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A Faroese cohort study

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Abstract – 249 words

Exposure to perfluoroalkyl substances (PFASs) has in some studies been associated with reduced anogenital distance (AGD) in newborns as a sensitive indicator of prenatal anti-androgenic exposure. The aim of this study was to investigate the association between maternal PFAS exposure and offspring AGD in a population with wide ranges of PFAS exposures.

Participants were recruited in the Faroe Islands in 2007-2009, and information on AGD and PFAS exposure was obtained from 463 mother-infant pairs. Perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) were measured in maternal pregnancy serum. Data were analyzed using multiple linear regression analysis adjusted for birth weight, child age at examination, parity, and maternal education level.

Among boys, higher maternal serum concentrations of PFOA, PFOS, PFNA and PFDA were significantly associated with a longer AGD, both with the exposure entered as a continuous variable and as quartiles. Boys in the highest quartile of PFOA, PFOS, PFNA and PFDA exposure had an increase in AGD of 1.2 mm (95% CI 0.1;2.2), 1.3 mm (95% CI 0.3;2.3), 1.0 mm (95%, CI 0.0:2.0) and 1.3 mm (95%, CI 0.3;2.4), respectively, when compared to boys in the lowest quartile of exposure ($p < 0.05$). No significant association was found between male AGD and PFHxS. No association was found for girls.

In conclusion, elevated maternal exposure to major PFASs was significantly associated with a longer AGD in boys. No significant associations were found among girls, thus suggesting a sex-dimorphic effect of PFAS exposure.

Keywords: Perfluorinated compounds; PFAS; Prenatal exposure; Anogenital Distance; Faroe Islands

1. Introduction

Perfluoroalkyl substances (PFASs) are a group of highly persistent synthetically manufactured chemicals used in fabrics and food packaging due to water-, stain-, and grease-resistant properties [1]. Human exposure to PFASs occurs through ingestion of contaminated food and drinking water, inhalation of indoor air and contact with other contaminated media [1-3]. Two of the previously most commonly used PFASs, erfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have been phased out by some of the major manufacturers [2]. Nonetheless, other PFAS, such as perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA), are still in production, and these PFASs have been studied to a lesser extent. PFASs cross the placental barrier and have been detected in human amniotic fluid and umbilical cord blood [3].

Among other important adverse effects, PFASs have potential endocrine-disrupting effects through affecting androgen receptor and estrogen receptor (ER) activity or altering the expression of estrogen-responsive genes [4, 5] leading to hormonal imbalance, and may thus play a central role in declining male reproductive health [6]. PFAS exposure has been associated with fetal growth, and a recent review found that most studies report that maternal exposure to PFOA and PFOS was associated with low birth weight [7, 8] but the mechanisms behind the influence of PFASs on fetal growth are largely unknown. Animal studies have reported that exposure to PFASs could impair the reproductive system during the developmental stage in male mice through an antiandrogen pathway[9, 10] and influence the expression of estrogen-responsive genes in animal studies[11], and PFAS-induced changes in sex hormone biosynthesis have been reported in vitro[12].

Anogenital distance (AGD), the distance from the anus to the genitalia, is routinely used in animal toxicology studies and is sensitive to anti-androgenic exposure. In rodents, AGD is a sensitive biomarker of androgen exposure during a critical embryonic window of testis development[13] and has been shown to reflect the amount of androgen to which a male fetus is exposed in early development where higher *in utero* androgen exposure results in a longer AGD [6, 14]. Two animal studies have found

that exposure to PFASs was associated with shorter AGD in male rat fetuses and wild male minks[15, 16]. The suggested mechanisms by which PFASs affects AGD is by increasing the ratio between estrogens and androgens through transcriptional induction of the aromatase enzyme while also being a direct agonist to the estrogen receptor (ER) [17-19]. Some of these findings have been reproduced in humans, e.g., in regard to prenatal exposure to phthalates, which are anti-androgenic, and which have been found to reduce AGD in boys [20]. Still, the potential effect on AGD is still poorly understood.

Three birth cohort studies have examined the association between maternal PFAS exposure and AGD in the offspring, the results have been somewhat unclear. A Danish study found higher PFOS, PFHxS, PFNA and PFDA concentrations to be associated with a shorter AGD in 3 month old girls, but a longer AGD in boys, suggesting a sex-dimorphic effect [21]. A Chinese study found that high maternal PFOS and PFDA exposure was associated with a shorter AGD in boys aged 1-3 days [22]. Lastly, a Canadian study found an association between high PFOA and longer AGD in boys but not in girls [23]. Thus, the limited evidence available shows somewhat unclear results and more studies are needed. We hypothesize that maternal exposure to PFASs has impact on the development of the reproductive tract and AGD in the offspring. Thus, the aim of this study was to investigate the association between maternal PFAS exposure and AGD in infants in a Faroese birth cohort, where ranges of PFAS exposure are wide.

2. Methods

2.1 Study population and data collection

The study is based on data from the Faroese Birth Cohort 5, a population-based prospective cohort of 490 children, recruited between October 2007 and April 2009 with a participation rate of 73% [24-26]. Obstetric information was obtained from midwife records and included maternal age, parity, pre-pregnancy weight, height, education level, alcohol and smoking during pregnancy, partner smoking during pregnancy, and birth weight.

Two weeks after expected term date, a pediatric examination was performed during which AGD were measured. The AGD was measured with Vernier calipers according to a standardized method described by Salazar-Martinez [27]. The infants were laid on their back with hips flexed and light pressure was placed on the thighs until the hand of the examiner touched the subject's abdomen.. In boys, AGD was determined as the distance from the center of the anus to the bottom of the scrotum (AGD_{AS}) and, in girls, as the distance from the center of the anus to the posterior convergence of the fourchette (AGD_{AF}). Measurement was repeated three times and a mean was calculated [27, 28]. All measurements were performed by the same trained pediatrician. During the examination, i.e., two weeks after expected term date, a sample of maternal blood was obtained for PFAS exposure assessment.

