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Adverse reproductive outcomes associated with fetal alcohol exposure: a systematic review

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Abstract

Fetal alcohol exposure results in well-characterised neurobehavioural deficits in offspring, which form the basis for diagnosing fetal alcohol spectrum disorder. However, there is increasing interest in the full range of health complications that can arise in children and adults with this disorder. We used a systematic review approach to locate all clinical and preclinical studies across a broad range of health outcomes in offspring exposed to prenatal alcohol. Our search encompassed four databases (PubMed, CINAHL, EMBASE and Web of Science) and titles/abstracts from retrieved studies were screened against strict inclusion/exclusion criteria. This review specifically evaluated studies reporting on reproductive outcomes in both males and females. A total of 23 studies were included, 5 clinical and 18 preclinical. Although there was a wide range in the quality of reporting across both clinical and preclinical studies, and variable results, trends emerged amongst the reproductive measures that were investigated. In females, most studies focussed on age at first menarche/puberty onset, with evidence for a significant delay in alcohol-exposed offspring. In males, offspring exposed to prenatal alcohol had altered testosterone levels, reduced testes and accessory gland weights and reduced sperm concentration and semen volume. However, further studies are required due to the paucity of clinical studies, the narrow scope of female reproductive outcomes examined and inconsistencies in outcomes across preclinical studies. We recommend that adolescents and individuals of reproductive age diagnosed with f-etal alcohol spectrum disorder be assessed for reproductive dysfunction to allow appropriate management of their reproductive health and fertility.

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Introduction

Prenatal alcohol exposure (PAE) has well-known teratogenic effects on the foetus, potentially resulting in growth restriction, congenital malformations, long-lasting neurological impairments and subsequent cognitive and behavioural deficits (Lange *et al.* 2017*b*). However, there is increasing interest in defining other adverse health outcomes in offspring with PAE, many of which may not manifest until adulthood. Recent conservative estimates of the prevalence of fetal alcohol spectrum disorder (FASD), a diagnosis encompassing a range of adverse physical, neurological, behavioural and social effects in individuals exposed prenatally to alcohol, are much higher than previously thought in the general population at around 5–7% (Lange *et al.* 2017*a*, May *et al.* 2018). Therefore, the societal and economic burden of these potentially preventable chronic health problems is a significant issue. In order to summarise knowledge to-date of the effects of PAE on other body systems, besides the brain and central nervous system, we conducted an extensive systematic review of available literature describing both clinical and preclinical studies. We intentionally kept our search strategy broad to encapsulate the range of health impacts. Studies were grouped according to the various domains of impairment, with in-depth analysis of study quality and outcomes conducted in separate publications for each domain (Fig. 1). This current review focuses on impacts on the reproductive system associated with PAE.

While direct effects of alcohol on the female and male reproductive systems are well known (Rachdaoui & Sarkar 2017 for review), effects of PAE on offspring reproductive function is less clear. Weinberg *et al.* (2008) provided a narrative review of the effect of PAE on the development and maturation of the hypothalamic–pituitary–gonadal axis (HPG) in males and females, as well as interactions with the hypothalamic–pituitary–adrenal (HPA) axis. However, the focus was more on gonadotropin hormones released from the pituitary, rather than gonadal steroid hormones and subsequent effects on reproductive function and fertility in adulthood. A more recent review by Caputo *et al.* (2016), examining effects of PAE on a variety of body systems, reported on only five original studies and eight reviews, none on the reproductive system. To our knowledge, this is the first systematic review to focus on long-term (i.e. beyond the neonate) effects of PAE on the reproductive system in males and females.

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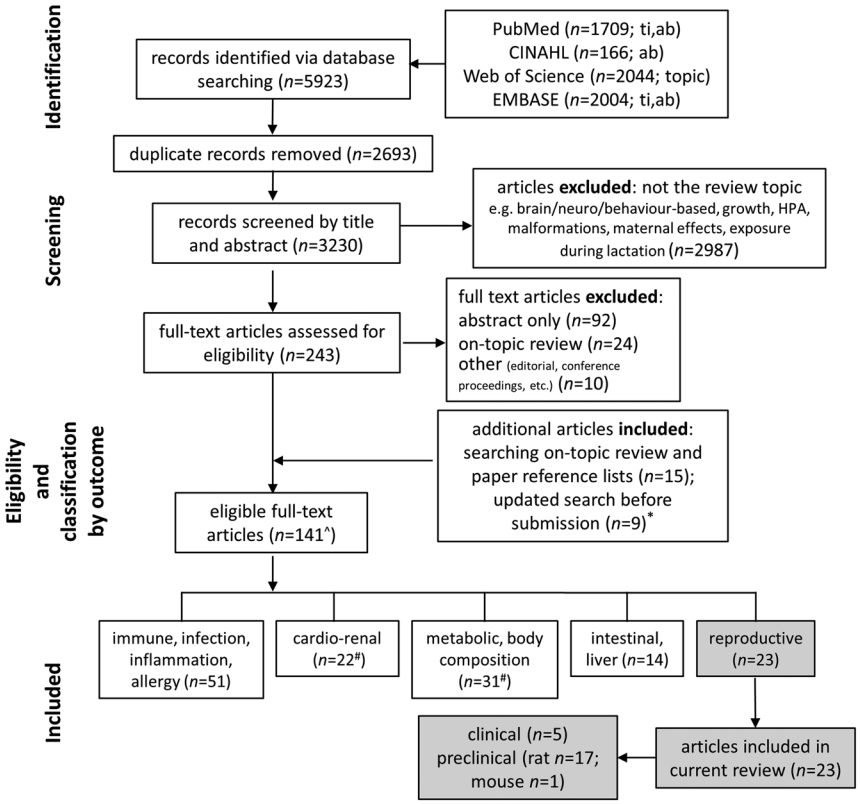

Figure 1 Flow diagram of the literature search strategy and study selection process (based on the PRISMA statement (Moher *et al.* 2009)). Studies were grouped by specific health outcomes examined in offspring exposed to alcohol during development. Data extraction and reporting was specifically done only on studies reporting on reproductive outcomes in the current review. *Search repeated October 2018. ^Only one study reported on structural and functional deficits in the lung so is not classified. #One of the papers contains data on both metabolic and cardio-renal outcomes so is counted in both of these groups. ab, abstract; ti, title.

Methods

Search strategy

Articles reporting on non-neurological/behavioural health impacts of PAE were identified through a broad systematic search as described in Akison *et al*. (under review). Briefly, four bibliographic databases were searched (PubMed, CINAHL, Web of Science and EMBASE) from inception to December 2017, with an updated search conducted closer to submission (October 2018). Search terms were maternal OR prenatal OR neonatal OR fetal OR foetal OR pregnancy OR pregnant OR fetal programming AND alcohol OR ethanol OR fetal alcohol OR foetal alcohol AND renal OR kidney OR cardiac OR cardio OR heart OR metabolic OR diabetes OR obesity OR respiratory OR lung OR immune OR reproductive OR endocrine. This systematic review conformed to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher *et al.* 2009) and was registered with the international prospective register of systematic reviews (PROSPERO; CRD42017082627).

Inclusion and exclusion criteria

Screening of title and abstracts was performed using the following criteria: (1) animal/*in vivo* study (any mammal) with fetal alcohol exposure or children diagnosed with FASD or PAE; (2) alcohol exposure occurred during pregnancy or during the third trimester-equivalent period in the rat (up to postnatal day (PD) 9); (3) included details of the alcohol exposure: for preclinical studies, alcohol concentration/dose and accurate timing of exposure must be provided (any route of administration); for clinical studies, children diagnosed with FASD using specified diagnostic criteria or suspected to have been exposed to moderate to heavy fetal alcohol exposure via a stated method of maternal alcohol consumption assessment; (4) a physical health impact of PAE was assessed in offspring (e.g. renal, cardiac, metabolic, reproductive, immune) and (5) included an appropriate unexposed control group for comparison. Studies were excluded if they (1) were not published in English; (2) only included assessments related to brain-based, neurological or behavioural outcomes; (3) only included outcomes related to malformations, growth rates or growth restriction; (4) only documented effects on embryos, foetuses or neonates during the third trimester-equivalent period in the rat; (5) only focussed on maternal effects of alcohol exposure rather than offspring effects or (6) were conference abstracts, PhD dissertations or editorials. Note that the third trimester-equivalent period in rodents, although early postnatal, has corresponding neurological (Workman *et al.* 2013) and ovarian (Sarraj & Drummond 2012) developmental milestones with human fetal development. The early postnatal period is therefore commonly included in preclinical models of early life alcohol exposure.

