acute inflammation, e.g. funisitis or chorioamnionitis, and acute placenta modification were excluded from the analysis. Subcategories of abnormalities in the histopathology of decidua were classified as: decidual vasculopathy, consisting of lack of physiologic conversion and persistence of arteriolar smooth muscle either partially or completely in the walls (untransformed arterioles) (Baergen, 2011), and superficial implantation site, that is characterized by persistent muscularization of basal plate arteries and increased placental site giant cells (Redline, 2017). Subcategories of abnormalities in the histopathology of villi were categorized as follow: presence of delayed villous maturation; crowded villi with increased angiogenesis, i.e. with clusters of richly capillarized, short, highly branched, and notched terminal villi, showing increased syncytial knots; distal villous hypoplasia, i.e. small terminal villi on cut surface, long and slender, with fewer branches compared to normal villi; chronic villitis (Baergen, 2011).

2.4. Statistical analysis

Descriptive statistics including central and dispersion measures for single PFAS were given as mean and standard deviation (SD) in cases of parametric distribution, as median and interquartile range (IQ that describes the range between the 25th and the 75th percentile). The type of distribution, whether parametric or non-parametric was calculated by the Stata sktest, that considers skeweness and kurtosis, and this data allowed us to understand whether parametric (meand and SD) or non-parametric (median and IQ) central and dispersion measures were most appropriate to describe the samples.

Toxicological results were reviewed in terms of the number of PFAS detected in each sample and quali-quantitative results for PFAS. Samples were classified into a binary PFAS category based on the number of PFAS detected, with categories defined by whether the count was above or below the 75th percentile. Since the 75th percentile corresponded to a count of 7 PFAS, the binary PFAS category was defined as detecting equal or more (≥) or less (<) than 7 PFAS.

Additionally, binary categories were used for PFAS that had detection rate around 60 % or less: detectable vs non-detectable PFHxA and detectable vs non-detectable PFBS concentrations.

Statistical associations between qualitative or categorical variables (clinical data of women, clinical data of newborns, results of histopathological analyses and binary PFAS categories) were attempted by Chi square test.

Quantitative PFAS results were evaluated as total PFAS values (sum of PFAS individual concentrations) in decidua and in villi extracts, as well as concentrations of the most occurred compounds (PFOA, PFOS, PFHxA, PFHxS, and PFBS) singularly taken in decidua and villi samples. For quantitative results, the following comparisons were performed by parametric or non-parametric *t*-test, according to the sktest result between:

- women with metabolic disorders, including cases of gestational diabetes and obesity (cases) and healthy women (controls);
- samples with (cases) and without (controls) abnormal histopathology of decidua, considering any abnormalities and subcategories of abnormalities.
- samples with (cases) and without (controls) abnormal histopathology of villi, considering any abnormalities and subcategories of abnormalities.

Statistical analyses, considered significant with p-values <0.05, were realized by Stata, while figures were created with Prism (GraphPad Software, LLC, version 9.0.0).

3. Results

3.1. Demographic, clinical data and histopathological results

The median age was 37 years (IQ = 29–39), ranging from 24 to 46 years. Maternal diseases were encountered in 43 % of subjects: 3 women had gestational diabetes, 3 had hypothyroidism, 2 had hypertension, and 2 were characterized by a body mass index >30 (obesity).

Newborns were 14 female (60.8 %) and 9 male (39.2 %), with a median weight of 3455 g (IQ = 2512–3550), ranging from 1850 to 4076 g. In 3 cases, fetal diseases were documented, including 2 cases of IUGR and one case of oligohydramnios. The placenta, cord, and membranes appeared normally on gross examination. Abnormalities in the decidua were observed in 7 samples, (30.5 %), all displaying decidual vasculopathy with untransformed arterioles and 3 cases (13.0 % of the total) additionally presenting superficial implantation site. Ten cases (43.5 % of the total) were characterized by abnormal histopathology of villi. The abnormalities, not mutually exclusive, included: delayed villous maturation (34.8 % of the cases), crowded villi with increased angiogenesis (30.4 % of all cases), distal villous hypoplasia (26.1 % of all cases) and chronic villitis (8.7 % of all cases). The relationships between clinical data of women and newborns, as well as with histopathological results are shown in the Sankey plot in Fig. 1, while histological examples of the detected decidual and villi abnormalities are shown in Fig. 2.

3.2. PFAS results

PFAS were detected in 95.7 % of decidua and villi samples. The median number of PFAS detected in decidua and villi extracts was 6 (IQ = 5–7) and 5 (IQ = 4–7), respectively. On the basis of the 75th percentile, samples were also classified into a binary category, depending on whether as equal or more (≥) or less (<) than 7 PFAS were detected. The most frequently detected PFAS were PFOS (88 %), followed by PFHxS (83 %), PFOA (83 %), perfluorobutane sulfonic acid (PFBS) (54 %) and PFHxA (54 %). The median total PFAS values in placenta was 1.0 ng/g (IQ = 0.50–1.66), ranging from 0.16 to 2.39 ng/g. The most abundant PFAS in both placental compartments were PFOS, followed by PFOA (0.08–0.22 ng/g), while PFHxA, PFHxS and PFBS showed lower concentrations. Detailed results for each PFAS are shown in Table 1.

No difference ($p > 0.05$) was observed by comparing decidua and villi extracts for the amounts of PFAS. Sex of the newborn did not statistically influence PFAS distribution in decidual and villi.

3.3. Clinical data results

The study found no observable link between maternal conditions such as gestational diabetes, obesity, hypothyroidism, or hypertension and adverse newborn outcomes or placental abnormalities. Similarly, cases of intrauterine growth restriction (IUGR) or low amniotic fluid (oligohydramnios) also showed no connection to placental changes, though these specific scenarios were limited in occurrence.

