

PERSPECTIVES

Impacts of bisphenol A (BPA) and phthalate exposures on epigenetic outcomes in the human placenta

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Abstract

The placenta guides fetal growth and development. Bisphenol A (BPA) and phthalates are widespread environmental contaminants and endocrine disruptors, and the placental epigenetic response to these chemicals is an area of growing research interest. Therefore, our objective was to summarize research linking BPA or phthalate exposure to placental outcomes in human pregnancies, with a particular focus on epigenetic endpoints. In PubMed, studies were selected for review (without limiting start date and ending on 1 May 2018) if they reported any direct effects of BPA or phthalates on the placenta in humans. Collectively, available studies suggest that BPA and phthalate exposures are associated with changes to placental micro-RNA expression, DNA methylation, and genomic imprinting. Furthermore, several studies suggest that fetal sex may be an important modifier of placental outcomes in response to these chemicals. Studies in humans demonstrate associations of BPA and phthalate exposure with adverse placental outcomes. Moving forward, more studies should consider sex differences (termed “placental sex”) in the measured outcomes, and should utilize appropriate statistical approaches to assess modification by fetal sex. Furthermore, more consistent sample collection and molecular outcome assessment paradigms will be indispensable for making progress in the field. These advances, together with improved non-invasive tools for measuring placental function and outcomes across pregnancy, will be critical for understanding the mechanisms driving placental epigenetic disruption in response to BPA and phthalates, and how these disruptions translate into placental and fetal health.

Key words: BPA; epigenetics; endocrine disrupting chemicals; phthalates; placenta

Introduction

The placenta's critical roles in nutrient transport and fetal growth are well-established. In humans, toward the end of the first trimester, deep trophoblast invasion and conversion of maternal spiral arteries allow for the uninterrupted supply of

nutrient-rich maternal blood to the placenta and fetus [1, 2]. Furthermore, hormones secreted by the human placenta include human placental growth hormone, human chorionic gonadotropin (hCG), progesterone, estradiol, and human

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chorionic somatomammotropin hormone/placental lactogen, all with critical functions for pregnancy and fetal development [3–10].

Maternal diet, environment, and lifestyle all impact fetal development, and the placenta has become an important element of the Developmental Origins of Health and Disease (DOHaD) hypothesis, which suggests that our experiences *in utero* program the way in which we respond to our postnatal environment [11]. Recently, the Human Placenta Project was launched to “understand the role of the placenta in health and disease,” stating: *The placenta is the least understood human organ but arguably one of the most important. It influences not just the health of a woman and her fetus during pregnancy, but also the lifelong health of both* [12]. The impact of environmental chemicals on the placenta is relatively unstudied in humans, despite the fact that reproductive-age women are exposed daily to a barrage of chemicals, including bisphenol A (BPA) and phthalates. Phthalates are stabilizers and plasticisers found in many consumer products, including toys and personal care products (perfumes, cosmetics, lotions, deodorants, and many others) [13–16]. They are also used in blood bags, medical devices, as well as in adhesives, solvents, and pesticides [17–19]. Exposures to bisphenols (including BPA), primarily come from polycarbonate plastics used for food packaging and storage containers, as well as from thermal paper receipts and some plastic toys [16, 18, 20–22]. Greater than 90% of pregnant women in the USA have measurable urinary levels of BPA and phthalate metabolites [23]. Specifically, reproductive-age women have higher concentrations of urinary phthalate metabolites than men, likely because of greater use of cosmetics and personal care products, which are important exposure sources [24]. Table 1 summarizes several phthalate parent compounds and their commonly measured metabolites [25]. While exposure to other phenols (e.g. triclosan) and parabens is equally widespread among pregnant women [26], at this time research linking these chemicals with fetal and placental outcomes remains too scarce to warrant a review. However, studies in humans (reviewed in [27]) suggest that maternal BPA and phthalate exposures are associated with numerous alterations in fetal and pregnancy outcomes, including increased risk of pregnancy loss, alterations in the timing of labor (either longer gestation or preterm birth), and changes to infant birth weights [28–31]. Importantly, as will be discussed throughout this review, there is recent research

proposing that the effects of these chemicals on the placenta may mediate these associations.

Epigenetic (“above” genetic) modifications alter gene transcription without affecting the underlying DNA code [32], which make them especially responsive to dietary and environmental cues [33]. Epigenetic modifications described here include genomic imprinting and other measures of DNA methylation, as well as the expression of non-coding RNAs. Imprinted genes are expressed solely from the maternal or paternal allele [34]. In early gestation, genomic imprinting regulates placental and fetal development (reviewed in [35]), and is maintained in the placenta by DNA methylation and histone modifications [36, 37]. Long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) are retrotransposons that, along with other repetitive DNA sequences, represent >50% of the human genome [38]. Because of their pervasive nature within the genome, the methylation of these repetitive sequences has been assessed to represent whole genome methylation in response to environmental exposures [39–41], and as a marker of placental epigenetic disruption. miRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression, and several miR clusters within the placenta have been shown to regulate placental development and function (reviewed in [42]). For example, the miR 17~92 and miR 106a~363 clusters were shown to target human cytochrome p45019A1/aromatase (CYP19A1) and glial cells missing 1 (GCM1) – a transcription factor important for mouse placental labyrinth development [43], while miR-367c was shown to induce trophoblast proliferation and invasion [44]. Although most downstream gene targets of these miRNAs have not yet been elucidated, these miR clusters have been proposed to be promising biomarkers of the effects of maternal chemical exposures on the placenta. While not much is known about other types of non-coding RNAs, long non-coding RNAs (lncRNAs) have been shown to epigenetically regulate gene transcription [45], with roles in development and disease [46, 47].

A critical question in the field of reproductive environmental epidemiology is whether there is placental epigenetic disruption in response to BPA and phthalate exposures that could be mediating fetal outcomes. Several studies have shown associations between phthalate or BPA exposure and altered placental gene expression patterns [48–51], and there is reason to hypothesize that the effects of environmental chemicals on the placental epigenome may impact placental and fetal growth. Early

Table 1: phthalates and their most commonly measured urinary metabolites

Parent phthalate compound (abbreviation)	Metabolite (abbreviation)
Dimethyl phthalate (DMP)	Monomethyl phthalate (MMP)
Diethyl phthalate (DEP)	Monoethyl phthalate (MEP)
Di- <i>n</i> -butyl phthalate (DBP)	Mono- <i>n</i> -butyl phthalate (MBP)
Di- <i>iso</i> -butyl phthalate (DIBP)	Mono- <i>iso</i> -butyl phthalate (MiBP)
Butylbenzyl phthalate (BBzP)	Monobenzyl phthalate (MBzP)
Di(2-ethylhexyl) phthalate (DEHP)	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)
	Mono(2-carboxy-hexyl) phthalate (MCMHP)
Di- <i>iso</i> -nonyl phthalate (DiNP)	Mono- <i>iso</i> -nonyl phthalates (MiNP)
	Mono(hydroxy- <i>iso</i> -nonyl) phthalate (MHiNP)
	Mono(oxo- <i>iso</i> -nonyl) phthalate (MOiNP)
	Mono(carboxy- <i>iso</i> -octyl) phthalate (MCiOP)

Adapted from [25].

placentation occurs under strict epigenetic control (reviewed in [52]), making it a sensitive process for disruption by maternal environmental exposures. Furthermore, as pregnancy progresses, toxicant-induced epigenetic modifications to genes involved in placental function have the potential to alter placental development and efficiency. For example, several studies described here have reported associations between chemical exposures and placental gene expression (Table 2), but additional studies will be needed to assess the epigenetic basis for these changes.

