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The role of exposure to phthalates in variations of anogenital distance: A systematic review and meta-analysis☆

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ABSTRACT

Environmental chemicals such as phthalate esters may have adverse effects on anogenital distance (AGD), but the evidence in both genders has not been reviewed systematically. The objective of the present study is to conduct a systematic review and meta-analysis of studies that analyzed the relationship between exposure to phthalates and AGD. English papers published up to March 2018 were searched in PubMed, Scopus, Clarivate-Web of Science, and Google scholar. We applied fixed-effects models to calculate pooled beta coefficient [β]. In the case of heterogeneity, random-effects models were used. Using the comprehensive search strategies, 313 papers were identified and after screening, 10 of them were included in this study. In primary analyses, we found that exposure to phthalates was not associated with short AGD (β = −0.11; 95% CI, −0.27, 0.06; $I^2 = 0\%$). However, results of subgroup analyses indicated that in boys, the sum of di-2-ethylhexyl phthalate (∑DEHP) metabolites had significant association with the risk of shortened anopenile distance (AGD_{AP}) (β = −0.915, 95% CI: 1.629, −0.2) and anoscrotal distance (AGD_{AS}) (β = −0.857, 95% CI: 1.455, −0.26). In addition, urinary monobutyl phthalate (MBP), monoethyl phthalate (MEP), and monoisobutyl phthalate (MiBP) were associated with short AGD_{AP}. We also observed significant association between monobenzylphthalate (MBzP) and anofourchette distance (AGD_{AF}) in girls. Our study provided findings on significant association of exposure to ∑DEHP metabolites, MBP, MEP, and MiBP with shortened AGD_{AP} in boys. The mechanisms of phthalates effect on AGD may involve receptors and enzymes involved in steroidgenesis, negative influence on Leydig cells, cell proliferation, gonocyte cell numbers, and testosterone production.

1. Introduction

Phthalate esters (PAEs) or phthalates are kind of plasticizer and a group of industrial chemicals that are mainly used in medical products, cosmetics, gelling agents, dispersants, adhesives, lubricants, emulsifying agents, and also found in many consumer goods. Therefore, they are considered as ubiquitous environmental contaminants. Human can be exposed to phthalates through dermal absorption, ingestion, or inhalation of contaminated products (Ji et al., 2010; Jurewicz and Hanke, 2011; Zarean et al., 2015; Zarean et al., 2016). These compounds are known as endocrine disrupting compounds (EDCs) with deleterious effects on hormonal balance (Zarean et al., 2016; Zarean et al., 2017). Phthalates are categorized into two distinct groups according to the length of their carbon chain including high molecular weight (HMW) and low molecular weight (LMW). HMW phthalates, such as di-iso-decyl phthalate (DiDP), di-iso-nonyl phthalate (DiNP), bis(2-ethylhexyl) phthalate (DEHP) and butyl benzyl phthalate (BBzP), are mostly used in the production of flexible vinyl plastics, medical devices and

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flooring. LMW phthalates, such as din-butyl phthalate (DnBP), di-iso-butyl phthalate (DiBP), diethyl phthalate (DEP) and dimethyl phthalate (DMP), are commonly used in the production of varnishes, lacquers, paints, and personal care products (Braun et al., 2013; Dong et al., 2017; Katsikantami et al., 2016). Because of short half-life of phthalate diesters, both LMW and HMW phthalates diesters are hydrolyzed into primary metabolite monoester phthalate (Marie et al., 2015). Phthalates can affect fetal growth and development directly or indirectly in pregnant woman by passing through the placental barrier (Liu et al., 2014). Fetuses may have been exposed to these compounds and their monoesters in the amniotic fluid of their mothers (Jurewicz and Hanke, 2011). The results of previous human studies show that prenatal exposure to phthalate at environmental levels could negatively affect male reproductive development (Liu et al., 2014; Marsee et al., 2006). In experimental animal studies, some phthalates induced reproductive tract developmental anomalies, including decreased anogenital distance (AGD), increased incidence of hypospadias, epididymal malformations, cryptorchidism, retention of thoracic nipple, delayedpreputial separation, and testicular lesions (Hauser and Calafat, 2005). AGD is an important clinical measure to health effects of EDCs in environmental toxicology and it has been identified as one of the endpoints in the US Environmental Protection Agency guidelines for reproductive toxicity studies (Liu et al., 2014). AGD is defined as the distance between the anus to genitalia that reflects the *in utero* androgenic action during the development of the reproductive system (Kita et al., 2016). In addition, AGD is a marker of fetal testosterone production by the testis; shorter AGD is observed in rats prenatally exposed to some phthalates (Braun et al., 2013). In addition, it is a marker of *in utero* endocrine disruption, and may also be a marker of hormonal status, fertility, and gonadal health in adulthood (Adibi et al., 2015).

