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Per- and polyfluoroalkyl substances and male reproductive health: a systematic review of the epidemiological evidence

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ABSTRACT

Exposure to environmental pollutants may produce impairment of male reproductive health. The epidemiological literature evaluating potential consequences of human exposure to per- and polyfluoroalkyl substances (PFAS) has grown in recent years with concerns for both pre- and postnatal influences. The aim of this systematic review was to assess available evidence on associations between PFAS exposures in different stages of life and semen quality, reproductive hormones, cryptorchidism, hypospadias, and testicular cancer. A systematic search of literature published prior to March 9th, 2020, was performed in the databases PubMed and Embase®. Predefined criteria for eligibility were applied by two authors screening study records independently. Among the 242 study records retrieved in the literature search, 26 studies were eligible for qualitative assessment. While several investigations suggested weak associations for single compounds and specific outcomes, a lack of consistency across studies limited conclusions of overall evidence. The current gap in knowledge is particularly obvious regarding exposures prior to adulthood, exposure to combinations of both PFAS and other types of environmental chemicals, and outcomes such as cryptorchidism, hypospadias, and testicular cancer. Continued efforts to clarify associations between PFAS exposure and male reproductive health through high-quality epidemiological studies are needed.

KEYWORDS

Per- and polyfluoroalkyl substances (PFAS); semen quality; reproductive hormones; male reproduction; systematic review

Introduction

The story of per- and polyfluoroalkyl substances (PFAS) has long been recounted as a nonstick nightmare. Following the introduction of PFAS in industrial products and processes in the 1940s, these chemicals quickly gained global usage as potent synthetic surfactants (IARC 2017; Olsen et al. 1998). Thus, PFAS were commonly found in coated cookware, food packaging, cosmetics, textiles, carpets, paints, lubricants, and firefighting foams (IARC 2017; Kotthoff et al. 2015). With the extensive production of especially perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), compounds eventually spread as bio-persistent environmental pollutants to air, dust, soil, and water around the world (EPA – United States Environmental Protection Agency 2018; IARC 2017). Exposure therefore, continues despite restrictions implemented in the last decades on production of several PFAS (Chemical Watch – Global risk and regulation news 2019).

In humans, ingestion is the primary source of systemic uptake with contributions from inhalation and dermal absorption (Bach et al. 2016; Itoh et al. 2016). Due to the innate strength of their fluorocarbon bonds, several common PFAS are not metabolized and subsequent elimination from the body is slow depending mainly upon excretion through bile and urine (Han et al. 2012). Humans have the highest renal tubular reabsorption of PFAS (99.94%) of all studied species and, therefore, also uniquely long elimination half-lives (4.8 years for PFOS and 3.5 years for PFOA) (Han et al. 2012; Olsen et al. 2007). Further, PFAS are known to readily cross the placental barrier and accumulate in the fetus and subsequently present in breast milk. Thus, exposure may begin *in utero* and typically continues through infancy and later life (Bach et al. 2016; IARC 2017).

Despite the extent of exposure, the toxic profile for PFAS remains incomplete with concerns for

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a range of adverse health effects (United Nations Environment Programme and the World Health Organization 2013). While PFAS are a large family of thousands of different chemicals, only few common compounds have routinely been included in risk assessments. These PFAS may exert damage especially to reproductive functions through endocrine disruption (Bonde et al. 2016). Additional suggested mechanisms of action include alterations in epigenetic inheritance and gene expression as well as direct cellular toxicity mediated by changes in membrane properties and, ultimately, induction of apoptosis (Itoh et al. 2016; Leter et al. 2014; Specht et al. 2012). In 2017, the International Agency for Research on Cancer (IARC) also classified PFOA as *possibly* carcinogenic in humans (group 2B) with positive associations observed for testicular cancer (IARC 2017). Evidence of effects of PFAS in animals include testicular Leydig cell hyperplasia and adenoma, changes in reproductive hormones and lower sperm counts (Bach et al. 2016; IARC 2017; Zhao et al. 2014).

In the past 70 years, the global rise in the production of synthetic environmental toxicants has been exponential (Sutton et al. 2012). During the same time, research has repeatedly confirmed an ominous and puzzling decline in several aspects of male reproductive function (Skakkebaek et al. 2016). While temporal trends in semen quality have been a matter of widespread controversy, the documented rise in testicular cancer in many areas of the world is indisputable (Skakkebaek et al. 2016). Additional concerns involve reports of lower testosterone levels and higher incidences of congenital malformations, especially cryptorchidism and hypospadias (Skakkebaek et al. 2016). Ultimately, these changes may add to the burden of male infertility, needs for assisted reproductive treatments, and involuntary childlessness. Increasing our understanding of potential, underlying etiological factors may help provide a rational basis for future prevention of male reproductive disorders.

The mounting literature on associations between exposure to PFAS and male reproductive health has been reviewed in the past with no clear verdicts reached (Bach et al. 2016; Bonde et al. 2016; Perry, Nguyen, and Porter 2016). With the addition of several epidemiological studies addressing this issue in recent years, the aim of this review is to reassess the evidence in a systematic review with the following three main hypotheses: (1) multiple PFAS contribute to risk of specific reproductive outcomes; (2) specific PFAS are associated with risk of specific reproductive outcomes; and (3) reproductive toxicity of PFAS is life stage-dependent (prenatal, childhood, adolescence, and adulthood exposures).

Materials and methods

This systematic review was conducted in accordance with the MOOSE guidelines for observational studies (Stroup et al. 2000).

Search strategy

A systematic search of literature in the databases PubMed and Embase® was performed. With the assistance of a trained research librarian, relevant publications were identified through combinations of index (MeSH and Emtree) and free text search terms for PFAS and selected male reproductive outcomes. A filter was applied in the search procedures limiting findings to original research involving human data published in English. Information on all studies identified prior to the final search on March 9th, 2020, was downloaded using EndNote X9 software (Clarivate Analytics, Boston, MA). The full search specification is presented in online Supplementary Table S1.

Eligibility criteria

Several criteria for inclusion of studies in the systematic review were defined *a priori*.

Exposures

All compounds belonging to the large family of PFAS qualified for assessment. Documentation of quantified environmental (including occupational) exposure to specific PFAS was required as direct measurements in biological samples such as blood, serum, urine, seminal fluid, amniotic fluid, breast milk, placenta and adipose tissue or application of individual level models or proxies. Exposures in all stages of life were included.

Outcomes

(1) Semen quality assessed through measures of semen volume, sperm concentration, total

sperm count, sperm motility, morphology, or DNA damage.
(2) Reproductive hormones including measures of testosterone, free androgen index, estradiol, sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, prolactin, and inhibin B.
(3) Congenital malformations of the genitals assessed through diagnoses of cryptorchidism and hypospadias given at birth or during childhood.
(4) Testicular cancer diagnosed at any age and regardless of histological classification. Based upon the high long-term survival for testicular cancer, only studies reporting measures of incidence or incident cases for this outcome were included.

Outcomes ascertained by clinical examination and analyzes of biological samples, self-reporting in questionnaires or interviews, retrieval of medical records, or data from health registries were considered eligible.

Associations

At least one quantitative estimate of association between exposure to PFAS and the selected outcomes was required to be provided such as risk estimates from regression analyses, correlation coefficients or differences in means.

Exclusion criteria

(1) *In vitro* and *in vivo* experimental studies in animals or humans.
(2) Publications of case reports, reviews, letters, editorials, and comments.