While experimental animal or cell studies assessing placental epigenetic disruption by BPA and phthalates are somewhat limited, there is evidence that these chemicals target the mammalian placental epigenome. A study in mice showed that BPA affected placental loss-of-imprinting and decreased both global and CpG-specific DNA methylation [53], while also in mice, DEHP was shown to increase maternal bias (via imprinting) of the *Rasgrf1* gene [54]. An *in vitro* study in 2 placental cell lines (HTR-8/Svneo and 3A) showed that BPA treatment affected 25 and 60 (respectively) miRs [55], while two studies in HTR-8/Svneo cells showed that treatment with MEHP (the primary oxidative metabolite of DEHP) increased the expression of numerous miRs, including miR-16, which was shown to mediate MEHP-induced decrease in the BCL-2/BAX ratio, a measure of cellular apoptosis [56, 57]. Given the findings from these mechanistic studies, and the established role of epigenetics in placental and fetal development, researchers have begun the arduous task of assessing associations between BPA/phthalate exposure and placental epigenetic disruption in humans. Here we present a comprehensive review of what is currently known about the impacts of BPA and phthalates on placental outcomes in humans, specifically focusing on epigenetic endpoints. In addition, we have provided examples and perspectives of future directions for the field of environmental placental epigenetics. To do this, PubMed was searched (through 1 May 2018) using any combination of the following terms: “BPA,” “bisphenol A,” “phthalate,” “phthalates,” and “placenta” or “placental.” Although numerous studies in non-human placental tissues or cells were identified, only studies with direct measurement of placental physiological or molecular endpoints in human pregnancies were selected for review ($n = 18$). Those only mentioning methodologies for chemical measurement within the placenta, the placenta’s role in fetal outcomes, or review papers (remaining $n = 38$) were not included. Information extracted from each study is reflected in the headings of Table 2.

Epigenetic Consequences of BPA or Phthalate Exposures in the Human Placenta

To date, ten studies focusing on associations between placental outcomes and BPA or phthalate exposures in humans have measured epigenetic endpoints (shaded light yellow in Table 2). As will be described in the sections below, these studies provide evidence that the placenta is an epigenetic target of these chemicals.

Placental Genomic Imprinting

One important and well-studied gene cluster linked to placental and fetal development is placental *IGF2/H19*. Disruption of methylation and imprinting status within the placental *IGF2/H19* domain has been associated with altered nutrient allocation and poor fetal growth [58]. Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer with widespread exposure in pregnant

women [59–61] that is used in food packaging, toys, medical devices, and other PVC-containing products, and human exposure occurs primarily from consuming processed and packaged foods [14, 16]. Exposure to DEHP is most often estimated by measuring the urinary concentrations of its major metabolites (molar sum of MEHP + MEHHP + MEOHP + MECPP = Σ DEHP) [62, 63]. A recent study in women with fetal growth restriction ($n = 80$) and healthy controls ($n = 101$) assessed phthalates in third-trimester first-morning urine, and observed that higher Σ DEHP and 2 of its individual metabolites (MEHHP, MEOHP) were associated with position-specific decreased methylation of placental *IGF2* at delivery in biopsies pooled from the maternal side. No associations were observed for MEHP, mBP (metabolite of dibutyl phthalate), or mMP (metabolite of dimethyl phthalate) [64]. After stratification of placentas by pregnancy outcome (fetal growth restricted vs. healthy pregnancies), the negative association between *IGF2* methylation with urinary MEHHP, MEOHP, and Σ DEHP persisted only in fetal growth restricted cases. While it is unclear from this study whether the methylome in fetal growth restricted pregnancies is especially sensitive to phthalate exposure when compared to healthy pregnancies (thus precluding a greater generalization to all pregnancies), this study does suggest that DEHP may be associated with disruption of placental DNA methylation.

In another study, placental imprinting was assessed at birth in placental samples ($n = 179$) collected from the upper layer of the placenta near the cord (primarily consisting of fetal cells) in relation to first-trimester concentrations of 11 urinary phthalate metabolites. Increases in either Σ phthalate metabolites or Σ low molecular weight phthalates were associated with decreased methylation of placental *H19* and *IGF2* differentially methylated region (*IGF2DMR0*), whereas increased deviation of allelic-specificity of the *H19* gene was associated with Σ DEHP and Σ high molecular weight phthalate metabolites. Furthermore, some findings were sex-specific, where increased MECPP, MEHHP, MEHP, and Σ DEHP were associated with decreased *IGF2DMR0* methylation in females only [65]. While these findings suggest that there may be a relationship between phthalate exposure and placental imprinting in humans, the discrepancy between gestational age at exposure assessment (first trimester) and methylation analysis (birth) is an important consideration when interpreting these results in terms of the direct effects of these chemicals on the placenta. There is evidence that placental methylation increases across pregnancy [66–68], and genomic imprinting may also be temporal. When compared to human third-trimester placentas from term healthy pregnancies, first-trimester placentas from elective terminations showed some level of loss-of-imprinting (biallelic expression) in the 14 genes tested, including *IGF2/H19* [69]. Moreover, *H19* has been shown to be biallelically expressed until week 10 in humans, when it becomes monoallelically expressed [70]. While these temporal differences in imprinting could be due to maternal contamination or could stem from a small number of cells with low baseline methylation, these timing considerations are especially critical for chemicals like BPA and phthalates that have short half-lives, and for which there is considerable day-to-day and within-day exposure variability [71–73]. Although challenging, future studies would ideally assess exposure at timepoints approximately age-matched to placental sampling.

