Do Perfluoroalkyl Compounds Impair Human Semen Quality?

Ulla Nordström Joensen, Rossana Bossi, Henrik Leffers, Allan Astrup Jensen, Niels E. Skakkebæk, and Niels Jørgensen

doi: 10.1289/ehp.0800517 (available at http://dx.doi.org/)
Online 2 March 2009
Do Perfluoroalkyl Compounds Impair Human Semen Quality?

Ulla Nordström Joensen¹, Rossana Bossi², Henrik Leffers¹, Allan Astrup Jensen³, Niels E. Skakkebæk¹, Niels Jørgensen¹.

¹ University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark
² National Environmental Research Institute, University of Aarhus, Roskilde, Denmark
³ FORCE Technology, Brøndby, Denmark

Address of institution where work was done:
University Department of Growth and Reproduction
Rigshospitalet, section 5064
Blegdamsvej 9
2100 Copenhagen
Denmark

Corresponding author:
Ulla Nordström Joensen, MD
University Department of Growth and Reproduction, Rigshospitalet
Blegdamsvej 9
2100 Copenhagen
Denmark
Telephone: +45 3545 5064
Fax: +45 3545 6054
E-Mail: ulla.nordstroem.joensen@rh.regionh.dk
Acknowledgements/grant support:

The authors greatly appreciate the support from the European Union (contract no. QLK4-CT-2002-00603), the Danish Agency for Science, Technology and Innovation (grant nos. 9700833 and 271070678), the Danish Ministry of Health (ISMF) (Grant no. 7-302-02-9/3), the Danish Environmental Protection Agency, and the University of Copenhagen.

The authors declare they have no competing financial or non-financial interests.

Running title: PFAAs and Human Semen Quality.

EHP article descriptor: Reproductive Health

Key words: Endocrine disruptors, male reproductive health, perfluoroalkyl compounds, PFAA, PFC, semen quality, sperm morphology, testosterone.

Abbreviations:

BMI: body mass index
CI: confidence interval
ESI: electrospray ionization
FAI: free androgen index
FSH: follicle stimulating hormone
HPLC: high performance liquid chromatography
LC-MS-MS: liquid chromatography-tandem mass spectrometry
LH: luteinizing hormone
LOD: limit of detection
PFAA: perfluoroalkyl acids
PFC: polyfluorinated compounds
PFDA: perfluorodecanoic acid
PFDoA: perfluorododecanoic acid
PFHpA: perfluoroheptanoic acid
PFHxS: perfluorohexane sulfonic acid
PFNA: perfluorononanoic acid
PFOS: perfluorooctane sulfonic acid
PFOSA: perfluorooctane sulfonamide
PFTrA: perfluorotridecanoic acid
PFUnA: perfluoroundecanoic acid
SHBG: sex hormone binding globulin
TDS: testicular dysgenesis syndrome
Abstract

**Background:** Perfluoroalkyl acids (PFAAs) are found globally in wildlife and humans, and are suspected to act as endocrine disruptors. There are no reports of PFAA levels in adult men from Denmark, and no reports of a possible association between semen quality and PFAA exposure. **Objectives:** To investigate possible associations between PFAAs and testicular function. The hypothesis was that higher PFAA levels would be associated with lower semen quality and lower testosterone levels. **Methods:** We included 105 Danish men (median age 19 years) from the general population, and analyzed serum samples for levels of 10 different PFAAs and reproductive hormones, and assessed semen quality. **Results:** Considerable levels of PFOS, PFOA and PFHxS were found in all young men (median 24.5, 4.9 and 6.6 ng/mL, respectively). Men with high combined levels of PFOS and PFOA had a median of 6.2 million normal spermatozoa in their ejaculate in contrast 15.5 million among men with low PFOS-PFOA (p=0.030). In addition we found non-significant trends with regard to lower sperm concentration, lower total sperm counts and altered pituitary-gonadal hormones among men with high PFOS-PFOA levels. **Conclusion:** High PFAA levels were associated with fewer normal sperms. Thus, high levels of PFAAs may contribute to the otherwise unexplained low semen quality often seen in young men. However, our findings need to be corroborated in larger studies.
Introduction