Study selection

With the full database search, 242 study records were retrieved. After removal of duplicates (n = 48), two authors screened titles and abstracts independently and identified 29 investigations requiring full reading. After full reading, 24 studies fulfilled all criteria for eligibility. Discrepancies in evaluations between authors were resolved by consensus. A subsequent hand search of bibliographies of retrieved records and previous systematic reviews was performed and resulted in identification of two additional studies. Unpublished literature was not retrieved. An overview of the study selection process is presented in Figure 1.

Data extraction

For each study included, descriptive characteristics and results were extracted (Tables 1–4 and S3-S5). Results were preferentially summarized as estimates of association with 95% confidence intervals (CIs) for specific compounds and outcomes. With the restricted time-frame available for this project, no authors were contacted in attempts to retrieve additional data.

The emerging heterogeneity among studies regarding especially choice of statistical analyzes, use of (un) transformed exposure and/or outcome scales and reported estimates rendered a quantitative assessment of data in a meta-analysis inappropriate (Rodriguez-Barranco et al. 2017).

Quality assessment

All studies were evaluated for sufficiency of reporting, bias, and confounding using a standardized form adapted from Bonde et al. (2016); Bonzini, Coggon, and Palmer (2007); Shamliyan, Kane, and Dickinson (2010). Two authors completed the forms separately and without blinding. Potential discrepancies in ratings were settled through consensus (Supplementary Tables A, B, and C).

First, sufficiency of reporting was assessed in the following 10 steps: (1) study design, (2) sampling frame and procedures, (3) inclusion and exclusion criteria, (4) population characteristics of exposed/unexposed or cases/referents, (5) response rates/study numbers reported or given implicitly, (6) methods for exposure assessment, (7) methods for outcome ascertainment, (8) detection level and precision for biological samples, (9) statistical analysis and (10) measures of association with 95% CIs. Items were assigned equal weights with a value of one given for adequate reporting. An added reporting score of ≥ 7 (or ≥ 6 with indirect measures of exposure) was considered sufficient.

Potential sources of bias and confounding were evaluated in 6 domains comprising: (1) reporting of

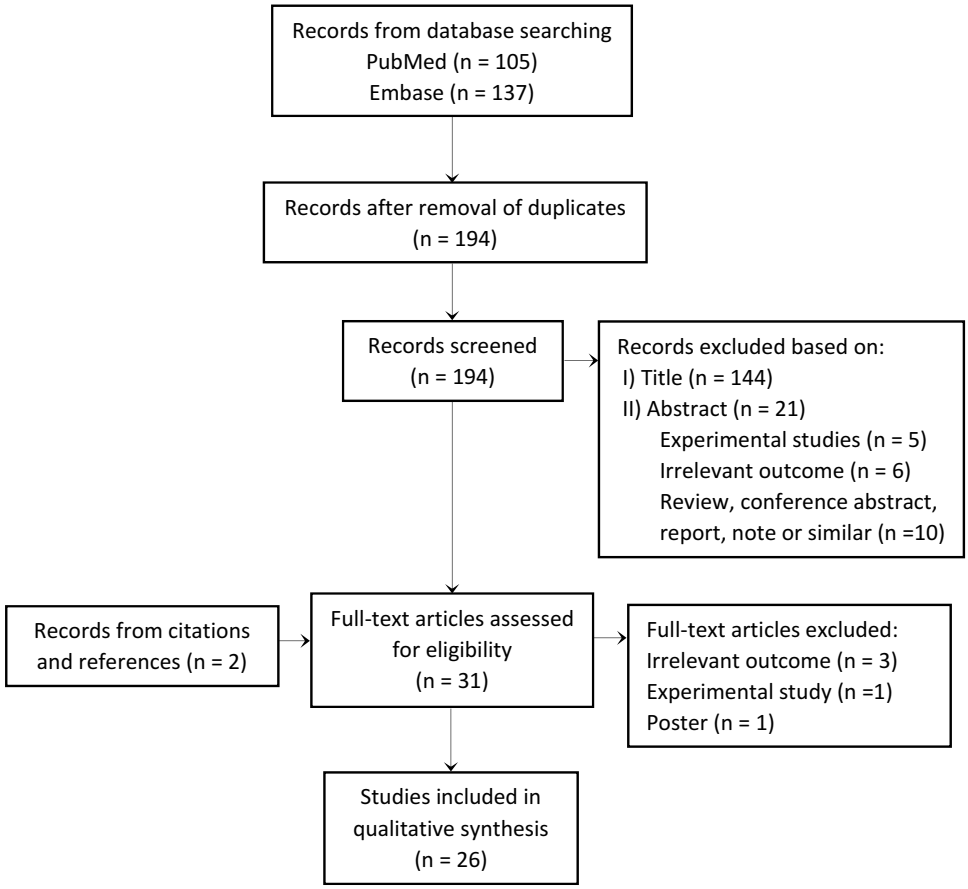

Figure 1.

tested hypotheses, (2) measurement of exposure through self-reporting or use of (in)direct measures, (3) reasonable exposure contrasts, (4) selection bias from loss to follow-up or lack of representativeness in a population sample, (5) information bias related to outcome ascertainment and (6) accounting for confounding of relevance to the individual outcome (factors are listed in Supplementary Table C). Domain 1, 4, 5 and 6 were considered the most important. Studies with two or more of these potential sources of bias were considered of higher risk of bias. The assigned quality assessment scores for the individual studies are presented in Table 1.

Results

Study characteristics

Selected characteristics of all 26 studies included in our qualitative analysis of associations between exposure to PFAS and male reproductive health are shown in Table 1. Nineteen studies were cross-sectional, 4 were case-control studies, and 3 were cohort studies. Twelve studies were conducted in Europe (including Greenland), 8 in the United States of America, and 6 in Asia. The included study populations were exposed either occupationally (Barry, Winquist, and Steenland 2013; Costa, Sartori, and Consonni 2009; Olsen et al. 1998; Sakr et al. 2007) or through other environmental sources. Most investigators reported measures of association for semen quality (n = 13) and/or reproductive hormones (n = 17). Only few contributed information on cryptorchidism, hypospadias, and testicular cancer (Tables 2–4).

Exposure measurements covered 20 different compounds in the PFAS family, from which only PFOS and/or PFOA were included consistently (Supplementary Table S3). Five studies also assessed combined measures for several PFAS (Governini et al. 2015; Huang et al. 2019; Joensen et al. 2009; Song et al. 2018; Yao et al. 2019). Reported median exposure levels ranged from

Table 1. Characteristics of studies on the association between exposure to per- and polyfluoroalkyl substances (PFAS) and male reproductive health.