Exposure to phthalates is related to life habits; fetuses are more vulnerable to exposure to EDCs including phthalates metabolites, AGD is an important measure in reproductive toxicity studies (Marie et al., 2015; Zarean et al., 2016). This study represents as systematic review and meta-analysis about exposure to phthalates and variations in AGD in both genders.

2. Methods

The present systematic review and meta-analysis was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Liberati et al., 2009).

2.1. Literature search

We used PubMed, Scopus and Clarivate-Web of Science databases for electronic search. All databases were searched till March 27, 2018. The following key words were used systematically in all mentioned databases: ("diethyl phthalate" OR "dimethyl phthalate" OR "dibutyl phthalate" OR "di (2-ethylhexyl) phthalate" OR "diisodecyl phthalate" OR "diisononyl phthalate" OR "benzyl butylphthalate" OR phthalate) AND ("anogenital distance" OR "anogenital index").

Furthermore, we searched Medline via Medical Subject Headings (MeSH) terms with following MeSH terms: ("bis (2-ethylhexyl) phthalate esterase" [Supplementary Concept] OR "Diethylhexyl Phthalate" [M esh] OR "di-n-octyl phthalate" [Supplementary Concept] OR "phthalate ester hydrolase" [Supplementary Concept] OR "phthalic acid" [Supplementary Concept]) AND ("anogenital distance" OR "anogenital index").

Moreover, we searched Google scholar for increasing the sensitivity of our search. The search was conducted on human studies, but it was not limited to title and abstract because our desired results or outcomes might have been considered as secondary aims of the studies and only mentioned in full text of articles. Limitations were applied to exclude conference papers, editorials, letters, commentaries, short surveys, and notes. We did not consider any time limitation.

2.2. Hand searching

We checked the reference list of the published studies to increase the sensitivity of our search, and to select more related studies.

2.3. Data management

We used EndNote software (version 6) for managing and handling extracted references that were searched from databases. Duplicates were removed and entered into a duplicate library.

2.4. Quality assessment

Quality of studies was conducted using the Newcastle-Ottawa Scale (NOS) for cohort studies (Wells et al., 2017). The NOS consists of three parts: selection (0–4 points), comparability (0–2 points), and exposure (or outcome assessment) (0–3 points). Scores of 7–9 were classified as high-quality studies, 4–6 as moderate quality studies, and 0–3 as low-quality studies. The NOS score was shown in Table 1 for included studies.

2.5. Selection criteria

Studies identified from the literature search were selected on the basis of the following predefined selection criteria:

2.5.1. Inclusion criteria

1) All observational studies (cross-sectional studies, case–control studies, cohort studies)
2) Studies that had evaluated the associations between phthalate exposure and AGD in girls or boys.
3) Studies with an exact definition and exact measuring the AGD and reporting quantitative measurements of associations.

2.5.2. Exclusion criteria

1) Conference papers, editorials, letters, commentaries, short surveys, and notes
2) Animal studies
3) Laboratory studies

2.6. Data extraction

Two independent reviewers (MZ and MK) screened the titles and abstracts of papers, which were identified by the literature search, for their potential relevance or assessed the full text for inclusion in the review. The following information were extracted from all studies: the name of author, year of publication, study design, study population, AGD measurements, phthalate metabolites, results, and beta coefficient (β) with 95% CIs (mm). In the case of disagreement, the discrepancy was resolved in consultation with an expert investigator (RK).

Table 1
Summary of studies included in the meta-analysis.