For the current review, quality assessment and data extraction were performed on those studies reporting on reproductive outcomes.

Study quality assessment

The methodological quality of the studies was assessed independently by authors NR and LA (with assistance by Melissa Wyllie, University of Queensland). Any disagreements

were resolved by discussion between assessors. The Downs and Black Checklist (Downs & Black 1998) was adapted by removing items relating to an intervention and applied to all clinical studies, assessing quality of reporting, internal and external validity and statistical power. The ARRIVE guidelines (Kilkenny *et al.* 2010) were used to assess the quality of reporting of animal studies.

Data extraction and synthesis

Data were extracted from each study for the following pre-defined parameters: study type (clinical/preclinical); species; description of PAE (timing/duration, dose, delivery method, assessment of PAE or FASD diagnosis); sample size; offspring age and sex; relevant assessment measures and key outcomes. Outcomes relating to males were tabulated separately to females. Sample sizes were estimated when accurate numbers per group were not available and were included as a measure of the potential scope or power of the study to detect significant differences if they were present. Preclinical studies were ordered in tables by the timing of alcohol exposure (i.e. stage of pregnancy) and their relative timing to each other presented in a graphical summary. Non-exposed offspring were considered controls and, wherever possible, isocaloric or pair-fed controls were the reference group. Given the large variability in timing/dose of the PAE and the outcomes assessed, meta-analysis was not possible and consequently a narrative synthesis method was used.

Results

Search results and classification of included studies by health outcomes

The initial search strategy resulted in 3230 articles (after duplicates were removed). Following title and abstract screening against the inclusion/exclusion criteria, 243 articles were eligible for full-text review. Reasons for exclusion are provided in the study flow diagram (Fig. 1). Following this review, and addition of further articles from an updated search and from the reference lists of included studies, a total of 141 articles encompassing five system domains were included. The current review focuses on the reproductive domain, which includes 23 studies: 5 clinical and 18 preclinical.

Quality of methodological reporting

The quality of reporting for the clinical studies was generally good, with one exception (Fig. 2A). Robe *et al.* (1979) was assessed as having a low score for reporting, but clearly stated that it was designed as a preliminary investigation only. However, this means that the results should be interpreted with caution, particularly as the source of the abstainers or control participants was not well defined. Common items that were not adequately addressed across all clinical studies were providing estimates of the random variability in the data (item 7),

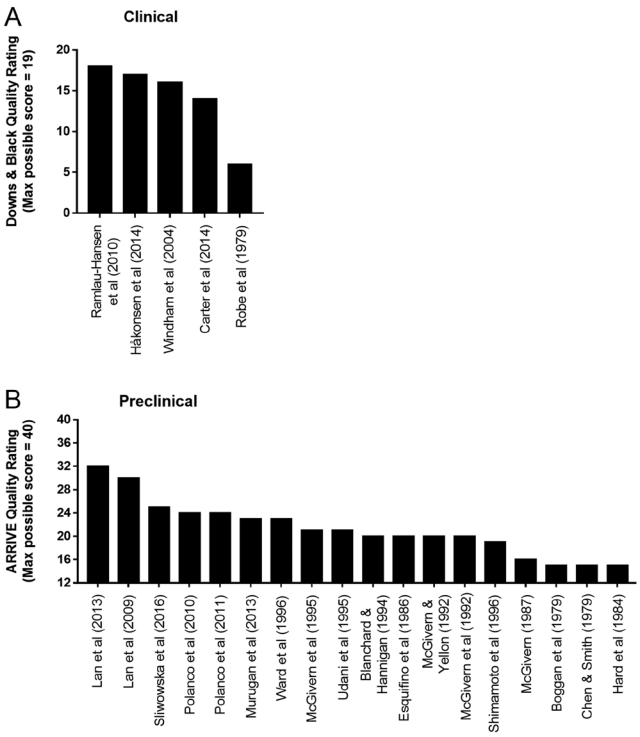

Figure 2 Total quality assessment scores for all included studies. (A) Clinical study scores based on the Downs and Black assessment criteria (Downs & Black 1998). Highest possible score is 19. (B) Preclinical study scores based on the ARRIVE guidelines (Kilkenny *et al.* 2010). Highest possible score is 40. Both (A) and (B) are ordered from the highest quality of reporting to the lowest. Refer to Supplementary data 1 for details of how scores were tallied.

mentioning if an attempt was made to blind assessors/researchers to the participants exposed to alcohol (item 15) and conducting *post hoc* analysis of the statistical power of the study based on the actual number of participants recruited (item 27) (Supplementary data 1A, see section on Supplementary data given at the end of this article).

There was a wide variation in the quality of reporting for the included preclinical studies, with scores ranging from 15 to 32 out of a possible score of 40 using the ARRIVE guidelines for animal reporting (Fig. 2B). Typical problems with the reporting of included preclinical studies were a general lack of detail on sample sizes, including the initial number of dams treated and then the number of pups used per dam for each experimental outcome; no mention of how dams were allocated to PAE or control/pair-fed diets; no baseline information on the dams assigned to each group (e.g. weights) before treatment began and no reporting of adverse events or why sample sizes given in the methods did not match sample sizes reported in the results (Supplementary data 1B). Note that older studies from the 1970s and 1980s tended to score lower. Although McGivern (1987) had relatively poor quality of reporting, this study was clearly identified by the author as a preliminary report.

Description of included studies

Details of the included clinical studies are summarised in Table 1. Two longitudinal cohorts from the United States were recruited from the San Francisco Bay area, CA, in the 1960s (Windham *et al.* 2004) and a predominantly African American antenatal clinic in Detroit, MI (Carter *et al.* 2014). There was also a retrospective cohort drawn from white, middle-class women from Long Island, NY, which included women recruited from Alcoholics Anonymous meetings (Robe *et al.* 1979). These three clinical studies predominantly reported on age at first menarche in females exposed prenatally to alcohol, although the study by Carter *et al.* (2014) also examined testosterone levels and pubertal development in males. Most studies assessed adolescents and young adults (14–21 years of age), although Robe *et al.* (1979) only included retrospective interviews with mothers about their daughter's age at first menarche and did not directly assess or interview PAE adolescents. There were also two studies reporting exclusively on male reproductive outcomes from a Danish cohort study (Ramlau-Hansen *et al.* 2010, Håkonsen *et al.* 2014). Out of all the included clinical studies, only one of the Danish cohort studies reported birth weight and a tendency for growth restriction in offspring as the number of drinks per week during pregnancy increased (Ramlau-Hansen *et al.* 2010). Numbers assessed in each study were quite variable, ranging from ~60 participants in the study by Carter *et al.* (2014), which only included a subset of participants from the larger longitudinal cohort, to >2500 participants (~400 in the PAE group) in one of the studies from the Danish cohort (Table 1).

The majority of studies reporting on reproductive outcomes were preclinical studies, predominantly in the rat (Table 2). A range of ages were assessed, including immediately post-weaning and peri-adolescent (~PD21–30) to adulthood (6 weeks to 12 months of age) (Table 2). Median age of adult offspring assessed was PD90. Eight studies reported exclusively on female reproductive outcomes, nine exclusively on male outcomes and four reported on both sexes. Sample sizes were generally low for each outcome assessed, with many studies reporting $n < 10$ (Table 2). For other studies with larger sample sizes, it was sometimes difficult to determine how many litters these were derived from (i.e. how many dams were initially treated per group). Two sets of studies appeared to report on subsets of offspring from the same treated dams (McGivern & Yellon 1992, McGivern *et al.* 1992, Polanco *et al.* 2010, 2011; Table 1 for details).

Details of alcohol exposure

Details regarding assessment of PAE for the clinical studies are summarised in Table 1. All the studies used retrospective interviews/questionnaires of maternal alcohol consumption during pregnancy. Most studies assessed maternal alcohol use prospectively at recruitment at antenatal visits. However, the study by Robe *et al.* (1979) did not question women about their consumption of alcohol during pregnancy until

Table 1 Clinical participant and study characteristics.