						Serum level (ng/mL)					
						PFOS		PFOA			
Study (year)	Location	Study design	Period	n	Population	Mean	Median	Mean	Median	CR [a]	Bias [b]
Olsen et al. (1998)	USA	Cross-sectional	1993, 1995	123	Workers at PFOA plant					6	1
Sakr et al. (2007)	USA, West Virginia	Cross-sectional	2004	782	Current workers at plant				0.5 [c]	8	0
Costa, Sartori, and Consonni (2009)	Italy	Cross-sectional	2000–2007	56	Workers at PFOA plant				5.7 [d]	9	1
Joensen et al. (2009)	Denmark	Cross-sectional	2003	105	Men reporting for military draft		24.5		4.9	9	1
					Testosterone profile: Low/High						
Raymer et al. (2012)	USA, North Carolina	Cross-sectional	2002–2005	256	Patients at infertility clinic	37.4	32.3 [e]	10.4	9.2	6	1
Specht et al. (2012)	Greenland, Poland and Ukraine	Cross-sectional	2002–2004	604	Partners of pregnant women	51.9	44.7	4.8	4.5	9	0
						18.6	18.5	5.1	4.8		
						8.1	7.6	1.8	1.3		
Toft et al. (2012)	Greenland, Poland and Ukraine	Cross-sectional	2002–2004	588	Partners of pregnant women		18.4		3.8	9	0
Barry, Winquist, and Steenland (2013)	USA, West Virginia and Ohio	Cohort	1952–2011	14,894	Community in contaminated area				24.2	8	1
					Workers at PFOA plant				112.7		
Joensen et al. (2013)	Denmark	Cross-sectional	2008–2009	247	Men reporting for military draft	8.5	7.79	3.5	3.0	9	0
Vested et al. (2013)	Denmark	Cohort	2008–2009	169	Adult sons of pregnancy cohort		21.2		3.8	10	0
Vieira et al. (2013)	USA, West Virginia and Ohio	Case-control	1996–2005	134 cases	Men with incident cancers					8	1
Jensen et al. (2014)	Denmark and Finland	Nested case-control	1997–2002	215	Danish-Finnish birth cohort		9.1		2.6	10	0
							5.2		2.1		
Leter et al. (2014)	Greenland, Poland and Ukraine	Cross-sectional	2002–2004	262	Partners of pregnant women	27.2		4.0		9	0
Governini et al. (2015)	Italy	Cross-sectional	-	59	Patients at sterility center					7	1
Den Hond et al. (2015)	Belgium	Case-control	-	163	Patients at four fertility clinics	8.7 [f]		2.4		8	1
Lewis, Johns, and Meeker (2015)	USA	Cross-sectional	2011–2012	857	National health survey participants		4.6–11.1 [g]		1.9–2.5	9	0
Louis et al. (2015)	USA, Michigan and Texas	Cross-sectional	2005–2009	462	Pregnancy planners: The LIFE study	20.4	20.4	4.9	5.2	8	1
Tsai et al. (2015)	Taiwan	Cross-sectional	2006–2008	210	Screening population, 1–12. grade	9.0		2.8		9	1
Itoh et al. (2016)	Japan	Cohort	2002–2005	83	Birth cohort: the Hokkaido Study		5.4		1.6	8	1
Lopez-Espinosa et al. (2016)	USA, West Virginia and Ohio	Cross-sectional	2005–2006	1,169	Children in contaminated area		22.4		34.8	9	1
Toft et al. (2016)	Denmark	Case-control	1980–1996	645	Pregnancy-screening registry					10	0
Zhou et al. (2016)	Taiwan	Cross-sectional	2009–2010	102	Childhood asthma control cohort		29.9		0.5	8	1
Petersen et al. (2018)	Faroe Islands	Cross-sectional	2007–2009	263	All men born 1981–1984		19.5		2.8	10	1
Song et al. (2018)	China, Guangdong	Cross-sectional	2012–2013	103	Patients at infertility clinic	120	96	9.1	7.9	6	1
Yao et al. (2019)	China, Shandong	Cross-sectional	2010–2013	180	Birth cohort		1.4		34.7	8	0
Huang et al. (2019)	China, Chongqing	Cross-sectional	2009–2010	57	Patients at fertility clinic	6.5	5.3	2.5	1.7	8	1

PFOS, Perfluorooctane sulfonate; PFOA, Perfluorooctanoate
Estimates with more than one decimal were rounded
[a] CR, Completeness of reporting on a scale from 0–10
[b] Bias and confounding: 0 = lower risk of bias and 1 = higher risk of bias
[c] ppm among current workers, [d] µg/mL among current workers in 2007, [e] Plasma levels, [f] Geometric means for cases, [g] Medians across age groups

Table 2. Summary of results from studies on the association between exposure to per- and polyfluoroalkyl substances (PFAS) and semen parameters.

Outcome	Study (year)	Age [a]	Outcome scale [b]	Outcome measure [c]	PFOS estimate	PFOA estimate
Semen volume	Joensen et al. (2009)	A	Ln (mL)	β (95% CI)	0.00 (−0.01, 0.01)	0.00 (−0.07, 0.07)
	Raymer et al. (2012)	A	mL	β (95% CI)	0.00 (−0.01, 0.01)	0.01 (−0.02, 0.05)
	Toft et al. (2012)	A	Ln (mL)	% diff (95% CI)	0 (−24, 25)	10 (−10, 29)
	Joensen et al. (2013)	A	Ln (mL)	β (95% CI)	0.02 (0.00, 0.03)	−0.01 (−0.04, 0.02)
	Vested et al. (2013)	P	Ln (mL)	β (SE)	0.00 (0.01)	−0.01 (0.02)
	Louis et al. (2015)	A	mL	β (95% CI)	0.06 (−0.20, 0.31)	−0.09 (−0.46, 0.27)
	Petersen et al. (2018)	A	Cubic Rt (mL)	β (95% CI)	0.13 (−0.05, 0.31)	−0.06 (−0.23, 0.12)
	Huang et al. (2019)	A	mL	β (95% CI)	0.01 (−0.04, 0.06)	0.00 (−0.09, 0.08)
Sperm concentration	Joensen et al. (2009)	A	Ln (10^6/mL)	β (95% CI)	−0.02 (−0.04, 0.01)	−0.08 (−0.23, 0.07)
	Raymer et al. (2012)	A	10^6/mL	β (95% CI)	0.16 (−0.23, 0.54)	0.14 (−1.24, 1.51)
	Toft et al. (2012)	A	Ln (10^6/mL)	% diff (95% CI)	22 (−21, 64)	15 (−17, 48)
	Joensen et al. (2013)	A	Cubic Rt (10^6/mL)	β (95% CI)	0.01 (−0.03, 0.05)	0.03 (−0.05, 0.11)
	Vested et al. (2013)	P	Ln (10^6/mL)	β (SE)	−0.01 (0.01)	**−0.11 (0.04)**
	Den Hond et al. (2015)	A	10^6/mL	β (SE)	0.50 (0.53)	0.22 (0.22)
	Louis et al. (2015)	A	10^6/mL	β (95% CI)	0.03 (−9.02, 9.07)	0.39 (−12.58, 13.36)
	Petersen et al. (2018)	A	Cubic Rt (10^6/mL)	β (95% CI)	0.22 (−0.90, 1,34)	−0.44 (−1.55, 0.67)
	Song et al. (2018)	A	10^6/mL	Spearman's ρ	0.08	−0.01
	Huang et al. (2019)	A	10^6/mL	β (95% CI)	0.15 (−3.45, 3.74)	−0.07 (−6.37, 6.23)
Total sperm count	Joensen et al. (2009)	A	Ln (10^6)	β (95% CI)	−0.02 (−0.05, 0.01)	−0.07 (−0.23, 0.09)
	Toft et al. (2012)	A	Ln (10^6)	% diff (95% CI)	18 (−32, 67)	22 (−17, 62)
	Joensen et al. (2013)	A	Cubic Rt (10^6)	β (95% CI)	0.05 (−0.01, 0.10)	0.03 (−0.08, 0.13)
	Vested et al. (2013)	P	Ln (10^6)	β (SE)	−0.02 (0.01)	**−0.20 (0.06)**
	Louis et al. (2015)	A	conc x 10^6/mL	β (95% CI)	9.74 (−20.24, 39.72)	0.68 (−42.30, 43.66)
	Petersen et al. (2018)	A	Cubic Rt (10^6)	β (95% CI)	0.82 (−0.98, 2.61)	−0.84 (−2.61, 0.94)
	Huang et al. (2019)	A	10^6	β (95% CI)	−0.55 (−9.85, 8.76)	−0.69 (−16.98, 15.61)
Motility	Joensen et al. (2009)	A	Ln (% motile)	β (95% CI)	−0.01 (−0.02, 0.01)	−0.03 (−0.11, 0.05)
	Raymer et al. (2012)	A	% motile	β (95% CI)	0.04 (−0.07, 0.16)	0.27 (−0.13, 0.67)
	Toft et al. (2012)	A	Ln (% motile)	% diff (95% CI)	−1 (−26, 25)	**19 (1,39)**
	Joensen et al. (2013)	A	Sq (% progressive)	β (95% CI)	−37.2 (−93.1, 18.7)	−4.43 (−109, 100)
	Vested et al. (2013)	P	Ln (% progressive)	β (SE)	0.00 (0.00)	−0.02 (0.01)
	Den Hond et al. (2015)	A	% motile	β (SE)	14.0 (7.39)	2.37 (3.20)
	Louis et al. (2015)	A	% motile	β (95% CI)	1.56 (−0.36, 3.47)	1.44 (−1.32, 4.19)
	Petersen et al. (2018)	A	Logit (% motile)	β (95% CI)	0.06 (−0.79, 0.91)	−0.25 (−1.13, 0.64)
	Song et al. (2018)	A	% progressive	Spearman's ρ	**−0.23**	**−0.21**
	Huang et al. (2019)	A	% progressive	β (95% CI)	0.08 (−1.04, 1.20)	−0.24 (−2.20, 1.72)
Morphology	Joensen et al. (2009)	A	% normal	β (95% CI)	−0.09 (−0.02, 0.03)	−0.54 (−1.2, 0.11)
	Toft et al. (2012)	A	Ln (% normal)	% diff (95% CI)	**−35 (−66, −4)**	10 (−13, 34)
	Joensen et al. (2013)	A	Sq Rt (% normal)	β (95% CI)	0.00 (−0.03, 0.04)	−0.01 (−0.07, 0.05)
	Vested et al. (2013)	P	Ln (% normal)	β (SE)	−0.01 (0.01)	−0.05 (0.03)
	Den Hond et al. (2015)	A	% normal	β (SE)	0.56 (0.51)	0.24 (0.25)
	Louis et al. (2015)	A	% normal	β (95% CI)	1.84 (−0.31, 3.98)	1.73 (−1.35, 4.80)
	Petersen et al. (2018)	A	% normal	β (95% CI)	−0.30 (−3.69, 3.09)	−1.49 (−5.06, 2.07)
	Huang et al. (2019)	A	% normal	β (95% CI)	0.00 (0.00, 0.01)	0.00 (−0.01, 0.01)
DNA fragmentation [d]	Louis et al. (2015)	A	%	β (95% CI)	−0.91 (−2.68, 0.85)	−0.71 (−3.18, 1.76)

