Per- and poly-fluoroalkyl substances (PFASs) in follicular fluid from women experiencing infertility in Australia

Authors: Young Ran Kim [1], Nicole White [1], Jennifer Bräunig [3], Soumini Vijayasarathy [3], Jochen F. Mueller[3], Christine L. Knox [2], Fiona A. Harden [4], Rosana Pacella [5], Leisa- Maree L. Toms [1]

1. School of Public Health and Social Work, Faculty of Health, Institute of Health and Biomedical Innovation, Queensland University of Technology, QLD, Australia
2. School of Biomedical Science, Faculty of Health, Institute of Health and Biomedical Innovation, Queensland University of Technology, QLD, Australia
3. Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, QLD, Australia
4. Hunter Industrial Medicine, Newcastle, NSW, Australia
5. Institute for Lifecourse Development, Faculty of Education, Health and Human Sciences, University of Greenwich, UK

Corresponding author: Young Ran Kim Tel. +61 424 054 551; email: youngran.kim@hdr.qut.edu.au Postal address: School of Public Health and Social Work, Faculty of Health, Queensland University of Technology (QUT), Victoria Park Road, Kelvin Grove, QLD 4059, Australia

Keywords

Per- and poly-fluoroalkyl substances (PFASs), Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA), Perfluorohexane sulfonate (PFHxS), Perfluorononanoic acid (PFNA), Australia, infertility

Abstract

Per- and poly-fluoroalkyl substances (PFASs) have been widely used and detected in human matrices. Evidence that PFAS exposure may be associated with adverse human reproductive health effects exists, however, data is limited. The use of a human matrix such as follicular fluid to determine chemical exposure, along with reproductive data will be used to investigate if there is a relationship between PFAS exposure and human fertility.

Objective

This study aims to: (1) assess if associations exist between PFAS concentrations and/or age and fertilisation rate (as determined in follicular fluid of women in Australia who received assisted reproductive treatment (ART)); and (2) assess if associations exist between PFAS concentrations and infertility aetiology.

Methods

Follicular fluids were originally collected from participants who underwent fully stimulated ART treatment cycles at an *in vitro* fertilisation (IVF) clinic in the period 2006-2009 and 2010-11 in Queensland, Australia. The samples were available for analysis of 32 PFASs including perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoic acid (PFNA) using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). 97 samples were matched with limited demographic data (age and fertilisation rate) and five infertility factors (three known female factors): 1) endometriosis, 2) polycystic ovarian syndrome (PCOS), and 3) genital tract infections - tubal/pelvic inflammation disease; as well as 4) male factor, and 5) idiopathic or unknown from either males or females. SPSS was used for linear regression analysis.

Results

PFASs were detected in all follicular fluid samples with the mean concentrations of PFOS and PFOA, 4.9, and 2.4 ng/ml, respectively. A lower fertilisation rate was observed at higher age when age was added as a covariate, but there was no relationship between PFAS concentrations and fertilisation rate. There were few statistically significant associations between PFAS concentrations in follicular fluid and infertility factors. Log-transformed PFHxS concentrations were lower in females with endometriosis (factor 1) than in women who had reported ‘male

factors' as a reason of infertility, while PFHpA was higher in women who had infertile due to female factors (factor 1-3) compared to those who had infertile due to male factor.

Conclusion: PFASs were detected in follicular fluid of Australian women who had been treated at an IVF clinic. PFAS exposure found in follicular fluids is linked to increased risk of some infertility factors, and increased age was associated with decreased fertilisation rate in our data. But there was no relationship between PFAS and ferlitisation rate. Further large-scale investigations of PFAS and health effects including infertility are warranted.

1. Introduction

Per- and poly-fluoroalkyl substances (PFASs), are chemicals that have been used widely as surfactants, lubricants, floor waxes, fire-fighting foams, denture cleaners, shampoos, pharmaceutical products, and in food packaging since the 1950s (Kantiani et al., 2010). The most common exposure route for PFAS is via ingestion, followed by dermal contact and inhalation (Quaak et al., 2016; D'Hollander et al., 2014; Jian et al., 2017).

Studies have shown potential associations between PFAS exposure and adverse health effects for metabolism, thyroid function, neurodevelopment, cancers, cardiovascular diseases, reproductive functions, and immunity (as reviewed by Kirk et al., 2018). Kirk et al. (2018) confirmed that while there are increasing numbers of studies investigating the health effects of exposure to PFASs, the results are limited or inconsistent. When looking specifically in terms of reproductive health outcomes, conflicting results have been observed (Fei et al., 2009; Fei et al., 2012; Whitworth et al., 2012; Jorgensen et al., 2014; Velez et al., 2015; Vestergaard et al., 2012; Buck Louis et al., 2013; Bach et al., 2015; Barrett et al., 2015). For example, lower levels of reproductive hormones, such as estradiol and progesterone, were related to higher concentrations of perfluorooctane sulfonate (PFOS), and perfluorooctane sulfonamide (PFOSA) in nulliparous women (women who have never given birth) (Barrett et al., 2015). However, the results were not consistent for other PFASs, including perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA) and perfluorohexane sulphonate (PFHxS) in parous women (women who have given birth) (Barrett et al., 2015). Fei et al. (2009; 2012) found lower fecundability (ability to achieve pregnancy), when comparing higher PFOS (26.1-43.2 ng/mL) to lower PFOS exposure (<26.1 ng/mL), when stratified by parity. There was an association between PFOS exposure and increased odds of infertility in three higher quartiles of PFOS concentrations when compared with the lowest quartile (Fei et al., 2009). No association was found between PFOS exposure and subfecundability or infertility (Velez et al., 2015; Bach et al., 2015).