Perfluoroalkyl acids (PFAAs) are degradation products of many man-made polyfluorinated compounds (PFCs) used in consumer and industrial products, for example for impregnation of carpets, textiles and paper (Jensen et al. 2008; Jensen and Leffers 2008; Kissa 2001). Studies of environment, wildlife and humans suggest widespread presence and exposure, as well as persistence in the environment and bioaccumulation (Giesy and Kannan 2001; Kannan et al. 2004). For perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS), three of the most abundant PFAAs, half-lives for humans have been estimated as 3.8, 5.4 and 8.5 years, respectively (Olsen et al. 2007). Some studies suggest that men may have higher serum concentrations of PFAAs than women, and younger individuals may have higher levels than older (Calafat et al. 2006). Thus, young men may have particularly high levels of exposure and may therefore be a group at risk for potential adverse effects of PFAAs.

PFAAs can cross the placental barrier and therefore have the potential to affect the fetus. In humans, levels of PFOS and PFOA in umbilical cord blood have been inversely related to birth weight (Apelberg et al. 2007). In addition, PFOS, PFOA and PFHxS have been detected in human seminal plasma samples (Guruge et al. 2005). However, data on effects in humans are sparse, and most come from studies of occupationally exposed individuals. These studies have not given conclusive evidence of adverse effects. A recent study, however, measured PFAA levels in early pregnancy found that higher levels of PFOS and PFOA was associated with significantly longer waiting time to pregnancy (Fei et al. 2009).

Animal studies provide some evidence for adverse reproductive effects on animals exposed as adults or in utero. Exposure of adult male rats to PFOA reduced their testosterone levels and increased their estradiol levels, which may partly explain earlier
findings of induction of Leydig cell hyperplasia and/or adenomas in the testes of exposed animals (Biegel et al. 1995; Cook et al. 1992).

Our objective was to investigate the associations between PFOS, PFOA, PFHxS and other PFAAs and testicular function. Our primary hypothesis was that high concentrations of PFAAs would be associated with low testosterone levels and secondly that high PFAA levels are negatively associated to semen quality variables.

Materials and methods

Study population. Since 1996, semen quality of young men in Denmark has been surveyed in a cross-sectional study (Andersen et al. 2000; Jørgensen et al. 2002). All young Danish men must report for military draft, and annually new cohorts of approximately 300 men from the Copenhagen area in Denmark have been included. They each provided one semen sample and had a venous blood sample drawn. Of the 546 men examined in 2003, we selected 105 for the investigation of associations between PFAAs and testicular function. The 105 men included the 53 men (group 1) with the highest testosterone levels (median 31.8 nmol/l, range 30.1 - 34.8), and the 52 men (group 2) with the lowest testosterone levels (median 14.0 nmol/l, range 10.5 - 15.5). We chose the men examined in 2003, as this was the latest year from which we had completed analyses of reproductive hormones. The median age of men in group 1 was 18,9 years (range 18,2 – 24,6), median age in group 2 was 19,0 years (range 18,2 – 25,1), and median age for all 105 young men was 19.0 years (range 18.2 - 25.2) years. Information on ejaculation abstinence period and hour of blood sampling was recorded. All samples of semen and blood were collected between 8.30 a.m. and 1.15 p.m. Serum was stored at -20ºC until chemical analysis.