Measures of Placental DNA Methylation

In a sub-sample from the study described above of women with fetal growth restricted ($n = 55$) and healthy pregnancies ($n = 64$),

Table 2: Relationships between BPA and phthalate exposure and placental endpoints in humans

Study design	BPA or phthalate assessment	Gestational age at placental sampling	Sampling site	Population size (n)	Sex	Outcomes	Ref. Year
Cohort	Phthalates in urine in early 3rd trimester	At delivery	Chorionic villi on the fetal side (avoiding membrane and decidua) – 1 sample proximal to the umbilical cord, 1 from closer to the edge	54	28 male/26 female, models adjusted for sex	<ul style="list-style-type: none"> No associations of log-phthalate metabolites with steroidogenesis genes (CYP19, 17β-HSD, p450sc, CYP11B1); but trend toward a U-shaped dose-response relationship for MnBP across quintiles Phthalate metabolites (MEHP, MEOHP, MnBP, MiBP, MBzP, ΣDEHP) associated with \downarrow trophoblast differentiation genes (PPARγ, AhR, HCG), with a suggested trend for a U-shaped dose-response relationship across quintiles for all metabolites except MEHP Interaction between genes and metabolites not significant for most phthalates, except a significant difference in patterns of steroidogenic genes across levels of MnBP and trophoblast target genes across levels of MnBP and MBzP 	[48] 2010
Cohort	Phthalates in urine in 1st trimester (<16 weeks)	At delivery	Upper layer near the cord, consisting of predominantly fetal cells	179	85 male/94 female, reported separately with significant sex X chemical interaction	<ul style="list-style-type: none"> No associations between individual phthalate metabolites and H19 or IGF2DMR2 methylation MEOHP or MEP associated with \downarrow IGF2DMR0 methylation (males and females) MECPP, MEHHP, MEHP associated with \downarrow IGF2DMR0 methylation (females only) No interaction between total phenols and phthalates on methylation LMW and Σphthalates associated with \downarrow H19 methylation LMW and Σphthalates, DEHP, associated with \downarrow IGF2DMR0 methylation DEHP metabolites associated with \downarrow IGF2DMR0 methylation (females only) HMW phthalates not associated with IGF2DMR0 methylation (males or females) No interactions between Σphthalates and infant sex on IGF2DMR2 methylation 	[65] 2014
Case-control				67 heterozygous for the IGF2 rs2585 SNP and 78 heterozygous for the H19 rs3741219 SNP		<ul style="list-style-type: none"> HMW phthalates and DEHP associated with 10% deviation from H19 allele-specific expression (males, not females) Allele-specific expression not associated with H19 or IGF2DMRs methylation (excluding 3 observations, deviation from allele-specific expression associated with \downarrow methylation) No associations between IGF2 mRNA and EDC burden Σphthalates associated with \downarrow H19 mRNA (males, not females) 	

(continued)

Table 2: (Continued)

Study design	BPA or phthalate assessment	Gestational age at placental sampling	Sampling site	Population size (n)	Sex	Outcomes	Ref. Year
Case-control	BPA in placental tissue	2nd trimester (13 to >24 weeks) therapeutic abortions. NR in healthy controls.	Villous core tissue; chorionic plate and decidua basalis were removed prior to analysis	40: healthy @term, 40: 2nd-trimester abortion for fetal malformation	Not specified for each exposure group	<ul style="list-style-type: none"> • 34 miRs (of 1349 profiled) had ≥ 2.5-fold expression difference between control and "malformed" fetuses (also reported to have higher BPA) • KEGG: 19 functions associated with miR146a target genes • \uparrowBPA associated with \uparrowmiR-146a expression ($r = 0.9789$) 	[78] 2015
Nested case-control	Phthalates and BPA in urine at the same times as an-giogenic plasma markers	Across pregnancy: median 10, 18, 26, and 35 weeks	Proposed placental biomarkers in plasma	130 from preterm and 352 from term deliveries	44% male; models not adjusted for sex	<ul style="list-style-type: none"> • IQR \uparrow BPA (not phthalates) associated with 7.08% \uparrowsFLT-1 • \uparrowMEHHP, MEOHP, MECPP, ΣDEHP associated with \uparrowPIGF • \uparrowMEP associated with \uparrowPIGF • \uparrowDEHP metabolites and BPA associated with \uparrowsFlt-1/PIGF • Fully adjusted estimates were attenuated but remained in the same direction 	[139] 2015
Cohort	BPA in placental tissue	At delivery	Villous parenchyma (no decidua basalis or chorionic plate)	63	NR for BPA analysis	BPA not associated with the 112 miRs assessed by microarray	[80] 2015
Cohort	BPA in placental tissue	2nd trimester	NR	12 placental tissues 2nd-trimester voluntary termination	NR	<ul style="list-style-type: none"> • Free BPA not associated with BPA-metabolism gene expression • Total or free BPA not correlated with average % methylation • 1 ng g^{-1} total BPA associated with 0.23% \uparrowLINE-1 methylation and 1 ng g^{-1} free BPA associated with a 0.27% \uparrowLINE-1 methylation (CpG site-specific variation) 	[75] 2015
Case-control	BPA in cord blood at delivery. Exposed: 153 Reference: 47	At delivery	Piece excised from the "central region" immediately after delivery	Exposed: 189-92; Reference: 56-60	Sexes assessed separately	<ul style="list-style-type: none"> • Males: \downarrowKISS1 mRNA, \downarrowleptin receptor mRNA • Females: \downarrowKISS1 and \downarrowleptin receptor mRNA • KISS1 mRNA different between males and females in reference group, but not exposed group • BPA positively correlated with KISS1, leptin, LRCs mRNA. 	[49] 2015
Cohort	Phthalates in 1st-trimester urine	1st- and 2nd-trimester serum	Circulating hCG as measures of placental production	541	266 male/275 female	<ul style="list-style-type: none"> • Males: \downarrowhCG with \uparrowMCOB, MnBP, and MBzP. \uparrowMnBP and MEHP associated with \downarrowAGD • Females: \downarrowhCG with \uparrowMCOB, MnBP, and MBzP. \uparrowMCOB associated with \uparrowAGD • Associations of phthalates with AGD partly explained by changes in hCG 	[141] 2015

(continued)

Table 2: (Continued)