Study	Study type: Country	Assessment of prenatal alcohol exposure (PAE)	Adolescents assessed			
			Age (years)	Sex	Control (*n*)	EtOH (*n*)
Carter *et al.* (2014)	Longitudinal cohort: USA	Retrospective interview of 2 weeks alcohol consumption at antenatal visits[a]	14	F/M	43[b]	19[b]
Håkonsen *et al.* (2014)[c]	Longitudinal cohort: Denmark	Retrospective questionnaire at 36 weeks gestation[d]	18–21	M	2348[e]	438[e]
Ramlau-Hansen *et al.* (2010)[c]					110[f]	237[f]
Robe *et al.* (1979)	Retrospective cohort: USA	Maternal retrospective interview after daughters' 1st menarche[g]	N/A[h]	F	131[g]	71[g]
Windham *et al.* (2004)	Longitudinal cohort: USA	Retrospective maternal report during pregnancy[i]	15–17	F	424	285

[a]Data on maternal alcohol consumption collected prospectively and was assessed using a timeline follow-back interview (Jacobson *et al.* 2002 for details). For the multiple regression analyses, where alcohol exposure was dichotomised, participants were split into heavy-to-moderate exposure (≥1.0 oz absolute alcohol (AA) per day across pregnancy or binge drinking monthly defined as ≥2.0 oz AA per occasion) and light-to-no exposure (<heavy-moderate) as controls; [b]sample sizes shown are only for the analysis of male reproductive outcomes where the alcohol exposure was dichotomised into low-to-no and heavy-to-moderate exposure. Analysis of female outcomes was via correlation analysis with alcohol exposure (average daily consumption) as a continuous variable; [c]adolescents were from the same study; [d]data on maternal alcohol consumption collected prospectively. Questionnaire contained fixed response categories for the number of drinks consumed per week or the number of binge drinking episodes (i.e. ≥8 drinks on a single occasion) during pregnancy. Refer to Olsen *et al.* (1989) for more details of this cohort; [e]adolescents were categorised into the following groups of prenatal binge alcohol exposure: 0 (control); 1–4 times, and >5 times (EtOH); [f]adolescents were categorised into the following groups of prenatal weekly alcohol exposure: <1.0 (control); 1.0–1.5, 2.0–4.0 and ≥4.5 (EtOH); [g]PAE classified as (1) abstainers (0 drinks); (2) rare drinkers (<1 drink/month); (3) moderate drinkers (average <1.5–3 drinks daily); (4) heavy drinkers (average >2 drinks daily and >5 drinks on occasion); and (5) alcoholics (>heavy drinkers). Control sample size includes (1) and (2); EtOH sample size is other groups (6 were alcoholics); [h]offspring were not assessed. Maternal report of daughter age at 1st menarche at some time after the event (time period not specified); [i]data on maternal alcohol consumption collected prospectively. PAE classified as >1 drink/week during pregnancy; controls were <1 drink/week. van den Berg *et al.* (1988) for more details of this cohort.
EtOH, ethanol; F, females; F/M, females and males; M, males; N/A, not applicable.

Table 2 Preclinical study characteristics.

Study	Species (strain)	Alcohol exposure	Isocaloric control	Offspring assessed				
				Age[#]	Sex	Control (*n*)[^]	EtOH (*n*)[^]	Growth restricted[+]
Preconception and throughout gestation								
Hard *et al.* (1984) (~4 months prior to pregnancy)	Rat (Wistar)	8–16% w/v EtOH in drinking water[a]	No	≥PD30	F/M[b]	48[c]	41[c]	No
Esquifino *et al.* (1986) (4–5 weeks prior to pregnancy)	Rat (Wistar)	36% EDC in liquid diet	Yes	≥PD28	F	15	26	NR
Throughout gestation								
Lan *et al.* (2009)	Rat (Sprague–Dawley)	36% EDC in liquid diet	Yes	≥PD28, PD90–120	F	27–49	22–49	Yes
Lan *et al.* (2013)	Rat (Sprague–Dawley)	36% EDC in liquid diet[d]	Yes	PD15–55	M	6	6	NR
Sliwowska *et al.* (2016)	Rat (Sprague–Dawley)	36% EDC in liquid diet	Yes	PD30, 35, 65	F	8–14[e]	8–14[e]	No[f]
Early-mid gestation (GD5–11)								
Boggan *et al.* (1979)	Mouse (C57Bl6/J)	30% EDC in liquid diet	Yes	≥PD28	F	6–18[g]	7–19[g]	No
Early gestation (GD5–7) to birth								
Blanchard & Hannigan (1994) (GD6–20)[h]	Rat (Long Evans)	35% EDC in liquid diet	Yes	PD85–100	M	31	31	Yes
Chen & Smith (1979)[i]	Rat (Long Evans)	10% w/v EtOH in 0.125% saccharin solution	No	≥PD45, PD75–150	M	9–18	14–15	No
McGivern *et al.* (1992)[j,k]	Rat (Sprague–Dawley)	35% EDC in liquid diet	Yes	≥PD28 (F), PD90–100	F/M	7–12	10–23	Yes
McGivern & Yellon (1992)[k]	Rat (Sprague–Dawley)	35% EDC in liquid diet	Yes	≥PD28	F	21	34	Yes
Shimamoto *et al.* (2006)	Rat (Sprague–Dawley)	36% EDC in liquid diet[l]	No[m]	PD14, 21	M	3–8	3–8	NR
Mid-gestation (GD10/11) to birth								
Murugan *et al.* (2013)	Rat (Sprague–Dawley)	35% EDC in liquid diet[n]	Yes	8 months	M	10	10	NR
Polanco *et al.* (2010)[o]	Rat (Sprague–Dawley)	35% EDC in liquid diet[p]	Yes	PD62–76	F	13	13	NR
Polanco *et al.* (2011)[o]				PD20, 40, 80	F	9	10	
Ward *et al.* (1996)	Rat (Sprague–Dawley)	36% EDC in liquid diet	Yes	PD90	M	43[q]	14[q]	Yes
Late gestation (GD13/14) to birth								
McGivern (1987) (from GD14)	Rat (Sprague–Dawley[r])	35% EDC in liquid diet	Yes	PD145	F/M	7–9	7–9	Yes
McGivern *et al.* (1992)[j] (from GD13)	Rat (Sprague–Dawley)	35% EDC in liquid diet	Yes	≥PD28 (F), PD90–100	F/M	7–12	7–29	No
McGivern *et al.* (1995) (from GD14)	Rat (Sprague–Dawley)	35% EDC in liquid diet	Yes	2, 6 and 12 months	F	8–10	8–16	NR
Early and mid-gestation to 3rd trimester equivalent								
Chen & Smith (1979)[i] (GD7–PD7)	Rat (Long Evans)	10% EtOH in 0.125% saccharin solution	No	≥PD45, PD75–150	M	9–18	8–14	No
Udani *et al.* (1985) (GD12–PD10)	Rat (Wistar)	36% EDC in liquid diet	Yes	PD55, 110	M	17	17	No[s]
3rd trimester equivalent only								
Chen & Smith (1979)[i] (birth-PD7)	Rat (Long Evans)	10% EtOH in 0.125% saccharin solution	No	≥PD45, PD75–150	M	9–18	17–18	No