PFASs are known as possible endocrine disrupting chemicals (EDCs) with adverse health effects on the endocrine system (Stahl et al., 2011; Caserta et al., 2013; DeWitt 2015). For example, the pituitary gland produces fertility hormones, including follicle stimulating hormones and luteinizing hormone, which are vital for ovulation and successful conception.

(Stach et al., 2011). Interference by PFASs or other chemicals on the endocrine system may cause reproductive health issues, such as infertility or hormone imbalance in women (Caserta et al., 2013; DeWitt 2015; Kim et al., 2019).

PFASs are persistent and bioaccumulate with concentrations detected in human samples worldwide (Cho et al., 2015 (South Korea); Stubleski et al., 2016 (Sweden); Whitworth et al., 2012 (Norway); Olsen et al., 2017 (USA); Gao et al., 2019 (China)). In Australia, PFAS were detected in human serum samples dating back to 2002 with levels similar to or higher than in European and Asian countries (Toms et al., 2014). There is recent interest in Australia and worldwide as to whether or not PFAS exposure may be linked to adverse health effects specifically in communities with PFAS exposure through drinking water and in occupationally exposed groups such as firefighters (Rotander et al., 2015).

Infertility, defined as the inability to conceive after one year of unprotected intercourse, is a global public health issue affecting about 15% of the population (Datta et al., 2016). Female fertility rate, defined as the average number of children born to a woman during her reproductive years, is likely to decrease with increasing age, and/ or an underlying medical condition that might affect ovulation or hormone imbalance, or cause blocked fallopian tubes (Barbieri 2018; Jaward et al., 2018). The most common medical conditions experienced by infertile women are endometriosis, polycystic ovarian syndrome, or pelvic inflammatory disease while poor semen quality is considered the main male cause (Hruska et al., 2000; Piotr et al., 2016; Skakkebaek et al., 2016; Sifakis et al., 2017; Barbieri 2018).

Human exposure to PFAS can be measured by analysis of food/drinking water, and through analysis of human matrices, such as blood serum, urine or breast milk. In this study, PFASs were examined in follicular fluid. This is a liquid in the ovarian follicle, which can be collected when a woman undergoes egg harvest during assisted reproductive technology (ART) treatment. Studies have used follicular fluid to measure PFASs, likely due to ease of collection, which is relatively non-invasive if carried out opportunistically (Governini et al., 2011; McCoy et al., 2017; Petro et al., 2014; Heffernan et al., 2018). Despite the determination of PFASs in follicular fluid, current data is limited to conclude whether associations exist between PFAS concentrations and adverse fertility effects.

Therefore, this study aims to assess if associations exist between: (1) PFAS concentrations and/or age and fertilisation rate (as determined in follicular fluid of women in Australia who received ART); and (2) PFAS concentrations and infertility aetiology.

2. Materials and Methods

2.1. Sample collection

Follicular fluid samples were collected from female participants who underwent fully stimulated ART treatment cycles at an IVF (*in vitro* fertilisation) clinic in Queensland in the period 2006-2009 and 2010-2011 (as part of the "Asymptomatic upper genital tract infections in infertile couples and assisted reproductive technology outcomes (ART)" and "Prevalent microorganisms detected in the female upper genital tract: the effect of these microorganisms on oocytes and on assisted reproductive technology outcomes" projects (Pelzer et al., 2013)). The samples were obtained when the participants were undergoing egg harvest for IVF as described previously (Pelzer et al., 2013). The data available included date of birth, infertility aetiology, fertilisation rate, and past clinical history of infertility. Study participants had been classified into groups depending on the aetiology of infertility for the couples including: three female factors with 1= endometriosis, 2= polycystic ovarian syndrome (PCOS), 3= genital tract infections (tubal/pelvic inflammation disease); 4= male factor (this is infertility due to only male partners issues, but detailed health information was not given); and 5= idiopathic or unknown. Factor 5, idiopathic, means causes of infertility were not identified from either the female or the male. We considered infertility aetiology 1, 2, and 3 as female case groups, and infertility aetiology factor 4 as a control group. Whilst factor 5 was included in the analysis it was not included as either a case group or a control group due to its unknown causes. ART treatment cycle(s) outcomes were also recorded for each couple. It should be noted that the date of sample collection was not supplied, only that the samples were collected between 2006 and 2010. In order to calculate an age at date of collection, we have taken a mid-point of 2008 and used participant date of birth to calculate an approximate age.

2.2 Ethics statement

We sought and received a waiver of consent to use follicular samples for analysis of PFAS from the Queensland University of Technology (QUT) ethics committee (approval number:

1800000016) and The University of Queensland Human Research Ethic Committee (approval number: 2018000550).