All protocols were approved by the Scientific Ethical Committee of the Faroe Islands and the institutional review board at Harvard T.H. Chan School of Public Health. Written informed consent was obtained from all mothers.

Table 1: Maternal characteristics (n=463) according to mean AGD (standard deviation) stratified by sex (n=232 males; n=231 females)

	N (%)	Males n=232		Females n=231	
		%	AGD_{AS} mean (SD)	%	AGD_{AF} mean (SD)
All children	463		25.1(3.1)		13.4(2.1)
Maternal age (years)					
<25	84 (18)	15	24.9 (2.8)	21	12.7 (1.8)*
25-29.9	135 (29)	30	25.5 (2.8)	28	13.4 (1.8)
30-35	175 (38)	38	25.0 (3.1)	37	13.2 (2.4)
>35	69 (15)	16	25.1 (3.5)	13	13.2 (1.8)
Parity					
0	141 (31)	25	24.9 (2.9)	36	13.2 (1.8)
1	160 (34)	37	25.0 (3.1)	33	13.7 (2.6)
2	102 (22)	26	25.5 (2.7)	18	13.0 (1.7)
3+	59 (13)	12	25.6 (3.7)	13	13.6 (1.7)
Maternal pre-pregnancy BMI					
<20	56 (12)	12	24.7 (2.8)	12	13.8 (2.1)
20-25	248 (54)	52	24.9 (3.2)	55	13.3 (1.8)
>25	159 (34)	36	25.7 (2.9)	33	13.5(2.5)
Gestational age at birth (weeks)					
<38	31 (7)	7	25.4 (4.5)	7	13.7 (1.3)*
38-40	302 (65)	58	25.2 (2.9)	72	13.2 (1.9)
>40	130 (28)	35	24.9 (3.0)	21	14.1 (2.6)
Birth weight (g)					
<2500	5 (1)	1	21.0 (.)*	2	14.3 (1.0)*
2500-4500	430(93)	92	25.1 (3.0)	93	13.3 (1.8)
>4500	28 (6)	7	26.7 (2.4)	5	15.0 (4.7)
Weight at examination (g)					
<3500	60 (13)	11	23.2 (2.9)*	15	12.4 (1.4)*
3500-4500	300 (65)	60	24.7 (2.7)	69	13.5 (1.8)
>4500	103 (22)	29	26.7 (3.0)	16	14.1 (3.1)
Age at examination (days)					
<14	100 (22)	20	24.3 (2.3)*	23	12.9 (1.5)
14-23	255 (55)	55	25.2 (3.2)	55	13.4 (2.3)
>23	108 (23)	25	25.8 (3.0)	22	13.7 (1.9)
Maternal education level					
Below high school	70 (16)	14	24.7 (2.6)	18	13.2 (2.1)
High school or above	378 (84)	86	25.2 (3.1)	82	13.4 (2.1)
Maternal alcohol use during pregnancy					
Yes	22 (5)	5	25.7 (3.4)	4	12.8 (1.3)
No	441 (95)	95	25.1 (3.0)	96	13.4 (2.1)
Maternal smoking during pregnancy					
Yes	73 (16)	15	25.2 (2.8)	17	13.4 (2.2)
No	389 (84)	85	25.2 (3.1)	83	13.4 (2.1)
Partner smoking during pregnancy					
Yes	152 (34)	33	25.0 (3.4)	34	13.3 (2.0)
No	299 (66)	67	25.3 (2.8)	66	13.5 (2.2)

Note: : AGD_{AF}, anofourchette distance; AGD_{AS}, anoscrotal distance; ; SD, standard deviation
Missing values: parity: 1, maternal pre-pregnancy BMI: 1, maternal education level: 15, smoking during pregnancy: 1, partner smoking during pregnancy: 12
*, marks a p-value <0.05 with one-way ANOVA test

2.2

Exposure assessment

Maternal serum concentrations of PFASs (PFOA, PFOS, PFHxS, PFNA and PFDA) were measured by online solid-phase extraction followed by high-pressure liquid chromatography with tandem mass spectrometry [29, 30]. To ensure accuracy and reliability of data, each analytical series included quality control serum samples, calibration standards, as well as reagent and serum blanks. Imprecision within and between batches was less than 3.0 % and 5.2 %, respectively [31].

2.3 Statistics

Data were presented as counts and percentages for categorical variables, mean and standard deviation (SD) or medians and interquartile range (IQR) for continuous variables. Pearson's correlation coefficient was used to evaluate correlation between the five measured PFASs. Data was assessed for normality by visual assessment using histograms and normal probability plots. One-way ANOVA-test was used for normally distributed covariates to test for differences in AGD in relation to the following covariates one at a time: maternal age, parity, maternal BMI, gestational age, birth weight, maternal education level, maternal alcohol use during pregnancy, maternal smoking during pregnancy, partner smoking during pregnancy, weight and age at examination adjusted to term-date. Differences in PFAS concentrations between the same covariates were tested with Kruskal-Wallis' test for skewed distributions. PFAS distributions were skewed and divided into quartiles, while also entered as a continuous variable after logarithmic transformation to approach normality.

Stepwise multiple linear regression was used to examine the associations between PFASs and AGD. Confounders included were factors known a priori to be important predictors of birth outcomes or AGD [28, 29]. Weight at examination and infant's age at examination were included in all analyses. The remaining covariates were gestational age (days), parity (0, 1, 2 and 3+), maternal smoking during pregnancy (yes/no), partner smoking during pregnancy (yes/no), maternal alcohol use during pregnancy (yes/no), maternal pre-pregnancy BMI (<20, 20–25, 25+ kg/m2), maternal education level (below high

school/high school or above). The only covariates that changed the beta estimate by at least 10 % were parity and maternal education level, and both were therefore included in the final regression model. Associations were reported in terms of beta estimates with 95% confidence intervals (95% CI), and statistical significance was reached at a p-value of <0.05. All data analyses were performed using STATA/IC 16®.