Studies are ordered based on the timing of alcohol exposure (summarised in Fig. 3).
[+]At birth/PD1; [^]samples sizes are per outcome measured. Controls were the isocaloric control group where available; [#]where '≥' is reported before age, this was when puberty onset was first examined and then continued to be monitored; [a]dams were given 8% w/v EtOH in drinking water from 16 days of age to 4 months of age prior to pregnancy. EtOH concentration was then increased to 16% w/v, and 2 weeks later, animals were tested daily for sexual receptivity and mated at first oestrous (no method given for this or time frame but can assume up to 4 days); [b]most assessments in males were behavioural; only anogenital distance at birth reported in Table 4; [c]represents litters rather than individual offspring; data from two males or females per litter were averaged for each litter; [d]gradually increasing concentration from 1/3 PAE:2/3 control diet (GD1), 2/3 PAE:1/3 control diet (GD2) and full diet by GD3; [e]exact numbers not given; range only provided; [f]reported in Uban *et al.* (2013). Both the isocaloric control and PAE pups had lower average birth weights than the chow controls; [g]sample sizes for each group unclear so estimates of the numbers assessed at each age are provided. A total of ten dams were included in the study so multiple offspring were used from each litter; [h]gestation was ~22 days; [i]three cohorts: (1) GD7 to birth; (2) GD7–PD7; (3) birth to PD7; [j]two cohorts: one group of dams treated from GD7 to birth; the other treated from GD13 to birth; [k]suspect same dams used and McGivern *et al.* (1992) reports on a subset of female offspring also reported in McGivern and Yellon (1992); [l]increased from 2% w/v EtOH GD2–3 to 3% w/v GD4 to 5% w/v by GD5. Assumed 5% w/v EtOH is equivalent to 36% EDC in liquid diet, as reported by Ward *et al.* (1996); [m]a cohort of dams were given the liquid diet without ethanol and classified as 'pair-fed' but there was no caloric substitution for the ethanol or mention of matching the consumption rates to the PAE-treated dams; [n]EtOH concentration increased from 1.7% (v/v) days 7–8 to 5.0% (v/v) days 9–10 to 6.7% (v/v) from day 11. The final concentration equates to 35% EDC in the liquid diet; [o]suspect same dams used and each study reports on different offspring outcomes; [p]EtOH concentration increased from 2.2% (v/v) days 7–8 to 4.4% (v/v) days 9–10 to 6.7% (v/v) from day 11. The final concentration equates to 35% EDC in the liquid diet; [q]additional groups were also subjected to prenatal restraint stress; [r]strain not reported but assumed from other studies by this author; [s]both the isocaloric control and PAE pups had lower average birth weights than the chow controls.
EDC, ethanol-derived calories in a Lieber–DeCarli liquid diet; EtOH, ethanol; F, females; F/M, females and males; GD, gestational day; M, males; NR, not reported; PD, postnatal day; w/v, weight/volume.

many years later, at an undisclosed time after their daughter's first menarche. Only the study by Carter *et al.* (2014) reported that three of the children assessed in their study had been previously diagnosed with fetal alcohol syndrome at 7.5 years of age. This study also dichotomised participants into heavy-to-moderate (PAE group) or light-to-no (control) exposure in a multiple regression analysis to look at male outcomes, but used a correlation analysis of reported female reproductive outcomes with alcohol exposure (amount per day) as a continuous covariate. Women in this study who did report drinking were generally heavy drinkers, with an average of ~5 standard drinks per occasion at conception and ~4 per occasion across pregnancy. They also reported drinking on ~12 days/month at conception and ~4 days/month across pregnancy. Similarly, almost half the participants in the study by Robe *et al.* (1979) reported heavy PAE (>2 drinks daily), with six participants identifying as 'alcoholics' during pregnancy.

For preclinical studies, the timing of alcohol exposure varied widely across gestation (Fig. 3 and Table 2). Several studies restricted alcohol exposure to late in gestation (from gestational day (GD) 14), a key time when the fetal gonad in the male is capable of producing testosterone and hormonal regulation of gonadal differentiation and development occurs (Warren *et al.* 1973). Chen and Smith (1979) included three different cohorts in their study in which they exposed offspring to alcohol from early in gestation to birth and/or during the early postnatal, 3rd trimester-equivalent period. Udani *et al.* (1985) also extended exposure to this early postnatal period. Note that for both these studies, only the dam was treated and not the pups directly; therefore, alcohol was transferred to the pups via lactation. Only three studies (all from the same author) used a chronic model of exposure throughout gestation, while many studies began exposure early or mid-gestation and continued until birth (Fig. 3). One study in the mouse used only an acute exposure from early-to-mid gestation (Boggan *et al.* 1979), while two studies also treated the dams for an extended period prior to mating (Hard *et al.* 1984, Esquifino *et al.* 1986).

Although the timing of exposure was quite variable, the dosage was very consistent, with most studies treating with 35–36% ethanol-derived calories (EDCs) in a Lieber–DeCarli style liquid diet (Lieber & DeCarli 1989) (Table 2). Previous reports have shown that this typically results in peak blood alcohol concentrations (BACs) of 100–150 mg/dL or 0.10–0.15% (Elton *et al.* 2002) and translates to around 3–5 drinks in 2 h in women (Leeman *et al.* 2010). Only two of the included studies directly measured maternal BAC (Udani *et al.* 1985, Ward *et al.* 1996), confirming previous reports. Udani *et al.* (1985) also measured BAC levels in the foetus (~150 mg/dL) and amniotic fluid (~180 mg/dL) at GD18 and found that they were significantly elevated compared to maternal circulating levels (~112 mg/dL). One study administered the ethanol (EtOH) via the drinking water at a concentration of 8% w/v prior to mating and 16% w/v thereafter (Hard *et al.* 1984), while one study delivered the EtOH at 10% w/v in a saccharin-sweetened solution (Chen & Smith 1979). BAC was not measured in either of these studies.

Details of control groups

Although the goal was to only include studies with a non-exposed control group, this was not always possible for the clinical studies. Following assessment of PAE, most clinical studies categorised the children

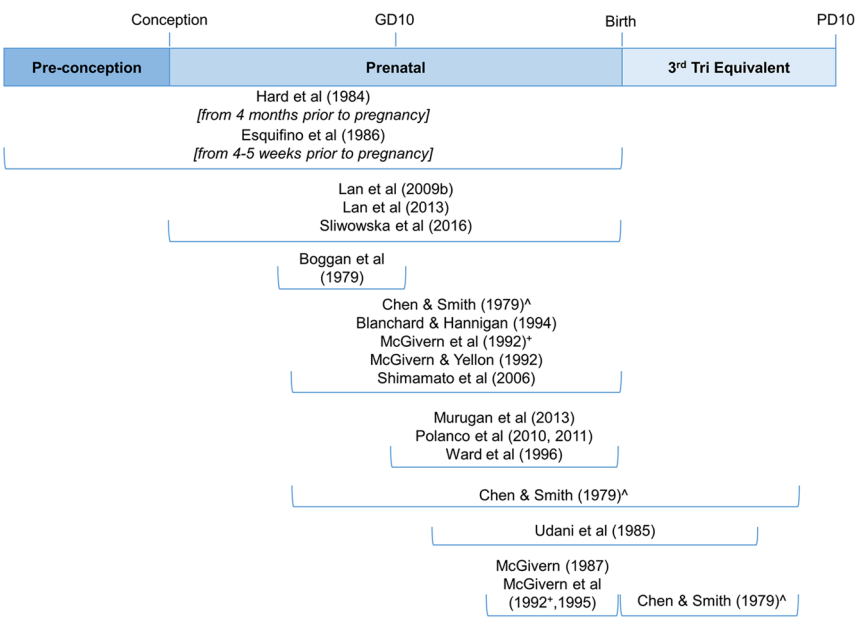

Figure 3 Summary of included preclinical studies relative to the timing of alcohol exposure. Further details are provided in Table 1. GD, gestational day; +McGivern *et al.* (1992) had two cohorts of pregnant dams treated with alcohol from different times during gestation; ^Chen & Smith (1979) had three cohorts of pregnant dams treated with alcohol.

based on the level of maternal alcohol exposure, with control groups including abstainers but also, typically, low level alcohol users. However, the definition of 'low level' alcohol use varied quite dramatically between studies, from less than 1 oz absolute alcohol (AA) per day (2 standard drinks) or less than one binge per month (Carter *et al.* 2014) to <1 drink per week (Windham *et al.* 2004, Ramlau-Hansen *et al.* 2010) or 0 prenatal binge episodes (Håkonsen *et al.* 2014) to <1 drink per month (Robe *et al.* 1979). This highlights the difficulty in recruiting sufficient participants with no alcohol exposure for the control groups, given that many women consume low levels of alcohol, particularly prior to pregnancy recognition (McCormack *et al.* 2017).