Study design	BPA or phthalate assessment	Gestational age at placental sampling	Sampling site	Population size (n)	Sex	Outcomes	Ref. Year
Case-control	Phthalates in 1st-morning urine in 3rd trimester	At delivery	Pool of 8 biopsies (2 from 4 quadrants) from the maternal side, 2 cm from umbilical cord, decidua removed	55 with fetal growth restriction and 64 healthy controls	26 male/29 female FGR, 34 male/30 female control; sex-adjusted	<ul style="list-style-type: none"> • \downarrowMEHHP, MEOHP, and ΣDEHP associated with \downarrowLINE-1 methylation in FGR cases (not controls) • Mediation effect did not reach statistical significance, but each natural-log unit \uparrowMEHHP and ΣDEHP associated with 0.015 kg and 0.012 (respectively) \downarrow in birth weight mediated through LINE-1 methylation 	[74] 2015
Cohort	Phthalates and BPA in 1st-trimester urine (<16 weeks)	NR (assumed to be at delivery, as with 2014 study)	Upper layer near the cord	179	85 male/94 female, reported separately with significant sex X chemical interaction	<ul style="list-style-type: none"> • $\downarrow$$\Sigma$LMW phthalates associated with \downarrow miR-185 • Infant sex did not modify association between any miRs and Σphthalates • Ten miRs associated w/ at least one phthalate: MEP (negative miR-185), MnBP (positive miR-143.3p), MBzP (positive miR-20a.5p), MNCP (positive miR-200c.3p), MCOP (positive miR-20a.5p, miR-17.5p, miR-16.5p, miR-106b.5p, miR-19a.3p), MCP (positive miR-17.5p, miR-106b.5p, miR-19a.3p), MEHP (positive miR-19a.3p, miR-143.3p, and negative miR-200c.3p, miR-128), and MEOHP (positive miR-143.3p) • BPA mainly reported as Σphenols, but numerous miRs positively associated w/ BPA • Interaction between Σphenols and Σphthalates not significant for any miRs • 19 biological pathways enriched among putative miR-185, miR-142.3p, and miR-15a-5p mRNA targets (analyzed because associated with phenols or phthalates) 	[81] 2016
Case-control	Phthalates in cord blood at delivery	At delivery	1 cm \times 1 cm tissue from both the maternal and fetal sides	124 in high-exposed group, 59 in low-exposed group	Some analyses separated by sex, but (n) for each sex not reported	<ul style="list-style-type: none"> • \downarrowMT-1A, \downarrowFATP1, \uparrowHFABP mRNA in high-exposed vs. low-exposed group • DMP: no significant correlation with MT, MT-1A, MT-2A, FATP1, HFABP mRNA • Males: \uparrowMT, \downarrowMT-1A, \uparrowMT-2A, \downarrowFATP1, \downarrowHFABP mRNA • Females: \downarrowMT, \downarrowMT-1A, \uparrowMT-2A, \downarrowFATP1, \downarrowHFABP mRNA • DEP: correlated with MT-2A, FATP1, and HFABP, but not MT or MT-1A • Males: \downarrowMT, \downarrowMT-1A, \downarrowMT-2A, \downarrowFATP1, \uparrowHFABP mRNA • Females: \downarrowMT, \uparrowMT-1A, \downarrowMT-2A, \downarrowFATP1, \downarrowFABP 	[50] 2016

(continued)

Table 2: (Continued)

Study design	BPA or phthalate assessment	Gestational age at placental sampling	Sampling site	Population size (n)	Sex	Outcomes	Ref. Year
Case-control	Phthalates in 1st-morning urine in 3rd trimester	At delivery	Pool of 8 biopsies (2 from 4 quadrants) from the maternal side, 2 cm from umbilical cord, decidua removed	80 with fetal growth restriction, 101 healthy controls	38 male/42 female (FGR), 55 male/46 female (control), adjustment for sex	<ul style="list-style-type: none"> • BBP: no significant correlation with MT, MT-1A, MT-2A, FATP1, HFABP mRNA • Males: -MT, -MT-1A, -MT-2A, -FATP1, -HFABP mRNA • Females: -MT, -MT-1A, -MT-2A, -FATP1, -HFABP mRNA • DEHP: correlated with MT-1A, but not MT, MT-2A, FATP1, HFABP • Males: -MT, -MT-1A, -MT-2A, -FATP1, -HFABP mRNA • Females: ↓MT, -MT-1A, ↓MT-2A, -FATP1, -HFABP mRNA • DNOP: correlated with MT, MT-2A, but not MT-1A, FATP1, HFABP • Males: -MT, -MT-1A, -MT-2A, -FATP1, -HFABP mRNA • Females: -MT, -MT-1A, -MT-2A, -FATP1, -HFABP mRNA 	[64] 2016
Cohort	Phthalates in urine in early 3rd trimester	At delivery	Fetal side, cutting into chorionic plate & sampling of fetal chorionic villi. Two biopsies - 1 close to cord insertion & 1 close to placental outer margin	180	90 male/90 female, using the (sex) X (phthalate) interaction to compute sex-specific & scenveo_unknown_entity_symbolmt_F062; estimates and confidence intervals	<ul style="list-style-type: none"> • HSD17B1: associations did not differ by sex, and no associations with phthalates • AHR: associations differed by sex for MiBP ($P = 0.04$). Generally, negatively associated with increasing MnBP and MiBP (in males only). • CGA: associations differed by sex for MnBP ($P = 0.01$), MBzP ($P = 0.05$), MEHP ($P = 0.02$), MiBP ($P < 0.01$), DEHP-oxo ($P = 0.04$). Overall associated with MnBP, MBzP, MEHP, MiBP, and DEHP-oxo (not MEP). Association with MnBP was opposite in direction between males and females. CGA expression was ↓ in males at the 3rd vs. 1st quartile of MnBP. In females, CGA expression was ↑ in the 3rd vs. the 1st quartile (the confidence interval included zero). 	[51] 2017

(continued)

Table 2: (Continued)

Study design	BPA or phthalate assessment	Gestational age at placental sampling	Sampling site	Population size (n)	Sex	Outcomes	Ref. Year
Cohort	Phthalates in spot urines in 1st, 2nd, and 3rd trimesters	At delivery	Only length, breadth, and thickness were measured	2725	1399 boys and 1326 girls. Several analyses separate by sex.	<ul style="list-style-type: none"> • CYP19A1: associations differed by sex for MnBP ($P = 0.08$), MBzP ($P = 0.07$), MiBP ($P < 0.01$), DEHP-oxo ($P = 0.09$), DBP-oxo ($P = 0.03$). Generally, negatively associated with increasing MiBP and DEHP-oxo (in males only). • SLC27A4: associations did not differ by sex, and no associations with phthalates • PTGS2: associations not different by sex, and no associations with phthalates • PPARG: associations not different by sex; negatively associated with MnBP (in males) • CYP11A1: associations differed by sex for MBzP ($P = 0.03$), MiBP (0.02), and DEHP-oxo ($P = 0.02$). Generally, negatively associated with increasing MnBP and MiBP (in males) • MCPP was increased in large-for-gestational-age pregnancies 	[113] 2018
Cohort	Phthalates in cord blood at birth	At delivery	Central area near cord, without serosa or amnion	207	Analyses controlled for fetal sex	<p>First trimester:</p> <ul style="list-style-type: none"> • \uparrowBreadth and surface area: \downarrowMBP • \downarrowDifference between length and breadth: \downarrowMMP, MBP, and LMWP <p>Second trimester:</p> <ul style="list-style-type: none"> • \uparrowThickness: \uparrowMMP, MBP, MEOHP, MEHHP, LMWP, and HMWP – associations with MBP, MEHP, MEHHP, and LMWP were driven by effects in males only <p>Third trimester:</p> <ul style="list-style-type: none"> • \uparrowThickness: \uparrowMBP and MEHP – driven by associations in males. Associations with MBP, MEHP, MEHHP, and LMWP were only significant in males. <p>Repeated measures analysis:</p> <ul style="list-style-type: none"> • \uparrowThickness: \uparrowMBP, MEHP, MEOHP, MEHHP, LMWP, and HMWP overall. Similar results in males. In females, \uparrowlength: \uparrowMBP, MEHP, and MEHHP. 	[111] 2018