3. Results

Of the cohort 490 mother-child pairs, a total of 463 pairs (94.4 % of the original cohort) had information on both PFAS exposure and AGD available and were thus included in the analyses, i.e. 27 mother-child pairs were excluded because of missing data. Mean maternal age at examination was 29.8 years and mean maternal pre-pregnancy BMI was 24.3kg/m^2. Most women were multiparous (69 %) and of Scandinavian origin (97 %). A total of 73 women (16 %) reported smoking and 22 (5 %) reported alcohol consumption during pregnancy. The means of birth weight and AGD were 3787 g and 25.1 mm for boys and 3612 g and 13.4 mm for girls, respectively. For both sexes, birth weight, weight at examination, and age at examination were significantly associated with the AGD while maternal age, gestational age at birth, and maternal education level were significantly associated with AGD only among the girls (Table 1).

All measured PFASs were found in quantifiable concentrations in serum from the 463 women (Supplementary table). PFOS had the highest average concentrations (8.3 µg/L), followed by PFOA (1.4 µg/L), PFNA (0.7 µg/L), PFDA (0.3 µg/L) and PFHxS (0.2 µg/L) and they were all mutually correlated (Pearson's correlation coefficient r between 0.40 and 0.85, p<0.001). Older women had significantly lower concentrations of all five PFASs while women with higher parity had significantly lower concentrations of PFOA, PFOS and PFHxS. Women with elevated PFOA exposure were more often smokers and gave birth to children of lower birth weight and a lower weight at examination (Supplementary table). Significant association was observed between exposure to all PFASs and birth weight (data not shown).

In multiple linear regressions, after adjusting for weight and age at examination (adjusted to term date), parity and maternal education, higher maternal exposure to PFOA, PFOS, PFNA and PFDA was significantly associated with a longer AGD_{AS} in boys, both with PFASs entered as continuous variable and as quartiles (Table 2). Boys in the highest quartile of exposure to PFOA, PFOS, PFNA and PFDA had an AGD_{AS} increase of 1.2 mm (95 % CI 0.1;2.2), 1.3 mm (95% CI 0.3;2.3), 1.0 (0.0:2.0) and 1.3 mm (95% CI 0.3;2.4), respectively. No association was seen for PFHxS. No associations were found between maternal PFAS exposure and AGD_{AF} in girls (Table 2).

Table 2: Linear regression analysis (β-coefficient and 95 % confidence intervals) on maternal pregnancy PFASs in quartiles, continuous (transformed by the use of natural logarithm) and AGD_{AS} and AGD_{AF}.

PFASs (ng/mL)	AGD_{AS} (mm) 232 males		AGD_{AF} (mm) 231 females	
	β	95 % CI	**β**	95 % CI
PFOA				
1st	Reference		Reference	
2nd	0.2	-0.8;1.1	0.8	-0.1;1.6
3rd	1.2*	0.1;2.2	0.3	-0.6;1.2
4th	1.1	0.0;2.3	0.2	-0.7;1.1
p-trend	0.02		0.85	
Continuous **	0.8*	0.1;1.5	0.0	-0.6;0.6
PFOS				
1st	Reference		Reference	
2nd	1.3*	0.3;2.4	0.1	-0.6;0.9
3rd	1.3*	0.2;2.3	0.4	-0.5;1.1
4th	1.3*	0.3;2.3	0.0	0.8;0.9
p-trend	0.02		0.81	
Continuous**	1.0*	0.1;1.8	0.1	-0.6;0.7
PFHxS				
1st	Reference		Reference	
2nd	-0.6	-1.6;0.4	0.3	-1.1;0.5
3rd	0.1	-0.9;1.2	0.00	-0.8:0.8
4th	0.5	-0.6;1.6	-0.5	-1.3;0.3
p-trend	0.18		0.39	
Continuous**	0.2	-0.3;0.7	-0.1	-0.4;0.3
PFNA				
1st	Reference		Reference	
2nd	0.2	-0.8;1.3	0.2	-0.7;1.0
3rd	1.0*	0.0;2.0	0.1	-0.8;0.9
4th	0.9	-0.2;1.9	-0.3	-1.1;0.4
p-trend	0.03		0.3	
Continuous**	1,1*	0.1;2.0	-0.1	-0.8;0.5
PFDA				
1st	Reference		Reference	
2nd	1.4*	0.4;2.5	0.3	-0.5;1.1
3rd	1.0*	0.0;2.1	-0.1	-0.9;0.7
4th	1.3*	0.3;2.4	-0.2	-1.0;0.6
p-trend	0.03		0.46	
Continuous**	0.8*	0.0;1,6	-0.3	-0.9;0.4

Note: Linear regression models are adjusted for parity, child age and weight at examination and mothers' level of education.
AGD_{AF}, anofourchette distance; AGD_{AS}, anoscrotal distance;;distance; PFASs, perfluoroalkyl substances Perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA).
Missing values: parity: 1; maternal education level: 15
*, marks a p-value <0.05
**, logarithmically transformed

197

198

199 **4. Discussion**

200 In this birth cohort study, maternal PFAS exposure was significantly associated with a longer AGD_{AS} in boys
201 aged 14 days after term, whereas no association was found in girls.