For the preclinical studies, all had a no PAE control group. Many of the preclinical studies included a pair-fed or isocaloric control group, in addition to a chow-fed control group, given that most were administering the EtOH using a liquid diet (Table 2). This ensured that the control group received the same daily caloric intake per body weight as a 'yoked' PAE dam, thus allowing the effects of the liquid diet alone to be disassociated from the specific effects of the EtOH. In some cases, the EtOH was replaced with sucrose (Boggan *et al.* 1979, McGivern & Yellon 1992, McGivern *et al.* 1992, 1995, Blanchard & Hannigan 1994) or maltose-dextrin (Udani *et al.* 1985, Esquifino *et al.* 1986, Lan *et al.* 2009, 2013, Murugan *et al.* 2013, Sliwowska *et al.* 2016), but in some cases, there was nothing added to substitute for the EtOH (McGivern 1987, Polanco *et al.* 2010, 2011), even though these studies classified their control as 'pair fed'. Chen and Smith (1979) delivered the EtOH in a saccharin solution to treated animals, but controls only received water. Hard *et al.* (1984) was the only study to deliver the EtOH in drinking water and had no isocaloric control group.

Summary of studies reporting on female reproductive outcomes

Outcomes related to female reproductive function are summarised in Table 3. Studies were limited in their range of assessments, with the majority examining age at first menarche via interview (clinical) or the equivalent measure in preclinical studies: puberty onset via age at vaginal opening. For clinical studies, results were quite variable across studies, with only one of the three clinical studies reporting an increased age at first menarche in females exposed to heavy levels of PAE (Robe *et al.* 1979). Mean age at menarche was increased from 12.3 years in mothers to 13.3 years in their daughters compared to no difference in abstainers/low level drinkers (range 12.4–12.9 years) and their daughters (12.5–12.8 years). However, this was also the only study that only interviewed the mothers and did not assess the offspring with PAE directly.

There was more evidence for delayed puberty onset from the preclinical studies, with six out of the eight studies measuring age at vaginal opening finding some degree of delay (Table 3). Most studies reported these data as the cumulative percentage of females displaying vaginal opening over time (usually starting from PD28 to PD30). For example, Esquifino *et al.* (1986) reported vaginal opening in 60% of pair-fed control offspring at PD34–36, but only in 38% of PAE offspring (chow-fed controls were 66%); and by PD40–42, vaginal opening had occurred in 93–100% of pair-fed/chow-fed offspring but only in 73% of PAE offspring. Similarly, McGivern and Yellon (1992) reported 100% vaginal opening in control groups at PD40, but only 73% in PAE females, with some PAE females taking until PD46 before puberty onset (median age for controls PD34–35 and PAE PD38–39). Given these studies were in different rat strains (Table 2), this shows general consistency in this phenotype in terms of effects of PAE on age at puberty onset. McGivern *et al.* (1992) included two PAE cohorts in their study, one beginning early and the other late in gestation, and although both showed delayed puberty onset, concluded that late gestation exposure appears most critical. However, the study by Boggan *et al.* (1979) only exposed mouse offspring to EtOH in early-to-mid gestation (GD5–11) and also reported delayed puberty onset.

Aside from puberty onset, oestrous cyclicity was evaluated in three studies using vaginal cytology (Table 3). Only McGivern *et al.* (1995) reported an increased incidence of acyclic females in PAE offspring, and only with increasing age (6 and 12 months but not 2 months of age, Table 3). The other two studies only assessed oestrous cycles at around 3.5–4 months of age. This suggests that PAE may shorten the reproductive lifespan. There were also three studies that measured ovary weight, with only one study finding an increase in relative ovary weight (corrected for body weight) when PAE began in early gestation (McGivern *et al.* 1992) (Table 3). Few studies (4 out of 14) measured ovarian steroid hormone levels, with only one preclinical study reporting increased circulating oestradiol (E2) levels in 10-week-old rats compared to controls during proestrous, just prior to ovulation (Table 3). Only one study (clinical) measured testosterone levels in saliva and found this was elevated in PAE adolescent females compared to controls (Table 3), although levels were still within the normal expected range for this age group. There was also one study that compared the mammary glands from prepubertal PAE and control offspring and found that those from the PAE group showed enhanced proliferation and altered growth and oestrogen signalling pathways that are hallmarks for increased susceptibility to tumour development (Polanco *et al.* 2011).

Interestingly, two studies (Lan *et al.* 2009, Sliwowska *et al.* 2016) reported quite similar hormonal and maturational changes in their PAE and pair-fed offspring

Table 3 Study outcomes related to female reproductive function.

Study	Relevant assessments	Key results*	Conclusion
Clinical			
Carter *et al*. (2014)	Saliva T levels; self-reported Tanner stages for pubertal development[a]; age at 1st menarche	↑T levels; ↔pubertal development or age at 1st menarche[b]	Despite increased salivary T levels, no changes during puberty
Robe *et al*. (1979)	Age at 1st menarche (compared filial to maternal and classified as 'early' or 'late')	↑Age at 1st menarche ('late') if PAE was heavy-alcoholic level	Heavy PAE may delay onset of puberty in daughters
Windham *et al*. (2004)	Age at 1st menarche	↔Age at 1st menarch	Daughters' age at menarche did not vary with PAE
Preclinical			
Preconception and throughout gestation			
Hard *et al*. (1984)	Vaginal opening (puberty onset); oestrous cycles (vaginal smears)[c]	↔Puberty onset or oestrous cycles	No effect of PAE on physiological measures of oestrous cycles
Esquifino *et al*. (1986)	Vaginal opening (puberty onset); plasma hormone levels (LH, PRL)	↑Age at vaginal opening; ↓LH and ↑PRL levels at puberty onset; only ↑PRL persisted into adulthood (PD120)	PAE caused delayed puberty onset and hormonal alterations that persisted into adulthood
Throughout gestation			
Lan *et al*. (2009)	Vaginal opening (puberty onset); oestrous cycles (vaginal smears); plasma hormone levels (E2, P, LH, PRL)	Slight delay in puberty onset[d]; ↔oestrous cycles; ↔E2 and LH but ↓P at proestrous[e]	Normal changes in most basal hormone levels over the oestrous cycle but puberty onset slightly delayed
Sliwowska *et al*. (2016)	Plasma hormone levels (E2, P, LH, PRL); puberty markers (vaginal opening, uterus/BW)	↔Puberty markers and hormone levels compared to pair-fed controls	PAE and pair-fed controls both showed altered maturation and hormone levels compared to controls, but some differential effects
Early-mid gestation			
Boggan *et al*. (1979)	Vaginal opening (puberty onset)	↑Age at vaginal opening	PAE resulted in delayed puberty onset
Early gestation to birth			
McGivern *et al*. (1992)[f]	Vaginal opening (puberty onset); ovary weight	↑Age at vaginal opening (~4 days); ↑ovary weight (corrected for BW)	See below for late gestation PAE cohort
McGivern & Yellon (1992)	Vaginal opening (puberty onset)	↑Age at vaginal opening (~4 days)	PAE resulted in delayed puberty onset
Mid-gestation to birth			
Polanco *et al*. (2010)	Serum E2 and IGF1 levels	↑E2 and ↔IGF1 levels during proestrous	PAE increased circulating E2 in 10 week-old, preovulatory offspring
Polanco *et al*. (2011)	Histology and molecular analysis (qPCR and IH) of mammary gland; serum IGF1/BP-3 levels	↑Epithelial cell proliferation[g,h]; ↑aromatase expression[h]; altered *Igf1/Bp5* expression[h]; ↔circulating IGFs; ↔mammary gland morphology	PAE advanced prepubertal mammary gland development via altered IGF/oestrogen signalling, potentially increasing the risk for mammary tumours
Late gestation to birth			
McGivern (1987)	Ovary weight	↔Ovary weight (absolute only given)	PAE restricted to late gestation does not impact on ovarian growth
McGivern *et al*. (1992)[f]	Vaginal opening (puberty onset); ovary weight	↑Age at vaginal opening (~4 days); ↔ovary weight (absolute or corrected for BW)	By assessing two PAE timings, late gestation exposure appears to be critical for subsequent age at puberty onset
McGivern *et al*. (1995)	Vaginal cytology (oestrous cycles)[i]	2 months: ↔oestrous cycles; 6 months: ↑number of acyclic females 12 months: all PAE females acyclic compared to 50% of controls	PAE may shorten the reproductive lifespan in female offspring

