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Prenatal perfluoroalkyl substances and newborn anogenital distance in a Canadian cohort

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ABSTRACT

Exposure to the man-made chemicals perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and perfluorohexanesulfonate (PFHxS) is widespread. These perfluoroalkyl substances (PFASs) have been associated with androgenic endocrine-disrupting properties; however, the evidence is equivocal and few human studies have examined the association between prenatal exposure to PFASs and markers of androgenic endocrine disruption such as changes in anogenital distance (AGD).

In the MIREC cohort, PFOA, PFOS and PFHxS were analyzed in first trimester maternal plasma. AGD was measured in 205 male and 196 female newborns. The change in estimate procedure was used to identify confounders by sex and AGD in multiple linear regression models.

Geometric mean plasma concentrations (95% CI) for PFOA, PFOS and PFHxS were 1.71 (1.61, 1.81), 4.40 (4.18, 4.64) and 1.15 (1.06, 1.25) μg/L, respectively. A one-unit increase in natural log transformed PFOA was associated with a 1.36 mm (95% CI 0.30, 2.41) increase in anoscrotal distance, adjusting for household income, active smoking status during pregnancy and gestational age. However, when examined by quartiles, a non-monotonic pattern was observed with wide confidence intervals. No consistent patterns were observed between maternal PFAS concentrations and female AGDs.

This study found no clear evidence that maternal plasma concentrations of PFOS, PFOA or PFHxS were associated with shorter infant anogenital distance in males or any change in AGD in females. Whether the positive association observed between longer anoscrotal distance and PFOA is real or would have any long-lasting effect on the reproductive health of males is unknown and needs to be investigated further.

1. Introduction

Perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and perfluorohexanesulfonate (PFHxS) are man-made chemicals that have been used for decades in the manufacture of stain- and water-resistant coatings for textiles and carpets; non-stick coatings on cookware; fire-fighting foams; and personal care products. PFOS is no longer manufactured, imported, sold, or used in Canada [1] but still persists in the environment as evidenced by the high detection rate in the Canadian population for PFOS as well as PFOA and PFHxS [2]. The estimated half-life for PFHxS (5.3 years) is considerably longer than for PFOS (3.4 years) and PFOA (2.7 years) with some evidence of more rapid elimination in women for PFHxS and PFOS, than in men [3].

An *in vitro* study has reported that some PFASs may act as androgen receptor antagonists [4], while another study reported no effect of PFASs on estrogen or androgen receptor activity [5]. There is some evidence from rodent models that PFOA and PFOS increase anogenital distance (AGD) in females [6] and PFOA may disrupt testicular Leydig cell development [7].

Anogenital distance is one of the battery of outcomes recommended

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Table 1
Characteristics of mother-infant pairs and plasma collection factors for participants from MIREC-ID birth assessment, according to median maternal PFAS concentrations (μg/L). (n = 403).