The Danish National Committee on Biomedical Research Ethics, Copenhagen Region, approved the research, and all young men gave written informed consent.
PFNA (perfluorononanoic acid), PFDA (perfluorodecanoic acid), PFUnA (perfluoroundecanoic acid), PFDa (perfluorododecanoic acid), and PFTrA (perfluorotridecanoic acid). One mL of serum was spiked with the surrogate standards \(^{13}\text{C}_8\)-PFOA, \(^{13}\text{C}_2\)-PFDA and \(^{13}\text{C}_4\)-PFOS and extracted according to the ion pairing method described previously (Hansen et al. 2001). Matrix-matched standards were prepared by spiking rabbit serum (Sigma Aldrich, Schnelldorf, Germany) with the analytes and the surrogate standards. Blank samples consisted of rabbit serum spiked with only surrogate standards. Standards and blanks were extracted together with each batch of samples. Instrumental analysis was performed by liquid chromatography-tandem mass spectrometry (LC-MS-MS) with electrospray ionization (ESI). The extracts (20 \(\mu\text{L}\) injection volume) were chromatographed on a C18 Betasil column (2.1 x 50 mm, Thermo Hypesil-Keystone, Bellafonte, PA) using an Agilent 1100 Series HPLC (Agilent Technologies, Palo Alto, CA). The high performance liquid chromatography (HPLC) was interfaced to a triple quadrupole API 2000 (Sciex, Concorde, Ontario, Canada) equipped with a TurboIon Spray source operating in negative ion mode. Chromatographic conditions and transition MS-MS ions have been described in details previously (Bossi et al. 2005). The limits of detection (LODs) ranged from 0.1 to 0.5 ng/mL.

Reproductive hormone analysis. Thawed serum samples were analyzed for the levels of testosterone, estradiol, SHBG (sex hormone binding globulin), LH (luteinizing hormone),
FSH (follicle stimulating hormone) and inhibin B as described previously (Paasch et al. 2008). Free androgen index (FAI) was calculated as [testosterone × 100 / SHBG]. Ratios between hormones were calculated by simple division.

Semen analysis. Semen volume was assessed by weight, and the sperm concentration by use of a Bürker-Türk haemocytometer. Total sperm count was calculated as semen volume x sperm concentration. The percentage of motile spermatozoa (WHO class A+B+C) was assessed on fresh samples. Sperm morphology slides were fixed, Papanicolaou stained and all assessed according to strict criteria (Menkveld et al. 1990) by one trained technician over a period of one week. Further details of the semen analysis can be seen in previously published work (Jørgensen et al. 2002).

Statistical analysis. Medians and 5-95th percentiles were used to describe the levels of PFAAs in group 1 and 2. Mann-Whitney U-test was used to compare the groups 1 and 2 with respect to PFAA levels, BMI (body mass index) and smoking status. Samples with values below LOD were set to 0 ng/mL. We used Pearson’s correlation coefficients and related p-values to describe correlations between levels of different PFCs. Univariate regression analysis was done for comparison of hormone levels between group 1 and 2, and to describe associations between PFAAs and hormones or semen variables. Sperm concentration, semen volume and total sperm count were adjusted for the effect of ejaculation abstinence period. Sperm motility was adjusted for time between ejaculation and assessment of motility. Sex hormone concentrations were adjusted for hour of blood sampling. Semen variables and hormone levels and ratios, except sperm morphology and total testosterone, were ln transformed to obtain normality of the residuals. Smoking and
BMI were tested for confounding effects but were found to be non-significant, and therefore not included in the final analyses.

We proceeded to analyze for associations between PFAAs and testicular function (hormone levels and semen quality) for the whole group of 105 men. We singled out PFOS and PFOA, and results were calculated as estimated changes in endpoint (reproductive hormones and semen variables) with a change in serum concentration of 1 ng/mL of PFOS, PFOA concentrations, and the summed concentration of PFOS and PFOA. We divided the men into three groups from the combined concentrations of PFOS and PFOA. Each sample was given a quartile score of 1 to 4 for PFOS and PFOA levels separately. Score 1 was given to samples with levels within the lowest quartile, and score 4 within the highest quartile. We then summed the quartile scores for PFOS and PFOA, giving each sample a possible score from 2 to 8. Samples were then divided into 3 quartile groups for PFOS and PFOA combined: “Low PFAA” group (N=29) with summed quartile score from 2 to 3, “intermediate PFAA” (N=48) group with score 4 to 6, and “high PFAA” (N=28) group with score 7 to 8. Analysis for association between quartile group and hormone levels or semen variables was done using univariate regression analysis, adjusted for the above-mentioned confounders.