(continued)

Table 2: (Continued)

Study design	BPA or phthalate assessment	Gestational age at placental sampling	Sampling site	Population size (n)	Sex	Outcomes	Ref. Year
Small pilot analysis	Phthalates in urine on day before or of delivery/C-section	At delivery	Chorionic plate near cord insertion site	10 twin (dichorionic/diamniotic) pregnancies	Some analyses controlled for fetal sex of each twin	<ul style="list-style-type: none"> • Most phthalate metabolites were correlated with placental lncRNAs • MCNP was consistently positively correlated with most lncRNAs • Strongest correlations: MHiBP and LOC91450. Others: lMiBP with lDPP10, HOTTIP. • AIRN, DACT3.AS1, DLX6, DPP10, HOTTIP, LOC143666, and LOC91450 were strongly correlated with the greatest number of phthalate metabolites <p>Controlling for IVF status and fetal sex:</p> <ul style="list-style-type: none"> • MBZP and MEHP positively associated with large number of lncRNAs • MEHP, MEHHP, MECPP, and MEOHP positively correlated with lncRNA IGF2.AS • MBZP, MCOP, MHiBP, and MiBP positively associated with lncRNA DACT3.AS1 	[94] 2018
Cohort	Phthalates in urine prior to procedure	1st-trimester elective termination	Villous tissue	49	Not considered	<p>Analyses in women with high vs. low total phthalates:</p> <ul style="list-style-type: none"> • 2214 differentially methylated single CpG sites, corresponding to 1460 genes. 282 differentially methylated regions, of which 245 correspond to unique genes. 39 genes identified as differentially methylated. • 163 differentially expressed genes (of 1543 tested). 124 genes were down-regulated and 39 were up-regulated. • There were 39 significant methylation-gene expression correlations, corresponding to 23 unique gene symbols. 29 out of 39 relationships were inversely correlated and genes with multiple significant correlations were all in the same direction. • Gene pathway analysis: Erb signaling pathway is the top pathway involved. • EGFR present in 18/51 identified pathways, and also identified in differentially methylated probes analysis 	[77] 2018

(continued)

Table 2: (Continued)

Study design	BPA or phthalate assessment	Gestational age at placental sampling	Sampling site	Population size (n)	Sex	Outcomes	Ref. Year
Small pilot analysis	Phthalates and phenols in urine at admission	Prior to delivery	Plasma extracellular micro-RNA identified as being uniquely placental	10 twin (divorced/diamniotic) pregnancies	Analysis between gender-concordant twins found no differences	<ul style="list-style-type: none"> Phthalates: only MBzP was positively correlated with miR_518e Methyl paraben: positively correlated with miR_518e, negatively correlated with miR_373-3p and miR_543 Ethyl paraben and propyl paraben: negatively correlated with miR_543 24-DCP, 25-DCP, and triclosan: negatively correlated with miR_543 The top correlated miRN did not differ by fetal sex in exploratory analysis 	[93] 2018

Studies assessing epigenetics outcomes are shaded in light yellow. AGD: anogenital distance; BBP: *n*-butyl benzyl phthalate; DEHP: di(2-ethylhexyl)phthalate; DEHP-oxo: sum DEHP oxidative metabolites; ΣDEHP: sum DEHP; DEP: diethyl phthalate; DMP: dimethyl phthalate; DNOP: di-*n*-octyl phthalate; IQR: interquartile range; LMWP: low-molecular weight phthalates; HMWP: high-molecular weight phthalates; MBzP: monobenzylphthalate; MCCOP: mono(carboxy) phthalate; MECP: mono(2-ethyl-5-carboxypentyl) phthalate; MEHP: mono(2-ethylhexyl) phthalate; MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP: mono(2-ethyl-5-oxohexyl) phthalate; MnBP: mono-*n*-butyl phthalate; MiBP: monoisobutyl phthalate; NR: not reported.

increased MEHHP and ΣDEHP metabolites were also associated with decreased placental LINE-1 methylation in women with fetal growth restriction (also assessed in 8 pooled biopsies collected at birth from the maternal side of the placenta) [74]. These results once again support the association of maternal DEHP exposure with altered placental DNA methylation. In a small study of 12 second trimester placentas obtained from a tissue bank (therefore lacking demographic and sampling site information), there was no relationship between the expression of BPA metabolism-related genes and placental BPA concentration, or between average global % methylation assessed by the LUMA assay and placental total or free BPA concentrations. However, global LINE-1 methylation in 4 adjacent LINE-1 promoter CpG sites assessed by pyrosequencing increased by 0.23% and 0.27% with every 1 ng g⁻¹ increase in total or free BPA (respectively) [75]. These studies suggest that LINE-1 methylation may be a sensitive measure of epigenetic disruption by BPA and phthalates, and is perhaps more sensitive than global methylation, as discussed by authors in the above study. Although these effects were rather small, a recent report suggests that caution may be needed when dismissing small-magnitude effect sizes in epigenetic outcomes in response to environmental chemicals [76]. Therefore, additional studies are needed to investigate the importance of these small EDC-induced epigenetic shifts for placental and fetal development.

A recent study compared the placental villous methylome in women who were characterized as having highest (*n* = 7) vs. lowest (*n* = 9) total phthalate urinary concentrations (as a sum of 23 urinary phthalate metabolites). 2214 CpG sites and 282 regions were differentially methylated between the high vs. low phthalate groups. Overall, there were 39 gene-methylation correlations, of which 29 had increased gene expression with decreased methylation, or vice versa. Gene expression and methylation pathway analysis identified the ErbB pathway, which has been shown to be critical for placental growth and development, as being the top pathway that differed between women in the high vs. low phthalate groups [77]. Although the population in this study was quite small, and it is unclear whether gestational ages were matched between the low vs. high phthalate groups, one major strength of this analysis was the use of placental tissues from elective terminations in the first trimester, and concomitant urine collection for phthalate assessment. While LINE-1 methylation appears to be a sensitive marker of epigenetic disruption in general, additional larger studies similar to the one above by Grindler *et al.* will continue to improve our understanding of how BPA and phthalates region-specifically impact the human placental methylome and whether these outcomes have direct downstream consequences for placental function and fetal development.