Author, country	Study population	AGD measurements and age of infants	Phthalates metabolites	Results	NOS score
Swan et al. (2005), USA	134 mothers–sons pairs	AGD_{AP} 13 months	MnBP, MBzP, MCPP, MEP, MiBP, MMP, MEHHP, MEHP, MEOHP	MnBP→AGDap↓ MEP→AGDap↓ MBzP→AGDap↓ MiBP→AGDap↓	7/9
Suzuki et al., 2012, Japan	111 mothers-sons pairs	AGD_{AP} At birth	MMP, MEP, MnBP, MBzP, MEHP, MEHHP, MEOHP	MEHP→AGDap↓	4/9
Bustamante-Montes (2013), Mexico	73 mothers–sons pairs	AGD_{AP}, AGD_{AS} At birth	MEHP, MBzP, MEP, MBP	Total phthalate→AGDap↓	7/9
Swan et al., 2015, USA	758 mothers-newborns pairs (371 boys, 387 girls)	AGD_{AP}, AGD_{AS}, AGD_{AF}, AGD_{AC} At birth	MEP, MnBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MCOP, MCNP, MCPP	in boys: MEHP→AGDas↓, MEOHP→AGDas↓, MEHHP→AGDas↓, in girls: no association	7/9
Bornehag et al., 2015, Sweden	225 mothers–sons pairs	AGD_{AP}, AGD_{AS} 19–21 months	MEP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MHiNP, MOiNP, MCOP	DiNPmetabolites→AGDas↓	7/9
Adibi et al., 2015, USA	541 mothers-newborns pairs	AGD_{AP}, AGD_{AS}, AGD_{AF}, AGD_{AC} At birth	MnBP, MBzP, MEHP, MEP, MiBP, MCPP, MCNP, MCOP	MnBP,MBzP,andmono-2-ethylhexyl phthalate,MEHP→AGD↓	7/9
Martino-Andrade et al., 2016, USA	370 mothers –sons pairs	AGD_{AP}, AGD_{AS} At birth	MEP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MCOP, MCNP, MCPP, MBP	DEHP metabolites in T1→AGD↓	7/9
Jensen et al., 2016, Denmark	245 mothers –sons pairs	AGD_{AP}, AGD_{AS} 3 months	MEP, MnBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MHiNP, MOiNP, MCiOP	No associations	6/9
Barrett et al., 2016, USA	754 mothers-newborns pairs (370 boys, 384 girls)	AGD_{AP}, AGD_{AS}, AGD_{AF}, AGD_{AC} At birth	MEHP, ΣDEHP, MEP, MnBP, MBzP, MEHHP, MEOHP, MECPP, MiBP, MCPP	In the lower stress group, first trimester ΣDEHP→AGD-AS↓ and AGD-AP↓	7/9
Wenzel et al., 2018, USA	187 African American and 193 white mothers	AGD_{AP}, AGD_{AS}, AGD_{AF}, AGD_{AC} At birth	MBP, MiBP, MBzP, MEHP, MEOHP, MEHHP, MEP, and MMP	for African Americans: MEHP → AGDap↓, AGDac↓, AGDaf↓ for white Americans: MnBP→AGDas↑ MEP → AGDac↑	5/9

2.7. Statistical analysis

The effect sizes were reported as regression coefficient and 95% CIs in all studies that were included in our meta-analysis. We used the generic inverse variance (IV) method in the framework of fixed effect approach we estimated an overall effect along with 95% CI based on the reported regression coefficients. To assess the heterogeneity of the results from individual studies, Cochrane's Q statistic, the I^2 statistic (I^2>50% as a threshold indicates significant heterogeneity) and P values ($P < 0.1$) for Cochrane's Q test; and in case of heterogeneity we used random effect meta-analysis for getting reliable and conservative results (Higgins et al., 2003).

We performed subgroup analyses to explore the sources of heterogeneity based on sex and combination of sex and AGD, also in this regard meta regression was performed for evaluating the studies sample size as a possible source of heterogeneity. In the current meta-analysis, publication bias was assessed by examining asymmetry in the Begg funnel plots, and conducting weighted Egger linear regression test (Begg, 1985). Trim-and-fill method also was used as a complementary approach to adjust the funnel plot asymmetry. The sources of publication bias were also evaluated using sensitivity analysis in which each individual study was removed from analyses one by one to evaluate their effect on the estimated overall regression coefficient.

3. Results

3.1. Study selection and characteristics

Fig. 1 presents the selection process. The databases search identified 313 studies. After initial screening of the title and abstract, 265 papers were excluded. After full-text review, 48 papers were included. Finally, 10 papers were included in the present systematic review and meta-analysis (Adibi et al., 2015; Barrett et al., 2016; Bornehag et al., 2015; Bustamante-Montes et al., 2013; Jensen et al., 2016; Martino-Andrade et al., 2016; Suzuki et al., 2012; Swan et al., 2015; Swan et al., 2005; Wenzel et al., 2018). The detailed overview of the ten studies is summarized in Table 1. All selected papers were from cohort studies. Seven studies were conducted in North-America, two in Europe, and one in Asia; their sample sizes varied from 73 to 758. Four studies comprised both boys and girls, and six of them were conducted only in boys. Exposure to phthalates was assessed by urinary phthalate ester metabolites. The metabolites of phthalates that were included in the current meta-analyses were as follows: Monobutyl phthalate (MBP), Monoethyl phthalate (MEP), Mono isobutyl phthalate (MiBP), Mono-benzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCPP), Mono-(2-ethylhexyl) phthalate (MEHP), Mono-(2-ethyl-5- hydroxyhexyl) phthalate (MEHHP), Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), Mono- (2-ethyl-5-carboxypentyl) phthalate (MECPP), ∑DEHP metabolites (MEHP, MEOHP, MEHHP, and MECPP). Overall, nine papers reported positive association of exposure to phthalates metabolites and shortened AGD (Adibi et al., 2015; Barrett et al., 2016; Bornehag et al., 2015; Bustamante-Montes et al., 2013; Martino-Andrade et al., 2016; Suzuki et al., 2012; Swan et al., 2015; Swan et al., 2005; Wenzel et al., 2018). Moreover, of the 10 papers included in the present systematic review, one study did not show any association between phthalate exposure and AGD (Jensen et al., 2016). All selected studies had collected urine sample to represent the individual's phthalate exposure.