	Frequency	Percentage	PFOA Median (IQR)	PFOS Median (IQR)	PFHxS Median (IQR)
Recruitment Site					
A	17	4.2	1.3 (0.7 - 1.6)*	4.4 (2.0 – 5.0)	0.9 (0.6 - 1.6)*
B	97	24.1	1.7 (1.1 - 2.5)	4.8 (3.8 - 5.9)	1.2 (0.8 - 1.7)
C	105	26.1	2 (1.4 - 2.8)	4.4 (3.3 - 6.4)	1.3 (0.9 – 2.0)
D	107	26.6	1.6 (1.1 - 2.5)	4.1 (3 - 5.5)	0.9 (0.6 - 1.6)
E	77	19.1	1.8 (1.1 - 2.3)	4.8 (3.2 - 6.7)	0.9 (0.6 - 1.6)
Maternal Education (missing = 2)					
≤High School	37	9.2	1.5 (1.1 - 1.9)*	3.8 (2.8 - 5.1)	1.1 (0.8 - 1.8)
Some College	23	5.7	1.6 (0.9 – 2.0)	4.0 (2.9 - 5.2)	1.0 (0.7 - 1.7)
College/ Trade School Diploma	99	24.7	1.9 (1.4 - 2.8)	4.8 (3.3 - 6.7)	1.3 (0.8 - 2.0)
University Degree	242	60.4	1.7 (1.1 - 2.6)	4.5 (3.4 - 6.1)	1.0 (0.7 - 1.6)
Household Income (CAD) (missing = 9)					
< = 50,000	82	20.8	1.6 (1.3 - 2.3)*	4.4 (3.0 - 6.1)	1.2 (0.7 - 1.9)
50,001-100,000	192	48.7	1.6 (1.0 - 2.4)	4.4 (3.4 - 5.9)	1.1 (0.7 - 1.8)
≥100,000	120	30.5	2.0 (1.2 - 2.7)	4.8 (3.4 - 6.7)	1.0 (0.7 - 1.7)
Parity					
Nulliparous	116	28.8	2.5 (1.8 - 3.6)*	5.3 (4.1 - 7.7)*	1.5 (0.9 - 2.3)*
Multiparous	287	71.2	1.4 (1.0 - 2.1)	4.2 (2.9 - 5.7)	1.0 (0.7 - 1.6)
Mother's Country of Birth					
Canada	347	86.1	1.8 (1.2 - 2.5)	4.5 (3.3 – 6.0)	1.1 (0.7 - 1.8)*
Other	56	13.9	1.5 (1.1 – 3.0)	4.3 (2.8 - 6.6)	0.9 (0.5 - 1.6)
Mother's Population Group					
White	369	91.6	1.8 (1.2 - 2.5)*	4.6 (3.4 - 6.3)*	1.1 (0.7 - 1.8)
Other	34	8.4	1.3 (1.0 - 1.7)	3.6 (2.4 - 4.8)	1.0 (0.5 - 1.5)
Mother's Smoking Status (Active) (missing = 24)					
Never	260	68.6	1.6 (1.1 - 2.5)	4.4 (3.3 - 6.1)	1.0 (0.7 - 1.6)
Former	95	25.1	1.9 (1.3 - 2.5)	4.8 (3.3 - 6.2)	1.2 (0.7 - 1.9)
Current (quit during pregnancy, occasional or daily)	24	6.3	1.9 (1.2 - 3.1)	4.8 (3.3 - 6.5)	1.5 (0.8 – 3.0)
Mother's Exposure to Environmental Tobacco Smoke during Pregnancy (Passive) (missing = 9)					
No	148	37.6	1.7 (1.1 - 2.5)	4.5 (3.0 - 6.0)	1.1 (0.7 - 1.7)
Yes	246	62.4	1.7 (1.2 - 2.5)	4.5 (3.4 - 6.3)	1.1 (0.7 - 1.8)
Pre-pregnancy Body Mass Index (kg/m^2) (missing = 37)					
< 25.00	220	60.1	1.6 (1.2 - 2.2)	4.4 (3.3 - 5.8)	1.0 (0.7 - 1.6)
25.00-29.99	77	21.0	2.0 (1.2 - 2.8)	4.8 (3.5 - 6.4)	1.1 (0.8 - 1.7)
≥30	69	18.9	1.6 (1.1 - 2.6)	4.4 (3.0 - 6.1)	1.1 (0.7 - 1.7)
Season of Plasma Collection					
Spring	102	25.3	1.9 (1.1 - 2.7)	4.8 (3.5 - 6.4)	1.3 (0.7 - 2.3)
Summer	89	22.1	1.7 (1.1 - 2.6)	4.4 (3.3 - 6.0)	1.1 (0.7 - 1.7)
Fall	135	33.5	1.9 (1.2 - 2.9)	4.8 (3.4 - 6.5)	1.3 (0.8 - 1.9)
Winter	77	19.1	1.6 (1.2 - 2.2)	4.4 (3.2 - 6.0)	1.1 (0.7 - 1.7)
Sex of Baby					
Female	205	50.9	1.8 (1.2 - 2.5)	4.5 (3.5 - 6.2)	1.2 (0.7 - 2.0)
Male	198	49.1	1.7 (1.1 - 2.5)	4.4 (3.0 - 6.1)	1.1 (0.7 - 1.7)

	Range	Mean (S.D.)	PFOA %Change (95%CI)	PFOS %Change (95%CI)	PFHxS %Change (95%CI)
Maternal Age (years)	[17.0, 42.0]	31.31 (4.78)	−1.0 (-2.2, 0.3)	0.1 (-0.1, 1.2)	−2.6 (-4.3, -1.0)**
Gestational Age at Birth (weeks)	[33.4, 42.0]	39.53 (1.41)	−0.1 (-4.4, 4.3)	0.01 (-3.7, 3.8)	−3.1 (-8.6, 2.7)
Gestational Age at Blood Collection (weeks)	[6.1, 14.5]	12.02 (1.53)	2.5 (-1.5, 6.7)	1.1 (-2.3, 4.7)	−0.4 (-5.7, 5.0)
Infant Age at Exam (days)	[0.0, 44.0]	3.41 (4.57)	2.1 (0.8, 3.5)**	2.1 (1.0, 3.3)**	2.2 (0.4, 4.0)**
Adjusted Infant age at exam (Gestational Age at Birth + Age at Exam) (weeks)	[36.1, 43.3]	40.01 (1.39)	3.2 (-1.2, 7.8)	3.4 (-0.5, 7.3)	0.2 (-5.6, 6.3)
Infant Weight at Exam (kg), (missing = 60)	[1.9, 4.6]	3.28 (0.47)	−5.9 (-18.4, 8.6)	−3.8 (-14.9, 8.7)	−17.8 (-32.1, -0.5)**
Infant Length at Exam (cm), (missing = 21)	[43.1, 59.1]	50.46 (2.48)	0.3 (-2.1, 2.7)	1.3 (-0.8, 3.4)	−0.5 (-3.8, 2.9)
Weight-for-Length Z-score[1], (missing = 84)	[-5.2, 2.8]	−0.48 (1.24)	−3.0 (-8, 2.3)	−3.8 (-8.0, 0.6)	−7.7 (-14.3, -0.5)**
Weight-for-Age Z-score[1], (missing = 60)	[-3.7, 2.5]	−0.08 (1.02)	−4.8 (-10.9, 1.7)	−4.1 (-9.4, 1.5)	−10.6 (-18.2, -2.4)**