Placental Expression of Non-Coding RNAs

Four studies in humans have reported associations between BPA or phthalate exposures and placental miRNAs. In one study, villous core placental samples were collected from healthy pregnancies at term in an unpolluted area of Italy (*n* = 40) or from second-trimester abortions due to fetal malformations in a highly polluted area of Salerno in southern Italy (*n* = 40) [78]. BPA was measurable only in placentas of women from the polluted area. Women from the polluted area had higher placental expression of miR-146a, and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis identified 19 biological functions associated with miR-146a target genes, including signal transduction, cell differentiation, and enzymatic activity [78].

The obvious challenges for interpreting these results are the gestational age differences between the exposed and unexposed placentas, and the presence of fetal malformations in association with the BPA-exposed placentas. Authors did discuss unpublished results suggesting the alteration of miR-146a with BPA exposure was also observed in age-matched placentas without fetal malformations, but additional data are needed to confirm these findings. Furthermore, the Salerno area has substantial toxic waste dumping and air pollution [79], so it is likely that BPA-exposed women were also concomitantly exposed to many other environmental chemicals, making it difficult to tease out contributions from BPA exposure alone.

Interestingly, a US study of 63 term vaginal births found no associations between placental villous parenchyma BPA concentrations and the 112 miRs found to be expressed in the placenta, including those within the placenta-specific chromosome 19 microRNA cluster (C19MC, discussed in more detail later) [80]. In another US-based study, BPA was assessed in 179 first-trimester urine samples, and placental samples were collected at birth in the upper layer near the cord. In this study, however, higher urinary BPA concentration was positively associated with the expression of six miRs (Table 2) [81]. The null findings in the Li *et al.* study and the positive associations observed in the LaRocca study may be attributed to a variety of factors, including the larger sample size in the LaRocca study (179 vs. 63), as well as the assessment of urinary first-trimester BPA vs. placental term BPA concentrations. The gestational timing of exposure assessment is critically important, but the decision to assess BPA in urine vs. within the placenta should also be carefully examined. It has been suggested that there may be preferential accumulation of BPA within the placenta [82, 83], making studies that use urinary vs. tissue BPA assessment difficult to compare. This may be another important caveat to consider in the Italian study discussed above. Given the major differences in both exposure assessment (in tissue vs. urine) and outcomes measured (different sets of miRs) between these studies, additional data are still needed to establish precise consequences of BPA exposure for placental miR expression.

The LaRocca study discussed above also assessed the association of urinary phthalate concentrations with placental miRs in 179 women. Fetal sex did not modify the associations between any phthalates and miRs. The sum of low molecular weight phthalate metabolites (MEP, MnBP, MiBP) from first-trimester urine samples was associated with a 0.10 (95% CI: -0.18, 0.01) decrease in the Δ Ct (normalized expression) of placental miR-185. When modeled individually, MEP seemed to drive this negative association (-0.08 Δ Ct; 95% CI: -0.15, -0.01). Other summative phthalate measures were not associated with placental miR expression, but 10 miRs were differentially associated with individual phthalates, and there were moderate associations between the expression of several miRs and the expression of selected placental genes [81]. The significance of these changes in miR expression for placental function in humans warrants future inquiry, as target genes for these miRs in the placenta are likely to be numerous and studies into the downstream functional roles of placental miRs are ongoing [84–87]. However, overall, these studies suggest that both phthalates and BPA may alter placental miRs, which likely has consequences for protein translation and the resulting downstream phenotypes.

One proposed approach for assessing placental function across pregnancy is to measure placenta-specific miR clusters (such as C19MC) that produce placentally exclusive miRs contained in circulating exosomes [88]. The 56 miRs produced from

C19MC are encoded by 46 genes [88], shown to be regulated by genomic imprinting [89], making it possible to distinguish placental exosomes from all others. These miRs have been proposed as good biomarkers of placental health because several miRs in the cluster have been linked to pregnancy complications [90], with studies suggesting roles of these miRs in regulating human trophoblast migration [91], as well as invasion and interaction with the extracellular matrix [92]. Data related to the use of exosomal miRs to assess associations of environmental chemicals with placental health are still limited. However, a recent small ($n = 10$ twin pregnancies) study assessed 3 circulating placentally sourced miRs (identified a priori in a literature search) in maternal plasma to assess associations with phthalates and phenols. Authors reported that MBzP, but not other phthalates, was positively associated with miR-518e, and that several phenols (Table 2) were positively or negatively associated with the 3 measured miRs [93]. These data are promising, and should be followed up by analyses in larger cohorts, assessing a wider array of placenta-specific miRs. miRs are only one type of non-coding RNAs, and a recent small study (from the same 10 twin pregnancies described above) suggests that placental long non-coding RNAs (lncRNAs) may also be targets of environmental chemicals. In this study, placental samples were collected at birth within the chorionic plate near the cord insertion site, and many maternal urinary phthalate metabolites, including MCNP, MEHP, MEHHP, MECPP, MEOHP, MBzP, MCOB, MHiBP, and MiBP were positively correlated with placental lncRNA expression [94]. lncRNAs have been shown to be important during cell differentiations and human early embryonic development [95–97]. Therefore, additional research in a larger and more diverse population that would allow for controlling for various demographic and lifestyle factors is warranted to follow-up on the findings from this small pilot study. This would allow assessment of whether lncRNAs mediate known associations between phthalate exposure and adverse pregnancy/developmental outcomes.

Sex-Specific Placental Outcomes

The placenta undergoes sex-specific development [98–100], which likely explains the observed sexually dimorphic placental responses to a variety of maternal factors, including diet [101–103], obesity [104], and stress [105, 106]. It is especially critical to consider placental sex when assessing the impact of external stressors on epigenetic endpoints, as there is evidence to suggest that associations between placental epigenetic markers and fetal outcomes may be sex-specific [107–109]. Human studies reporting sex differences in the placental response to BPA or phthalates are limited, but they do suggest that placental/fetal sex is a critical factor to consider when interpreting placental outcomes in response to these and other environmental chemicals. As is summarized in Table 2, however, of the 18 studies discussed here, only 4 utilized appropriate statistical approaches for assessing sex-specific placental responses to BPA or phthalates, while 4 others did not. Five other studies did not specify sex at all, and 5 controlled for fetal sex, making comparisons between sexes impossible.