PFOS, Perfluorooctane sulfonate; PFOA, Perfluorooctanoate
Estimates for the highest exposure groups shown. Estimates with more than two decimals were rounded
[a]Exposure age; A = Adult and *P* = Prenatal
[b]Transformation: ln, natural logarithm; Cubic Rt, cubic root; Sq, squared; Sq Rt, square root
[c]β, regression coefficient; diff, difference
[d]Specht et al. (2012), Leter et al. (2014), Governini et al. (2015) reported additional measures of sperm DNA damage

1.4 ng/mL to 96 ng/ml for PFOS and 0.5 ng/mL to 5700 ng/mL for PFOA (Table 1). Although exposures were predominantly measured or modeled in adults, a number of investigations involved prenatal (Itoh et al. 2016; Toft et al. 2016; Vested et al. 2013), birth (Jensen et al. 2014; Yao et al. 2019), childhood (Lopez-Espinosa et al. 2016), and adolescence (Lewis, Johns, and Meeker 2015; Tsai et al. 2015; Zhou et al. 2016) exposure levels. Study periods spanned the years 1952–2013 with large variations in sample sizes from 56 to 14,894 subjects.

In the quality assessment, the completeness of reporting was generally high with only three studies scoring below the sufficiency limit (Olsen et al. 1998; Raymer et al. 2012; Song et al. 2018) (Table 1). On the other hand, more than 60% of investigations (n = 16) were considered at higher risk of bias according to our predefined criteria. Potential

Table 3. Summary of results from studies on the association between exposure to per- and polyfluoroalkyl substances (PFAS) and reproductive hormones.