Animal studies are ordered based on the timing of alcohol exposure.
*Key results are in alcohol-exposed offspring compared to non-exposed, isocaloric (where available) controls; [a]Tanner stage drawings (Marshall & Tanner 1969) were used to assess breast/pubic hair development in girls; [b]only adjusted means (no S.D.) given for T levels across three alcohol exposure levels (<0.10 oz absolute alcohol (AA)/day, 0.10–0.24 oz AA/day and ≥0.25 oz AA/day); correlation coefficients only provided for pubertal data and age at 1st menarche; [c]also reported on signs of behavioural oestrous but this was beyond the scope of the primary outcomes reported in this review. See text for more details; [d]PAE females had a significantly lower % of vaginal opening in females at PD32 compared to both pair-fed and chow-fed controls but was only delayed compared to the chow-fed group at PD33, 35 and 36. 100% of vaginal opening occurred by PD40; [e]no difference to chow control; [f]two cohorts with different timings of alcohol exposure were used; [g]cell proliferation measured by BrdU staining; [h]changes seen at PD20 and 40 but not PD80; [i]measured as % of females with two normal (4–5 days) oestrous cycles in succession over a 15-day period.
BW, body weight; BP, binding protein; E2, oestradiol; IGF, insulin-like growth factor; IH, immunohistochemistry; LH, luteinising hormone; P, progesterone; PAE, prenatal alcohol exposure; PD, postnatal day; PRL, prolactin; T, testosterone.

compared to chow-fed controls, although there were some subtle differential effects. For example, Sliwowska *et al*. (2016) reported that despite both groups having much reduced prolactin (PRL) levels compared to the controls, PAE females still showed the expected age-related increase in progesterone (P), whereas this was absent in the pair-fed group.

Summary of studies reporting on male reproductive outcomes

Outcomes related to male reproductive function are summarised in Table 4. There were a greater variety of assessments in male versus female offspring, ranging from serum hormone levels, puberty onset, testes and accessory organ weights, semen analysis/sperm counts and cytoarchitecture of the testis and prostate gland. In the clinical studies, Carter *et al*. (2014) measured testosterone levels in saliva, finding elevated levels in PAE adolescents compared to controls. However, even for offspring with the heaviest alcohol exposure, testosterone levels were still within the normal range for unexposed offspring, providing a possible explanation for why no PAE-induced changes were detected in pubertal development. The two studies from the Danish cohort reported a trend for delayed pubertal development in males with PAE compared to controls and reduced sperm concentration and semen volume, particularly with heavy PAE (Table 4). However, there were no changes in serum hormone levels or sperm motility/morphology, suggesting that PAE affected Sertoli rather than Leydig cell function in the testis. Only one of the preclinical studies measured sperm count and found no difference between PAE males and controls (McGivern *et al*. 1992). Similarly, only one preclinical study examined puberty onset (Chen & Smith 1979), as indicated by preputial separation (Korenbrot *et al*. 1977). This study included three cohorts with different durations of exposure (Table 2), with only the two offspring cohorts exposed to EtOH during the postnatal, 3rd trimester-equivalent period showing precocious puberty onset (average GD44.8) compared to the third PAE cohort and control group (GD46.4–46.9).

Testes weights were measured in six preclinical studies, with four of these studies also measuring accessory organ weights, with variable results (Table 4). Half of these studies reported reduced testes weights in PAE males while the other half found no change. Blanchard and Hannigan (1994) saw reduced testes weight in both PAE and pair-fed offspring compared to controls, suggesting that nutritional deficits rather than alcohol-specific mechanisms may be important. Only Udani *et al*. (1985) reported a reduction in accessory gland weight, expressed as the ratio of prostate-seminal vesicle weights, in PAE offspring.

Circulating testosterone levels (in plasma or whole blood) were measured in six preclinical studies, with only one study reporting elevated testosterone levels in PAE offspring at weaning (Shimamoto *et al*. 2006) and one study reporting reduced testosterone levels into adulthood (Udani *et al*. 1985). Only the latter study also measured testes weight and found a concomitant decrease in PAE offspring.

Many preclinical studies also reported on anogenital distance (AGD) at birth, a sensitive marker used by reproductive toxicologists to measure potential disruptions to androgen levels during male development (McIntyre *et al*. 2001, Hsieh *et al*. 2008). Although technically measured during the 3rd trimester equivalent which is outside the scope of this review, this was reported along with other primary outcomes in postnatal life (i.e. >PD10) and may be an early indicator of long-term reproductive dysfunction. AGD is sexually dimorphic, being 2–2.5-fold longer in males than females, and several studies reported that AGD was reduced in PAE offspring compared to controls (Table 4). However, other studies reported no change. Note that although Chen and Smith (1979) used three different timings for PAE, AGD was reported as an average for all EtOH treatment groups. Most studies reported both absolute AGD as well as AGD index (relative to body length or weight), given that pups are often growth restricted at birth in preclinical models of PAE and AGD is known to be highly correlated with body weight (Graham & Gandelman 1986).

Only one study by Lan *et al*. (2013) investigated the cytoarchitecture of the testes during early development and maturation. Although outside the time frame of this review, they found significantly fewer gonocytes (male primordial germ cells) attached to the basal lamina at the periphery of the seminiferous tubule in PAE offspring testes just after birth (PD5), suggesting delayed establishment of spermatogenesis in neonatal males. This was accompanied by smaller relative testes weight and delayed maturation around weaning age and delayed spermatogenesis at PD55. However, this study reported no changes in testosterone.

Finally, one study by Murugan *et al*. (2013) examined the histopathology and oestrogenic components of the prostate gland and found molecular and cellular changes in PAE offspring at 8 months of age, indicative of an increased risk for tumour development. As per the study reported above in the mammary gland, this included evidence for increased proliferation (via Ki-67 staining) and oestrogen production and activity, as well as inflammatory cell infiltration and cell hyperplasia.

Discussion

Despite growing interest in the range of adverse health outcomes arising from PAE, relatively little attention has focussed on potential impacts on male and female reproductive function and fertility. This systematic review highlights the relative paucity of data in this domain,

Table 4 Study outcomes related to male reproductive function.

Study	Relevant assessments	Key results*	Conclusion
Clinical			
Carter *et al.* (2014)	Saliva T levels; self-reported Tanner stages for pubertal development[a]	↑T; ↔pubertal development[b]	PAE-induced increase in T suggests decreased T responsiveness in tissues involved in pubertal development
Håkonsen *et al.* (2014)	Retrospective self-reported pubertal development[c]	Trend for ↑age at first nocturnal emission and voice break, particularly in response to binge drinking	Tendency for delayed pubertal development in boys with PAE
Ramlau-Hansen *et al.* (2010)	Semen analysis (volume, sperm motility and concentration); serum T, E2, SHBG and inhibin B[d]	↓Sperm concentration (at highest level of PAE); ↓semen volume; ↔sperm motility, morphology or hormone levels	PAE predicted to affect Sertoli cell function and therefore sperm concentration
Preclinical			
Preconception and throughout gestation			
Hard *et al.* (1984)	AGD at birth[e]	↔AGD (no relative index reported)	Although PAE did not impact on AGD, there were behavioural signs of feminisation in PAE males
Throughout gestation			
Lan *et al.* (2013)	Morphometric analysis of testes; testes weight; serum T levels	↔T levels; ↓relative testes weight; ↓% of seminiferous tubules with open lumen; ↓primary spermatocytes and spermatids; delayed spermatogenesis	PAE delayed seminiferous epithelial maturation and onset of spermatogenesis, persisting at least until young adulthood
Shimamoto *et al.* (2006)	Whole blood T levels[f]	↔T at PD14; ↑T at PD21	Suggests PAE increases testosterone biosynthesis at weaning
Early gestation to birth			
Blanchard and Hannigan (1994)	AGD at birth; testes weight in adulthood	↓AGD[m]; ↔testes weight[g]	PAE may affect early testes development but long-term effects in adulthood are more likely due to nutritional deficits
McGivern *et al.* (1992)[h]	Right testis, epididymis, prostate, and seminal vesicle weights; plasma T levels; sperm count	↓Testis weight but ↑testis weight/BW; ↓tubule weight; ↔accessory gland weights; ↔T levels or sperm counts	Some subtle changes in testis and tubule weights but generally no long-term effects of PAE on the male reproductive system
Mid-gestation to birth			
Murugan *et al.* (2013)	Histopathology and molecular analysis (IH, WB, ELISA, EIA) of prostate gland	Abnormal cytoarchitecture of the ventral lobe[i]; ↑oestrogenic components (ERα, E2, aromatase)	Molecular and cellular changes in the prostate of PAE offspring may predispose to enhanced tumour susceptibility
Ward *et al.* (1996)	Serum T and LH levels	↔T and LH	No effect of PAE on hormone levels in adulthood
Late gestation to birth			
McGivern (1987)	AGD at birth; testes and seminal vesicle weights	↓AGD but ↔AGD corrected for BW; ↔organ weights	PAE restricted to late in gestation does not impact on growth of male reproductive organs
McGivern *et al.* (1992)[h]	Right testis, epididymis, prostate and seminal vesicle weights; plasma T levels	↓Testis weight and tubule weight; ↔accessory gland weights; ↔T levels or sperm counts	Some subtle changes in testis and tubule weights but generally no long-term effects of PAE on the male reproductive system
Mid-gestation to 3rd trimester equivalent (PD10)			
Udani *et al.* (1985)	AGD at birth; testes, prostate, seminal vesicle weights; serum T and LH levels	↓AGD[m]; ↓testes and accessory organ weights[n]; ↓T and LH	PAE reduced phenotypic masculinisation and T and LH levels into adulthood. This reduced mating performance/motivation
Early gestation to birth and/or 3rd trimester equivalent			
Chen and Smith (1979)	AGD at birth; testes, penis and accessory gland weights; plasma T levels; preputial separation[o] (puberty onset); penile reflex[j]	↓AGD[k]; ↓age at puberty onset (PD EtOH groups only); ↓penile reflex in naïve males[l]; ↔T and testes, penis and accessory gland weight	PAE, particularly during the 3rd trimester equivalent period, accelerated the age at puberty without long-term effects on testosterone production