202 To date, only three epidemiological studies have investigated the association between
203 prenatal PFAS exposure and AGD, although showing somewhat uneven results (Table 3). Our findings of a
204 longer AGD in boys exposed to PFAS is in agreement with a study from the Canadian Maternal-Infant
205 Research on Environmental Chemicals Cohort (MIREC) that investigated the association between AGD_{AS},
206 anoscrotal distance, AGD_{AP}, anopenile distance, AGD_{AC}, anoclitoral distance, and $AGD_{AF,}$ anofourchette
207 distance, and PFOA, PFOS, and PFHxS in 403 children [23]. They found that elevated PFOA was significantly
208 associated to a longer $AGD_{AS,}$ as was PFOS, albeit not significantly so. No association was observed with
209 AGD_{AS} or among girls and PFASs. The Canadian study population resembled ours regarding maternal age
210 and parity but had a higher level of education and with children at a lower average birth weight (Table 3).
211 The PFOA concentration was similar, but the Canadian study measured PFAS in first trimester as opposed
212 to a median of 19 days post-partum in our cohort. Interestingly, a Danish study (n=547) from the Odense
213 Child Cohort (OCC) [21] also found increased AGD_{AS} in boys exposed to PFOS, PFNA and PFDA. Further, they
214 reported a significant association between higher PFOS, PFHxS, PFNA and PFDA exposure and decreased
215 AGD_{AC} in girls (Table 3). The Faroese and Danish studies are comparable regarding maternal characteristics
216 and serum-PFAS concentrations although they measured PFAS in first trimester. The third study from the
217 Shanghai-Minhang Birth Cohort, included 439 boys [22] and found significant associations between higher
218 exposure to PFOS and PFDA and shorter AGD_{AS} , though not AGD_{AP} 1-3 days post-partum, i.e., findings

219 inconsistent with our results. However, the PFAS exposure in the Chinese boys was much higher compared
220 with our study (Table 3), and 84 % of the Chinese mothers were nulliparous which may have affected the
221 differences in results. Besides the three studies above, to our knowledge, only two animal studies have
222 investigated the association between PFAS exposure and AGD and with consistent associations. A reduction
223 AGD in male rat fetuses after exposure to PFOS was observed[15] and PFOS, PFDA, PFUA, and total PFASs
224 were related to shorter AGD in wild male minks[16]. Thus, our results are inconsistent with the findings in
225 animals.
226 In addition to AGD, a number of studies, both human and animal, have investigated the
227 effects of PFAAs on reproductivity and development[32]. In humans increases in exposure to PFOA and
228 PFOS has been associated with lower birth weight[7, 8], a finding supported by animal studies where
229 increased PFOA, PFOS, PFNA, and PFDA is associated with lower birthweight[1]. This is in accordance with
230 our study as we see that higher levels of all measured PFAS were significantly associated with lower
231 birthweight.
232
233

Table 3: A comparison of studies on PFAS exposure and AGD in infants

Study population	№ of participants	PFAS concentrations in ng/mL	AGD measurements	Age at AGD-measurement	Confounders adjusted for	Results				
						PFOA	**PFOS**	**PFHxS**	**PFNA**	**PFDA**
Odense Child Cohort	316 boys 231 girls	Gestational age of 5-12 weeks: PFOA 1.7 PFOS 8.1 PFHxS 0.3 PFNA 0.7 PFDA 0.3	AGD_{AS} AGD_{AP} AGD_{AF} AGD_{AC}	3.5 months (range 2.1–6.8 months)	Age at examination, weight for age Z-score, pre-pregnancy BMI, parity, smoking	NA	$AGD_{AC}\downarrow$ * $AGD_{AS}\uparrow$ ʌ	$AGD_{AC}\downarrow$ * Ω $AGD_{AS}\downarrow$ *	$AGD_{AC}\downarrow$ * Ω $AGD_{AS}\uparrow$ *	$AGD_{AC}\downarrow$ ʌ Ω $AGD_{AS}\uparrow$ ʌ Ω
Shanghai-Minhang Birth Cohort Study[1]	439 boys 0 girls	At gestational age of 12-16 weeks: PFOA 20.1 PFOS 10.7 PFHxS 2.8 PFNA 1.8 PFDA 2.1	AGD_{AS} AGD_{AP}	1-3 days and 6 months	Age at examination, birth weight, parity, maternal education level, maternal age at delivery, gestational age, pre-pregnancy BMI	NA	$AGD_{AS}^{1\text{-}3d}\downarrow$ * $AGD_{AS}^{6mo}\downarrow$ *	NA	NA	$AGD_{AS}^{1\text{-}3d}\downarrow$ * $AGD_{AP}^{1\text{-}3d}\downarrow$ *
Maternal-Infant Research on Environmental Chemicals Cohort	198 boys 205 girls	At gestational age of <15 week: PFOA 1.7 PFOS 4.5 PFHxS 1.1	AGD_{AS} AGD_{AP} AGD_{AF} AGD_{AC}	Mean 3.41 days	Varied, but could include recruitment site, education, gestational age, weight-for-length Z-score, active smoking status and household income	$AGD_{AS}\uparrow$ *	NA	NA		
Faroese birth cohort, Cohort 5	232 girls 231 boys	14 days after expected birth[§]: PFOA 1.4 PFOS 8.3 PFHxS 0.2 PFNA 0.7 PFDA 0.3	AGD_{AS} AGD_{AF}	Mean 19.3 days	Age at examination, birth weight, parity, maternal education level	$AGD_{AS}\uparrow$ *ʌ Ω	$AGD_{AS}\uparrow$ *ʌ Ω	$AGD_{AS}\uparrow$ ʌ	$AGD_{AS}\uparrow$ *ʌ Ω	$AGD_{AS}\uparrow$ *ʌ Ω

Note: NA, no association; AGD_{AF}, anofourchette distance; AGD_{AS}, anoscrotal distance AGD_{AP}, anopenile distance; AGD_{AC}, anoclitoral distance; PFASs, perfluoroalkyl substances; Perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA)

1: Did not investigate separate quartiles

**: p <0.05 as continuous variable*

ʌ: p <0.05 in higher quartiles (q3 and/or q4)

Ω: p-trend <0.05

§ Mean 19.3 days after expected date of birth

234

235 The four epidemiological studies strongly indicate that PFASs exert a sex-dimorphic effect,

236 likely through endocrine disruption. This disruption of PFASs has also been studied in vitro and in animals.