3.2. Association between phthalate exposure and anogenital distance

Fig. 2 depicts the beta coefficient as the overall estimate of regression coefficients for the associations between phthalate exposure and AGD in boys and girls. Our pooled results showed significant association between phthalates metabolite and AGD in boys −0.22 (−0.43, −0.01). Heterogeneity was found among studies

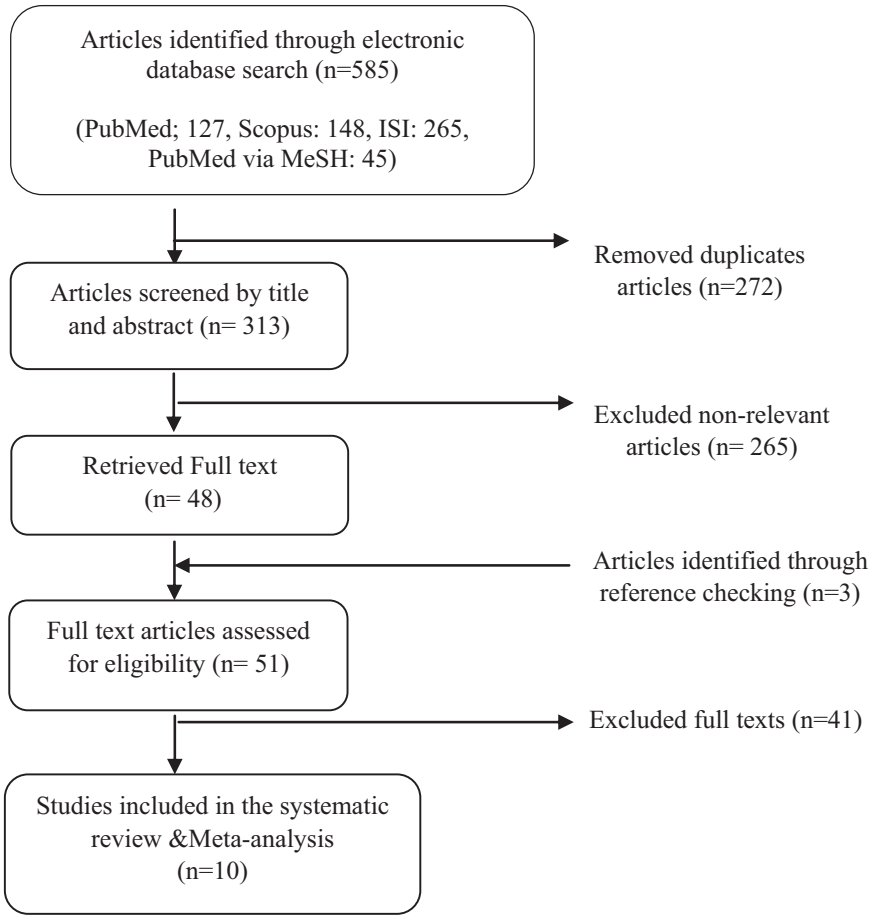

Fig. 1. Literature search and review flowchart for study selection.