When analysing PFAS levels, decidua samples from women with metabolic disorders exhibited slightly elevated concentrations of PFOA, PFHxA, and PFBS compared to healthy individuals. While the combined levels of these three substances showed a marginal difference, no definitive relationship emerged between metabolic conditions and PFAS distribution patterns.

In Table 2 a focus on PFAS results for women with metabolic diseases and IUGR fetuses is shown.

3.4. Histopathology results

Cases with decidual vasculopathy or superficial implantation sites had a variable number of PFAS. Cases with decidual histological abnormalities, such as vasculopathy with untransformed arterioles or superficial implantation sites did not show higher concentration of PFOA, PFOS, PFHxS and PFHxA neither for the villi nor the decidua. Samples with decidual vasculopathy and untransformed arterioles more frequently presented detectable levels of PFBS in decidua extracts, and the association was significant ($p = 0.021$) (Table 3), while the association with superficial implantation site tested non-significant.

Considering quantitative variables, samples with decidual histological abnormalities, such as vasculopathy with untransformed arterioles

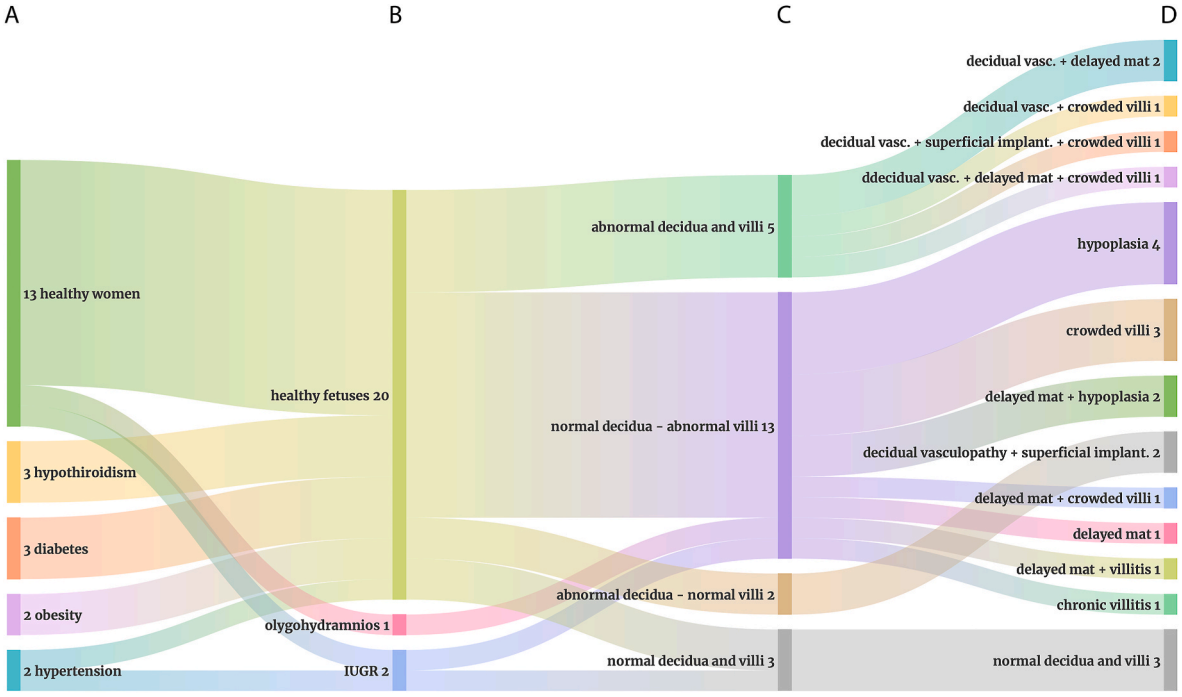

Fig. 1. Sankey plot describing clinical data on women (A), clinical data on newborns (B), histopathology results (C) and detailed histopathology (D). delayed mat: delayed maturation; superficial implant: superficial implantation site.