2.3 Chemical Analysis for PFASs

Analysis of the follicular fluid samples was undertaken at the Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland. Samples were analyzed for 32 PFASs; perfluorobutanoic acid (PFBA), perfluoropetanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorohepatanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutane sulphonate (PFBS), perfluoropentane sulphonate (PFPeS), perfluorohexane sulphonate (PFHxS), perfluoroheptane sulphonate (PFHpS), perfluorooctane sulfonate (PFOS), perfluorononane sulfonate (PFNS), perfluordecane sulphonate (PFDS), perfluordodecane sulphonate (PFDoDS), sodium 1H,1H,2H,2H-perfluorohexane sulfonate (4:2) (8:2 FTS), sodium 1H,1H,2H,2H-perfluorohexane sulfonate (4:2) (4:2 FTS), Sodium 1H,1H,2H,2H-perfluorooctane sulfonate (6:2) (6:2 FTS), sodium 1H,1H,2H,2H-perfluorodecane sulfonate (8:2) (8:2 FTS), perfluoroethylcyclohexane sulfonate (PFECHS), perfluoro-1-octane sulfonamide (FOSA), n-ethylperfluoro-1-octane sulfonamidoacetic acid (NEtFOSAA), n-methylperfluoro-1-octane sulfonamidoacetic acid (NMeFOSAA), n-methylperfluoro-1-octane sulfonamide (NMeFOSA), n-ethylperfluoro-1-octane sulfonamide (NEtFOSA), 2-(N-methylperfluoro-1-octane sulfonamido)-ethanol (NMe FOSE), 2-(N-ethylperfluoro-1-octane sulfonamido)-ethanol (NEt FOSE) (Supplementary information Table S1). A 200 µl aliquot of follicular fluid was transferred to a 2 ml Eppendorf tube, followed by addition of the internal standards. Proteins were precipitated with acetonitrile, centrifuged, filtered (2 µm GHP membrane; Pall, East Hills, NY, USA), and concentrated to 200µl under a gentle stream of nitrogen. Samples were reconstituted to 500µl with 5 mM ammonium acetate in water and spiked with recovery standards prior to analysis via high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) using a Nexera HPLC (Shimadzu Corp., Kyoto, Japan) coupled to a Triple Quad 6500+ mass spectrometer (Sciex, Melbourne, Australia) with electrospray ionization (ESI) interface operating in negative mode. Chromatographic separation of the analytes was achieved with a Gemini C18 column (50 x 2.0 mm, 4 µm;

Phenomenex, Torrance, CA), maintained at 45°C, with a flow rate of 0.3 mL/min and injection volume of 5 µL. Mobile phases consisted of methanol water (1:99, v/v) (A), and methanol: water (95:5, v/v) (B), with 5mM ammonium acetate in both phases. An isolator column (Phenomenex) was included inline directly after the mobile phase mixing chamber to delay elution of solvent-derived background PFASs contamination. Data acquisition and processing was carried out using analyst® TF 1.6 and MultiQuantTM software (Sciex). If the PFASs were detected in less than 60% of samples, they were excluded from statistical analysis (eg: PFBA, and PFDoDA, 38.4% and 4% respectively). Linear PFAS congeners were determined for the current study.

Quality control

Laboratory blanks (MilliQ water) were extracted and analyzed in parallel with each batch of samples. Batches included inter-batch replicates which generally showed CV < 15%. The method limit of quantification (LOQ) was calculated by multiplying the SD obtained from injecting the lowest calibration standard seven times by 10. Percentage average recovery ranged from 70 to 110%.

2.4 Data analysis

Descriptive statistics were calculated to summarize overall cohort characteristics and PFAS concentrations in follicular fluid samples. Evidence of associations between PFAS concentrations and fertility variables were evaluated using linear modelling. A linear regression model was fitted to examine the influence of PFAS concentrations and age on expected fertilisation rates, which was defined as number of oocytes fertilized by ART. This model specified fertilisation rate as the dependent variable, with participant age (added as a covariate) and individual PFAS concentrations included as continuous independent variables. To assess whether PFAS concentrations varied by aetiology infertility factors, a one-way analysis of variance (ANOVA) was fitted, with log-transformed concentrations as the dependent variable, and aetiology factor as a categorical independent variable. Our decision to apply a log transformation was informed by findings of preliminary analysis to satisfy residual assumptions. Linear modelling outcomes were summarized by parameter estimates and corresponding 95% confidence intervals. Hypothesis testing of estimates was conducted to determine if associations were statistically significant, assuming a significance level of 0.05. All statistical analyses were undertaken in SPSS version 25.

3. Results

The mean age of female participants was 35.4 years, and the mean fertilisation rate was 63% (Table 1). Most common aetiology factors of females participating in ART treatment in this study was 'idiopathic (24.7%)', and male partner's factors of infertility (22.7%)' (Table 1). In total, 97 follicular samples matched with demographic information were analysed for 32 PFASs. 8 PFASs were detected in most samples (PFOS, PFOA, PFHxS, PFNA, PFDA, PFHpS, PFUnDA, and PFHpA) and were included in further analysis. The concentrations from highest to lowest were: PFOS (Mean = 4.8; Range = 0.7-22.4 ng/ml), PFOA (2.4; 0.3-14.5 ng/ml), PFHxS (1.7; 0.2-21.3 ng/ml), PFNA (0.5; 0.08-2.0 ng/ml), PFDA (0.2; 0.05-0.9 ng/ml), PFHpS (0.1; 0.05-1.1 ng/ml), PFUnDA (0.1; <LOD-0.4 ng/ml), and PFHpA (0.01; <LOD-0.6 ng/ml) (Table 2). The remaining 24 PFASs were detected in a small number of samples or at <LOD), thus they were not discussed further, details are available in the SI.