Outcome	Study (year)	Exposure age	Outcome scale	Outcome measure [a]	PFOS estimate	PFOA estimate
Testosterone	Olsen et al. (1998)	Adult	ng/dL	Pearson's r		1993: 0.01 1995: 0.02
	Sakr et al. (2007)	Adult	-	β (p)		**0.6 (0.03)**
	Costa, Sartori, and Consonni (2009)	Adult	ng/mL	β (95% CI)		−0.01 (−0.02, 0.01)
	Joensen et al. (2009)	Adult	nmol/L	β (95% CI)	−0.09 (−0.32, 0.15)	−0.98 (−2.33, 0.37)
	Raymer et al. (2012)	Adult	ng/mL	Spearman's ρ (p)	−0.01 (0.83)	0.05 (0.44)
	Joensen et al. (2013)	Adult	Ln (nmol/mL)	β (95% CI)	**−0.01 (−0.02, 0.00)**	0.00 (−0.02, 0.02)
	Vested et al. (2013)	Prenatal	Ln (nmol/L)	β (SE)	0.00 (0.00)	0.00 (0.01)
	Den Hond et al. (2015)	Adult	ng/dL	β (SE)	−0.07 (0.05)	0.01 (0.03)
	Lewis, Johns, and Meeker (2015)	12–20 years	ng/dL	% diff (95% CI)	7.9 (−9.1, 28.1)	17.3 (−9.4, 51.8)
		20–40 years			-1.2 (−5.1, 2.9)	-0.5 (−5.0, 4.1)
		40–60 years			-2.7 (−7.3, 2.2)	-1.4 (−7.9, 5.5)
		60–80 years			4.9 (−1.9, 12.1)	7.2 (−1.9, 17.1)
	Tsai et al. (2015)	12–17 years	Ln (ng/dL)	Mean (SD)	6.34 (0.31)	6.48 (0.30)
		18–30 years			6.33 (0.05)	6.28 (0.09)
	Itoh et al. (2016)	Prenatal	pg/mL	β (95% CI)	−0.03 (−0.37,0.31)	−0.16 (−0.47, 0.15)
	Lopez-Espinosa et al. (2016)	6–9 years	Ln (ng/dL)	% diff (95% CI)	**−5.8 (−9.4, −2.0)**	**−4.9 (−8.7, −0.8)**
	Toft et al. (2016)	Prenatal	nmol/L	% diff (95% CI)	**0.16 (0.09, 0.23)**	
	Zhou et al. (2016)	13–15 years	Ln (nmol/L)	β (95% CI)	**−0.003 (−0.006, 0.000)**	−0.05 (−0.12, 0.01)
	Petersen et al. (2018)	Adult	Log (nmol/L)	β (95% CI)	0.11 (−0.16, 0.38)	−0.11 (−0.39, 0.16)
	Yao et al. (2019)	Birth	Log (ng/mL)	β (95% CI)	0.12 (−0.04, 0.28)	0.00 (−0.11, 0.11)
Free testosterone or FAI	Olsen et al. (1998)	Adult	ng/dL	Pearson's r		1993: 0.09 1995: 0.01
	Joensen et al. (2009)	Adult	Ln	β (95% CI)	−0.01 (−0.02, 0.00)	−0.04 (−0.09, 0.01)
	Raymer et al. (2012)	Adult	pg/mL	Spearman's ρ (p)	−0.01 (0.84)	**0.16 (0.02)**
	Joensen et al. (2013)	Adult	Ln	β (95% CI)	**−0.02 (−0.03, −0.01)**	0.01 (−0.01, 0.04)
	Vested et al. (2013)	Prenatal	Ln (nmol/L)	β (SE)	0.00 (0.00)	0.01 (0.01)
	Den Hond et al. (2015)	Adult	ng/dL	β (SE)	0.04 (0.10)	0.06 (0.05)
	Petersen et al. (2018)	Adult	Log (pmol/L)	β (95% CI)	−0.03 (−0.30, 0.25)	−0.28 (−0.56, 0.00)
Estradiol	Olsen et al. (1998)	Adult	pg/mL	Pearson's r		1993: 0.12 1995: 0.15
	Sakr et al. (2007)	Adult	-	β (p)		**22.3 (0.02)**
	Costa, Sartori, and Consonni (2009)	Adult	pg/mL	β (95% CI)		−0.01 (−0.08, 0.06)
	Joensen et al. (2009)	Adult	Ln (pmol/L)	β (95% CI)	0.00 (−0.01, 0.01)	−0.01 (−0.05, 0.03)
	Raymer et al. (2012)	Adult	pg/mL	Spearman's ρ (p)	0.02 (0.77)	0.02 (0.75)
	Joensen et al. (2013)	Adult	Ln (pmol/L)	β (95% CI)	−0.01 (−0.02, 0.00)	0.00 (−0.02, 0.02)
	Vested et al. (2013)	Prenatal	Ln (nmol/L)	β (SE)	0.00 (0.00)	0.02 (0.01)
	Den Hond et al. (2015)	Adult	pg/mL	β (SE)	−0.02 (0.04)	0.01 (0.02)
	Itoh et al. (2016)	Prenatal	ng/mL	β (95% CI)	**0.37 (0.06, 0.69)**	−0.13 (−0.44, 0.17)
	Lopez-Espinosa et al. (2016)	6–9 years	Ln (pg/mL)	% diff (95% CI)	**−4.0 (−7.7, −0.1)**	4.3 (−0.4, 9.1)
	Zhou et al. (2016)	13–15 years	Ln (pmol/L)	β (95% CI)	0.002 (−0.001, 0.006)	**0.09 (0.02, 0.17)**
	Petersen et al. (2018)	Adult	Log (nmol/L)	β (95% CI)	0.07 (−0.15, 0.30)	−0.01 (−0.25, 0.23)
	Yao et al. (2019)	Birth	Log (pg/mL)	β (95% CI)	0.02 (−0.05, 0.09)	0.04 (−0.01, 0.08)
SHBG	Olsen et al. (1998)	Adult	-	Pearson's r		1993: −0.07 1995: 0.03
	Joensen et al. (2009)	Adult	Ln (nmol/L)	β (95% CI)	0.00 (−0.01, 0.01)	−0.01 (−0.07, 0.05)
	Specht et al. (2012)	Adult	nmol/L	Mean (95% CI)		27.0 (23.6, 31.0)
	Joensen et al. (2013)	Adult	Ln (nmol/L)	β (95% CI)	0.01 (0.00, 0.02)	−0.01 (−0.04, 0.01)
	Vested et al. (2013)	Prenatal	Ln (nmol/L)	β (SE)	0.00 (0.00)	−0.01 (0.01)
	Den Hond et al. (2015)	Adult	nmol/L	β (SE)	−0.03 (0.06)	−0.03 (0.03)
	Tsai et al. (2015)	12–17 years	Ln (nmol/L)	Mean (SD)	3.46 (0.39)	3.79 (0.39)
		18–30 years			3.16 (0.08)	3.10 (0.14)
	Itoh et al. (2016)	Prenatal	nmol/L	β (95% CI)	−0.05 (−0.17, 0.06)	0.01 (−0.10, 0.12)

(*Continued*)

Table 3. (Continued).

Outcome	Study (year)	Exposure age	Outcome scale	Outcome measure [a]	PFOS estimate	PFOA estimate
	Petersen et al. (2018)	Adult	Log (nmol/L)	β (95% CI)	**0.31 (0.02, 0.60)**	0.23 (−0.08, 0.53)
LH	Olsen et al. (1998)	Adult	-	Pearson's r		1993: −0.06 1995: 0.13
	Joensen et al. (2009)	Adult	Ln (IU/L)	β (95% CI)	0.00 (−0.01, 0.01)	−0.01 (−0.08, 0.06)
	Raymer et al. (2012)	Adult	mIU/mL	Spearman's ρ (p)	0.12 (0.06)	**0.16 (0.01)**
	Joensen et al. (2013)	Adult	Ln (IU/L)	β (95% CI)	0.01 (−0.01, 0.02)	0.01 (−0.02, 0.03)
	Vested et al. (2013)	Prenatal	Ln (IU/L)	β (SE)	0.00 (0.00)	**0.04 (0.02)**
	Den Hond et al. (2015)	Adult	mU/mL	β (SE)	−0.05 (0.07)	−0.04 (0.03)
	Itoh et al. (2016)	Prenatal	mIU/mL	β (95% CI)	−0.24 (−0.64, 0.16)	0.07 (−0.30, 0.44)
	Petersen et al. (2018)	Adult	Log (IU/L)	β (95% CI)	**0.35 (0.02, 0.68)**	−0.11 (−0.46, 0.24)
FSH	Olsen et al. (1998)	Adult	-	Pearson's r		1993: −0.12 1995: −0.13
	Joensen et al. (2009)	Adult	Ln (IU/L)	β (95% CI)	0.00 (−0.13, 0.22)	−0.04 (−0.14, 0.06)
	Raymer et al. (2012)	Adult	mIU/mL	Spearman's ρ (p)	0.04 (0.58)	0.04 (0.58)
	Joensen et al. (2013)	Adult	Ln (IU/L)	β (95% CI)	0.01 (−0.01, 0.03)	0.02 (−0.01, 0.06)
	Vested et al. (2013)	Prenatal	Ln (IU/L)	β (SE)	0.01 (0.01)	**0.06 (0.02)**
	Den Hond et al. (2015)	Adult	mU/mL	β (SE)	−0.13 (0.08)	−0.05 (0.04)
	Tsai et al. (2015)	12–17 years 18–30 years	Ln (mIU/mL)	Mean (SD)	**0.76 (0.29)** 1.26 (0.08)	1.49 (0.36) 1.13 (0.15)
	Itoh et al. (2016)	Prenatal	mIU/mL	β (95% CI)	−0.03 (−0.32, 0.27)	−0.14 (−0.41, 0.13)
	Petersen et al. (2018)	Adult	Log (IU/L)	β (95% CI)	0.23 (−0.25, 0.72)	0.32 (−0.19, 0.83)
Progesterone	Itoh et al. (2016)	Prenatal	ng/mL	β (95% CI)	**−0.34 (−0.68, −0.01)**	0.26 (−0.06, 0.57)
	Toft et al. (2016)	Prenatal	nmol/L	% diff (95% CI)	**0.21 (0.14, 0.29)**	
Prolactin	Olsen et al. (1998)	Adult	µg/L	Pearson's r		1993: 0.04 1995: −0.04
	Raymer et al. (2012)	Adult	ng/mL	Spearman's ρ (p)	0.10 (0.11)	0.06 (0.35)
	Itoh et al. (2016)	Prenatal	ng/mL	β (95% CI)	−0.13 (−0.34, 0.08)	0.04 (−0.15, 0.24)
Inhibin B	Joensen et al. (2009)	Adult	Ln (pg/mL)	β (95% CI)	−0.00 (−0.21, 0.12)	0.01 (−0.08, 0.11)
	Joensen et al. (2013)	Adult	Ln (pg/mL)	β (95% CI)	0.00 (−0.01, 0.02)	−0.01 (−0.03, 0.02)
	Vested et al. (2013)	Prenatal	Ln (pg/mL)	β (SE)	0.00 (0.00)	−0.02 (0.02)
	Den Hond et al. (2015)	Adult	pg/mL	β (SE)	0.12 (0.08)	0.07 (0.04)
	Itoh et al. (2016)	Prenatal	pg/mL	β (95% CI)	**−0.44 (−0.62, −0.26)**	**0.20 (0.01, 0.38)**
	Petersen et al. (2018)	Adult	Log (pg/mL)	β (95% CI)	0.09 (−0.51, 0.69)	0.07 (−0.56, 0.69)