Statistical analysis was performed using SPSS statistical software version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Levels of PFAAs in serum. The serum levels and number of samples above LOD for all PFAAs are shown for the low- and high testosterone groups and the entire group of 105 men (Table 1). Except for PFOSA, which was detected in only 56 men, there was no significant difference in levels of any PFAA between group 1 and 2. The median PFOS,
PFOA and PFHxS concentrations for the whole group of men were 24.5, 4.9 and 6.6 ng/mL, respectively, and only these were included in the final regression analyses. The remaining PFAAs were found in much lower concentrations, and therefore these results are not discussed further. PFOS levels were positively correlated with PFOA (r = 0.594, p < 0.0005) and PFHxS (r = 0.304, p = 0.002) levels. PFOA and PFHxS levels were positively correlated, but not statistically significantly (r = 0.136, p = 0.2).

**PFAAs and semen variables.** In the whole group of 105 men, there was a tendency toward reduced levels of all semen variables in the “high” PFAA quartile group compared to the “low” group (groups constructed to include PFOS and PFOA levels, see statistical analysis), Table 2. The difference in percentage of morphologically normal spermatozoa as well as in the total number of normal spermatozoa (total sperm count x % morphologically normal sperms) was statistically significant (p = 0.037 and 0.030, respectively). In the high PFOS-PFOA group the median number of normal spermatozoa in the ejaculate was 6.2 million vs. 15.5 million in the low group (Figures 1 and 2).

When analyzing associations of semen variables to PFOS and PFOA separately, as well as the simple summed concentration of PFOS and PFOA, estimated changes in semen variables with a change in serum PFAA concentration of 1 ng/mL indicated negative but non-significant associations between the PFAAs and semen variables (Table 3).

**PFAAs and reproductive hormones.** There was no significant association between testosterone levels and PFAA levels, and no significant difference in PFAA levels between the high- and low-testosterone groups. For the whole group of 105 men, adjusted medians for the hormones point to a negative association to PFAA levels - however, none of these tendencies were statistically significant (Table 2).
Estimated associations between reproductive hormones and PFOS and PFOA separately and the summed concentration of PFOS and PFOA, showed no significant associations (Table 4).

*Smoking and BMI.* 35% of the 105 men were smokers, and there were more smokers in the high testosterone group compared to the low testosterone group (49% and 21% smokers, respectively, p = 0.003). Smoking status was significantly associated to higher testosterone, lower estradiol and higher SHBG when entered as a confounder in univariate analyses for these three variables only (p = 0.004 to 0.009). Smoking was not associated with any semen quality variables (p = 0.1 to 0.4), or levels of any PFAA (p = 0.1 to 1.0). Including smoking as a confounder did not considerably change the presented results or significance levels. Therefore, smoking was not included in the final analyses. BMI was not associated to levels of any PFAA (p = 0.1 to 1.0), nor was there any confounding effect on any semen variables (e.g. p = 0.5 for morphologically normal sperms, and p = 0.9 for total number of morphologically normal sperms in analyses for difference between high and low PFAA groups).

**Discussion and conclusions**

This study examined PFAA levels in young adults. High serum concentrations of PFAAs were significantly associated with reduced numbers of normal spermatozoa. In addition, sperm concentration, total sperm count and sperm motility showed some tendency towards lower levels in men with high PFAA levels, although not at a statistically significant level. A tendency toward lower inhibin B/FSH ratio with high PFAA levels was in agreement with these findings, as these hormones reflect the spermatogenetic activity.
Testosterone, FAI, testosterone/LH ratio and testosterone/estradiol ratio could suggest a poorer Leydig cell function in the “high” compared to the “low” PFAA quartile group. However, the associations between reproductive hormones and PFAAs were not completely consistent, and all were non-significant. This study therefore cannot demonstrate an adverse effect of PFAAs on Leydig cell function.

We singled out PFOS and PFOA, as there are no data that specifically support PFHxS as an endocrine disruptor, and divided the men into three groups from the combined concentrations of PFOS and PFOA to account for a potential different effect at same concentration levels. As we could find no significant association between testosterone levels and PFAA levels, and no significant difference in PFAA levels between high- and low-testosterone groups, we could analyze associations between PFOS-PFOA levels and reproductive hormones or semen variables for the whole group. Controlling for confounding effect of smoking or BMI did not change estimates or significance levels. To our knowledge, there have been no consistent reports of associations between PFAA levels and smoking or BMI.