Table 1. Characteristics and fertility outcomes of participating women for ART (N=97)

Variable	**Mean (SD); Range**
Age (years)	35 (4); 23 - 42
Fertilisation rate (%)	63 (22); 20-100
Aetiology of infertility	**n (%) of 97 participants**
Endometriosis (factor 1)	18 (18.6)
Polycystic ovarian syndrome (factor 2)	18 (18.6)
Genital tract infections (factor 3)	15 (15.5)
Male factors of infertility (factor 4)	22 (22.7)
Idiopathic (factor 5)	24 (24.7)

SD: standard deviation

Table 2. Descriptive data of 8 PFASs in follicular fluids (97 demographic information matched samples)

Ng/mL	Detection frequency (%)	Minimum	Maximum	Mean	S.D
PFOS	98	0.7	22.4	4.8	3.1
PFHxS	98	0.2	21.3	1.7	2.7
PFHpS	96	0.05	1.1	0.1	0.1
PFOA	98	0.3	14.5	2.4	1.7
PFNA	97	0.08	2.0	0.5	0.3
PFDA	98	0.05	0.9	0.2	< LOD
PFHpA	90	< LOD	0.6	0.01	0.008
PFUnDA	97	< LOD	0.4	0.1	0.007

LOD: Limit of detection

3.1. PFAS concentrations, and fertilisation rate and age

Fertilisation rate is defined as the number of oocytes fertilized by ART divided by the total number of oocytes collected. Information was available for 92 samples (5 missing data points). It was found that for every increase of one standard deviation in age, fertilisation rate decreased by 1.49 (Estimate= -1.49, 95%CI; -2.64-0.35, p= 0.013). Inconsistent results were observed in the relationship between fertilisation rate, age and PFAS concentrations. The concentration of selected PFASs in follicular fluid was positively (eg; PFHpA, PFOA, PFUnDA) or negatively (eg; PFDA, PFHpS) associated with fertilisation rate, however high levels of uncertainty in parameter estimates meant that none of these associations were statistically significant (Table 3).

Table 3. Associations between PFAS concentrations and fertilisation rate and age CI: Confidence interval

Variables	Estimate	95% CI	Test statistic	p-value
Age	-1.49	-2.64—0.35	-2.609	0.013
PFOS	2.279	-0.556-5.114	1.599	0.114
PFHxS	0.692	-0.855-2.239	0.890	0.376

PFHpS	-48.371	-111.980-15.238	-1.512	0.134
PFOA	0.706	-2.219-3.631	0.480	0.633
PFNA	15.647	-18.849-50.143	0.902	0.370
PFDA	-60.830	-129.250-7.590	-1.768	0.081
PFHpA	17.390	-65.276-100.055	0.418	0.677
PFUnDA	73.581	-3.413-150.574	1.900	0.061

3.2. PFAS concentrations and infertility by aetiology factors

Analysis of PFAS concentrations by aetiology factor provided mixed results, with statistically significant differences observed for PFHpA (test statistic = 2.4; p-value = 0.04) and PFHxS (test statistic = 4.7; p-value = 0.002) (Table 4). For PFHxS, average log transformed concentrations were seen to be lowest for aetiology factor 1 (Mean = -0.3; SE = 0.1) and highest for aetiology factor 2 (Mean = 0.3; SE = 0.1). For PFHpA, average concentrations were observed to be lowest for aetiology factor 4 (Mean =-1.5; SE = 0.1) and highest for aetiology factor both 3, and 5 (Mean = -1.2; SE = 0.1). When female aetiology factors of infertility (factors 1-3) were compared to male factor of infertility (factor 4), the concentrations of PFHpA were lower in the male infertility factor than the female infertility factors, and the concentrations of PFHxS were lower in only the female infertility factor of endometriosis (Table 4).

Table 4. Association between PFAS concentrations and infertility by 5 aetiology factors. Summary statistics are presented for log-transformed concentrations. SE: Standard error.