[1] World Health Organization, Child Growth Standards. WHO Anthro (version 3.2.2, January 2011) and macros. http://www.who.int/childgrowth/software/en/.
*p < 0.05 Kruskal Wallis to test for differences in PFASs between categorical variables.
**p < 0.05 Linear Regression to test for change in PFASs according to continuous variables.

by the Organisation for Economic Co-operation and Development as an indicator of developmental toxicity in rodents [8]. A shortened AGD is a marker of anti-androgenic effects in males while a longer AGD is a marker of androgenic effects in females. Although the evidence suggests that exposure to endocrine disrupting chemicals is pervasive in Canada (https://www.canada.ca/en/health-canada/services/environmental-workplace-health/environmental-contaminants/human-biomonitoring-environmental-chemicals.html#a1) and the US (https://www.cdc.gov/exposurereport/index.html), their role in changes in infant AGD remains unclear. Of the few human studies of infant AGD, most have focused on maternal urinary concentrations of phthalate metabolites. While our previous analysis reported no association between maternal diethylhexyl phthalate (DEHP) urinary concentrations and shorter AGD in male infants [9], an earlier meta-analysis of 5 studies published up to mid-2016 reported DEHP metabolites were associated with decreased AGD in boys [10]. Studies which have examined associations between other non-persistent or persistent chemicals and AGD are limited, with many suffering from methodological challenges [11].

Only a few epidemiologic studies have examined the association between prenatal exposure to PFASs and AGD and results have been

Table 2
Association between anogenital distances in newborns and perfluoroalkyl substances (ln transformed and quartiles) in 1 st trimester maternal plasma, expressed as a beta-coefficient (change in mm) with 95% CI from an adjusted linear regression model.

PFAS (μg/L)	ACD (mm) [1]		AFD (mm) [2]		APD (mm) [3]		ASD (mm) [4]	
	Beta	95% CI	Beta	95% CI	Bata	95% CI	Beta	95% CI
PFOA								
ln PFOA (continuous)	0.78	(-0.25, 1.82)	0.06	(-1.20, 1.32)	0.1	(-0.94, 1.14)	1.36*	(0.30, 2.41)
Q1 (0.05 - 1.10)	Ref		Ref		Ref		Ref	
Q2 (1.11 - 1.70)	0.88	(-0.79, 2.54)	−0.69	(-2.66, 1.28)	−0.76	(-2.65, 1.12)	0.23	(-1.67, 2.13)
Q3 (1.71 - 2.50)	0.48	(-1.22, 2.17)	0.73	(-1.27, 2.74)	−0.02	(-1.91, 1.88)	−0.43	(-2.34, 1.47)
Q4 (2.51 - 11.00)	1.06	(-0.65, 2.76)	−0.56	(-2.60, 1.48)	−0.51	(-2.50, 1.48)	1.77	(-0.23, 3.77)
p-value for trend [5]	0.3153		0.9388		0.8072		0.1484	
PFOS								
ln PFOS (continuous)	0.07	(-1.03, 1.18)	−0.29	(-1.62, 1.04)	0.13	(-1.13, 1.38)	1.05	(-0.24, 2.35)
Q1 (0.15 - 3.30)	Ref		Ref		Ref		Ref	
Q2 (3.31 - 4.50)	−0.06	(-1.70, 1.58)	−0.12	(-2.09, 1.85)	−0.97	(-2.81, 0.87)	−0.87	(-2.78, 1.04)
Q3 (4.51 - 6.10)	0.17	(-1.50, 1.85)	0.89	(-1.12, 2.90)	−1.28	(-3.22, 0.66)	0.33	(-1.67, 2.33)
Q4 (6.11 - 19.00)	−0.05	(-1.68, 1.57)	−0.33	(-2.31, 1.65)	0.22	(-1.68, 2.13)	0.49	(-1.47, 2.46)
p-value for trend [5]	0.977		0.9907		0.9077		0.3936	
PFHxS								
ln PFHxS (continuous)	0.3	(-0.47, 1.07)	0.14	(-0.79, 1.07)	0.24	(-0.52, 1.01)	0.22	(-0.54, 0.98)
Q1 (0.10 - 0.73)	Ref		Ref		Ref		Ref	
Q2 (0.74 - 1.10)	1.01	(-0.56, 2.59)	1.23	(-0.66, 3.13)	−0.91	(-2.74, 0.91)	−0.08	(-1.99, 1.83)
Q3 (1.11 - 1.80)	0.31	(-1.40, 2.02)	−0.51	(-2.56, 1.54)	0.64	(-1.23, 2.51)	0.13	(-1.80, 2.06)
Q4 (1.81 - 40.00)	0.92	(-0.94, 2.79)	0.52	(-1.71, 2.75)	0.57	(-1.30, 2.44)	0.57	(-1.33, 2.46)
p-value for trend [5]	0.4827		0.9597		0.2786		0.5313	

[1] Models adjusted for: recruitment site, education, gestational age, weight-for-length Z-score.
[2] Models adjusted for: recruitment site, weight-for-length Z-score.
[3] Models adjusted for: recruitment site, education, active smoking status, gestational age.
[4] Models adjusted for: household income, active smoking status, gestational age.
[5] p-value for trend across quartiles using linear regression.
* $p < 0.05$.
ACD: anoclitoris distance; AFD: anofourchette distance; APD: anopenile distance; ASD: anoscrotal distance.

inconsistent. A Danish pregnancy study observed that women who had higher serum PFOS concentrations had female infants with shorter AGD, while there was no association with male AGD [12]. A study of male infants from China reported that maternal plasma PFOS was associated with shorter anoscrotal distance at birth [13].