Our material included only 105 men, and in addition, we had selected the men based on their serum testosterone values. The selection of two groups with high and low testosterone was done to test our primary hypothesis and therefore affects the homogeneity of the group when correlations are analyzed for the group as a whole. This could potentially influence the subsequent analysis of semen quality by bias or confounding, and may affect the general applicability of the results. A larger follow-up study should preferably include randomly selected men from the general population.

Our study is, to our knowledge, the first report of a correlation between semen quality and PFAAs. Very few studies of other endocrine disruptors (e.g. phthalates and pesticides) have previously demonstrated such an association (Duty et al. 2003; Hauser et
al. 2003; Hauser et al. 2007; Meeker et al. 2008; Swan et al. 2003). If the results from our preliminary study of an association between high levels of PFAAs and decreased number of normal sperms are confirmed, then high levels of PFAAs may be regarded as another endocrine disrupting factor contributing to the low semen quality seen among many young men. However, the importance of mixture effects of low-dose exposure to multiple compounds is becoming evident from animal studies but remains to be studied in humans (Hass et al. 2007; Kortenkamp et al. 2007).

The mode of action by PFAAs is not clear and only a few animal studies have explored mechanistic issues. These show decreased testosterone levels and reduced expression of steroidogenesis genes associated with Leydig cell hyperplasia in adult animals (Biegel et al. 1995; Shi et al. 2007), suggesting a direct testicular effect. Recent studies have indicated that the fetal gonad is particularly sensitive to exogenous factors (Skakkebæk et al. 2001). However, our results could indicate that exposures later in life may contribute to impairment of semen quality, in line with other recent studies (Hauser et al. 2007). We speculate that morphology is perhaps more susceptible to this than sperm concentration or total sperm count. Sperm morphology has proven an important indicator of semen quality and fertility in a clinical setting, even in men with normal sperm concentration (Guzick et al. 2001). Interestingly, a recent study showed higher levels of maternal PFOS and PFOA levels in early pregnancy was associated with significantly longer waiting time to pregnancy (Fei et al. 2009). We speculate that men and women living together may have similar exposure to PFAAs and that decreased semen quality caused by high PFAA levels may contribute to the longer waiting time to pregnancy found in that study.

The use and emission polyfluorinated compounds continue to increase, and they are not readily cleared from the environment (Jensen et al. 2008; Prevedouros et al. 2006).
Therefore, humans and wildlife worldwide will be exposed for years to come. We found positive correlations between levels of different PFAAs, as has been found previously (Apelberg et al. 2007; Calafat et al. 2007; Fei et al. 2007), suggesting common sources of exposure. The PFAAs levels we have detected are comparable to those found in other countries like Sweden and the Faroe Islands (Kärrman et al. 2007; Weihe et al. 2008), but lower than earlier results from Denmark from 1996-2002 (Fei et al. 2007). Thus, the effects we have indicated may also be true for other than the Danish population.

In conclusion, our results indicate that higher PFAA levels were associated with lower numbers of normal sperms. In addition, we found non-significant negative associations between PFAA levels and other semen variables and reproductive hormones. Thus, high levels of PFAAs may contribute to the otherwise unexplained low semen quality seen in many young men. However, results from this first and preliminary study should be corroborated in larger studies.
References


Http://www.mst.dk/Udgivelser/Publications/2008/10/978-87-7052-845-0.htm [accessed 10 February 2009].


Skakkebæk NE, Rajpert-De Meyts E, Main KM. 2001. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 16:972-978.

Table 1

PFAA median concentrations (5-95\textsuperscript{th} percentiles) for high and low testosterone groups and all study subjects (ng/mL), and p-values for difference between the two groups.