237 Affected physiological pathways have been studied in vitro [11, 33-35]. One pathway is through PFOA and

238 PFOS decreasing testosterone levels by inducing aromatase activity through transcriptional activation of

239 CYP19, the aromatase enzyme and also induction of *CYP11B2*, coding for aldosterone synthase, which may

240 disrupt several of the functions related to aldosterone. Further, studies have found some PFASs to bind

241 directly to the ER with low affinity relative to endogenous estrogen [11, 33] and thereby exerting an
242 estrogenic effect and PFOA and PFOS are found to enhance the effect of estradiol on estrogen-responsive
243 genes [34]. In one animal study, blockage of androgen stimulation within a sensitive programming window
244 in fetal development resulted in de-masculinization of male genitalia including a shorter AGD, increased risk
245 of both hypospadias and cryptorchidism [14]. Generally, PFASs are suspected to cause endocrine disruption
246 through an increase in the fetal estrogen/androgen ratio. Following such disruption, one would expect a
247 shorter AGD in male offspring. . The suggested pathways which disrupt endocrine homeostasis found in
248 vitro and animal studies, support our findings of a sex-dimorphic effect of PFAS.
249 In humans, changes in the AGD relate to the symptom complex of diseases in male
250 reproductive system referred to as the testicular dysgenesis syndrome (TDS) [6], a syndrome causing
251 decreased fertility through a complex of symptoms such as decreased sperm quality, and an increased risk
252 of cryptorchidism and hypospadias [24, 36, 37]. The incidence of TDS is increasing and is hypothesized to be
253 linked to environmental pollutants, such as PFASs, that disrupt normal endocrine signaling, thereby causing
254 abnormal androgen action which alters the in utero development of the reproductive organs [38, 39].
255 Shorter AGD has been associated with, e.g., poorer semen quality [40, 41], genital malformations in men
256 [42, 43], and an increased risk of testicular germ cell tumor development [44]. Besides, longer AGD has
257 been suggested to be associated with higher sperm concentration, total sperm count, and total motile
258 sperm count in adult men and with fatherhood and may predict normal male reproductive potential [36,
259 45, 46]. In females, the long-term implications of AGD on reproductive health have only been studied
260 sporadically. In our study and the Canadian study [44], increased PFAS exposure was associated with longer
261 AGD, which may seem to contradict the TDS hypothesis, but cannot be considered as harmless, as the long-
262 term implications of longer AGDs are currently unknown.
263 Our study has several strengths. First, it is fairly large and comprised of a population-based
264 birth cohort with a high participation rate [24-26], and thus the cohort is likely to be representative of the
265 general population, thereby limiting selection bias. Further, the birth outcomes were objectively reported

and AGD was measured three times by the same trained pediatrician to eliminate inter-examiner variance and reduce imprecision. However, some weaknesses need consideration. PFASs were measured approximately two weeks post-partum, i.e., several months after the hypothesized masculinization programming window occurring between 8- and 14 weeks of gestation [14]. Exposure misclassification may have occurred, as maternal serum concentrations of PFOS, PFOA, and PFNA average higher in the first trimester, as compared to the second and third trimester [47, 48]. However, due to the long half-life of PFASs, one can assume that PFAS concentrations, measured postpartum after the initiation of breastfeeding, were likely comparable or even lower than PFAS concentrations during the relevant stage of development [37]. The Faroese women were exposed to lower PFAS concentrations than the Chinese women, whereas the exposure levels were relatively similar to Danish and Canadian pregnant women (table 3). Additionally, unknown confounders associated with PFAS exposure and intra-uterine growth may exist. e.g. lifestyle, and exposure to other chemicals, including PCBs and methylmercury, that the Faroese population are highly exposed to [49]. Lastly, the number of endocrine disruption chemicals (EDCs) that the mothers have been exposed to is not limited to the five PFASs measured, and serum-PFAS concentrations could conceivably function as a surrogate for other EDCs that may be highly correlated. Of note, studies exist that associate other EDCs with shorter AGD, e.g. phthalates [6], which is opposite to the findings of the present study.

5. Conclusions

In conclusion, we found that maternal PFAS exposure was significantly associated with a longer AGDs in boys. No significant associations were found among girls, suggesting sex-dimorphic effects of PFASs. Whether the observed association with longer ASD and PFAS influences the reproductive health of males is unknown and needs to be investigated further.

Conflict of interest

The authors declare that they have no competing financial interests.

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Supplementary table: Maternal characteristics (n=463) according to median (inter quartile range) maternal serum concentrations of PFASs (ng/ml)