We postulate that developmental exposure to perfluorinated compounds could adversely impact androgen-dependent development of the reproductive tract and thus, could adversely affect anogenital distance (AGD) in newborns. The objectives of the current study were to examine associations between prenatal exposure to PFOS, PFOA and PFHxS and anogenital distances in male and female neonates in a Canadian cohort exposed to background levels of these chemicals.

2. Materials and methods

2.1. Study population

The population for this study came from the prospective pregnancy cohort study Maternal-Infant Research on Environmental Chemicals (MIREC) [14]. Pregnant women were recruited in 2008–2011 from multiple sites across Canada during the 1st trimester of pregnancy and followed through delivery. Plasma samples were collected at recruitment, questionnaires were administered at each trimester to collect information on lifestyle and socio-economic status and clinical information was abstracted from medical records. Gestational age was derived using both the woman's last menstrual period (LMP) and ultrasound dating. If early ultrasound and LMP dates differed by ≤ 7 days, gestational age estimate was based on LMP date; if > 7 days, early ultrasound was used to estimate gestational age.

As there were long delays in obtaining ethics approval at some of the recruitment sites and insufficient funds and period of funding to recruit from the entire cohort, the study population (called MIREC-ID) was limited to five of the 10 sites. As one of the objectives of MIREC-ID was to study infant behavior and development, the study population was restricted to singleton infants without any major congenital birth defects or neurological disorders.

The study was reviewed and approved by the Health Canada Research Ethics Board and ethics committees at each recruitment site. All women provided informed consent.

2.2. Infant anthropometry and anogenital distances

Shortly after birth (mean 3.5 days), anogenital distances were measured in male and female infants along with their weight and length. Weight and length were measured during the physical exam using an infant scale (seca 727®) and infantometer (seca 416®) (seca, Hamburg Germany). Initially, two measures for weight and length were obtained. If the two measures for weight differed by greater than 5 g, then a third measurement was taken. Similarly, if the two measures for length differed by greater than 3 mm, then a third measurement was taken.

In females, the distance (in mm) from the center of the anus to the posterior convergence of the fourchette (anofourchette distance or AFD) or the base of the clitoris (anoclitoris distance or ACD) was measured using metric dial Vernier calipers. Similarly, for males, the distances (in mm) between the base of the scrotum (junction of the smooth perineal skin and the rugated skin of the scrotum) and the mid-anus (anoscrotal distance or ASD) and between the centers of the anus to the cephalad (superior) base of the penis (anopenile distance or APD) were measured. The caliper was properly calibrated and set to zero prior to each measurement. Two measurements were taken and reported; if there was a > 2 mm difference between the 2 measures of AGD, then a third measurement was taken.

For all metrics, the mean of the two measurements was calculated and used for this analysis; if a third measurement was taken, then the mean of the 2 closest measures was calculated and used in the analysis. All measurements were taken by trained study examiners, who had no knowledge of maternal plasma PFAS levels.