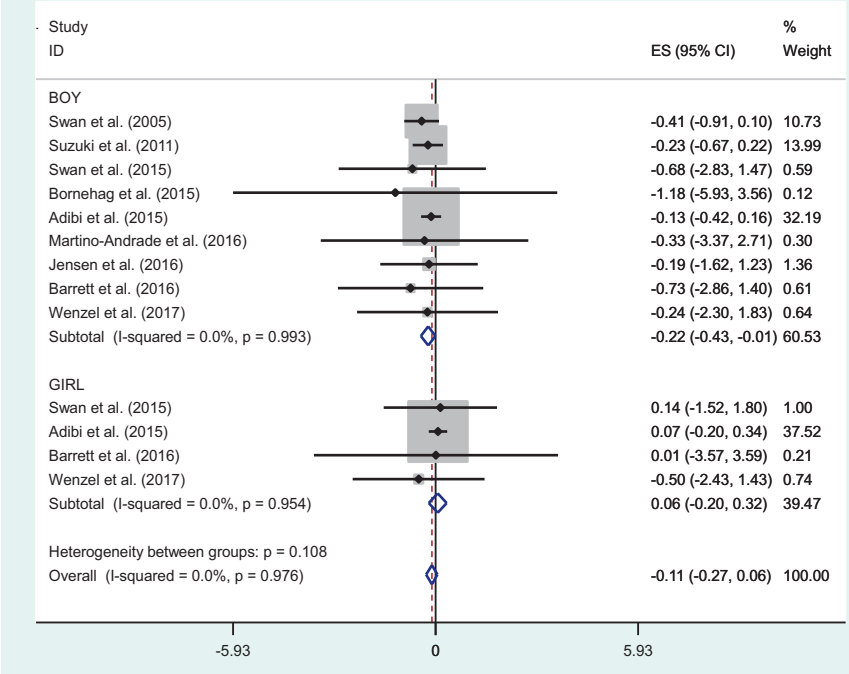

Fig. 2. Forest plot of the association between exposure to phthalates and shortened anogenital distance.

as ($I^2 = 0.0\%$; $P_{heterogeneity} = 0.993$), but these associations were not statistically significant neither in girls nor in the total population studied. The summary measure of association (95% CI) from 10 studies was −0.11 (−0.275, 0.057). Heterogeneity was documented among studies ($I^2 = 0.0\%$; $P_{heterogeneity} = 0.976$).

Also, we conducted a new analysis and extracted the age of infants in each study (Table 1). We excluded three studies (Swan et al., 2005; Bornehag et al., 2015; Jensen et al., 2016), because these were not based on the measurement of AGD at birth. Thereafter, we estimated new analysis based on genders. Among remaining studies, seven had measured AGD in boys and four in girls. The new forest plot (Supplemental Material, Fig. S1) depicts the beta coefficient as the overall estimate of regression coefficients for the associations between phthalate exposure and AGD in boys and girls at birth. The evaluated associations were not statistically significant neither in boys [-0.18 (−0.41, 0.06)] nor in girls [0.06 (−0.2, 0.32)]. But the summary measure of association (95% CI) from 7 studies was similar to our previous results.

Subgroup analyses by different phthalate types or AGD definition (Supplemental Material, Table S1) separately for boys and girls were performed and the results are presented in Tables 2 and 3, respectively (Supplemental Material, Figs. S2–S11). As presented in Table 2, significant relationship was observed between MBP and MEP levels with shortened AGD_{AP} in boys: β of −0.62 (95% CI: 0.997, −0.263) and −0.288 (95% CI: 0.522, −0.054), respectively. The estimated regression coefficients, as indicator for the associations between MECPP and $AGD_{AP,}$ was −0.738 (95% CI: 1.460, −0.016), and −0.721 (95% CI: 1.325, −0.118) for AGD_{AS}. Results showed that exposure to ∑DEHP had the highest significant association with the risk of shortened AGD_{AP} and AGD_{AS} in boys (β = −0.915, 95% CI: 1.629, −0.2 and β = −0.857, 95% CI: 1.455, −0.26, respectively). MEHP, MEHHP, and MEOHP levels were also significantly associated with shortened AGD_{AP} and AGD_{AS}). Table 3 demonstrates the estimated regression coefficients between different phthalate types with AGD_{AP} and AGD_{AS}. Only the associations between MBzP and shortened AGD_{AF} was statistically significant and the overall estimated β for the associations between MBzP and AGD_{AF} was 0.178 (95% CI: 0.045, 0.311).

3.3. Publication bias, meta regression and sensitivity analysis

Begg's funnel plots were plotted to assess the publication bias; also Begg's rank correlation and Egger's regression analyses were applied to formally test the publication bias. There was no evidence of publication bias (or asymmetry) in funnel plots and none of the formal statistical tests led to significant results indication no publication bias for the all evaluated association between exposure to phthalates and the risk of shortened AGD. The results of sensitivity analyses did not show any significant change in the overall estimated effect sizes of all studied exposures after excluding studies one by one; indicating that none of the included studies had notable and different impacts on the estimated regression confections. We used meta-regression models to detect the source of heterogeneity. In this regard, the sample size was used as a covariate. The fitted models for all studied different phthalate types showed no significant estimated slope (the coefficient for the association of sample size and AGD_{AP} and AGD_{AS} in the meta-regression framework).