PFOS, Perfluorooctane sulfonate; PFOA, Perfluorooctanoate; FAI, free androgen index; SHBG, sex hormone-binding globulin; LH, luteinizing hormone; FSH, follicle-stimulating hormone

Estimates for the highest exposure groups shown. Estimates with more than two decimals were rounded (except for Zhou et al.)

[a]β, regression coefficient; diff, difference

Specht et al. (2012) performed analyses using general linear models for testosterone, estradiol, SHBG, LH, FSH and inhibin B, found no associations with PFAS exposure and reported estimates only for SHBG.

Table 4. Summary of results from studies on the association between exposure to per-and polyfluoroalkyl substances (PFAS), testicular cancer and congenital genital malformations.

Outcome	Study (year)	Exposure age	Exposure assessment	Cases	Outcome measure	PFOS estimate	PFOA estimate
Testicular cancer	Barry, Winquist, and Steenland (2013)	≥ 20 years	Modeled PFOA	17	HR (95% CI)	-	**1.34 (1.00, 1.79)**
	Vieira et al. (2013)	-	Modeled PFOA	134	OR (95% CI)	-	1.00 (0.6, 1.8)
Cryptorchidism	Toft et al. (2016)	Prenatal	Amniotic fluid PFOS	270	OR (95% CI)	0.99 (0.75, 1.30)	-
	Jensen et al. (2014)	Birth	Cord blood serum	107	OR (95% CI)	0.83 (0.39–1.78)	0.46 (0.20–1.02)
Hypospadias	Toft et al. (2016)	Prenatal	Amniotic fluid PFOS	75	OR (95% CI)	0.87 (0.57, 1.34)	-

PFOS, Perfluorooctane sulfonate; PFOA, Perfluorooctanoate; HR, Hazard ratio; OR, Odds ratio

issues with residual confounding were the main concern in these studies with large variations in covariates included in analyzes. A full overview of the available covariates in studies of semen quality and reproductive hormones is presented in online Supplementary Tables S4 and S5.

Semen characteristics

An overview of results from studies on semen quality is presented in Table 2. Overall, there were no consistent indications of an association between exposure to PFAS and semen quality measured through either semen volume, sperm concentration, total sperm count, sperm motility, morphology, or DNA damage. In the 8 investigations reporting estimates for PFAS exposure and semen volume, results were all close to unity. Sperm concentration was assessed in 10 studies (Table 2). While Huang et al. (2019) noted a positive association between exposure to perfluorohexane sulfonate (PFHxS) and sperm concentrations, earlier studies did not support this finding. In the study by Vested et al. (2013), prenatal exposure to PFOA was associated with a numerically lower sperm concentration and sperm count among young Danish men. Total sperm count was determined in 6 additional studies with no observed significant associations for PFAS exposures measured in adulthood.

Across the 10 investigations reporting measures of sperm motility, estimates tended to point in opposite directions (Table 2). While Toft et al. (2012) found a higher % motile sperm with exposure to PFOA, the remaining studies did not support the existence of a positive association. In the study by Joensen et al. (2013), exposure to perfluoroheptane sulfonate (PFHpS) was associated with a lower % progressively motile sperm. This compound was, however, not assessed in any of the remaining studies in this review (Supplementary Table S3). Song et al. (2018) analyzed several other PFAS in a high-level exposure setting in the Guangdong province in China and detected positive correlations between perfluorobutanoate (PFBA), perfluorobutane sulfonate (PFBS), and perfluoropentanoate (PFPeA) measured in blood and progressive sperm motility. Song et al. (2018) also measured analytes in semen and here all correlations were negative for PFBA, PFBS, PFPeA, perfluorohexanoate (PFHxA), PFOS, and PFOA. The strongest negative correlation appeared for the overall sum of PFAS versus progressive sperm motility (Song et al. 2018).

Eight studies examined sperm morphology (Table 2). While Joensen et al. (2009) demonstrated a lower mean % of morphologically normal sperm in men with higher combined exposure to PFOS and PFOA, this relationship was not significant in regression analyses. PFOS and PFHxS exposure was associated with a reduced % of morphologically normal sperm in a study by Toft et al. (2012), but these findings were not corroborated by several later studies (Den Hond et al. 2015; Huang et al. 2019; Joensen et al. 2013; Louis et al. 2015). In addition to overall results, Louis et al. (2015) reported associations for a range of specific morphological sperm changes (i.e. specific head or tail abnormalities) not included in this review.

Four investigators assessed various measures of sperm DNA damage (Governini et al. 2015; Leter et al. 2014; Louis et al. 2015; Specht et al. 2012). Specht et al. (2012) noted no significant relationship between widely varying PFAS exposure levels in a large cohort from three countries and markers of sperm DNA damage or apoptosis. Subsequently, Leter et al. (2014) examined a subset of the same cohort with no evidence of a consistent association with markers of DNA global methylation levels. In the study by Louis et al. (2015), perfluorooctane sulfonamide (PFOSA) and 2-(N-methyl perfluorooctane sulfonamido) acetate (MePFOSAA) exposure was related with a decreased % of sperm with high DNA stainability (indicating immature chromatin structure). Finally, Governini et al. (2015) found higher DNA fragmentation and aneuploidy rates in sperm from Italian men positive for PFOS and/or PFOA exposure. This study was based upon a small number of participants (n = 59) with a crude exposure contrast and a higher risk of bias according to our score.

Reproductive hormones

The main results from studies on reproductive hormones are shown in Table 3. Altogether, no consistent associations were detected between exposure to PFAS and reproductive hormone

levels. Testosterone levels were determined in 16 studies. Seven of these investigations also reported estimates for measured or calculated free testosterone or free androgen index. The earliest three studies by Olsen et al. (1998), Sakr et al. (2007) and Costa, Sartori, and Consonni (2009) all represented occupational settings with very high PFOA exposures. Only the largest of these studies by Sakr et al. (2007) indicated an association with higher testosterone in workers (Tables 1 and 3). While Raymer et al. (2012) later noted a positive correlation between PFOA and especially free testosterone in a population of infertile patients, studies in non-occupational settings generally provided little support for these observations. In the study by Joensen et al. (2013), PFOS exposure was associated with numerically lower total and free testosterone. The remaining results for PFOS exposures and testosterone levels measured in adults were, however, mixed.