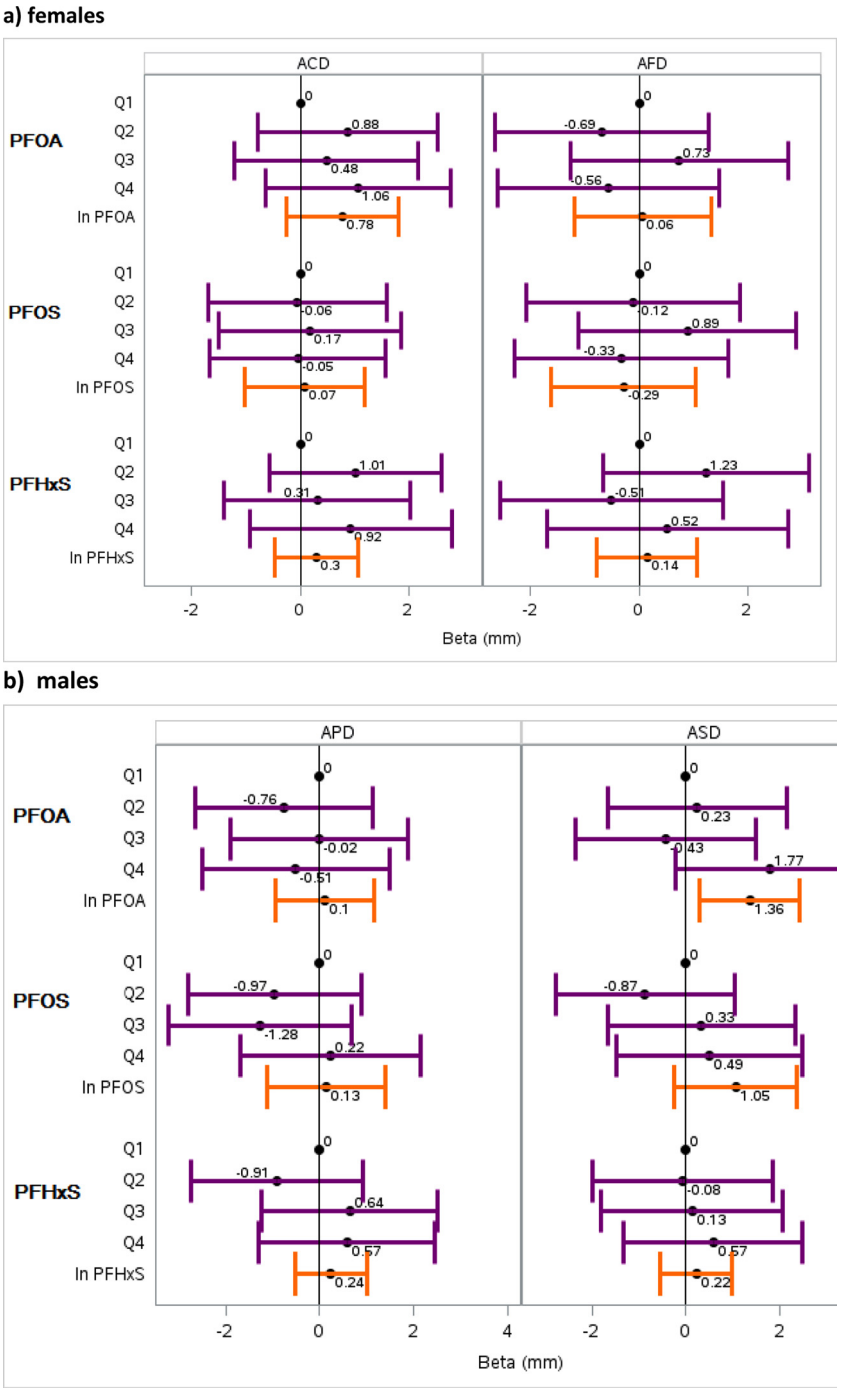

Fig. 1. Associations between newborn anogenital distances and ln-transformed or quartiles of perfluoroalkyl substances in 1 st trimester maternal plasma, expressed as a beta-coefficient (change in mm).
ACD: anoclitoris distance; AFD: anofourchette distance; APD: anopenile distance; ASD: anoscrotal distance.

2.3. Maternal plasma perfluoroalkyl substances

The three perfluoroalkyl substances were measured in first trimester plasma from 403 pregnant women as the hypothesized masculinization programming window is believed to be between 8 and 14 weeks' gestation. The analytes were extracted at alkaline pH with methyl tert-butyl ether and ion-pairing with tetrabutylammonium hydrogensulfate, evaporated to dryness and dissolved in the mobile phase at the Centre de toxicologie du Québec, Institut National de Santé Publique du Québec (INSPQ), Québec, Canada. They were analyzed by Waters Acquity UPLC-MS-MS operated in the MRM mode with an electrospray ion source in negative mode. The limit of detection (LOD) was 0.1, 0.3 and 0.2 (μg/L) for PFOA, PFOS and PFHxS, respectively. Any values below the limit of detection were substituted by one half of the LOD.

2.4. Covariates

The following maternal variables were considered as potential covariates: age (continuous), population group (White, other), country of birth (Canada, other), pre-pregnancy body mass index (BMI) (< 25, 25–29, ≥30), education (high school or less, some college, college/trade school, university degree), annual household income (≤50,000,