Animal studies are ordered based on the timing of alcohol exposure.
*Key results are in alcohol-exposed offspring compared to non-exposed, isocaloric (where available) controls; [a]Tanner stage drawings (Marshall & Tanner 1970) were used to assess genital/pubic hair development in boys; [b]only adjusted means (no S.D.) given for T levels across three alcohol exposure levels (<0.10 oz absolute alcohol (AA)/day, 0.10–0.49 oz AA/day and ≥0.50 oz AA/day); correlation and regression coefficients only provided for pubertal data; [c]included age at first nocturnal emission, voice break, acne, regular shaving; [d]serum luteinising hormone (LH) and follicle stimulating hormone (FSH) levels were also tested; [e]also many behavioural measures that were outside the scope of the outcomes reported in this review. See text for more details. This study also included female reproductive outcomes (reported in Table 3); [f]measured using a novel method combining HPLC with UV detection. Shimamoto *et al.* (2006) for more details; [g]when compared with pair-fed control; [h]two cohorts with different timings of alcohol exposure were used; [i]inflammatory cell infiltration, increased stromal thickness, focal hyperplasia, epithelial atypia, increased proliferation (via Ki-67 staining), reduced epithelial cell integrity (via E-cadherin staining and WB); [j]penile reflex test was as described by Davidson *et al.* (1978); [k]all alcohol cohorts combined; absolute AGD only reported; [l]only a subset of males exposed to alcohol from GD7 to PD7 were compared against a subset of non-exposed controls; [m]both absolute AGD and AGD index (corrected for body length and/or weight) were reduced in PAE males; [n]expressed as prostate-seminal vesicle weight ratio only; [o]preputial separation is the separation of the prepuce from the glans penis and is an external sign of androgen-dependent puberty onset in male rats (Korenbrot *et al.* 1977).
AGD, anogenital distance; E2, oestradiol; EIA, enzyme-linked immunoassay; ELISA, enzyme-linked immunosorbent assay; ERα, oestrogen receptor α; EtOH, ethanol; GD, gestational day; IH, immunohistochemistry; PAE, prenatal alcohol exposure; PD, postnatal day; T, testosterone; WB, Western blot.

particularly the number of clinical studies. It has also identified the narrow range of outcomes that have been studied, particularly in females. The main deficits that have been identified in both male and female offspring exposed to PAE are summarised in Fig. 4. However, it should be noted that there was variability in outcomes across studies, with many reporting no changes to some of these parameters in PAE offspring compared to appropriate controls. Also, evidence for these deficits often only come from one or two studies, sometimes with poor quality of reporting and potential for bias. Therefore, the deficits summarised in Fig. 4 should be considered a starting point for further hypothesis testing in this domain.

Of the clinical studies included, all but one (Robe *et al.* 1979) used self-reports during gestation. The reliability of self-reported alcohol consumption has been called into question, with different patterns of drinking behaviour, respondent characteristics and assessment timing potentially affecting response accuracy and bias (Del Boca & Darkes 2003). The specific social context for reporting pregnancy drinking has obvious consequences for self-report accuracy. One study found that retrospective reporting by mothers of early school age children was biased toward under-reporting when compared to gestational reporting by the same women (Eichler *et al.* 2016). However, another study found the opposite was true when assessing alcohol consumption during pregnancy by both antenatal interview and during a 14-year follow-up retrospective interview (Hannigan *et al.* 2010). Use of multiple data sources, such as collateral reporting (Maisto & Connors 1992) or biomarkers for metabolites of alcohol exposure during late gestation such as ethyl glucuronide (EtG) in meconium (Bakdash *et al.* 2010) or phosphatidylethanol in neonatal screening cards (Bakhireva *et al.* 2013), should potentially be considered in future clinical studies.

The evidence for reproductive dysfunction that we report here for offspring with PAE (Fig. 4) is similar to what has been reported for the direct effects of alcohol consumption (Rachdaoui & Sarkar 2017 for review). In females, alcohol use can result in irregular menstrual/oestrous cycles, anovulation, early menopause and elevated E2 levels in both women and rodents. In males, alcohol use results in well-documented decreases in testosterone levels; lower semen volume, sperm count and sperm motility; and increased morphological abnormalities in sperm. It also increases the activity of aromatase, the enzyme that converts androgens to oestrogens, and so E2 levels can be abnormally elevated. High alcohol use in adolescent boys also results in reduced testosterone, luteinising hormone (LH) and follicle stimulating hormone (FSH), although the impact on age at puberty onset has not been reported. Therefore, impacts of PAE on male offspring also generally parallel those seen from direct alcohol exposure, although effects on testosterone levels and age at puberty onset appear more variable in PAE males (Fig. 4), warranting further investigation. There is also, as yet, no evidence for effects on sperm motility or morphology.

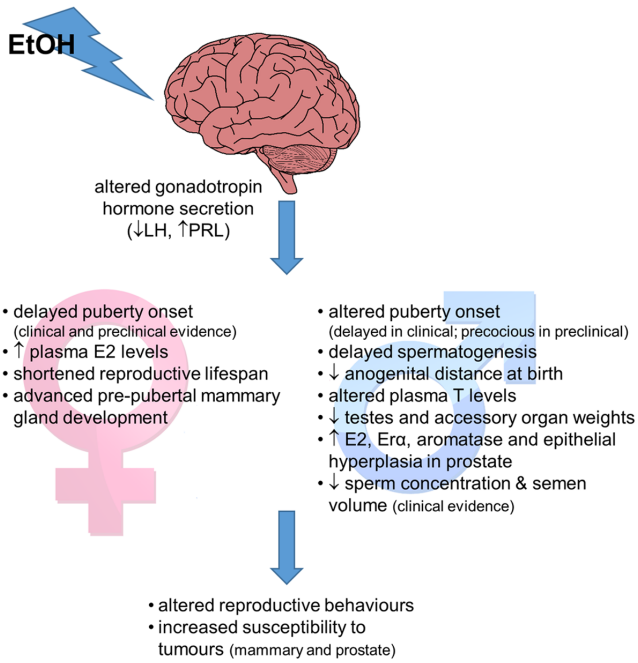

Figure 4 Summary of female and male reproductive deficits reported in offspring exposed to prenatal alcohol exposure. These mainly arise due to the teratogenic effect of alcohol on the brain, affecting the hypothalamic–pituitary–gonadal axis and production of gonadotropic hormones that regulate gonadal function. Alterations in gonadal hormones may in turn alter reproductive behaviours, such as lordosis response in females and mating behaviour in males.