PFAS (log transformed ng/ml))	**Mean (SE)**					
	Aetiology factor 1 Endometriosis	**Aetiology factor 2 Polycystic ovarian syndrome**	**Aetiology factor 3 Genital tract**	**Aetiology factor 4 Male factors of**	**Aetiology factor 5 Idiopathic**	**Test statistic (p-value)**

			infections	infertility		
PFOS	0.6 (0.04)	0.7 (0.1)	0.5 (0.04)	0.6 (0.03)	0.5 (0.06)	2.8 (0.26)
PFHxS	-0.3 (0.1)	0.3 (0.1)	0.02 (0.04)	0.01 (0.03)	-0.02 (0.1)	4.7 (0.002)
PFHpS	-0.8 (0.04)	-0.7 (0.06)	-0.9 (0.04)	-0.8 (0.03)	-0.9 (0.05)	3.12 (0.15)
PFOA	0.3 (0.1)	0.4 (0.1)	0.2 (0.1)	0.3 (0.03)	0.3 (0.04)	1.7 (0.15)
PFNA	-0.3 (0.1)	-0.3 (0.1)	-0.3 (0.1)	-0.3 (0.02)	-0.3 (0.02)	0.21 (0.93)
PFDA	-0.7 (0.1)	-0.8 (0.1)	-0.6 (0.1)	-0.7 (0.03)	-0.7 (0.04)	0.11 (0.97)
PFHpA	-1.4 (0.1)	-1.3 (0.1)	-1.2 (0.1)	-1.5 (0.1)	-1.2 (0.1)	2.4 (0.04)
PFUnDA	-1.04 (0.7)	-0.9 (0.1)	-0.9 (0.1)	-0.9 (0.03)	-0.9 (0.04)	0.64 (0.63)

4. Discussion

In this study, PFASs were detected in follicular fluid in women experiencing infertility. We observed association between PFAS, such as PFHpA, or PFHxS, and the 5 aetiology factors of infertility. From the limited studies investigating follicular fluid and fertility effects, McCoy et al. (2017) found no significant associations between ovarian response measures and PFAS concentrations, and also found decreased blastocyst conversion rate in follicular fluid exposed to perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA). Heffernan et al. (2018) conducted a study using serum and follicular fluid of women with and without polycystic ovarian syndrome (PCOS) undergoing fertility treatment and found higher serum PFOS concentrations in PCOS cases than controls, and in women with irregular menstrual cycles compared to women with regular menstrual cycles. Governini et al. (2011) reported that PFASs were present in human follicular fluid and suggested PFAS concentrations had a potentially detrimental effect on oocyte fertilisation capacity but the sample size was limited (n=16).

Higher levels of PFNA have also been associated with increased risk of infertility in women (Jorgensen et al., 2014). While in another study, the presence of higher PFASs in human follicular fluid (a linear combination of PFOA, PFOS, PFNS and PFHxS) had a higher chance of an oocyte developing into a high-quality embryo (Petro et al., 2014).

Associations were found between PFHxS, and PFHpA, and 5 aetiology factors of infertility. Epidemiological studies have reported an association between PFASs, and infertility caused by endometriosis (Vagi et al., 2014; Campbell et al., 216; Wang et al., 2017). In Chinese females seeking ART treatment due to endometriosis, plasma levels of perfluorobutane sulfonic acid (PFBS), which were excluded in this current study due to low detection rate, was related to an increased risk of infertility (Wang et al., 2017). In the United States of America, women with endometriosis had higher levels of PFNA, PFOA and PFOS (Campbell et al., 2016). There was also evidence of a relationship between PCOS and PFASs exposure. Vagi et al (2014) reported higher serum levels of PFOA and PFOS in females with PCOS compared to females with no PCOS. Although incidence of genital tract infections in both females and males is related to risk of infertility, the effects of PFAS exposure on genital tract infections have not been well understood (Pellati et al., 2008). Further epidemiology studies are needed to identify if these disease-causing infertilities are associated with PFAS exposure.

In the current study, we used individual samples of follicular fluid, and were therefore able to measure the range of PFAS concentrations (minimum to maximum) from individual persons. This is advantageous as the body of exposure data on PFAS in Australia uses pooled blood serum data which has the limitation of not being able to identify extreme concentrations, but rather provides a mean of the concentrations of the individuals in that pool (Toms et al. 2019). Individual's serum samples have been analysed for PFASs in pregnant women from Western Australia (Callan et al., 2016), community residents (Bräunig et al., 2017), and workers exposed to PFASs (Rotander et al., 2015) in Australia. In the current study, we were able to calculate the inter quartile range (IQR) for PFASs in Australia which showed little variation in concentrations among the 97 women. This little variation was also observed in 98 pregnant women in the Western Australian study by Callen et al. (2016) (PFOS 0.45-8.1 ng/L, PFHxS 0.06-3.3 ng/L, PFOA 0.21-3.1 ng/L). PFOS, PFOA, and PFHxS were detected at the highest concentrations in the follicular fluid as has been seen in human serum in Australia (Toms et al.,

2009). This finding is consistent with follicular fluid studies from Belgium (Petro et al., 2014), the United States of America (McCoy et al., 2017), and the United Kingdom (Heffernan et al., 2018).

In terms of age effects on PFASs, there were no trends in any of the PFAS concentrations by age. As is expected from samples from females of child-bearing age, the age range is reasonably narrow making the assessment of trends difficult. Age trends have been identified in previous studies of Australian serum covering the full lifecycle where different PFAS concentrations varied with age (Toms et al., 2019). We found decreased fertilisation rate in follicular fluids is related to increased age, confirming accepted evidence that fertilisation rate decreases with age (Barbieri 2018).

Limitations

Limited demographic information available on participants and a small sample size limited the interpretation of this dataset. Another limitation arose from simplifying causes of infertility to five infertility aetiology factors, as human infertility is influenced by various factors, not only physiologically, or pathogenically, but also environmentally.