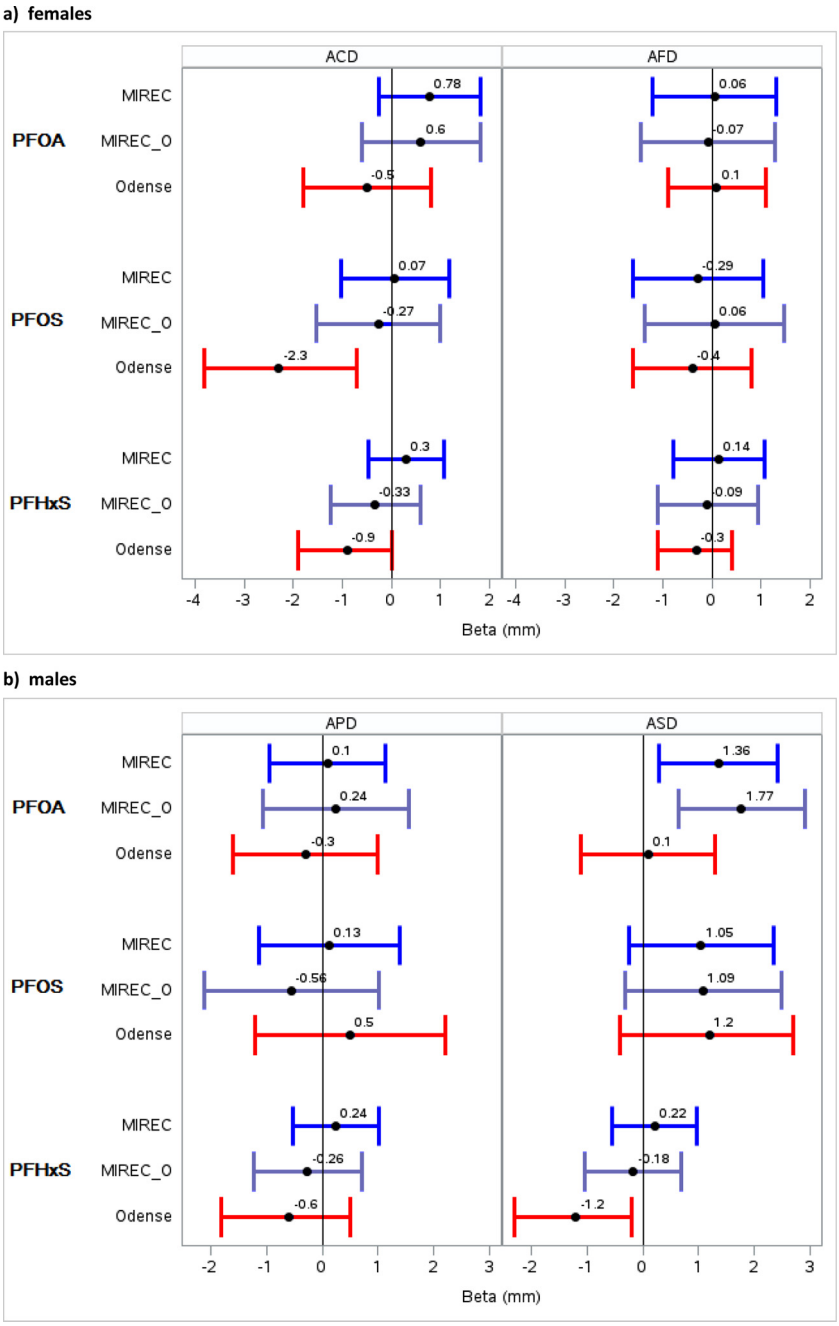

Fig. 2. Comparison between the MIREC and Odense [12] study results of the association between infant anogenital distances and ln-transformed Perfluoroalkyl Substances (PFASs) in 1 st trimester maternal plasma/serum, expressed as a beta-coefficient (change in mm).
MIREC: Results adjusted for significant covariates**; MIREC_O:** Results from the MIREC study adjusted for the same covariates as in the Odense study**; Odense:** Results from the Odense study.
ACD: anoclitoris distance; AFD: anofourchette distance; APD: anopenile distance; ASD: anoscrotal distance.

50,001–100,000, > 100,000 CAD), parity (nulliparous, multiparous), active exposure to tobacco smoke (never, former, current) and passive smoke exposure (yes, no). In addition, we considered gestational age at plasma collection, recruitment centre, gestational age at birth, adjusted gestational age at examination (sum of gestational age at birth (in days) and the age of the child at the AGD measurements (in days)), age, weight and length at exam, weight for age z-score, and lastly, a weight-for-length Z-score which was calculated based on the standards set out by the World Health Organization [15]. As transportation in cold temperatures before processing may decrease PFOS plasma concentrations by approximately 30% [16], we also considered season of blood collection. However, as our blood collections were done in the clinic and the protocol required that bloods needed to be processed immediately after the visit, with the tube processed within 1 h after collection and all the aliquots frozen within 2 h after collection, delays and transportation of unprocessed bloods are unlikely.

2.5. Statistical analysis

Descriptive statistics for each AGD measure and the PFAS analytes were calculated. To test whether there are differences in PFAS concentrations according to the potential covariates, the Kruskal Wallis test was used for the categorical variables and linear regression was used to test for an association with the continuous variables. Multiple linear

Table 3
Comparison of studies of prenatal PFAS concentrations and AGD in infants.

Study Population	No. of Participants	PFAS Concentrations	Age at AGD	Confounders Adjusted	Result		
					PFOS	PFHxS	PFOA
Odense Denmark[1]	316 boys, 231 girls	Median at 5-12 wks: PFOA 1.7 PFHxS 0.3 PFOS 8.1 μg/L	3.5 months	age at examination, weight for age z-score, pre-pregnancy BMI, parity, smoking.	↓ACD	↓ACD ↓ASD	NA
China[2]	550 boys	Median at 12-16 wks: PFOA 20.13 PFHxS 2.84 PFOS 10.70 μg/L	1-3 days	maternal age at delivery, gestational age, maternal education, parity, pre-pregnancy BMI, infant age at physical examination, birth weight	↓ASD	NA	NA
MIREC	204 boys, 195 girls	Median at < 15 wks: PFOA 1.7 PFHxS 1.1 PFOS 4.5 μg/L	Mean: 3.41 days	Varied by sex and AGD, but could include: recruitment site, education, gestational age, weight-for-length Z-score, active smoking status, and household income	NA	NA	↑ASD

[1] [12].
[2] [13].
NA: no association – confidence intervals of beta included 0; ACD: anoclitoris distance; AFD: anofourchette distance; APD: anopenile distance; ASD: anoscrotal distance.

regression models were created to examine the relationship between each AGD and plasma PFAS concentrations, including significant confounders as identified from the change-in estimate (CIE) procedure [17], where variables are selected based on relative or absolute changes in the estimated exposure effect (10%). Significant confounders were identified from the change-in estimate procedure and included in the multivariable linear models. For all models, the regression assumptions were examined using residual plots and were illustrated by plotting a normal probability curve and quantile-quantile plots. Adjusted models were also checked for collinearity using the variance inflation factors (VIFs) of each variable. As the PFAS chemicals were skewed to the right, they were natural log transformed before being included as a continuous variable in the linear models. The transformations led to more normally and evenly distributed residuals. In addition, the chemical concentrations were categorized into quartiles and tested for a linear trend using multiple linear regression models.