In a study from Southeast China, cord blood and placenta (from both the maternal and fetal sides) were collected from women after delivery ($n = 124$ in high-exposed group and $n = 59$ in low-exposed group) to assess the mRNA expression of a family of placental metallothionein (MT) proteins, a fatty acid transporter (FATP1), and a fatty acid binding protein (HFABP). This study showed that dimethyl phthalate (DMP) and diethyl

phthalate (DEP) did not differ by sex in their associations with any genes tested. However, DEHP was associated with MT and MT-2A only in female placentas [50]. While this study highlights the importance of considering placental/fetal sex, several factors must be taken into account when interpreting these findings. First, phthalate diesters in this study were measured in cord blood, but the recommended approach for assessing phthalate exposure is to measure their metabolites in urine because (i) the diesters have been shown to be non-persistent in humans; they are oxidized quickly and the monoesters are excreted in urine, and (ii) parent phthalate compound contamination is difficult to avoid in the laboratory setting, and may overshadow any parent compounds in blood [110]. A similar limitation was also present in a recent study of 207 women from Southwest China, reporting associations of higher cord blood concentrations of DiBP, DBP, and DEHP with higher placental (sampled near the cord) PPARG protein at delivery, higher DEHP with higher cord blood estradiol, and higher DBP and DEHP with lower cord blood estriol [111]. More importantly for assessing sex-specific outcomes, unlike the studies by LaRocca et al., modification by fetal sex (interaction between exposure and fetal sex) was not assessed prior to testing associations stratified by sex. The Xu et al. 2015 study discussed next has a similar statistical limitation. In women from an electronic-waste recycling town ($n = 189$ – 192) and from a reference town ($n = 56$ – 60), BPA was assessed in cord blood, and placental tissue was collected at delivery from the central region to assess the mRNA expression of leptin receptor and KISS1, which has been shown to be important for placental invasion and gonadotropin release [112]. Both transcripts were higher in BPA-exposed female placentas relative to the reference group, while only KISS1 was higher in placentas of BPA-exposed males [49]. A recent study using a more appropriate statistical approach and study design assessed the mRNA expression of eight placental (from the fetal chorionic villi) genes at delivery and the concentration of nine third-trimester urinary phthalate metabolites in 90 male and 90 female placentas. Overall, associations between gene expression and phthalate metabolites were strongest for MnBP and MiBP, and tended to be stronger for males than females (see Table 2 for additional detail) [51]. While more studies using appropriate analytical and statistical approaches are needed, these studies do suggest that there may be sexually dimorphic associations between exposure to BPA and phthalates and placental outcomes.

While the mechanisms behind these sex-specific associations are not well-understood, a recent large ($n = 2725$) cohort study reported that urinary phthalate metabolites (MBP, MMP, MEHP, MEOHP, MEHHP, SumLMWP, and SumHMWP) were associated with changes to placental breadth (the maximal diameter orthogonal to the maximal length of the placenta surface), surface area, thickness, and the difference between length and breadth. Importantly, many of these associations were only observed in male or female placentas. For example, positive associations of placental thickness with phthalates in the second trimester (MBP, MEHP, MEHHP, and LMWP) and third trimester (MBP and MEHP) were driven by effects in males. Furthermore, in repeated measures analyses, increased MBP, MEHP, MEOHP, LMWP, and HMWP concentrations were associated with larger placental thickness in males, while higher MBP, MEHP, and MEHHP concentrations were associated with longer placental length in females [113]. David Barker was one of the first to suggest that placental morphology (size, width, breadth) is an important predictor of fetal development [114–116]. Given the clear associations between placenta morphology and fetal

development, this study may provide an exciting opportunity for future research to investigate whether these measures, together with upstream epigenetic disruption, mediate known sex-specific associations between phthalate or BPA exposure and fetal outcomes.

Summary and Conclusions

Placental Epigenetic Disruption by BPA and Phthalates

The studies reviewed here provide evidence that exposures to BPA and phthalates are associated with changes to placental epigenetic endpoints (e.g. genomic imprinting, global DNA methylation, and miR expression). Given the fundamental role of the placenta in maternal and fetal health, substantive additional research is needed to understand how environmental toxicants drive human placental epigenetic shifts. Specifically, more research is needed in humans to understand whether these chemicals interact with hormone receptors to disrupt downstream molecular signaling and alter the chromatin state of placental cells, or whether they create a microenvironment within the placenta that is more conducive to epigenetic disruption [for example by increasing reactive oxygen species (ROS) or inflammation]. While certain dietary components (e.g. folate) indirectly contribute to DNA methylation by participating in 1-carbon (methyl group) metabolism [117], and the metabolic status of a cell drives the modifications on histone tails [118–120], little insight is available regarding the precise epigenetic actions of EDCs. The reasons for this are numerous, but include the fact that exposure assessment across pregnancy is challenging, and investigating direct relationships between chemical exposures and placental epigenetic disruption are complicated by the placenta's unique epigenetic signature. This includes temporal shifts in epigenetic marks across gestation [121], relative genomic hypomethylation driven by the placenta's unique methylation machinery signature [122–125], differential genomic imprinting [126], and seemingly unique intragenic vs. gene body methylation patterns [127] when compared to other tissues (reviewed extensively in [128]). If the goal is to more-deeply interrogate the epigenetic effects of environmental chemicals on the placenta, entirely unique approaches are needed for both the design of studies (e.g. timing of exposure and endpoint assessment) and the interpretation of placental epigenetic outcomes (e.g. sexual-dimorphism and epigenetic shifts across pregnancy). Although modeling human exposures and development in experimental animal and cell models is unquestionably challenging, such models could be helpful for establishing mechanistic hypotheses that can be tested in humans.

Limitations of Current Studies and Future Directions

The major challenges for interpreting results from currently available research are as follows:

1. *Discordant timing of exposure and placental endpoint analysis, especially as it relates to measurement of epigenetic endpoints.*
2. *Differences in the matrix (tissue vs. urine vs. plasma) used for exposure analysis.* As discussed in the previous sections, the non-persistent nature of bisphenols and phthalates means that the interpretation of results depends on the biological matrix used for exposure analysis.
3. *Absence of sex-specific analyses, or use of inappropriate statistical analysis to assess sex differences.* Future placental studies should include data from both sexes to accurately assess the

potential for sex-specific associations of chemical exposure with placental outcomes. Furthermore, caution may be warranted when drawing conclusions from currently available studies that do not distinguish between the sexes, that include only one sex in the measured placental outcomes, or that do not utilize appropriate statistical approaches to assess sex differences.