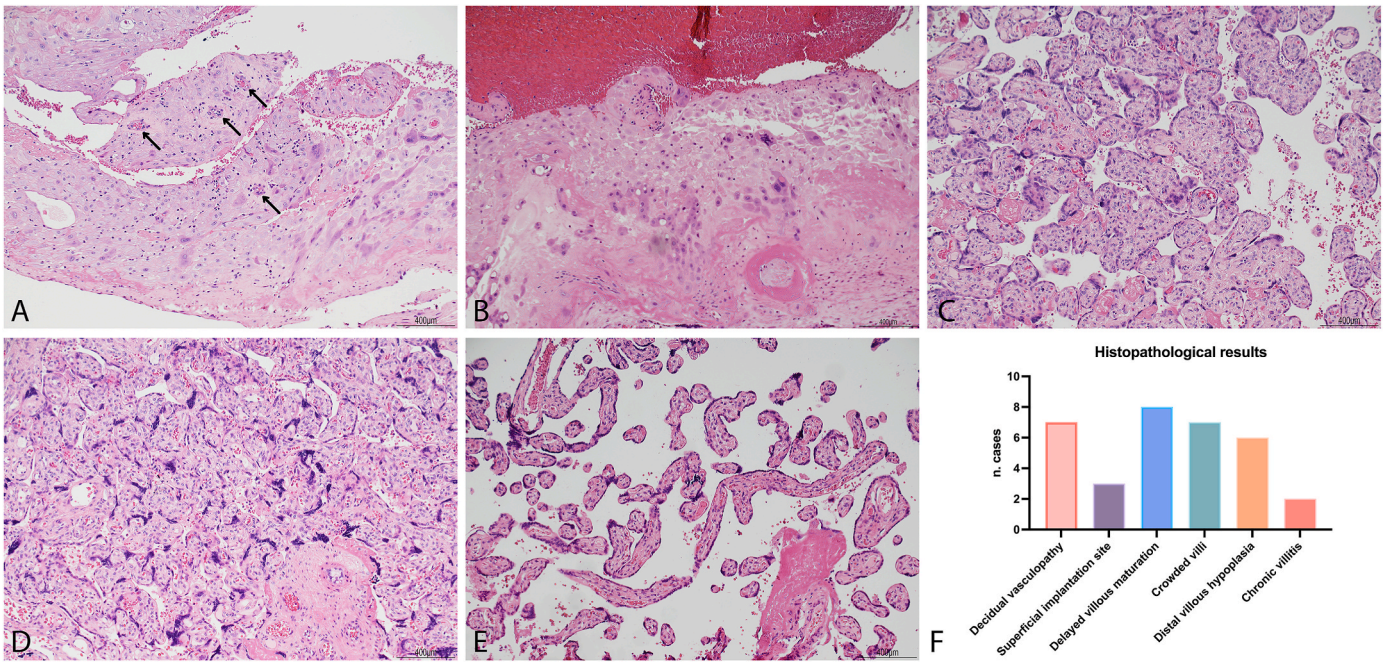

Fig. 2. Example of histopathological alteration encountered in our casuistry. A: decidual vasculopathy with untransformed arterioles (arrows). In B: superficial implantation site with untransformed arterioles and abundant implantation trophoblast In C: delayed villous maturation, showing enlarged villi with reduced vasculo-syncytial membranes In D: crowded villi with enhanced branching angiogenesis: the villi are packed together with increased capillaries and syncytial knots. In E: distal villous hypoplasia: the villi are slender and in cross sections, appear with a reduced diameter. In F: a scheme with the number of cases showing decidual or villi histopathology abnormalities.

Table 1
Detection rate, mean (together with SD), median (together with IQ) and distribution for each parameter. n = number; IQ = interquartile range; SD = standard deviation.

Analyte or parameter	Tissue	Detection	Mean (SD) [ng/g	Median (IQ) [ng/g]	Distribution
n. PFAS	decidua	At least 1 PFAS 95.7 %	5.74 (2.36)	6 (5–7)	non-parametric
	villi	At least 1 PFAS 95.7 %	5.87 (2.28)	5 (4–8)	non-parametric
Total PFAS value	decidua	100 %	0.52 (0.31)	0.46 (0.27–0.71)	non-parametric
	villi	91.3 %	0.59 (0.45)	0.49 (0.23–0.77)	non-parametric
PFOS	decidua	95.7 %	0.25 (0.18)	0.20 (0.15–0.29)	parametric
	villi	91.3 %	0.30 (0.24)	0.22 (0.15–0.39)	non-parametric
PFOA	decidua	87.0 %	0.08 (0.06)	0.08 (0.04–0.11)	parametric
	villi	87.0 %	0.12 (0.13)	0.08 (0.03–0.17)	parametric
PFHxS	decidua	91.3 %	0.04 (0.03)	0.03 (0.02–0.06)	non-parametric
	villi	87.0 %	0.04 (0.05)	0.03 (0.02–0.06)	parametric
PFBS	decidua	65.2 %	0.01 (0.02)	0.01 (0–0.02)	parametric
	villi	56.5 %	0.01 (0.01)	0.00 (0–0.02)	non-parametric
PFHxA	decidua	56.5 %	0.07 (0.11)	0.02 (0–0.09)	parametric
	villi	56.5 %	0.04 (0.05)	0.03 (0.02–0.06)	parametric

Table 2
PFAS results regarding women with metabolic diseases and IUGR fetuses.

Cases	n. PFAS in decidua - villi	Total value of PFAS median (IQ) [ng/g]	PFOS concentration	PFOA concentration	PFHxS concentration	PFBS detection	PFHxA detection
	median (IQ)		median (IQ) [ng/g]	median (IQ) [ng/g]	median (IQ) [ng/g]	%	%
Women with metabolic diseases	decidua: 6 (3–7)	decidua: 0.46 (0.35–0.86)	decidua: 0.15 (0.15–0.23)	decidua: 0.12 (0.07–0.14)	decidua: 0.03 (0.00–0.04)	decidua: 80 %	decidua: 60 %
	villi: 7 (4–7)	villi: 0.96 (0.49–1.21)	villi: 0.39 (0.15–0.62)	villi: 0.18 (0.08–0.19)	villi: 0.02 (0.00–0.03)	villi: 60 %	villi: 60 %
IUGR Fetuses	decidua: 4.5 (2–7)	decidua: 0.15 (0.04–0.25)	decidua: 0.09 (0–0.19)	decidua: 0.01 (0–0.01)	decidua: 0.00 (0.00–0.01)	decidua: 0 %	decidua: 50 %
	villi: 4.5 (4–5)	villi: 0.33 (0.32–0.33)	villi: 0.19 (0.19–0.20)	villi: 0.07 (0.06–0.09)	villi: 0.03 (0.02–0.04)	villi: 0 %	villi: 100 %

n = number; IQ = interquartile range.

Table 3
Statistically significant associations between detectable PFBS in decidua extracts and decidual vasculopathy with untransformed arterioles.

	Decidual vasculopathy	Normal decidua
Non-detectable PFBS in decidua	0 (0 %)	8 (50 %)
Detectable PFBS in decidua	7 (100 %)	8 (50 %)
Total	7 (100 %)	16 (100 %)
$p = 0.021$		

or superficial implantation site, did not show higher number of PFAS, total value of PFAS nor individual concentrations of PFOA, PFOS, PFHxS and PFHxA in decidua extracts (Fig. 3). PFBS concentration in decidua was slightly higher in samples with decidual vasculopathy (mean = 0.02 g/g, SD = 0.01) (mean = 0.01 g/g, SD = 0.00) (Fig. 3F) and significantly higher in samples with histopathological hallmarks of superficial implantation site ($p = 0.023$) compared to samples with normal histology (Fig. 3M).