<table>
<thead>
<tr>
<th>Samples &gt; LOD</th>
<th>High testosterone N=53</th>
<th>Low testosterone N=52</th>
<th>Whole group N=105</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFHxS</td>
<td>105</td>
<td>6.6 (4.0 - 13.0)</td>
<td>6.6 (3.5 - 12.1)</td>
<td>6.6 (4.0 - 12.1)</td>
</tr>
<tr>
<td>PFHpA</td>
<td>98</td>
<td>0.2 (0.01 - 0.9)</td>
<td>0.3 (0.00 - 1.3)</td>
<td>0.2 (0.00 - 1.1)</td>
</tr>
<tr>
<td>PFOA</td>
<td>105</td>
<td>4.4 (2.6 - 7.0)</td>
<td>5.0 (2.7 - 7.5)</td>
<td>4.9 (2.7 - 7.2)</td>
</tr>
<tr>
<td>PFOS</td>
<td>105</td>
<td>25.5 (14.2 - 39.6)</td>
<td>23.9 (12.8 - 45.2)</td>
<td>24.5 (14.2 - 42.1)</td>
</tr>
<tr>
<td>PFOSA</td>
<td>56</td>
<td>0.1 (0.00 - 3.7)</td>
<td>0.00 (0.00 - 3.5)</td>
<td>0.06 (0.00 - 3.5)</td>
</tr>
<tr>
<td>PFNA</td>
<td>105</td>
<td>0.8 (0.4 - 1.8)</td>
<td>0.8 (0.4 - 2.0)</td>
<td>0.8 (0.4 - 1.8)</td>
</tr>
<tr>
<td>PFDA</td>
<td>104</td>
<td>0.9 (0.2 - 1.1)</td>
<td>0.8 (0.4 - 1.2)</td>
<td>0.9 (0.3 - 1.1)</td>
</tr>
<tr>
<td>PFUnA</td>
<td>101</td>
<td>0.1 (0.04 - 0.3)</td>
<td>0.2 (0.00 - 0.4)</td>
<td>0.1 (0.02 - 0.4)</td>
</tr>
<tr>
<td>PFDoA</td>
<td>102</td>
<td>0.08 (0.04 - 0.8)</td>
<td>0.08 (0.02 - 0.8)</td>
<td>0.08 (0.04 - 0.8)</td>
</tr>
<tr>
<td>PFTrA</td>
<td>7</td>
<td>0.00 (0.000 - 0.4)</td>
<td>0.00 (0.00 - 0.06)</td>
<td>0.00 (0.00 - 0.2)</td>
</tr>
</tbody>
</table>
Table 2

Adjusted means (95% CI) for PFAA quartile groups, and p-value for difference between the low and high PFAA quartile groups.

<table>
<thead>
<tr>
<th>Sex hormonesa</th>
<th>Low PFAA</th>
<th>Intermediate PFAA</th>
<th>High PFAA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N = 29</td>
<td>N = 48</td>
<td>N = 28</td>
<td></td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>25.2 (21.7 - 28.7)</td>
<td>22.3 (19.6 – 25.0)</td>
<td>22.3 (18.8 - 25.8)</td>
<td>0.2</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>77.6 (70.3 - 85.8)</td>
<td>72.4 (67.0 – 84.7)</td>
<td>76.6 (69.3 - 84.7)</td>
<td>0.9</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>27.8 (24.0 - 32.2)</td>
<td>25.4 (22.6 – 28.5)</td>
<td>26.1 (22.5 - 30.3)</td>
<td>0.5</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>3.4 (2.8 - 4.1)</td>
<td>2.9 (2.5 – 3.4)</td>
<td>3.7 (3.0 - 4.4)</td>
<td>0.6</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>2.7 (2.1 - 3.5)</td>
<td>2.7 (2.2 – 3-3)</td>
<td>3.0 (2.3 - 3.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>Inhibin-B (pg/mL)</td>
<td>181 (142 - 232)</td>
<td>175 (144 - 212)</td>
<td>152 (119 - 195)</td>
<td>0.3</td>
</tr>
<tr>
<td>FAI</td>
<td>84.1 (74.1 - 95.5)</td>
<td>80.2 (72.6 – 88.7)</td>
<td>77.8 (68.5 - 88.3)</td>
<td>0.4</td>
</tr>
<tr>
<td>Testosterone/LH</td>
<td>6.9 (5.6 - 8.4)</td>
<td>7.0 (5.9 - 8.2)</td>
<td>5.5 (4.5 - 6.8)</td>
<td>0.1</td>
</tr>
<tr>
<td>FAI/LH</td>
<td>24.7 (19.9 - 30.7)</td>
<td>27.4 (23.1 – 32.5)</td>
<td>21.2 (17.1 - 26.4)</td>
<td>0.3</td>
</tr>
<tr>
<td>Estradiol/Testosterone</td>
<td>3.3 (3.0 - 3.7)</td>
<td>3.6 (3.2 – 3.9)</td>
<td>3.8 (3.4 - 4.2)</td>
<td>0.1</td>
</tr>
<tr>
<td>Inhibin/FSH</td>
<td>66.8 (42.1 - 106.0)</td>
<td>66.0 (45.9 – 94.8)</td>
<td>51.3 (32.3 - 81.4)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Semen qualityb