In adolescents, Zhou et al. (2016) demonstrated lower testosterone with exposure to PFHxS, PFOS, perfluorononanoate (PFNA), and perfluorodecanoate (PFDA). However, Lewis, Johns, and Meeker (2015) and Tsai et al. (2015) performed studies in overlapping age groups with no evidence of any negative associations for testosterone (Table 3). Lopez-Espinosa et al. (2016) examined PFAS in younger boys and found lower testosterone with exposure to both PFOS and PFOA. Toft et al. (2016) noted that prenatal exposure to PFOS was correlated with higher testosterone levels measured in amniotic fluid samples. While the two remaining studies on prenatal exposures offered no support for this finding, these investigators both assessed hormone levels at later stages, at birth and in adults respectively (Itoh et al. 2016; Vested et al. 2013).

Occupational exposure to high levels of PFOA was associated with higher estradiol levels in the study by Sakr et al. (2007) (Table 3). A positive correlation with this outcome was also observed for both PFOA and PFHxS exposure by Zhou et al. (2016) among adolescents. In contrast, 12 other studies assessing estradiol found no significant association between PFOA and estradiol levels. Most of these studies also showed no marked relationship between levels of PFOS and estradiol. Lopez-Espinosa et al. (2016) reported lower estradiol in boys exposed to PFOS, while Itoh et al. (2016) noted higher estradiol levels at birth following prenatal exposure to the same compound.

Across the 9 studies examining SHBG, Petersen et al. (2018) were alone in reporting a positive association with exposure to PFOS (Table 3). Petersen et al. (2018) also reported a positive association between PFOS and LH, while Raymer et al. (2012) and Vested et al. (2013) noted a positive correlation between PFOA and LH. Vested et al. (2013) demonstrated a similar positive relationship between prenatal exposure to PFOA and FSH, while Tsai et al. (2015) reported a negative association for PFOS and FSH in adolescents. In the two studies addressing progesterone in relation to prenatal PFOS exposure, estimates indicated opposite directions (Itoh et al. 2016; Toft et al. 2016) (Table 3). For inhibin B, Itoh et al. (2016) found a negative association with PFOS and a positive relationship with PFOA. In the remaining 5 studies assessing inhibin B and 3 investigations on prolactin, no consistent associations were detected.

Cryptorchidism and hypospadias

In the two identified case-control studies assessing pre- and perinatal PFAS exposure in relation to cryptorchidism and/or hypospadias, no indications of any positive associations were found (Table 4) (Jensen et al. 2014; Toft et al. 2016). The first study by Jensen et al. (2014) was nested in a joint Danish and Finnish birth cohort with exposure to several PFAS measured at birth in cord blood serum. Cases of cryptorchidism (n = 107) were determined at birth through medical examination. A second study by Toft et al. (2016) utilized data from a Danish pregnancy-screening registry with measures of PFOS from amniotic fluid samples collected during pregnancy. Diagnoses of cryptorchidism and hypospadias (n = 270 and n = 75) were retrieved from the Danish National Patient Register.

Testicular cancer

Two studies examined the correlation between exposure specifically to PFOA and testicular cancer (Table 4). Both studies investigated exposures from a chemical plant in West Virginia emitting large quantities of PFOA into the Ohio River and

ambient air (Barry, Winquist, and Steenland 2013; Vieira et al. 2013). In the study by Barry, Winquist, and Steenland (2013), a mixed cohort of 14,894 male plant workers, residents, and school attendants in the contaminated area were followed in the years 1952–2011. Here, exposure was modeled using historical data and diagnoses of cancer were ascertained from survivors via self-reporting in interviews conducted from 2008–2011. Based upon 17 cases, the incidence of testicular cancer was increased in this cohort with indications of a dose-response trend.

Vieira et al. (2013) retrieved information on incident cancer cases and cancer controls for selected counties from 1996–2005 in cancer registries in Ohio and West Virginia. Exposure status was assigned from records of residency and assumed water intake. With 134 cases, no overall increase in testicular cancer was observed among the exposed (Table 4). A non-significant excess was found for this outcome only in the very high exposure group in the Ohio data (Vieira et al. 2013).

Discussion

In this first systematic review of the epidemiological evidence linking PFAS exposures in all stages of life to male reproductive health, no clear and consistent associations between either individual or combined concentrations of compounds and semen quality, reproductive hormone levels, cryptorchidism, hypospadias, or testicular cancer were detected. With the small amount of data available on risk following PFAS exposures prior to adulthood, concerns regarding reproductive toxicity in especially early stages of development remain. In addition, the few studies addressing cryptorchidism, hypospadias and testicular cancer mostly provided information on exposure to just one compound rendering the evidence of associations for these outcomes highly limited.

The main strength in this systematic review was our extensive and transparent literature search followed by application of predefined eligibility and quality assessment tools. Twenty-six studies were identified providing relevant estimates of risk and a qualitative evaluation of all outcomes was completed. Ten of the identified studies were not included in a previous systematic review on PFAS and male reproduction (Bach et al. 2016). Unfortunately, the available data were not suitable for a quantitative assessment in a meta-analysis. Especially the inconsistency in transformations and analyzes applied to data across studies rendered summary estimates of associations invalid. Additional limitations involved a lack of high-quality studies on all outcomes. Further, not including non-English and unpublished studies introduced a potential language and publication bias. The latter may have been limited by especially the cost of PFAS and outcome analyses (Bach et al. 2016).

Exposures

Despite the wide range of specific PFAS represented in exposure assessments, the narrow focus on PFOS and/or PFOA in many analyses impeded adequate evaluation of associations for other compounds. Apart from specific occupational settings, chemical exposures rarely occur in isolation (Hipwell et al. 2019). Potential health effects may, therefore, not be entirely attributable to single compounds and rather depend on mixtures of PFAS present in relatively low concentrations (Hipwell et al. 2019). With only 5 studies attempting to combine PFAS measures, no clear answers on the potentially additive or synergistic interactions between these compounds were available in relation to male reproduction at this point (Governini et al. 2015; Huang et al. 2019; Joensen et al. 2009; Song et al. 2018; Yao et al. 2019).

Exposure assessments were based almost exclusively upon direct measurements in various body fluids with only two studies modeling exposure levels from historical data (Barry, Winquist, and Steenland 2013; Vieira et al. 2013). Applying proxies for exposure (i.e. occupation, assumed water consumption, and residency in contaminated areas) may have resulted in errors of misclassification in these studies. On the other hand, direct measures of PFAS levels were predominantly performed on whole blood, serum, or plasma. As PFAS bind strongly to albumin and therefore accumulate predominantly in blood, concentrations measured here may not correlate sufficiently with those present in target tissues (Ng and Hungerbuhler 2013). In the three studies providing measures of analytes in both blood and semen, correlations between concentrations

achieved in these media differed widely among compounds (Governini et al. 2015; Raymer et al. 2012; Song et al. 2018). Thus, mechanisms for disrupting and crossing the blood-testis barrier (BTB) are likely highly selective with specific affinities for individual PFAS (Li et al. 2016). As suggested by Song et al. (2018), exposures measured in semen may correlate more closely with semen quality outcomes than concentrations in blood.