4. Conclusion

In conclusion, we identified PFAS in follicular fluid of Australian women who had been treated at an IVF clinic. PFOS, PFOA, and PFHxS were detected in the highest concentrations in the follicular fluids. Increased age was associated with decreased fertilisation rate in our data. There were significant differences in PFAS concentrations between female infertility factors and the control group that showed links between PFAS exposures and increased risk of infertility factors. Further studies are needed to investigate the relationship between PFAS levels and health effects including human infertility.

Acknowledgement

YR is funded by a Postgraduate research scholarship from Queensland University of Technology.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Aylward, L., Green, E., Porta, M., Toms, L., Den Hond, E., Schulz, C., … Mueller, J. (2014). Population variation in biomonitoring data for persistent organic pollutants (POPs): An examination of multiple population-based datasets for application to Australian pooled biomonitoring data. *Environment International*, *68*, 127–138.

Bräunig, J., Baduel, C., Heffernan, A., Rotander, A., Donaldson, E., & Mueller, J. (2017). Fate and redistribution of perfluoroalkyl acids through AFFF-impacted groundwater. *Science of the Total Environment*, *596-597*, 360–368. https://doi.org/10.1016/j.scitotenv.2017.04.095

Bach, C., Bech, B., Nohr, E., Olsen, J., Matthiesen, N., Bossi, R., … Henriksen, T. (2015). Serum perfluoroalkyl acids and time to pregnancy in nulliparous women. *Environmental Research*, *142*, 535–541. https://doi.org/10.1016/j.envres.2015.08.007

Barbieri, R. (2018). Female Infertility. In *Yen & Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management: Eighth Edition* (pp. 556–581.e7). https://doi.org/10.1016/B978-0-323-47912-7.00022-6

Barrett, E., Chen, C., Thurston, S., Haug, L., Sabaredzovic, A., Fjeldheim, F., … Thune, I. (2015). Perfluoroalkyl substances and ovarian hormone concentrations in naturally cycling women. *Fertility and Sterility*, *103*(5), 1261–1270.e3. https://doi.org/10.1016/j.fertnstert.2015.02.001

Buck Louis, G., Sundaram, R., Schisterman, E., Sweeney, A., Lynch, C., Gore-Langton, R., … Barr, D. (2013). Persistent Environmental Pollutants and Couple Fecundity: The LIFE Study. *Environmental Health Perspectives (Online)*, *121*(2), 231–236. https://doi.org/10.1289/ehp.1205301

Callan, A., Rotander, A., Thompson, K., Heyworth, J., Mueller, J., Odland, J., & Hinwood, A. (2016). Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in offspring. *Science of the Total Environment*, *569-570*, 1107–1113. https://doi.org/10.1016/j.scitotenv.2016.06.177

Campbell, S., Raza, M., & Pollack, A. (2016). Perfluoroalkyl substances and endometriosis in US women in NHANES 2003–2006. *Reproductive Toxicology*, *65*, 230–235. https://doi.org/10.1016/j.reprotox.2016.08.009

Caserta, D., Bordi, G., Ciardo, F., Marci, R., La Rocca, C., Tait, S., … Moscarini, M. (2013). The influence of endocrine disruptors in a selected population of infertile women. *Gynecological Endocrinology*, *29*(5), 444–447. https://doi.org/10.3109/09513590.2012.758702

Cho, C., Lam, N., Cho, B., Kannan, K., & Cho, H. (2015). Concentration and correlations of perfluoroalkyl substances in whole blood among subjects from three different geographical areas in Korea. *Science of the Total Environment*, *512-513*, 397–405. https://doi.org/10.1016/j.scitotenv.2015.01.070

D'Hollander, W., Bruyn, L., Hagenaars, A., Voogt, P., & Bervoets, L. (2014). Characterisation of perfluorooctane sulfonate (PFOS) in a terrestrial ecosystem near a fluorochemical plant in Flanders, Belgium. *Environmental Science and Pollution Research*, *21*(20), 11856–11866. https://doi.org/10.1007/s11356-013-2449-4

Datta, J., Palmer, M., Tanton, C., Gibson, L., Jones, K., Macdowall, W., … Wellings, K. (2016). Prevalence of infertility and help seeking among 15 000 women and men. *Human Reproduction (Oxford, England)*, *31*(9), 2108–2118. https://doi.org/10.1093/humrep/dew123

DeWitt, J. (2015). *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances* (1st ed. 2015.). https://doi.org/10.1007/978-3-319-15518-0

Fei, C., McLaughlin, J., Lipworth, L., & Olsen, J. (2009). Maternal levels of perfluorinated chemicals and subfecundity. *Human Reproduction*, *24*(5), 1200–1205. https://doi.org/10.1093/humrep/den490

Fei, R., Weinberg, R., & Olsen, R. (2012). Commentary: Perfluorinated Chemicals and Time to Pregnancy: A Link Based on Reverse Causation? *Epidemiology*, *23*(2), 264–266. https://doi.org/10.1097/EDE.0b013e3182467608