| Volume (mL)         | 4.0 (3.2 - 5.0) | 3.4 (2.9 – 4.1) | 3.5 (2.9 - 4.4) | 0.3     |
| Concentration (mio./mL) | 59 (36 - 96) | 51 (35 – 74) | 40 (25 - 64) | 0.2     |
| Total count (mio.)  | 228 (134 - 389) | 172 (114 - 261) | 143 (86 - 237) | 0.1     |
| Motile sperms (%)   | 73 (69 - 77)   | 70 (66 - 73)    | 71 (66 - 75)    | 0.4     |
| Morphologically normal (%) | 8.8 (7.2 - 10.4) | 7.7 (6.4 – 9.0) | 6.3 (4.6 - 8.0) | 0.037*  |
| Total morphologically normal (mio.) | 15.5 (7.3 - 33.0) | 10.0 (5.6 – 17.9) | 6.23 (3.0 - 12.8) | 0.030*  |

a Hormone levels are adjusted for time of blood sampling.

b Volume, concentration and total count are adjusted for duration of abstinence. Motility is adjusted for time between ejaculation and semen analysis. Morphology is not adjusted for confounders.
Table 3

Estimated change in semen variables\(^a\) with a change in PFAA of 1 ng/mL (95% CI). All 105 men included.

<table>
<thead>
<tr>
<th></th>
<th>PFOS</th>
<th>PFOA</th>
<th>PFAA sum(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ln Volume</td>
<td>0.000 (-0.012 - 0.011)</td>
<td>-0.002 (-0.070 - 0.066)</td>
<td>0.000 (-0.010 - 0.010)</td>
</tr>
<tr>
<td>ln Concentration</td>
<td>-0.020 (-0.044 - 0.005)</td>
<td>-0.080 (-0.230 - 0.066)</td>
<td>-0.018 (-0.040 - 0.004)</td>
</tr>
<tr>
<td>ln Total count</td>
<td>-0.018 (-0.045 - 0.010)</td>
<td>-0.074 (-0.230 - 0.086)</td>
<td>-0.016 (-0.041 - 0.008)</td>
</tr>
<tr>
<td>ln Motility</td>
<td>-0.006 (-0.019 - 0.007)</td>
<td>-0.027 (-0.110 - 0.053)</td>
<td>-0.006 (-0.018 - 0.007)</td>
</tr>
<tr>
<td>Morphology</td>
<td>-0.085 (-0.200 - 0.026)</td>
<td>-0.540 (-1.200 - 0.110)</td>
<td>-0.082 (-0.181 - 0.018)</td>
</tr>
</tbody>
</table>

\(^a\) Volume, concentration and total count are adjusted for duration of abstinence. Motility is adjusted for time between ejaculation and semen analysis. Morphology is not adjusted for confounders.

\(^b\) “PFAA sum” is PFOS and PFOA mass concentrations summed (ng/mL).
Table 4

Estimated change in reproductive hormones\(^a\) with a change in PFAA concentration of 1 ng/mL (95% CI). All 105 men included.