Samples displaying abnormal histopathology of villi (cases) presented a number of PFAS equal or above 7 (75th percentile) very frequently and the association showed significant ($p = 0.027$). To explain this association, statistical analyses were repeated taking into consideration subcategories of histopathological abnormality in villi according to different number of PFAS compound, and distal villous hypoplasia was most frequently characterized by a number of PFAS equal or above 7 (75th percentile) ($p = 0.022$) (Table 4).

According to the chi-square test, samples with abnormal villi, and those with distal villous hypoplasia were also characterized by significantly higher number of PFAS in samples ($p = 0.013$ and $p = 0.001$). Results of all quantitative comparisons between samples with (cases) and without (controls) delayed villous maturation, distal villous hypoplasia and crowded villi with increased angiogenesis is given in Fig. 4. Samples with abnormal villi (cases) did not show statistically higher total PFAS concentrations, even when analyzing individual PFAS or considering each subcategory of histopathological abnormality. The only exception was noted for PFHxA, which showed significantly higher concentrations in samples with increased villi angiogenesis ($p = 0.031$) compared to normal villi (Fig. 4U).

4. Discussion

While the mechanisms underlying PFAS effects on pregnancy—and how pregnancy outcomes may be influenced by PFAS-mediated modulation of placental functions and metabolism—are challenging to investigate due to confounding factors, studying histopathological changes in the placenta associated with PFAS exposure could serve as an important first step. Our study represents a first preliminary study to highlight possible influence of PFAS presence to placental morphology also in relation to clinical aspects.

Toxicological analysis confirmed detection of PFAS in the placenta which aligns with other studies (Hall et al., 2022), with at least 1 compound confirmed in both decidua and villi in 96 % of cases with many samples displaying positive up to 6 different compounds. Among the 23 PFAS congeners, five compounds displayed occurrence above 50 %, namely PFOS, PFOA, PFHxS, PFHxA and PFBS and were further considered in relation to histopathology. All these compounds are characterized by a chain length of $C \leq 8$, demonstrating the high permeability of shorter C-F deep into human tissues with respect to molecules at higher molecular weight of the PFAS family. No differential distribution has been observed between decidua and villi counterparts for the sum of PFAS, as already described in our previous study (Pascali et al., 2023), however, differential partitioning should be considered in relation to single PFAS because of the huge heterogeneity of this class of compounds. PFAS concentrations for individual molecules in our cohort aligned with levels reported in non-high-risk populations. The most frequently detected congeners—PFOS (median: 0.21 ng/g), PFOA (median 0.08 ng/g), and PFHxS (median 0.03 ng/g)—showed comparable levels to those observed in Israeli women from the Groisman et al. (2023) cohort. In contrast, our findings differed significantly from studies involving high-risk pregnancy cohorts (Bangma et al., 2020), where concentrations of PFOS and PFHxS were up to an order of magnitude higher for extreme values and exhibited elevated median levels. Notably, the absence of extreme outliers in our sample (range: 0.23–0.77 ng/g total PFAS) suggests minimal exposure to acute point-source contamination. One of the most well-documented associations of PFAS exposure in pregnancy is with gestational diabetes and preeclampsia. Particularly, PFNA, PFOA, perfluoroheptanoic acid (PFHpA), perfluorododecanoic acid (PFDoDA), and PFHxS, have been associated to metabolic disorders such as gestational diabetes and impaired glycemic status of the mothers (Rahman et al., 2019; Szilagyi et al., 2020).

Decidua, the maternal side of placenta, displayed higher concentrations of PFOA, PFHxA and PFBS in cases of women affected by metabolic diseases (diabetes and obesity) compared to healthy ones; it is worth noting that women with metabolic diseases also tended to have a higher number of PFAS in villi and at higher concentrations, particularly of PFOS and PFOA.

Maternal diabetes is often characterized, on histopathological examination, by villous immaturity and delayed maturation (Baergen, 2011), but even this pathology was observed in our samples, the association was not statistically confirmed, likely due to the low sample size.

More in general, there was no statistical association between maternal-fetal diseases and histopathological abnormalities, as well as between clinical data and PFAS number or concentrations in the extracts, likely due to the low number of pathological cases (e.g. only 5 participants displayed metabolic diseases, or only 2 IUGR cases). Another documented association concerns prenatal exposure to PFAS, particularly PFOS, which has been linked to an increased risk of preterm birth. Associations have also been observed with low birth weight, small for gestational age, and miscarriage (Gao et al., 2021; Gui et al., 2022).In our study, although only a few fetuses presented with intrauterine growth restriction (IUGR), we observed higher PFOS concentrations in the placental villi of these cases.

However, some associations between subclinical histopathological abnormalities and PFAS exposure were observed: samples with histopathological signs of maternal or fetal malperfusion presented either a greater number of detected PFAS or higher concentrations in villi or decidua extracts.

Placental histopathological signs of malperfusion are often seen in a spectrum of obstetric disorders, including pre-eclampsia and IUGR, even combined one another, e.g. decidual vasculopathy and distal villous hypoplasia, and, although not pathognomonic, these findings could occur long before clinical manifestations. Studies on twins demonstrated a significant association between prenatal PFAS exposure and maternal malperfusion, a category that included multiple histopathological abnormalities including decidual vasculopathy and distal villous hypoplasia (Park et al., 2024).