Table 2
Subgroup analysis of the association between different urinary metabolites of phthalates and shortened AGD in boys.

Phthalates Metabolite	Number	I^2 (p-value)	Pooled β (95% CI)*
MBP			
AGD_{AP}	6	0.0% (0.849)	**−0.620 (-0.997,-0.243)**
AGD_{AS}	6	47.6% (0.089)	−0.236 (−0.706,0.233)
MEP			
AGD_{AP}	6	0.0% (0.599)	**−0.288 (-0.522,-0.054)**
AGD_{AS}	6	0.0% (0.591)	−0.061 (−0.155,0.034)
MiBP			
AGD_{AP}	4	0.0% (0.704)	**−0.601 (-1.025,-0.176)**
AGD_{AS}	5	26.6% (0.245)	−0.123 (−0.327,0.08)
MBzP			
AGD_{AP}	6	0.0% (0.685)	−0.221 (−0.537,0.095)
AGD_{AS}	6	41.4% (0.129)	−0.024 (−0.155,0.108)
MCPP			
AGD_{AP}	3	0.0% (0.467)	0.068 (−0.404,0.541)
AGD_{AS}	3	0.0% (0.740)	−0.03 (−0.139,0.076)
MEHP			
AGD_{AP}	7	36.3% (0.128)	**−0.294 (-0.487,-0.101)**
AGD_{AS}	6	8.2% (0.367)	**−0.316 (-0.459,-0.172)**
MEHHP			
AGD_{AP}	5	44.5% (0.094)	**−0.655 (-1.035,-0.274)**
AGD_{AS}	4	41.1% (0.132)	**−0.657 (-1.144,-0.169)**
MEOHP			
AGD_{AP}	5	53.2% (0.046)	**−0.643 (-1.030,-0.255)**
AGD_{AS}	4	33.8% (0.182)	**−0.856 (-1.366,-0.346)**
MECPP			
AGD_{AP}	3	20.4% (0.285)	**−0.738 (-1.460,-0.016)**
AGD_{AS}	3	0.0% (0.615)	**−0.721 (-1.325,-0.118)**
∑DEHP			
AGD_{AP}	3	43.6% (0.131)	**−0.915 (-1.629,-0.200)**
AGD_{AS}	3	16.6% (0.309)	**−0.857 (-1.455,-0.260)**

*Significant associations are highlighted in bold.
Note (Phthalate metabolites and abbreviations): monobutyl phthalate (MBP), monoethyl phthalate (MEP), monoisobutyl phthalate (MiBP), monobenzyl phthalate(MBzP), mono-3-carboxypropyl-phthalate (MCPP), monoethylhexyl phthalate (MEHP), mono(2-ethyl-5-carboxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5- carboxypentyl) phthalate (MECPP).

Table 3
Subgroup analysis among urinary metabolites of phthalates and shortened AGD in girls.

Phthalates Metabolite	Number	I^2 (p-value)	Pooled β (95% CI) *
MBP			
AGD_{AF}	4	0.0% (0.394)	0.071 (−0.090,0.231)
AGD_{AC}	3	0.0% (0.486)	0.322 (−0.289,0.933)
MEP			
AGD_{AF}	4	0.0% (0.997)	0.046 (−0.047,0.140)
AGD_{AC}	3	23.6% (0.270)	−0.021 (−0.386,0.343)
MiBP			
AGD_{AF}	3	39.5% (0.192)	0.08 (−0.368, 0.528)
AGD_{AC}	2	0.0% (0.608)	−0.429 (−1.201, 0.342)
MBzP			
AGD_{AF}	4	34.6% (0.205)	**0.178 (0.045,0.311)**
AGD_{AC}	3	38.6% (0.196)	0.038 (−0.532,0.607)
MCPP			
AGD_{AF}	3	0.0% (0.912)	−0.016 (−0.112,0.081)
AGD_{AC}	2	0.0% (0.967)	−0.430 (−0.902,0.042)
MEHP			
AGD_{AF}	4	0.0% (0.448)	0.039 (−0.103,0.182)
AGD_{AC}	3	0.0% (0.449)	−0.331 (−0.927,0.265)
MEHHP			
AGD_{AF}	3	12.4% (0.319)	−0.002 (−0.448,0.445)
AGD_{AC}	3	0.0% (0.672)	−0.451 (−1.057,0.156)
MEOHP			
AGD_{AF}	3	8.9% (0.334)	0.081 (−0.399,0.560)
AGD_{AC}	3	0.0% (0.574)	−0.167 (−0.809,0.475)
MECPP			
AGD_{AF}	2	0.0% (0.957)	0.245 (−0.303,0.793)
AGD_{AC}	2	0.0% (0.977)	−0.430 (−1.123,0.263)
∑DEHP			
AGD_{AF}	2	0.0% (0.947)	0.270 (−0.322,0.862)
AGD_{AC}	2	0.0% (0.990)	−0.335 (−1.085,0.415)