<table>
<thead>
<tr>
<th></th>
<th>PFOS</th>
<th>PFOA</th>
<th>PFAA sum(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>-0.087 (-0.32 - 0.15)</td>
<td>-0.98 (-2.33 - 0.37)</td>
<td>-0.093 (-0.303 - 0.116)</td>
</tr>
<tr>
<td>ln Estradiol</td>
<td>-0.001 (-0.008 - 0.005)</td>
<td>-0.012 (-0.051 - 0.027)</td>
<td>-0.001 (-0.007 - 0.005)</td>
</tr>
<tr>
<td>ln SHBG</td>
<td>0.002 (-0.007 - 0.012)</td>
<td>-0.009 (-0.067 - 0.048)</td>
<td>0.002 (-0.007 - 0.011)</td>
</tr>
<tr>
<td>ln LH</td>
<td>0.000 (-0.014 - 0.012)</td>
<td>-0.010 (-0.084 - 0.064)</td>
<td>0.000 (-0.012 - 0.010)</td>
</tr>
<tr>
<td>ln FSH</td>
<td>0.004 (-0.13 - 0.22)</td>
<td>-0.037 (-0.14 - 0.064)</td>
<td>0.003 (-0.013 - 0.018)</td>
</tr>
<tr>
<td>ln Inhibin-B</td>
<td>-0.004 (-0.21 - 0.12)</td>
<td>0.012 (-0.084 - 0.11)</td>
<td>-0.003 (-0.018 - 0.012)</td>
</tr>
<tr>
<td>ln FAI</td>
<td>-0.006 (-0.015 - 0.002)</td>
<td>-0.038 (-0.087 - 0.011)</td>
<td>-0.006 (-0.014 - 0.001)</td>
</tr>
<tr>
<td>ln Testosterone/LH</td>
<td>-0.003 (-0.017 - 0.011)</td>
<td>-0.038 (-0.087 - 0.011)</td>
<td>-0.003 (-0.016 - 0.009)</td>
</tr>
<tr>
<td>ln FAI/LH</td>
<td>-0.006 (-0.020 - 0.009)</td>
<td>-0.028 (-0.114 - 0.058)</td>
<td>-0.005 (-0.018 - 0.008)</td>
</tr>
<tr>
<td>ln Estradiol/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.003 (-0.005 - 0.010)</td>
<td>0.035 (-0.010 - 0.081)</td>
<td>0.003 (-0.004 - 0.010)</td>
</tr>
<tr>
<td>ln Inhibin/FSH</td>
<td>-0.009 (-0.039 - 0.022)</td>
<td>0.049 (-0.13 - 0.23)</td>
<td>-0.006 (-0.034 - 0.022)</td>
</tr>
</tbody>
</table>

\(^a\) Hormone levels are adjusted for time of blood sampling.

\(^b\) “PFAA sum” is PFOS and PFOA mass concentrations summed (ng/mL).
Figure legends

Figure 1.
Morphologically normal spermatozoa (%) and PFAA quartile groups (adjusted means and 95% CI). All 105 men included.

* p = 0.037 for difference compared to Low PFAA quartile group.

Figure 2.
Total morphologically normal spermatozoa (mio.)\(^\text{a}\) and PFAA quartile groups (adjusted means and 95% CI). All 105 men included.

\(^\text{a}\) Total morphologically normal spermatozoa is adjusted for duration of abstinence.

* p = 0.030 for difference compared to Low PFAA quartile group.
Morphologically normal spermatozoa (%) and PFAA quartile groups (adjusted means and 95% CI).

All 105 men included.

* $p = 0.037$ for difference compared to Low PFAA quartile group.

201x285mm (150 x 150 DPI)
Total morphologically normal spermatozoa (mio.)a and PFAA quartile groups (adjusted means and 95% CI). All 105 men included.

a Total morphologically normal spermatozoa is adjusted for duration of abstinence.

* p = 0.030 for difference compared to Low PFAA quartile group.

201x285mm (150 x 150 DPI)