One of the factors connected with adverse pregnancy outcomes is inadequate placentation expressed as decidual vasculopathy or superficial implantation site (Redline, 2017). In healthy pregnancies, the trophoblast infiltrates the arterial beds of the decidua and superficial myometrium, substitutes the muscular walls of the arterioles with fibrinoid, making the arteries enlarge and incapable of vasoconstrictive response to the various vasoactive mediators. In our study, cases of poor trophoblastic invasion of the myometrial branches with superficial implantation sites were characterized by higher PFBS decidual concentrations. This is consistent with previous human studies showing an association between PFBS in umbilical cord and hypertensive disorders and pre-eclampsia (Huang et al., 2019). In rabbits an association between prenatal PFBS exposure, adverse pregnancy outcomes and placenta weight was also shown (Crute et al., 2022), though no effect on histopathological placenta morphology was seen. Preeclampsia and

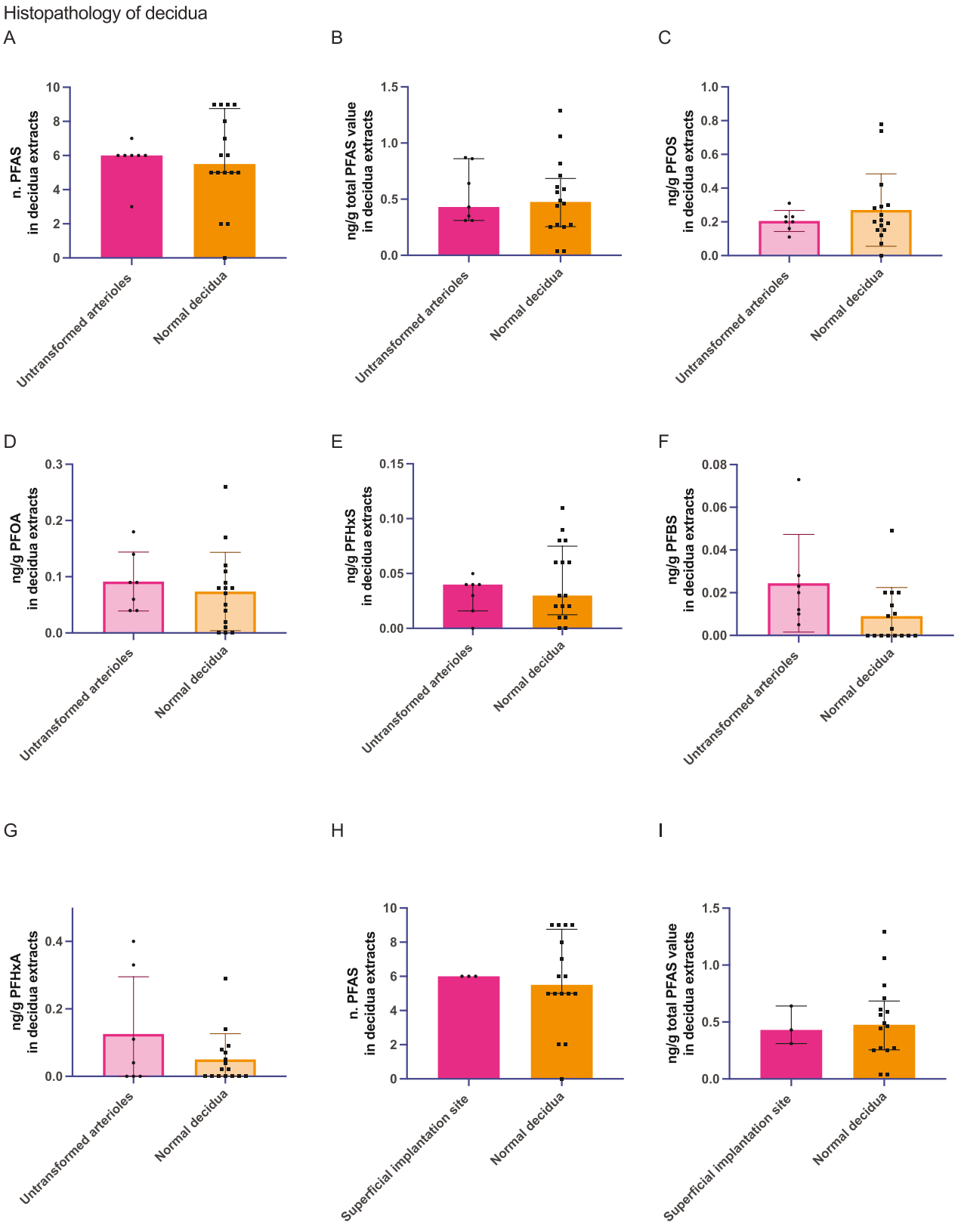

Fig. 3. Results of comparison between samples with (cases) and without (controls) abnormal histopathology of decidua. Particularly, A-G show comparison of samples with decidual vasculopathy with untransformed arterioles vs normal decidua, H-N comparison of samples with superficial site implantation vs normal decidua. Cases and controls are compared on the basis of number (n.) of PFAS (A, H), total PFAS value (B, I), PFOS (C, J), PFOA (D, K), PFHxS (E, L), PFBS (F, M), and PFHxA (G, N) concentrations. Significant results are given with p value. Mean and standard deviation (in lighter colours) or median and interquartile range (in darker colour) are shown according to parametric or non-parametric distribution.