Separate models were created for each AGD to allow the data to drive the selection and for potential sex differences in the confounders to be identified, as the relationships between confounding factors and outcomes may differ by sex, and failure to account for these differences may result in false estimates of effect [18,19].

To compare our results with those from the Danish cohort [12], we also produced models adjusting for the same set of covariates (age at examination (gestational age at birth + age at examination), weight for age z-score, pre-pregnancy BMI, parity and smoking).

The CIE procedure was automated using augmented backward elimination for selecting confounders using R 3.2.4. The descriptive statistics and linear modelling was performed using SAS EG 5.1.

3. Results

The median anogenital distance in 204 males was longer (APD 43.7 mm) than that for 195 females (ACD 33.4 mm) (Table S1). The geometric mean concentrations of PFOA, PFOS and PFHxS were 1.71, 4.40 and 1.15 μg/L, respectively with high (> 97%) rates of detection (Table S2). A total of 403 mother-infant dyads had maternal plasma PFAS measurements and at least one infant AGD measure. The mean maternal age was 31.3 years with 60% of the women having a university degree, 14% born outside Canada, 30% with a household income over $100,000 CAD, 29% nulliparous and 6% currently smoking (Table 1). Demographics in the MIREC-ID population were similar to those in the entire cohort [9]. Women with less education or lower income had lower PFOA median concentrations compared to those in the highest category, while all three median PFAS levels tended to be higher among women who were born in Canada, were White or nulliparous. There were no differences in maternal PFAS concentrations by season of blood collection, sex of the infant, maternal smoking or pre-pregnancy BMI (Table 1). Metrics of infant size (e.g., weight-for-length z-score and weight-for-age z-score) were associated with significant changes in AGD (Table S3).

No consistent patterns of association were observed between maternal PFAS concentrations and female AGDs (Table 2, Fig. 1). Among male infants, prenatal PFOA examined as a continuous variable was associated with longer ASD (adjusted beta = 1.36; 95% CI 0.30, 2.41). Although the test for trend was not significant, maternal PFOA exposure in the highest quartile (2.51–11.00 μg/L) was associated with 1.77 (95% CI -0.23, 3.77) mm increase in ASD (Fig. 1).

Using the same set of covariates in the models as in the Danish study [12], did not substantially change our results (Fig. 2).

4. Discussion

In this prospective cohort study we found some evidence that maternal ln-transformed PFOA plasma concentration was associated with longer (masculinized) ASD in male infants; however, the test for trend by quartile was not significant (p = 0.15). Still, endocrine disrupting chemicals have been frequently associated with non-monotonic dose-response curves [20]. No consistent patterns were observed among female infants. Geometric mean PFAS concentrations in maternal plasma in MIREC were similar to those for females of reproductive age in the 2009–2011 Canadian population (PFOA 1.71 vs. 1.5; PFHxS 1.15 vs. 0.86; PFOS 4.40 vs. 4.4 μg/L, respectively) [2].

Only three previous epidemiologic studies have investigated associations between PFASs and anogenital distance, each with different findings (Table 3). In the Odense Denmark cohort, prenatal exposure to ln-transformed PFOS or PFHxS was associated with reduced ACD, while PFHxS was associated with reduced ASD [12]. While median PFOA concentrations were the same in both cohorts, PFOS was higher in Odense, while PFHxS was lower than in MIREC. Both MIREC and Odense studies reported a longer ASD associated with PFOS levels, but both had confidence intervals straddling the null value (Fig. 2). Neither study found evidence of an association between PFASs and AFDs.

In the largest study conducted to date, maternal plasma PFOS was associated with shorter ASD at birth [13]. No associations were observed between AGD and PFHxS or PFOA. However, the association between PFOS and shorter ASD was no longer apparent when AGDs were measured at 12 months of age in the same infants. Median maternal PFAS concentrations were substantially higher in this Chinese study compared to MIREC (Table 3).

Possible reasons for the disparity in results between the 3 pregnancy

cohort studies of PFASs and AGD include: differences in age of the infants at testing, the small effect sizes (1–2 mm measured per ln-logarithm PFAS) making it difficult to achieve good statistical power and increasing the likelihood of measurement errors, the concentrations and proportions of individual PFASs in maternal blood differing in some studies, residual or unmeasured confounding, differences in population genetics, and likely exposure to other chemical mixtures.

In a cross-sectional study in Italy of young adults, significantly shorter ASDs were observed among males from the exposed region compared to a control region [21]. However, when the analysis was restricted to the males with serum PFAS data, no significant differences in ASD were observed but PFASs in plasma and seminal fluid were positively correlated with circulating testosterone. The males from the exposed region also had lower mean testicular volume, shorter penile length, poorer semen quality and higher semen pH [21].