	N (%)	PFOA	PFOS	PFHxS	PFNA	PFDA
All women	463	1.4 (1.0-2.0)	8.3 (6.2-11.7)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.4)
Maternal age (years)						
<25	84 (18)	1.7 (1.4-2.3)*	8.0 (6.2-10.0)*	0.2 (0.1-0-3)*	0.6 (0.5-0.8)*	0.2 (0.1-0.3)*
25-29.9	135 (29)	1.4 (1.0-2.0)	8.7 (6.6-10.7)	0.2 (0.1-0.3)	0.6 (0.5-0.8)	0.3 (0.2-0.3)
30-35	175 (38)	1.1 (0.9-1.8)	8.0 (5.6-10.4)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.4)
>35	69 (15)	1.2 (0.9-1.8)	9.4 (6.3-12.8)	0.3 (0.2-0.4)	0.8 (0.6-1.0)	0.3 (0.2-0.4)
Parity						
0	141 (31)	2.0 (1.5-2.5)*	8.6 (7.0-10.8)*	0.2 (0.2-0.4)*	0.7 (0.5-0.9)	0.2 (0.2-0.3)
1	160 (34)	1.3 (1.0-1.7)	8.6 (6.3-10.6)	0.2 (0.1-0.3)	0.6 (0.5-0.8)	0.2 (0.2-0.3)
2	102 (22)	1.1 (0.8-1.6)	8.0 (5.2-10.8)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.4)
3+	59 (13)	0.9 (0.7-1.1)	7.0 (5.1-9.6)	0.1 (0.1-0.3)	0.7 (0.5-0.8)	0.3 (0.2-0.4)
Maternal pre-pregnancy BMI						
<20	56 (12)	1.4 (0.9-2.0)	7.8 (5.8-10.2)	0.2 (0.1-0.3)	0.7 (0.5-0.8)	0.3 (0.2-3.3)
20-25	248 (54)	1.3 (0.9-2.0)	8.1 (6.0-10.6)	0.2 (0.1-0.3)	0.6 (0.5-0.9)	0.3 (0.2-0.4)
>25	159 (34)	1.4 (1.0-1.9)	8.7 (6.7-11.2)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.2 (0.2-0.4)
Gestational age at birth (weeks)						
<38	31 (7)	1.7 (0.9-1.9)	9.0 (6.3-11.1)	0.3 (0.2-0.3)	0.7 (0.6-0.8)*	0.3 (0.2-0.3)
38-40	302 (65)	1.4 (0.9-2.0)	8.9 (6.3-10.5)	0.3 (0.1-0.3)	0.8 (0.5-0.9)	0.3 (0.2-0.4)
>40	130 (28)	1.5 (1.0-1.8)	8.5 (5.3-11.1)	0.2 (0.1-0.3)	0.7 (0.5-0.8)	0.3 (0.2-0.3)
Birth weight (g)						
<2500	5 (1)	1.8 (1.8-2.0)*	9.0 (6.9-11-5)	0.2 (0.2-0.3)	0.7 (0.7-0.7)	0.3 (0.3-0.4)
2500-4500	430 (93)	1.4 (1.0-2.0)	8.4 (6.2-10.8)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.4)
>4500	28 (6)	0.9 (0.7-1.4)	7.6 (5.4-9.3)	0.2 (0.1-0-3)	0.6 (0.5-0.8)	0.3 (0.2-0.3)
Weight at examination (g)						
<3500	60 (13)	1.6 (1.0-2.4)*	8.9 (7.0-11.0)	0.3 (0.2-0.4)*	0.7 (0.6-1.0)*	0.3 (0.2-0.4)
3500-4500	300 (65)	1.4 (1.0-2.0)	8.3 (6.2-10.6)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.3)
>4500	103 (22)	1.1 (0.8-1.6)	7.7 (5.6-9.9)	0.2 (0.1-0.3)	0.6 (0.5-0.8)	0.3 (0.2-0.3)
Age at examination (days)						
<14	100 (22)	1.7 (1.0-2.4)	8.9 (7.1-11.1)	0.3 (0.2-0.4)	0.7 (0.6-1.0)	0.3 (0.2-0.4)
14-23	255 (55)	1.4 (1.0-1.9)	8.2 (6.2-10.6)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.3)
>23	108 (23)	1.1 (0.8-1.6)	7.7 (5.6-9.9)	0.2 (0.1-0.3)	0.6 (0.5-0.8)	0.3 (0.2-0.3)
Maternal level of education						
Below high school	70 (16)	1.6 (1.1-2.2)*	8.2 (6.2-11.0)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.2 (0.2-0.3)
High school or above	378 (84)	1.3 (0.9-1.9)	8.2 (6.2-10.5)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.4)
Maternal alcohol use during pregnancy						
Yes	22 (5)	1.5 (0.9-1.7)	8.4 (6.8-11.2)	0.2 (0.1-0.3)	0.6 (0.5-0.9)	0.3 (0.2-0.4)
No	441 (95)	1.4 (1.0-2.0)	8.3 (6.2-10.6)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.4)
Maternal smoking during pregnancy						
Yes	73 (16)	1.6 (1.1-2.0)*	8.8 (6.8-10.6)	0.2 (0.2-0.3)*	0.6 (0.5-0.9)	0.3 (0.2-0.4)
No	389 (84)	1.3 (0.9-1.9)	8.2 (6.1-10.6)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.4)
Smoking partner during pregnancy						
Yes	152 (34)	1.5 (1.1-2.1)*	8.7 (6.2-11.2)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.3)
No	299 (66)	1.3 (0.9-1.9)	8.2 (6.2-10.5)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	0.3 (0.2-0.4)

Note: AGD, anogenital distance; PFASs, perfluoroalkyl substances; Perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA)*p<0.05, Kruskal Wallis' test.

References

[1] ATSDR. (2018). *Toxicological Profile for Perfluoroalkyls*. Available: https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237

[2] A. B. Lindstrom, M. J. Strynar, and E. L. Libelo, "Polyfluorinated compounds: past, present, and future," *Environ Sci Technol,* vol. 45, pp. 7954-61, Oct 1 2011.

[3] E. M. Sunderland, X. C. Hu, C. Dassuncao, A. K. Tokranov, C. C. Wagner, and J. G. Allen, "A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects," *Journal of Exposure Science and Environmental Epidemiology,* vol. 29, pp. 131-147, 2019.

[4] B. Spachmo and A. Arukwe, "Endocrine and developmental effects in Atlantic salmon (Salmo salar) exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids," *Aquat Toxicol,* vol. 108, pp. 112-24, Feb 2012.

[5] L. S. Kjeldsen and E. C. Bonefeld-Jorgensen, "Perfluorinated compounds affect the function of sex hormone receptors," *Environ Sci Pollut Res Int,* vol. 20, pp. 8031-44, Nov 2013.

[6] T. K. Jensen, "Endocrine disrupters, semen quality and anogenital distance," *Current Opinion in Endocrine and Metabolic Research,* vol. 7, pp. 34-42, 2019/08/01/ 2019.