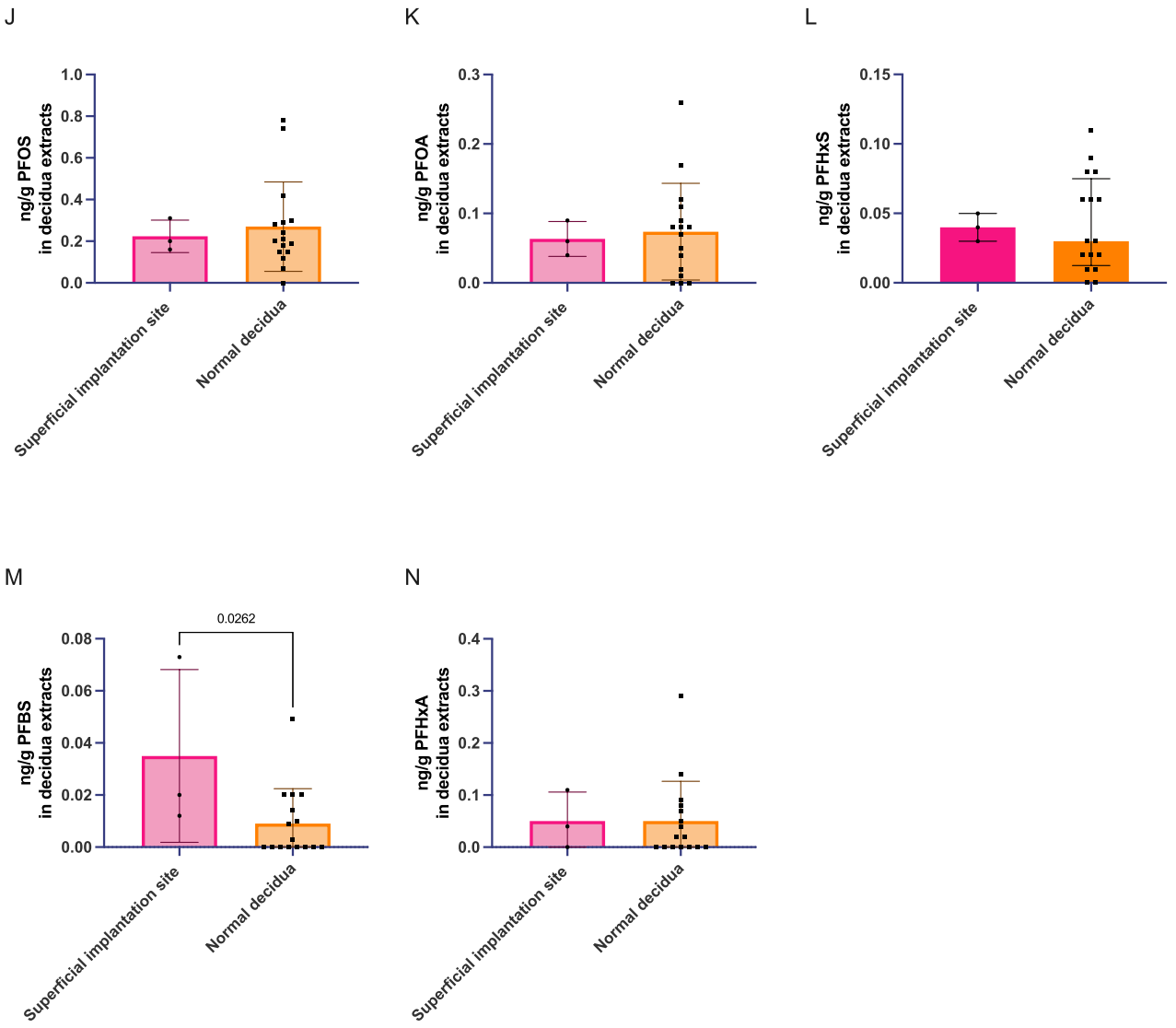

Fig. 3. (*continued*).

Table 4
Statistically significant associations between number of PFAS, whether < or ≥7 (75th percentile), and abnormal histopathology of villi as well as distal villous hypoplasia.

	Abnormal histopathology of villi	Normal villi
<7 PFAS in villi	8 (44.4 %)	5 (100 %)
≥7 PFAS in villi	10 (55.6 %)	0 (0 %)
Total	18 (100 %)	5 (100 %)
p = 0.027		
	Distal villous hypoplasia	**Normal villi**
<7 PFAS in villi	1 (16.7 %)	12 (70.6 %)
≥7 PFAS in villi	5 (83.3 %)	5 (29.4 %)
Total	6 (100 %)	17 (100 %)
p = 0.022		

IUGR are also associated to a failure of the angiogenesis and altered remodeling processes during pregnancy involving fetoplacental vasculature and angiogenesis, which influences the number and shapes of villi. A recent study suggested that PFAS, particularly PFOA, could alter placental arterial vasculature in term human placentae, reducing the surface vasculature (Chowdhury et al., 2024). In our sample, both terminal villous deficiencies, also called villous hypoplasia, and samples with crowded villi and increased angiogenesis were noted at histopathology examination. Particularly, distal villous hypoplasia is a severe underdevelopment of the peripheral placental vascular bed, that is associated with IUGR, when extensive, or to maternal vascular malperfusion when more limited. Poorly developed, filiform terminal villi with minimal branching are thought to be a reflection of postplacental hypoxia, where an increase in vascular resistance and a reduction in the oxygen extraction from maternal blood results in high oxygen level in the placenta, that inhibits ramification (Baergen, 2011; Park et al., 2024). In our study, distal villous hypoplasia was observed in samples with a number of PFAS above the 75th percentile (equal or more than 7 different PFAS), suggesting a cumulative impact of these contaminants on the vascular resistance and blood flow, ultimately affecting the morphology of villi. Rather than a reduction of the angiogenesis, some cases demonstrated crowded villi with increased angiogenesis, and cases displaying this abnormality also presented significantly higher PFHxA concentrations compared to normal villi samples. It is known that one of the most typical triggers for angiogenesis is represented by hypoxia, via expression of various proangiogenic factors. For example, conditions of chronic fetal hypoxia, as in cases of fetal hyperglycaemia and hyperinsulinemia with maternal diabetes, are characterized by hypervascularization (Cvitic et al., 2014). Elevated angiogenic biomarkers