Gao, K., Zhuang, T., Liu, X., Fu, J., Zhang, J., Fu, J., … Jiang, G. (2019). Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) and Association between the Placental Transfer Efficiencies and Dissociation Constant of Serum Proteins-PFAS Complexes. *Environmental Science & Technology*, *53*(11), 6529–6538. https://doi.org/10.1021/acs.est.9b00715

Governini, L., Orvieto, R., Guerranti, C., Gambera, L., Leo, V., & Piomboni, P. (2011). The impact of environmental exposure to perfluorinated compounds on oocyte fertilisation capacity. *Journal of Assisted Reproduction and Genetics*, *28*(5), 415–418. https://doi.org/10.1007/s10815-011-9548-2

Heffernan, A., Cunningham, T., Drage, D., Aylward, L., Thompson, K., Vijayasarathy, S., … Sathyapalan, T. (2018). Perfluorinated alkyl acids in the serum and follicular fluid of UK women with and without polycystic ovarian syndrome undergoing fertility treatment and associations with hormonal and metabolic parameters. *International Journal of Hygiene and Environmental Health*, *221*(7), 1068–1075. https://doi.org/10.1016/j.ijheh.2018.07.009

Høyer, B., Ramlau-Hansen, C., Vrijheid, M., Valvi, D., Pedersen, H., Zviezdai, V., … Toft, G. (2015). Anthropometry in 5- to 9-Year-Old Greenlandic and Ukrainian Children in Relation to Prenatal Exposure to Perfluorinated Alkyl Substances. *Environmental Health Perspectives*, *123*(8), 841–846. https://doi.org/10.1289/ehp.1408881

Hruska, S., Furth, A., Seifer, B., Sharara, I., & Flaws, A. (2000). Environmental Factors in Infertility. *Clinical Obstetrics and Gynecology*, *43*(4), 821–829. https://doi.org/10.1097/00003081-200012000-00014

Jawad, A. H., Ibrahim, S. A., Hadi, D. M., & Jawad, Z. H. (2018). A Study of the Correlation of Some Sex Hormone with Obesity in Women with Secondary Infertility. Al-Nahrain Journal of Science, 18(2), 44-49. Retrieved from http://anjs.edu.iq/index.php/anjs/article/view/328

Jian, J., Guo, Y., Zeng, L., Liang-Ying, L., Lu, X., Wang, F., & Zeng, E. (2017). Global distribution of perfluorochemicals (PFCs) in potential human exposure source–A review. *Environment International*, *108*, 51–62. https://doi.org/10.1016/j.envint.2017.07.024

Jørgensen, K., Specht, I., Lenters, V., Bach, C., Rylander, L., Jönsson, B., … Bonde, J. (2014). Perfluoroalkyl substances and time to pregnancy in couples from Greenland, Poland and Ukraine. *Environmental Health : a Global Access Science Source*, *13*(1), 116. https://doi.org/10.1186/1476-069X-13-116

Kantiani, L., Llorca, M., Sanchís, J., Farré, M., & Barceló, D. (2010). Emerging food contaminants: a review. *Analytical and Bioanalytical Chemistry*, *398*(6), 2413–2427. https://doi.org/10.1007/s00216-010-3944-9

Kirk M,1 Smurthwaite K,1 Bräunig J,5 Trevenar S,1 D'Este C,1 Lucas R,1 Lal A,1 Korda R,1 Clements A,2 Mueller J,5 Armstrong B.3,4 The PFAS Health Study: Systematic Literature Review. Canberra: The Australian National University. 2018.

Kim, Y., Pacella, R., Harden, F., White, N., & Toms, L. (2019). A systematic review: Impact of endocrine disrupting chemicals exposure on fecundity as measured by time to pregnancy. *Environmental Research*, *171*, 119–133. https://doi.org/10.1016/j.envres.2018.12.065

McCoy, J., Bangma, J., Reiner, J., Bowden, J., Schnorr, J., Slowey, M., … Parrott, B. (2017). Associations between perfluorinated alkyl acids in blood and ovarian follicular fluid and ovarian function in women undergoing assisted reproductive treatment. *Science of the Total Environment*, *605-606*, 9–17. https://doi.org/10.1016/j.scitotenv.2017.06.137

Okada, E., Sasaki, S., Saijo, Y., Washino, N., Miyashita, C., Kobayashi, S., … Kishi, R. (2012). Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious

diseases in infants. *Environmental Research*, *112*, 118–125. https://doi.org/10.1016/j.envres.2011.10.003

Olsen, G., Mair, D., Lange, C., Harrington, L., Church, T., Goldberg, C., … Ley, C. (2017). Per- and polyfluoroalkyl substances (PFAS) in American Red Cross adult blood donors, 2000–2015. *Environmental Research*, *157*, 87–95. https://doi.org/10.1016/j.envres.2017.05.013

Pellati, D., Mylonakis, I., Bertoloni, G., Fiore, C., Andrisani, A., Ambrosini, G., & Armanini, D. (2008). Genital tract infections and infertility. *European Journal of Obstetrics and Gynecology*, *140*(1), 3–11. https://doi.org/10.1016/j.ejogrb.2008.03.009

Pelzer, E., Allan, J., Waterhouse, M., Ross, T., Beagley, K., & Knox, C. (2013). Microorganisms within Human Follicular Fluid: Effects on IVF. *PLoS One*, *8*(3), e59062. https://doi.org/10.1371/journal.pone.0059062