Given the level of exposure was relatively consistent across studies, particularly across preclinical studies (Table 2), the timing across gestation would appear the most obvious determinant of outcome. However, deficits were reported in studies that spanned all stages of gestation and the early postnatal period (Fig. 3), suggesting the timing of exposure may be important for specific outcomes. Many authors suggested that exposure late in gestation had the greatest impact on puberty onset in both males and females. This outcome was extensively investigated in females, and while most studies did have exposure spanning late gestation, the study by Boggan *et al.* (1979) restricted exposure to early-mid gestation (GD5–11) and still reported pubertal delay. In contrast, only one preclinical study looked at puberty onset in males (Chen & Smith 1979), presumably as this is much more difficult than that in female rats. They found a late exposure increased the age of puberty (by ~2 days), in contrast to both clinical and preclinical results in females. However, this study scored very low in terms of quality of reporting, and therefore, the results should be considered with caution for potential bias. Clearly, more studies need to investigate puberty onset in males and subsequent impacts on male fertility.

While the underlying mechanisms for how alcohol exerts its effects on the reproductive system are not entirely understood, it is clear that the effects of alcohol on the hypothalamus and pituitary are important due to their role in regulation of reproductive function via gonadotropins. Although the effects of PAE on the brain were outside the scope of this review, we consider it here as an underlying mechanism for the changes reported in gonadal function. The preclinical study by Sliwowska *et al.* (2016) measured kisspeptin (*Kiss1*) expression in the hypothalamus and saw changes in PAE offspring that affected reproductive maturation and function. In their earlier study (Sliwowska *et al.* 2014), ovariectomised PAE female offspring, treated exogenously with E2 and P, showed altered responses of kisspeptin/ERα immunoreactive neurons within the hypothalamus, which are involved in the hormonal feedback loops regulating the oestrous cycle. Two studies included in this review (Udani *et al.* 1985, Esquifino *et al.* 1986), as well as others outside of the scope of this review (Handa *et al.* 1985, Morris *et al.* 1989, Wilson & Handa 1997), report changes in LH and FSH secretion in both male and female offspring in adult life in response to PAE. Typically these were reduced, unlike prolactin (PRL), which was increased in PAE offspring (Esquifino *et al.* 1986). This is consistent with effects of chronic alcohol exposure in rats (Sanchis *et al.* 1985) and in men and women with alcohol use disorder (AUD) (Rachdaoui & Sarkar 2017). As menses onset is under the control of the HPG (Plant 2015), it is perhaps not surprising that prenatal alcohol, with known effects on the brain and central nervous system, result in the alterations to puberty onset. Interestingly, a study by Creighton-Taylor and Rudeen (1991) found that the delay in puberty onset could be restored in PAE offspring by injection with an opiate antagonist (naltrexone), suggesting that pubertal delay in PAE animals is not due to permanent pathology, but increased inhibition of gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus. This is supported by McGivern and Yellon (1992) who found no differences in the number of GnRH-immunoreactive neurons in the hypothalamus due to PAE.

The other potential mechanism underlying the observed impact of PAE on postnatal reproductive parameters, at least in males, are hormonal changes during development. Appropriate perinatal androgen exposure is essential for male development. Preclinical studies have reported effects of PAE on the prenatal testosterone surge in males (Kakihana *et al.* 1980, McGivern *et al.* 1988, 1993, 1998, Kelce *et al.* 1989, Ward *et al.* 2002*a*,*b*, 2003). The majority of these studies report PAE-induced suppression of the prenatal testosterone surge, suggesting this may be a mechanism for alcohol to cause long-term impacts on male health and fertility. However, Shimamoto *et al.* (2006) suggests that the timing of exposure during gestation is important. In normal rats, the fetal gonads are capable of testosterone secretion in the rat at ~GD14–15 and the plasma testosterone surge typically occurs at GD18 (Warren *et al.* 1973, Weisz & Ward 1980). Therefore, when PAE begins before GD14, the testosterone surge is increased (Ward *et al.* 2002*a*, 2003), while the testosterone surge is suppressed in the foetus and newborn when PAE commences from GD14 (McGivern *et al.* 1988, 1993). This is potentially due to early PAE exposure effecting Leydig cell differentiation, while later exposure results in suppression of Leydig cell testosterone production.

Although this review did not focus on neurobehavioural outcomes, some of the included preclinical studies reported on reproductive behaviours, primarily mating performance in males. Only one study examined female mating receptivity. Hard *et al.* (1984) saw no effect of PAE on the onset of physiological measures of oestrous (vaginal opening and smears), but did report a delay in the onset of the appropriate lordosis response (the arched back normally displayed when receptive to mating). This study also reported an increased lordosis response in PAE males in response to other males, suggesting feminisation, but no changes in masculine sexual behaviours. Udani *et al.* (1985) showed reduced motivation to mate and subsequent performance in PAE males. However, the majority of other studies were negative, with Blanchard and Hannigan (1994) finding PAE did not result in demasculinisation of play behaviour; Chen and Smith (1979) found no impacts on mating behaviour over their three PAE models encompassing both the prenatal and early postnatal (3rd trimester equivalent) period; and Ward *et al.* (1996) found no effect on copulatory potential and mating behaviour. Interestingly, this study only found an effect on male mating behaviour when PAE was combined with a restraint stress during pregnancy, recoverable via testosterone injection. In a previous study by the same authors (Ward *et al.* 1994), PAE resulted in feminisation as shown by an enhanced lordosis response in adult male offspring. Therefore, the effects on male reproductive behaviours and potential feminisation reported in preclinical studies are variable, no doubt reflecting the variable effects of PAE on testosterone levels.

Most of the preclinical studies attempted to control for the reduced food intake that is typical in EtOH treatment by including a pair-fed control group, using a control diet with maltose-dextrin or sucrose substituted for the calories derived from ethanol in the same amount consumed by a 'yoked' EtOH animal on the same day of gestation. However, several studies reported that this pair-feeding had the same effect as PAE, with both groups differing from the chow-fed controls for some of the reproductive outcomes. This suggests that nutritional effects of the diet, rather than the effect of alcohol *per se*, may be mediating some of the effects. The 'pair-feeding' effect has been reviewed by Weinberg *et al.* (2008), suggesting that even if there are no apparent differences in hormonal responsiveness or downstream

reproductive behaviour between these groups, there may still be different underlying mechanisms to these phenotypes. Therefore, the limitations inherent in the control groups for PAE exposure need to be considered and may underlie some of the variability in outcomes.

Two studies reported PAE-induced changes in the mammary gland and prostate (Polanco *et al.* 2011, Murugan *et al.* 2013) that enhanced susceptibility to the development of tumours in response to exposure to a carcinogen. Our initial search strategy could not specifically identify all studies examining cancer or tumour susceptibility in PAE offspring. However, a recent study in the rat has reported similar tumour susceptibility in the pituitary glands of PAE offspring (Jabbar *et al.* 2018). A systematic review by Latino-Martel *et al.* (2010) found a significant association between PAE and childhood acute myeloid leukaemia, suggesting that cancer susceptibility is not just restricted to animal studies.

Conclusion

The current review provides some evidence that PAE can have an impact on both male and female reproductive parameters. However, inconsistencies between studies highlight the importance of further preclinical studies, with different timing of exposure, to clarify the precise impacts at different stages during the reproductive lifespan. In addition, a much broader range of outcomes needs to be examined, particularly in females, to obtain a more complete picture of the potential implications of PAE on future fertility. Although impacts on age at first menarche/puberty onset seem quite clear in females, no studies have examined other critical aspects of fertility, such as ovarian reserve, folliculogenesis, ovulation, oviduct/fallopian tube function, fertilisation/implantation success or live birth rates. Also, few studies have investigated age at menopause/reproductive senescence. The small number of clinical studies with appropriate controls in this domain is also of concern. We recommend that adolescents and individuals of reproductive age diagnosed with FASD are assessed for potential reproductive dysfunction as early as possible, to allow for future family planning and management of reproductive health.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/REP-18-0607.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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