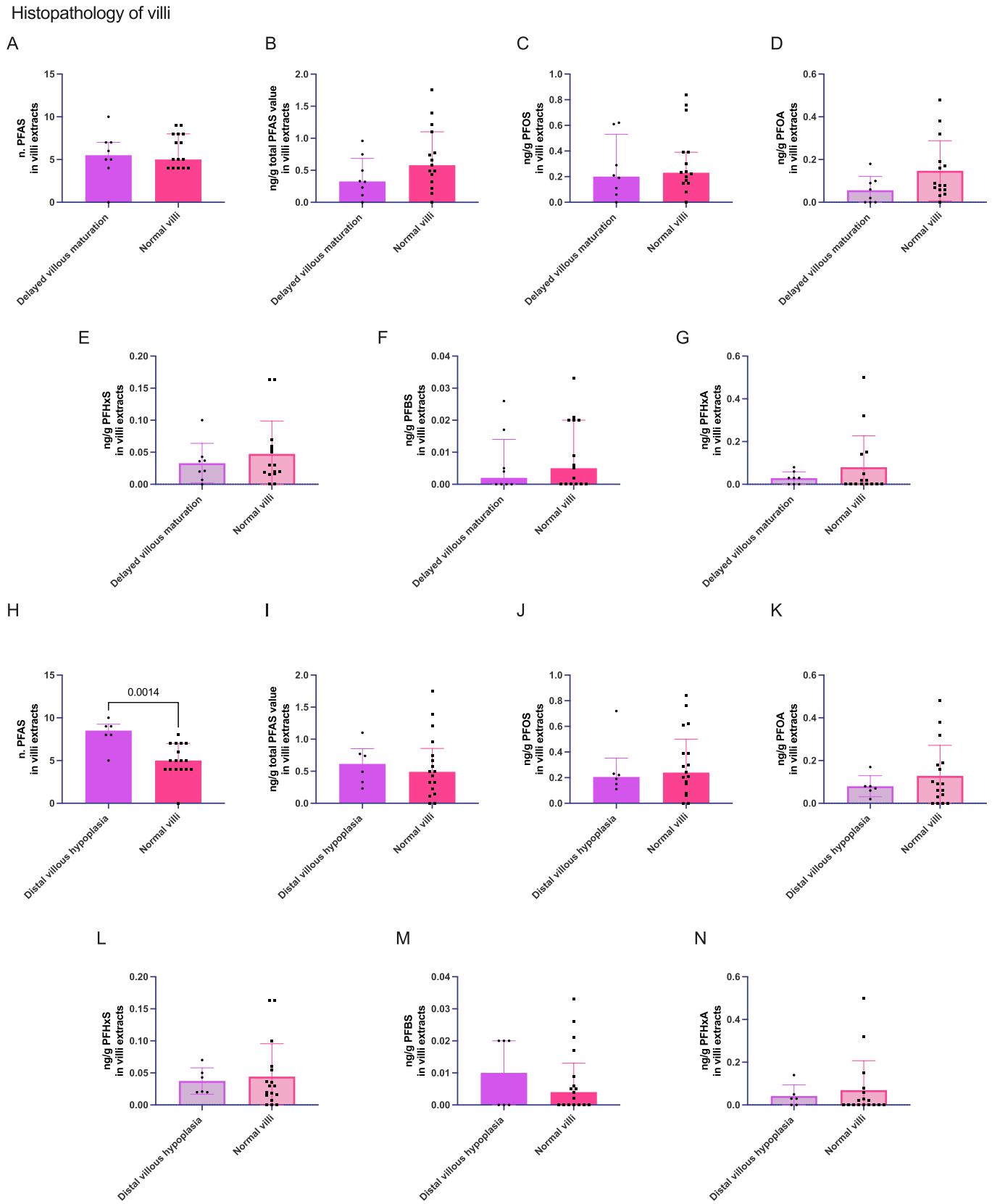

Fig. 4. Results of comparison between samples with (cases) and without (controls) abnormal histopathology of villi. Particularly, A-G show comparison of samples with delayed villous maturation vs normal villi, H-N comparison of samples with distal villous hypoplasia vs normal villi, O-U comparisons of samples with crowded villi vs normal villi. Cases and controls are compared on the basis of number (n.) of PFAS (A, H, O), total PFAS value (B, I, P), PFOS (C, J, Q), PFOA (D, K, R), PFHxS (E, L, S), PFBS (F, M, T), and PFHxA (G, N, U) concentrations. Significant results are given with p value. Mean and standard deviation (in lighter colours) or median and interquartile range (in darker colour) are shown according to parametric or non-parametric distribution.