Petro, E., D&Apos;Hollander, W., Covaci, A., Bervoets, L., Fransen, E., De Neubourg, D., … Bols, P. (2014). Perfluoroalkyl acid contamination of follicular fluid and its consequence for in vitro oocyte developmental competence. *Science of the Total Environment*, *496*, 282–288. https://doi.org/10.1016/j.scitotenv.2014.07.028

Piotr Szkodziak, Slawomir Wozniak, Piotr Czuczwar, Ewa Wozniakowska, Paweł Milart, Artur Mroczkowski, & Tomasz Paszkowski. (2016). Infertility in the light of new scientific reports – focus on male factor. *Annals of Agricultural and Environmental Medicine*, *23*(2), 227–230. https://doi.org/10.5604/12321966.1203881

Quaak, I., de Cock, M., de Boer, M., Lamoree, M., Leonards, P., & van de Bor, M. (2016). Prenatal Exposure to Perfluoroalkyl Substances and Behavioral Development in Children. *International Journal of Environmental Research and Public Health*, *13*(5). https://doi.org/10.3390/ijerph13050511

Rotander, A., Toms, L., Aylward, L., Kay, M., & Mueller, J. (2015). Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF). *Environment International*, *82*, 28–34. https://doi.org/10.1016/j.envint.2015.05.005

Sifakis, S., Androutsopoulos, V., Tsatsakis, A., & Spandidos, D. (2017). Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. *Environmental Toxicology and Pharmacology*, *51*, 56–70. https://doi.org/10.1016/j.etap.2017.02.024

Skakkebaek, N., Rajpert-De Meyts, E., Louis, G., Toppari, J., Andersson, A., Eisenberg, M., … Juul, A. (2016). Male Reproductive Disorders and Fertility Trends: Influences of Environment and

Genetic Susceptibility. *Physiological Reviews*, *96*(1), 55–97. https://doi.org/10.1152/physrev.00017.2015

Stahl, T., Mattern, D., & Brunn, H. (2011). Toxicology of perfluorinated compounds. *Environmental Sciences Europe*, *23*(1), 1–52. https://doi.org/10.1186/2190-4715-23-38

Stubleski, J., Salihovic, S., Lind, L., Lind, P., van Bavel, B., & Kärrman, A. (2016). Changes in serum levels of perfluoroalkyl substances during a 10-year follow-up period in a large population-based cohort. *Environment International*, *95*, 86–92. https://doi.org/10.1016/j.envint.2016.08.002

Toms, L., Calafat, A., Kato, K., Thompson, J., Harden, F., Hobson, P., … Mueller, J. (2009). Polyfluoroalkyl chemicals in pooled blood serum from infants, children, and adults in Australia. *Environmental Science & Technology*, *43*(11), 4194–4199. https://doi.org/10.1021/es900272u

Toms, L., Thompson, J., Rotander, A., Hobson, P., Calafat, A., Kato, K., … Mueller, J. (2014). Decline in perfluorooctane sulfonate and perfluorooctanoate serum concentrations in an Australian population from 2002 to 2011. *Environment International*, *71*, 74–80. https://doi.org/10.1016/j.envint.2014.05.019

Toms, L., Braunig, J., Vijayasarathy, S., Phillips, S., Hobson, P., Aylward, L., … Mueller, J. (2019). Per- and polyfluoroalkyl substances (PFAS) in Australia: Current levels and estimated population reference values for selected compounds. *International Journal Of Hygiene And Environmental Health*, *222*(3), 387–394. https://doi.org/10.1016/j.ijheh.2019.03.004

Vestergaard, S., Nielsen, F., Andersson, A., Hjøllund, N., Grandjean, P., Andersen, H., & Jensen, T. (2012). Association between perfluorinated compounds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive. *Human Reproduction*, *27*(3), 873–880. https://doi.org/10.1093/humrep/der450

Vélez, M., Arbuckle, T., & Fraser, W. (2015). Maternal exposure to perfluorinated chemicals and reduced fecundity: the MIREC study. *Human Reproduction*, *30*(3), 701–709. https://doi.org/10.1093/humrep/deu350

Vagi, S., Azziz-Baumgartner, E., Sjödin, A., Calafat, A., Dumesic, D., Gonzalez, L., … Azziz, R. (2014). Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol a in polycystic ovary syndrome: a case-control study. *BMC Endocrine Disorders*, *14*(1), 86. https://doi.org/10.1186/1472-6823-14-86

Wang, B., Zhang, R., Jin, F., Lou, H., Mao, Y., Zhu, W., … Zhang, J. (2017). Perfluoroalkyl substances and endometriosis-related infertility in Chinese women. *Environment International*, *102*, 207–212. https://doi.org/10.1016/j.envint.2017.03.003

Whitworth, W., Haug, S., Baird, D., Becher, A., Hoppin, P., Skjaerven, P., … Longnecker, P. (2012). Perfluorinated Compounds and Subfecundity in Pregnant Women. *Epidemiology*, *23*(2), 257–263. https://doi.org/10.1097/EDE.0b013e31823b5031