A few studies have examined prenatal PFAS exposure and associations with infant steroid hormones. A Danish study has reported that prenatal exposure to PFOA was associated with lower sperm count and higher levels of luteinizing and follicle stimulating hormone levels [22]. A Japanese study of 185 mother-infant pairs reported that maternal 2nd trimester serum concentrations of PFOS were positively associated with cord dehydroepiandrosterone (DHEA) levels while prenatal PFOA was negatively associated with DHEA; associations were stronger among boys than girls [23]. Furthermore, prenatal PFOS was associated with a decrease in the cord glucocorticoid/androgenic hormone ratio, indicating that PFOS may shift steroidogenesis to androgenic hormones [23]. Fetal programming by prenatal exposure to PFOS, PFOA or PFHxS has also been suggested by a small study which found these chemicals to be associated with higher testosterone levels in girls at age 15 [24].

An *in vitro* study, has observed that PFHxS, PFOS and PFOA significantly induced the estrogen receptor transactivity, whereas these same chemicals (and other PFASs) significantly antagonized the androgen receptor activity in a concentration dependent manner [4]. Only a few animal toxicology studies have been published which have examined PFASs exposure and effects on reproductive toxicity, with no consistent pattern emerging [6,25,26]. However, reported sex differences in the rate of elimination of PFOA [27] and PFHxS [28] in rats and species differences (rats and mice may be more effective at eliminating PFOS [29] or PFHxS [30] than monkeys) may make it difficult to extrapolate findings on PFASs from rodents to humans [28]. In humans, PFASs can be efficiently transported across the placenta [31,32] and have been measured in umbilical cord blood, albeit at lower levels than in maternal blood [33]. Higher placental transfer of PFOA and PFOS (as measured by maternal to cord blood ratio) have been reported in the female compared to the male fetus [34]. In another study, the placenta to maternal serum ratios of PFOS and PFOA were higher in pregnancies with male fetuses compared to female fetuses [35].

Under the testicular dysgenesis syndrome (TDS) hypothesis, “abnormal testis development (dysgenesis), which could have numerous primary causes, leads secondarily to hormonal or other malfunctions of the Leydig and/or Sertoli cells during male sexual differentiation, leading in turn to increased risk of the reproductive disorders” (at birth: cryptorchidism, hypospadias, shorter AGD, or in young adulthood: testicular germ cell cancer and low sperm count) [36]. Any disruption in testosterone production or action in fetal life could lead to the downstream TDS disorders such as shorter AGD in males. Exposure to endocrine disrupting chemicals has been linked with the increasing incidence of male reproductive disorders including poor semen quality, testicular malignancies and congenital developmental defects such as hypospadias and cryptorchidism [37].

The clinical significance of infant AGDs on longer term reproductive health in humans is still to be determined. Cross-sectional studies have suggested that longer ASD in adult men is associated with higher sperm concentration, total sperm count, and total motile sperm count [38,39], while men in the lower 10th percentile of ASD have a higher risk of being in the sub-fertile range for either sperm concentration or morphology compared to men with ASDs above the median [40]. A shorter AGD in women has been associated with increased risk for gynecological morbidities [41].

Jain and colleagues have suggested that AGD in humans, like animals, is fixed in early gestation (likely during the hypothesized masculinization programming window between 8 and 14 weeks) and is unaffected by androgen levels thereafter [42]. However, in a rat study, while in utero exposure to DEHP was associated with shorter AGD in male offspring, pubertal exposure was still associated with minor effects on AGD [43]. In addition, a prospective study of Chinese infants reported that the association observed between prenatal PFASs exposure and shorter AGD in males at birth, was not present when the infants were 12 months of age [13]. While only very few longitudinal studies have been conducted to determine whether AGDs at birth correlate with those in adulthood, the evidence so far does not support this hypothesis. One UK study that measured AGD in full-term males (n = 463) and females (n = 426) at birth and then at 3, 12, 18, and 24 months of age reported low correlations between AGDs at birth and subsequent measurements in boys (r = 0.30 to 0.15) and lower still for girls (0.26 to 0.07) [44]. A Danish study measured AGD in males (n = 407) and females (282) at 3 and 18 months of age and found the AGD z-score for each child was significantly correlated between the two examinations (intra-class correlation coefficients of 0.63 and 0.35 for ASD and APD, respectively; however, the correlation coefficients were lower for females (0.26 for AFD and 0.19 for ACD) [45].

Some of the strengths of this study are the collection of biomarkers of exposure during the critical window of development and prior to measurement of the outcome, and the multi-site study population. This research is limited by the sample size and associated reduced statistical power. In addition, as newborns lose about 6–7% of their body weight within the first week of life [46], measuring AGD shortly after birth may have resulted in some AGD measurement errors, which can be difficult to quantify. Unmeasured confounding and concomitant exposure to other potential endocrine disrupting chemicals, including other PFASs may also be factors. Our results may not be generalizable to other populations, given MIREC participants tended to be older, more educated, more likely to be born in Canada, married and less likely to be a current smoker than the Canadian population giving birth during the same time period [14].

In summary, this study found no clear evidence that maternal plasma concentrations of PFOS, PFOA or PFHxS were associated with shorter infant anogenital distance in males or any change in AGD in females. Whether the positive association observed between longer ASD and PFOA is real or would have any long-lasting effect on the reproductive health of males is unknown and needs to be investigated further. Given the likelihood of measurement error using current methods, future studies should consider using new technologies allowing more precise measurement of AGD in infants.

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.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.reprotox.2020.03.011.

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