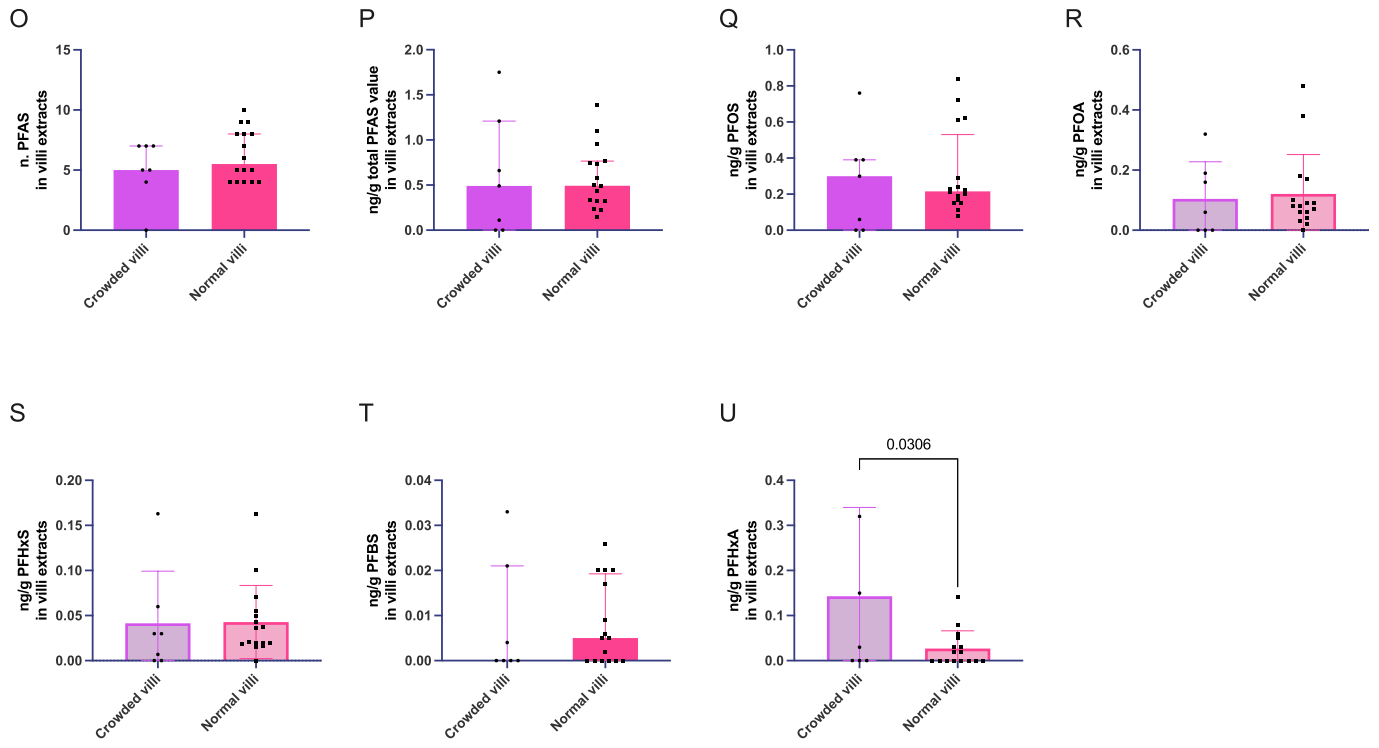

Fig. 4. (*continued*).

and disruption of VEGF signaling have been observed in women affected by pre-eclampsia and the markers were associated with PFOS and PFHpS exposure (Forsthuber et al., 2022; Mishra et al., 2024). Based on our results, it could be hypothesized that PFAS and particularly PFHxA also exert an adverse influence on placental angiogenesis, due to pre-placental hypoxia stimulating angiogenesis and resulting in increased ramification and number of branches. One of the main limitations of our study is the small sample size, which likely reduced the statistical power and limited the generalizability of our findings to the broader population. This preliminary data may encourage us to proceed with the study including also miscarriage and or stillbirths. The second limitation is related to the sampled material, which may be not representative of the whole organ, both in terms of toxicology and histopathology. PFAS and histopathological features may be localized in specific areas, causing only a limited impact on the overall functionality of the placenta. This also contributes to the challenges in studying and interpreting their effects. However, despite the relatively limited number of enrolled subjects and potential concerns about sample representativeness, the study required multiple analyses for each participant. This made the overall research process both time-consuming and costly.

To summarize the results, this study provided preliminary insights into the influence of PFAS exposure on placental morphology and its potential links to clinical outcomes. Toxicological analysis confirmed the widespread presence of PFAS, even in general populations, with multiple compounds detected in nearly all samples. Short-chain PFAS ($C \leq 8$) were particularly prevalent, highlighting their deep tissue permeability. While no statistically significant associations were found between PFAS concentrations and maternal-fetal diseases, subclinical histopathological abnormalities, such as maternal and fetal malperfusion, were linked to higher PFAS levels. PFBS concentrations were frequently observed in cases of decidual vasculopathy, at higher concentrations in cases of superficial implantation site, aligning with previous studies on hypertensive disorders. Additionally, PFHxA higher concentration was observed in samples with increased angiogenesis and altered villous morphology, suggesting a potential impact on vascular resistance and blood flow.

5. Conclusion

Despite the study's limitations, including sample size constraints and the challenge of tissue heterogeneity, our findings highlight a potential link between PFAS exposure and placental vascular abnormalities. The observed histopathological alterations, particularly in cases of malperfusion and angiogenesis changes, suggest that PFAS may contribute to placental dysfunction, potentially affecting pregnancy outcomes. Future research with larger cohorts, including cases of miscarriage and stillbirth, is necessary to further elucidate the role of PFAS in placental pathology and maternal-fetal health.

CRediT authorship contribution statement

Arianna Giorgetti: Writing – original draft, Data curation. **Arianna Fornasari:** Formal analysis, Data curation. **Maria Paola Bonasoni:** Writing – original draft, Methodology, Formal analysis. **Alice Ferretti:** Resources, Formal analysis. **Anna Seidenari:** Resources, Formal analysis. **Maria Sech:** Resources, Data curation. **Elena Piva:** Validation, Software, Resources, Formal analysis, Data curation. **Jennifer P. Pascali:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Paolo Fais:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Paolo Fais reports financial support was provided by Ministry of Education and Science Italy. None If there are other authors, they declare

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121846.

Data availability

Data will be made available on request.

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