*Significant associations are highlighted in bold.
Note (Phthalate metabolites and abbreviations): monobutyl phthalate (MBP), monoethyl phthalate (MEP), monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), mono-3-carboxypropyl-phthalate (MCPP), monoethylhexyl phthalate (MEHP), mono(2-ethyl-5-carboxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5- carboxypentyl) phthalate (MECPP).

4. Discussion

4.1. Summary of evidence

In this meta-analysis, we identified 10 papers reporting on the association of exposure to specific phthalates and AGD. We found that exposure to DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) had strong positive associations with both shortened AGD_{AP} and AGD_{AS} in boys. Our results revealed associations between biomarkers of exposure to specific phthalates and shortened AGD_{AP} (MBP and MEP). In addition, BBzP exposure was positively associated with an increased risk of shortened AGD_{AF} in girls. The greatest risk estimates were observed in the association between exposure to $\sum$DEHP metabolites and shortened AGD_{AP} and AGD_{AS}. We did not observe any significant association between exposure to phthalates and AGD_{AC} in girls.

The mechanisms of the effects of phthalates on the childhood reproductive development are unclear. A majority of studies focused on MEHP, which has known anti-androgenic effects (Foster et al., 2001). Exposure to DEHP/MEHP during gestation caused a decrease in testosterone production as well as shortened AGD in the male offspring of rats (Ge et al., 2007). Several studies have shown that one of the possible mechanisms of the anti-androgenic effect of prenatal DEHP exposure is low expression of receptors and enzymes involved in steroidgenesis (Borch et al., 2006; Howdeshell et al., 2008). According to *in vitro* studies, the main targets of toxicity in DEHP-administered rodents are Leydig cells and therefore phthalates have a negative influence on Leydig cells, increasing cell proliferation and disrupting testosterone production (Ge et al., 2007). These effects contribute to testicular dysgenesis syndrome, which includes a variety of conditions that involve the male reproductive system, including undescended testes, changes in the timing of puberty, hypospadias, fertility and testicular cancer (Green, 2000). The results of study (Johnson et al., 2011) attributed phthalate-induced rat fetal testosterone inhibition associated with reduced activity of lipid metabolism and cholesterologenesis in Leydig cells. Another possibility is that in cultured foetal rat testis cells, MEHP significantly decreased gonocyte cell numbers and testosterone production (Chauvigné et al., 2009; Ji et al., 2010). Human chorionic gonadotropin (hCG) has a role in almost every physiologic process in pregnancy including implantation, immune tolerance, angiogenesis, and uterine quiescence. In fact, the shortened AGD measure was significantly associated with hCG (Dean and Sharpe, 2013). The findings of cross-sectional studies suggested the same link between prenatal anti-androgen exposure and adult reproductive function, which have found associations between AGD and adult serum testosterone and semen quality (Eisenberg et al., 2011; Eisenberg et al., 2012; Mendiola et al., 2011). In other words, the shortened AGD may be a member of the symptom complex of the testicular dysgenesis syndrome (TDS). Then, TDS symptoms might result from a disturbance in the Leydig cell and Sertoli cell differentiation during fetal life, leading to impaired testosterone production and decreased virilization (Mendiola et al., 2011; Skakkebaek et al., 2001; Thankamony et al., 2014).