The specific timing of exposure assessments provided additional uncertainties in several investigations. The critical window of time in prenatal male reproductive development presents at 7–15 weeks of gestation followed by a gradual descent of the testes to their final scrotal position prior to birth (Holland, Nassar, and Schneuer 2016; Skakkebaek et al. 2016). In all three studies applying prenatal assessments, exposures were measured in mid- or late pregnancy (Itoh et al. 2016; Toft et al. 2016; Vested et al. 2013). In addition, Jensen et al. (2014) and Yao et al. (2019) assessed PFAS in cord blood serum at birth as a proxy of prenatal exposure. While the long elimination half-lives for many PFAS may enable measures to represent longer exposure intervals in adults, the radical changes in body composition and PFAS concentrations occurring during pregnancy limit options for extrapolation of exposure levels to both previous and coming stages prenatally (Mamsen et al. 2019). In young children and adolescents, rapid growth also produces dilution and potentially decreasing PFAS serum concentrations over time (Koponen et al. 2018). However, concerns regarding temporal changes in PFAS concentrations were limited by the purely cross-sectional designs applied in studies in these age groups (Lewis, Johns, and Meeker 2015; Lopez-Espinosa et al. 2016; Tsai et al. 2015; Zhou et al. 2016).

The spectrum of exposure levels present both within and between studies was relatively wide. In addition, the large span in study periods enabled evaluation of temporal changes in contributions from individual compounds. With the implemented restrictions on production of several PFAS in the US, Europe and Japan in the last decade, exposure levels are declining in many temperate regions with a contrasting expected rise in Artic areas until 2030 (Leter et al. 2014; Yao et al. 2019). While calendar time and geographical coverage served as key determinants of PFAS levels in many of the studied settings, occupation was by far the most contributing source of exposure with very high serum PFOA concentrations measured in both former and current workers (Costa, Sartori, and Consonni 2009; Sakr et al. 2007). Most studies applied exposure contrasts on continuous scales in analyses with no risk of misclassifying measurements. Potential misclassification of exposure levels was also limited in investigations stratifying exposures by tertiles or quartiles, while the two-tier approach based upon detectable/undetectable PFAS used in a single study was less robust (Governini et al. 2015).

Outcomes

In studies assessing semen quality and/or reproductive hormones, all outcomes were measured directly in biological samples. Despite a substantial intra-individual variability in several of these measures (Wilcox 2010), repeated sampling was only applied in one study on reproductive hormones and two studies on semen quality (Den Hond et al. 2015; Louis et al. 2015; Olsen et al. 1998).

As variability in reproductive hormone levels mainly stems from circadian and pulsatile secretion, failure to account for time of sampling during the day complicates interpretation of several hormonal measures (Zhou et al. 2016). Only 6 studies on reproductive hormones were able to consider the specific timing of outcome ascertainment in analyses (Den Hond et al. 2015; Joensen et al. 2009, 2013; Lopez-Espinosa et al. 2016; Petersen et al. 2018; Vested et al. 2013). While the most important determinant of semen quality, abstinence time, was considered either through adjustment in analyses or sampling criteria in most investigations on semen quality, parameters such as time from ejaculation to analysis, spillage, season, and analysis site were included only sporadically.

Assessment of cryptorchidism, hypospadias, and testicular cancer predominantly relied on clearly defined diagnoses from standardized clinical examinations or validated registers. In the study by Barry, Winquist, and Steenland (2013), the use of self-reported cancer diagnoses with a recall period of up to almost 60 years may, however, have introduced both potential errors through misclassification and a substantial survivorship bias.

Remaining methodological issues

Bias from residual confounding was a major concern across most of the included studies. While virtually all investigations accounted for potential effects of age and body mass index (BMI) through either application of covariates in analyses or study population criteria (i.e. narrow age intervals), inclusion of additional factors varied immensely. Despite the documented importance to reproductive development and health later in life, parental characteristics were, thus, rarely considered in analyzes (Wilcox 2010).

Multiple comparisons across multiple groups were performed in most of the studies on especially semen quality, reproductive hormones and cancer. Testing a large number of hypotheses on the same data, the chance of producing a purely coincidental, statistically significant finding increases (Christensen and Kampmann 2011). As only two of the included studies attempted to correct for this issue, many of the lone significant results presented in this review may indeed be chance findings (Huang et al. 2019; Yao et al. 2019)

Further, the selection of specific study populations potentially compromised generalizability of results and, hereby, the external validity of several investigations. In studies of semen quality and reproductive hormones, inclusion was often based upon predetermined reproductive profiles focusing on either fertile or infertile men (Den Hond et al. 2015; Governini et al. 2015; Huang et al. 2019; Joensen et al. 2009; Leter et al. 2014; Raymer et al. 2012; Song et al. 2018; Specht et al. 2012; Toft et al. 2012). Assessment of cryptorchidism and hypospadias was also limited to offspring from suspected high-risk pregnancies in the study by Toft et al. (2016) (indications for amniocentesis). In addition, participation rates were modest in most of the cross-sectional and cohort studies (Barry, Winquist, and Steenland 2013; Itoh et al. 2016; Joensen et al. 2009, 2013; Leter et al. 2014; Louis et al. 2015; Petersen et al. 2018; Specht et al. 2012; Tsai et al. 2015). Bias from selection was particularly concerning in studies where participation potentially depended upon the outcome of interest. In the study by Barry, Winquist, and Steenland (2013), having cancer may have affected the motivation for participating in contamination-related health surveys. However, participation may also have depended upon knowledge of or suspected exposure status in contaminated areas or occupational settings. Given the overall quality of the studies included in this review, findings must be interpreted with caution and consideration for the complexity of research in this field.

Perspectives

Adding to the complexity of evaluating PFAS in relation to reproductive health, virtually all humans are simultaneously exposed to a wide range of other environmental pollutants (Bonde et al. 2016). As exposure to different types of chemicals correlate, separating contributions from individual compounds might prove difficult (Bonde et al. 2016; Zhou et al. 2016). Future research may, therefore, benefit from development of methods to account for overall chemical body burden to supplement individual PFAS measurements. With the ongoing phase-out and replacement of several PFAS, awareness of potential toxicity in relation to new compounds is also required (Chemical Watch – Global risk and regulation news 2019).

Further, both sources and other determinants of PFAS levels measured in biological samples need clarification and consideration (Lindh et al. 2012). With diet serving as one of the major determinants of PFAS in humans, the presence of these compounds can to some extent also be interpreted as a proxy of health behavior (Joensen et al. 2013; Lindh et al. 2012; Tsai et al. 2015). In addition, genetic variability in or actual conditions restricting renal clearance lead to accumulation of PFAS and, thus, higher internal levels (Foresta, Tescari, and Di Nisio 2018). Such unknown or unmeasured factors may ultimately confound observed associations with reproductive health.

Finally, efforts to reduce heterogeneity between future studies are recommended. The standardization of methods and especially effect measures used in this particular field may enable quantitative assessment in a meta-analysis and, hereby, strengthen the overall epidemiological evidence. A complete risk assessment for PFAS also requires integration of both observational and experimental evidence from human and animal studies.

While experimental studies may provide potential mechanisms for reproductive damage and

substantiate associations for specific outcomes, an overview of these data is beyond the scope of this review.

Conclusion

Despite the growing literature on PFAS exposure and male reproductive health, evidence for an actual association remains limited. The current gap in knowledge is particularly obvious when it comes to exposure prior to adulthood, exposure to combinations of both PFAS and other types of environmental chemicals, and certain outcomes such as cryptorchidism, hypospadias and testicular cancer. While several investigations suggest weak associations for single compounds and specific outcomes, a lack of consistency across studies limits interpretation of causality. Continued efforts to clarify relations between PFAS and aspects of male reproduction through additional large, high-quality epidemiological studies are recommended.

Authors contributions

All authors participated in designing the study. KKH, SDH, LD, SST, JRL, KUP, and EMF performed the data collection, extraction, and evaluation. All authors participated in the interpretation of results. KUP and JRL drafted the manuscript and the remaining authors subsequently revised it critically, approved the final version and accepted responsibility for the contents. SST supervised the process and was overall responsible for the study.

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