[7] P. I. Johnson, P. Sutton, D. S. Atchley, E. Koustas, J. Lam, S. Sen*, et al.*, "The Navigation Guide - evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth," *Environ Health Perspect,* vol. 122, pp. 1028-39, Oct 2014.

[8] C. C. Bach, B. H. Bech, N. Brix, E. A. Nohr, J. P. Bonde, and T. B. Henriksen, "Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: a systematic review," *Crit Rev Toxicol,* vol. 45, pp. 53-67, Jan 2015.

[9] H. Zhang, Y. Lu, B. Luo, S. Yan, X. Guo, and J. Dai, "Proteomic analysis of mouse testis reveals perfluorooctanoic acid-induced reproductive dysfunction via direct disturbance of testicular steroidogenic machinery," *J Proteome Res,* vol. 13, pp. 3370-85, Jul 3 2014.

[10] H. T. Wan, Y. G. Zhao, M. H. Wong, K. F. Lee, W. S. Yeung, J. P. Giesy*, et al.*, "Testicular signaling is the potential target of perfluorooctanesulfonate-mediated subfertility in male mice," *Biol Reprod,* vol. 84, pp. 1016-23, May 2011.

[11] A. D. Benninghoff, W. H. Bisson, D. C. Koch, D. J. Ehresman, S. K. Kolluri, and D. E. Williams, "Estrogen-like activity of perfluoroalkyl acids in vivo and interaction with human and rainbow trout estrogen receptors in vitro," *Toxicol Sci,* vol. 120, pp. 42-58, Mar 2011.

[12] G. Du, H. Huang, J. Hu, Y. Qin, D. Wu, L. Song*, et al.*, "Endocrine-related effects of perfluorooctanoic acid (PFOA) in zebrafish, H295R steroidogenesis and receptor reporter gene assays," *Chemosphere,* vol. 91, pp. 1099-106, May 2013.

[13] A. Thankamony, V. Pasterski, K. K. Ong, C. L. Acerini, and I. A. Hughes, "Anogenital distance as a marker of androgen exposure in humans," *Andrology,* vol. 4, pp. 616-25, Jul 2016.

[14] M. Welsh, P. T. Saunders, M. Fisken, H. M. Scott, G. R. Hutchison, L. B. Smith*, et al.*, "Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism," *J Clin Invest,* vol. 118, pp. 1479-90, Apr 2008.

[15] B. Zhao, L. Li, J. Liu, H. Li, C. Zhang, P. Han*, et al.*, "Exposure to perfluorooctane sulfonate in utero reduces testosterone production in rat fetal Leydig cells," *PLoS One,* vol. 9, p. e78888, 2014.

[16] S. Persson and U. Magnusson, "Environmental pollutants and alterations in the reproductive system in wild male mink (Neovison vison) from Sweden," *Chemosphere,* vol. 120, pp. 237-45, Feb 2015.

[17] N. Ding, S. D. Harlow, J. F. Randolph, Jr., R. Loch-Caruso, and S. K. Park, "Perfluoroalkyl and polyfluoroalkyl substances (PFAS) and their effects on the ovary," *Hum Reprod Update,* vol. 26, pp. 724-752, Sep 1 2020.

[18] L. Kjeldsen and E. C. Bonefeld-Jørgensen, "Perfluorinated compounds affect the function of sex hormone receptors," *Environmental science and pollution research international,* vol. 20, 06/14 2013.
[19] C. Liu, Y. Du, and B. Zhou, "Evaluation of estrogenic activities and mechanism of action of perfluorinated chemicals determined by vitellogenin induction in primary cultured tilapia hepatocytes," *Aquat Toxicol,* vol. 85, pp. 267-77, Dec 30 2007.
[20] M. Zarean, M. Keikha, A. Feizi, M. Kazemitabaee, and R. Kelishadi, "The role of exposure to phthalates in variations of anogenital distance: A systematic review and meta-analysis," *Environmental Pollution,* vol. 247, pp. 172-179, 2018.
[21] D. V. Lind, L. Priskorn, T. H. Lassen, F. Nielsen, H. B. Kyhl, D. M. Kristensen*, et al.*, "Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort," *Reprod Toxicol,* vol. 68, pp. 200-206, Mar 2017.
[22] Y. Tian, H. Liang, M. Miao, F. Yang, H. Ji, W. Cao*, et al.*, "Maternal plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances during pregnancy and anogenital distance in male infants," *Hum Reprod,* vol. 34, pp. 1356-1368, Jul 8 2019.
[23] T. E. Arbuckle, M. S., W. G. Foster, S. sathyanarayana, f. M., M. P.*, et al.*, "Prenatal perfluoroalkyl substances and newborn anogenital distance in a