A positive exposure–response relationship between exposure to some phthalates and risk of variation in AGD was observed in the current study. Further studies are needed to examine the potential mechanisms by which prenatal exposure to phthalates could lead to the childhood reproductive development. The present study shows that the majority of the studies have reported that DEHP metabolites are associated to shorten AGD (Adibi et al., 2015; Barrett et al., 2016; Bustamante-Montes et al., 2013; Martino-Andrade et al., 2016; Suzuki et al., 2012; Swan et al., 2015; Swan et al., 2005). LMW phthalates are generally considered to be less toxic, however, some animal studies reported their adverse effects, e.g. reduced BW and shortened duration of gestation after DEP exposure (Fujii et al., 2005; Marie et al., 2015). Some review studies conducted to evaluate the effects of phthalates. Braun et al. (2013) studied exposure to phthalate and children's health. Some studies pointed to few information and was examined other effects on the children health including gestational length and infant size at birth, physical growth, asthma and allergy, and neurodevelopment (Braun et al., 2013; Suzuki et al., 2012; Swan et al., 2005). Review studies of Hauser and Calafat (2005), Jurewicz and Hanke (2011), Dean and Sharpe (2013), Marie et al. (2015), Liu et al. (2014), Foster et al. (2017), Katsikantami et al. (2016) also reported some information about association of phthalates and AGD. Results of the papers included in our study also indicated that the impact of exposure to phthalate esters on AGD might differ between boys and girls. Several studies have reported significant reductions in AGD in rats after prenatal exposure at high doses to DEHP, DBP, and BzBP (Barlow et al., 2004; Foster et al., 2001; Gray Jr et al., 2000; Nagao et al., 2000; Parks et al., 2000; Tyl et al., 2004). It should be noted that none of these studies have assessed the role of medications as a source of exposure to phthalate in pregnant women, infants or children. The US Food and Drug Administration has published guidelines for urgeing drug manufacturers to remove DEHP or DBP from excipient formulations in medications (Food and Administration, 2012). Experimental and clinical studies have documented adverse effects of phthalates on AGD, which may occur through multiple mechanisms, including receptors and enzymes involved in steroid genesis, Leydig cells, cell proliferation, gonocyte cell numbers, and testosterone production (Ge et al., 2007).

4.2. Limitations and strengths

Although meta-analysis is a beneficial tool to estimate the causal relationships by combining results from different studies, outcomes can be constrained by several limitations of the original studies (Chen et al., 2015). The small number of studies and the lack of accurate exposure assessment methodologies are its major limitations. Time of sample collection is an important and effective factor in studies. Most studies had collected samples in first trimester (Adibi et al., 2015; Barrett et al., 2016; Bornehag et al., 2015; Martino-Andrade et al., 2016; Swan et al., 2015; Swan et al., 2005), except three of them that collected samples in the third trimester (Bustamante-Montes et al., 2013; Jensen et al., 2016; Suzuki et al., 2012). The other limitation of the present study is that most published studies are from the North American and Western European populations and limited data are available for non-Western populations. Exposure to phthalates varies according to educational level, socioeconomic status, geographic location, and work environment. Phthalates exposure exists at general level and is considered as a potential harm for pregnant women. For the risk assessments of phthalate-induced reproductive toxicity, phthalates should be considered as a class and including exposures from several sources (Gray Jr et al., 2000; Swan et al., 2005). Phthalates exposure can be increased during pregnancy by changes in life habits such as more extensive use of body care products and changes in dietary habits. In addition, these compounds cross from maternal blood and enter into the developing fetus via placental transfer (Wittassek and Angerer, 2008).

The main strength of current study included a congener specific meta-analyses that enabled us to identify the potential risk associated with phthalates and AGD, moreover, the heterogeneity among the significant results was low, the included studies were of good quality, and most of them have used both the primary and the secondary metabolites of DEHP for providing a better evaluation of

DEHP exposure. The recent systematic review of Dorman et al. reported associations between exposure to phthalates and AGD. Although, the authors searched both human and animal studies, they did not study the effects of phthalates on AGD in females, and their study was performed on the association between phthalates and AGD only on male sex. Our study investigated the effects of six types of phthalates (including DBP, DEP, DiBP, Di-benzyl phthalate (DBzP), di-n-octyl phthalate (DOP), and DEHP) on the variation of AGD. Moreover, while Dorman et al. studied the effects of only one type of phthalates (DEHP) on AGD, we considered four metabolites including MEHP, MEHHP, MEOHP, and MECPP. It is suggested that one of the primary goals of the systematic review and meta-analysis studies is to find comprehensive results of the published or even unpublished studies (gray literature). Another difference between our study and that of Dorman et al. (2018) is the method of literature search. In 2017, Bramer et al. reported that to guarantee adequate and efficient coverage and to have a robust systematic review on medical studies, the minimum databases to be searched include Embase, Medline, Web of Science, and Google Scholar (Bramer et al., 2017), which have been considered in our study.

5. Conclusion

The results of this meta-analysis demonstrate that exposure to some phthalates metabolites including ∑DEHP, MBP, MEP, MiBP are associated with shortened AGD_{AP} in boys. Reducing the usage of phthalates in various products, and limiting the exposure to phthalates should be emphasized, especially in the prenatal period.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.01.026.

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