Canadian cohort," *Reproductive Toxicology,* vol. 94, pp. 31-39, 2020.
[24] C. A. G. Timmermann, E. Budtz-Jorgensen, M. S. Petersen, P. Weihe, U. Steuerwald, F. Nielsen*, et al.*, "Shorter duration of breastfeeding at elevated exposures to perfluoroalkyl substances," *Reprod Toxicol,* vol. 68, pp. 164-170, Mar 2017.
[25] B. M. Kim, A. L. Choi, E. H. Ha, L. Pedersen, F. Nielsen, P. Weihe*, et al.*, "Effect of hemoglobin adjustment on the precision of mercury concentrations in maternal and cord blood," *Environ Res,* vol. 132, pp. 407-12, Jul 2014.
[26] C. Dalgard, M. S. Petersen, U. Steuerwald, P. Weihe, and P. Grandjean, "Umbilical Cord Serum 25-Hydroxyvitamin D Concentrations and Relation to Birthweight, Head Circumference and Infant Length at Age 14 Days," *Paediatr Perinat Epidemiol,* vol. 30, pp. 238-45, May 2016.
[27] E. Salazar-Martinez, P. Romano-Riquer, E. Yanez-Marquez, M. P. Longnecker, and M. Hernandez-Avila, "Anogenital distance in human male and female newborns: a descriptive, cross-sectional study," *Environ Health,* vol. 3, p. 8, Sep 13 2004.
[28] S. H. Swan, S. Sathyanarayana, E. S. Barrett, S. Janssen, F. Liu, R. H. Nguyen*, et al.*, "First trimester phthalate exposure and anogenital distance in newborns," *Hum Reprod,* vol. 30, pp. 963-72, Apr 2015.
[29] L. S. Haug, C. Thomsen, and G. Becher, "A sensitive method for determination of a broad range of perfluorinated compounds in serum suitable for large-scale human biomonitoring," *J Chromatogr A,* vol. 1216, pp. 385-93, Jan 16 2009.
[30] P. Grandjean, E. W. Andersen, E. Budtz-Jorgensen, F. Nielsen, K. Molbak, P. Weihe*, et al.*, "Serum vaccine antibody concentrations in children exposed to perfluorinated compounds," *Jama,* vol. 307, pp. 391-7, Jan 25 2012.
[31] P. Grandjean, C. Heilmann, P. Weihe, F. Nielsen, U. B. Mogensen, A. Timmermann*, et al.*, "Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years," *J Immunotoxicol,* vol. 14, pp. 188-195, Dec 2017.
[32] ATSDR, "Toxicological profile for Perfluoroalkyls. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.," 2021.
[33] Y. Gao, X. Li, and L. H. Guo, "Assessment of estrogenic activity of perfluoroalkyl acids based on ligand-induced conformation state of human estrogen receptor," *Environ Sci Technol,* vol. 47, pp. 634-41, Jan 2 2013.

[34] P. Sonthithai, T. Suriyo, A. Thiantanawat, P. Watcharasit, M. Ruchirawat, and J. Satayavivad, "Perfluorinated chemicals, PFOS and PFOA, enhance the estrogenic effects of 17beta-estradiol in T47D human breast cancer cells," *J Appl Toxicol,* vol. 36, pp. 790-801, Jun 2016.

[35] J. S. Kang, J. S. Choi, and J. W. Park, "Transcriptional changes in steroidogenesis by perfluoroalkyl acids (PFOA and PFOS) regulate the synthesis of sex hormones in H295R cells," *Chemosphere,* vol. 155, pp. 436-443, Jul 2016.

[36] M. L. Eisenberg, M. H. Hsieh, R. C. Walters, R. Krasnow, and L. I. Lipshultz, "The relationship between anogenital distance, fatherhood, and fertility in adult men," *PLoS One,* vol. 6, p. e18973, May 11 2011.

[37] D. Mondal, R. H. Weldon, B. G. Armstrong, L. J. Gibson, M. J. Lopez-Espinosa, H. M. Shin*, et al.*, "Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids," *Environ Health Perspect,* vol. 122, pp. 187-92, Feb 2014.

[38] N. E. Skakkebaek, E. Rajpert-De Meyts, and K. M. Main, "Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects," *Hum Reprod,* vol. 16, pp. 972-8, May 2001.

[39] J. S. Xing and Z. M. Bai, "Is testicular dysgenesis syndrome a genetic, endocrine, or environmental disease, or an unexplained reproductive disorder?," *Life Sci,* vol. 194, pp. 120-129, Feb 1 2018.

[40] J. Mendiola, R. W. Stahlhut, N. Jorgensen, F. Liu, and S. H. Swan, "Shorter anogenital distance predicts poorer semen quality in young men in Rochester, New York," *Environ Health Perspect,* vol. 119, pp. 958-63, Jul 2011.

[41] M. Eisenberg, M. Hsieh, R. Walters, R. Krasnow, and L. Lipshultz, "The Relationship between Anogenital Distance, Fatherhood, and Fertility in Adult Men," *PloS one,* vol. 6, p. e18973, 04/01 2011.

[42] M. H. Hsieh, B. N. Breyer, M. L. Eisenberg, and L. S. Baskin, "Associations among hypospadias, cryptorchidism, anogenital distance, and endocrine disruption," *Curr Urol Rep,* vol. 9, pp. 137-42, Mar 2008.

[43] P. M. Foster, "Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters," *Int J Androl,* vol. 29, pp. 140-7; discussion 181-5, Feb 2006.

[44] D. Moreno-Mendoza, E. Casamonti, A. Riera-Escamilla, S. Pietroforte, G. Corona, E. Ruiz-Castane*, et al.*, "Short anogenital distance is associated with testicular germ cell tumour development," *Andrology,* vol. 8, pp. 1770-1778, Jul 19 2020.

[45] M. L. Eisenberg and L. I. Lipshultz, "Anogenital distance as a measure of human male fertility," *J Assist Reprod Genet,* vol. 32, pp. 479-84, Mar 2015.

[46] J. Mendiola, M. Melgarejo, M. Monino-Garcia, A. Cutillas-Tolin, J. A. Noguera-Velasco, and A. M. Torres-Cantero, "Is anogenital distance associated with semen quality in male partners of subfertile couples?," *Andrology,* vol. 3, pp. 672-6, Jul 2015.

[47] C. Fei, J. K. McLaughlin, R. E. Tarone, and J. Olsen, "Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort," *Environ Health Perspect,* vol. 115, pp. 1677-82, Nov 2007.

[48] A. Glynn, U. Berger, A. Bignert, S. Ullah, M. Aune, S. Lignell*, et al.*, "Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010," *Environ Sci Technol,* vol. 46, pp. 9071-9, Aug 21 2012.

[49] P. Weihe and P. Grandjean, "Cohort studies of Faroese children concerning potential adverse health effects after the mothers' exposure to marine contaminants during pregnancy," *Acta Veterinaria Scandinavica,* vol. 54, pp. S7-S7, 2012.