4. *Differences in placental sampling paradigms (especially important for consistently assessing molecular endpoints).* While most human studies reviewed here carefully reported their tissue collection protocols, the sampling procedures differed greatly across studies (Table 2). A study of full-term human placentas assessed the expression of two genes disrupted by phthalates in other tissues (CYP19 and PPAR α). Samples were collected from 12 placental regions, at various times after delivery, and mRNA expression data were normalized to two housekeeping genes or total RNA. When expression from all 12 sampling sites was considered, within-placenta variability was actually greater than between-placenta variability (63–99% vs. 1–37%, respectively). The expression of both transcripts decreased within 25 min of delivery, which the authors suggested may be due to increased hypoxia, and variability differed greatly depending on the housekeeping gene selected for normalization (with 18S determined to be a better internal control). The authors concluded that sampling site, timing of analysis after delivery, and the selection of an appropriate internal control for qPCR analysis must be optimized to best assess the impact of external factors, including chemical exposures, on the placenta [129]. These considerations are also critical for assessing large-scale placental epigenetic outcomes, including DNA methylation and other nucleic-acid based analyses [130–133]. To limit both within- and between-study variability, it may be necessary to establish widely accepted sampling and analytical quality standards, which include consistent sampling location (distance from the cord, biopsy depth from the surface) and time from placental delivery to sampling, as well as selection of appropriate analytical controls (e.g. housekeeping genes) for gene expression and other molecular analyses.
5. *Small sample sizes in some studies.*
6. *Lack of information about placental epigenetic outcomes in early pregnancy.*

To circumvent some of these challenges, in addition to the suggestions provided above, non-invasive approaches would allow for the assessment of placental function throughout pregnancy, for the concurrent measurement of both exposures and placental function, and for the measurement of a variety of molecular, genetic, and epigenetic markers to correlate with both exposure and fetal growth measures. Circulating miRs have long-been considered as promising biomarkers for the detection of cancer and other diseases [134]. As discussed in the previous section, potentially more important for assessing effects of environmental exposures on placental function and/or epigenetic disruption is the recent interest in circulating vesicles/exosomes (and the miRs/other material they contain) as noninvasive biomarkers of placental function [42, 121, 135, 136]. Numerous challenges and uncertainties still exist for using these circulating biomarkers, and while their assessment cannot replace animal/cell models for studying mechanisms associated with environmental exposures, their presence in blood provides a potentially exciting method for non-invasively assessing the effects of environmental chemicals on the human placenta across pregnancy. There is recent evidence that these

biomarkers also exist in urine, and the feasibility of assessing placental exosomes in maternal urine is also being explored (unpublished findings from the Illinois Children's Environmental Health Research Center).

Other less direct approaches for the non-invasive temporal measurement of placentally relevant endpoints may also contribute to our knowledge of the effects of environmental chemicals on placental function. While these markers do not directly measure the epigenetic state of the placenta in response to environmental exposures, they are important for developing future epigenetic studies based on established mechanisms of actions of BPA and phthalates within the placenta. In a study of mothers who delivered preterm ($n = 130$) or at term ($n = 352$), urinary BPA and phthalates were assessed at up to 4 points across pregnancy, as were two plasma angiogenic markers: placental growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family important for placental vascularization [137], and soluble fms-like tyrosine kinase-1 (sFlt-1), a VEGF receptor splice variant associated with decreased angiogenesis [138]. No associations were found for plasma sFlt-1 and urinary phthalates, however, increased urinary MECPP and Σ DEHP were significantly associated with decreased PlGF, while increased mono-ethyl phthalate (MEP) was significantly associated with increased PlGF. Furthermore, an increase in urinary MEHHP, MECPP, and Σ DEHP was significantly associated with an increased plasma sFlt-1/PlGF ratio. BPA was positively associated with sFlt-1 and the ratio of sFlt-1/PlGF in both crude and adjusted models [139]. Placental hormones may also be important non-invasive markers of placental health in response to environmental chemicals. In 541 women, maternal serum hCG, which is released from the placenta to initiate testosterone production in the male fetus [140], was positively associated with MnBP, MBzP, and MCOP in women carrying a female fetus, and negatively associated with these chemicals in women carrying a male fetus [141]. Furthermore, hCG was shown to partially mediate associations between maternal urinary phthalate concentrations and anogenital distance, a marker of androgen status in the fetus [142].

In conclusion, the development of non-invasive measures of placental function in humans should be a high priority, as has been clearly highlighted as part of the The Human Placenta Project [12]. Such approaches will be needed to further our understanding of the impacts of prenatal chemical exposure on placental outcomes and to begin unraveling the proposed role of epigenetic changes in mediating these associations. Regardless of the types of assays or approaches that become available, the studies reviewed here suggest that moving forward, it will be critical to assure measurement accuracy and relevance of the selected placental biomarkers, as well as consideration of placental sex. Such progress will also be indispensable in expanding our understanding of the placenta's role in mediating relationships between the environment and fetal health.

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References

- Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biol Reprod* 2003;**69**: 1–7.
- Desforges M, Sibley CP. Placental nutrient supply and fetal growth. *Int J Dev Biol* 2010;**54**:377–90.
- Lacroix MC, Guibourdenche J, Frenzo JL, Muller F, Evain-Brion D. Human placental growth hormone – a review. *Placenta* 2002;**23**(Suppl A):S87–S94.
- Newbern D, Freemark M. Placental hormones and the control of maternal metabolism and fetal growth. *Curr Opin Endocrinol Diabetes Obes* 2011;**18**:409–16.
- Albrecht ED, Pepe GJ. Estrogen regulation of placental angiogenesis and fetal ovarian development during primate pregnancy. *Int J Dev Biol* 2010;**54**:397–408.
- Padmanabhan RA, Laloraya M. Estrogen-initiated protein interactions during embryo implantation. *Am J Reprod Immunol* 2016;**75**:256–62.
- Vannuccini S, Bocchi C, Severi FM, Challis JR, Petraglia F. Endocrinology of human parturition. *Ann Endocrinol (Paris)* 2016;**77**:105–13.
- Cole LA. Biological functions of hCG and hCG-related molecules. *Reprod Biol Endocrinol* 2010;**8**:102.
- Douglas AJ, Johnstone LE, Leng G. Neuroendocrine mechanisms of change in food intake during pregnancy: a potential role for brain oxytocin. *Physiol Behav* 2007;**91**:352–65.
- Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF, Petraglia F. Inflammation and pregnancy. *Reprod Sci* 2009;**16**: 206–15.
- Gillman MW, Barker D, Bier D, Cagampang F, Challis J, Fall C, Godfrey K, Gluckman P, Hanson M, Kuh D et al. Meeting report on the 3rd International Congress on Developmental Origins of Health and Disease (DOHaD). *Pediatr Res* 2007;**61**: 625–9.
- NICHD THPP. 2016; <https://www.nichd.nih.gov/research/HPP/Pages/default.aspx> (6 August 2018, date last accessed).
- Braun JM, Just AC, Williams PL, Smith KW, Calafat AM, Hauser R. Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic. *J Expo Sci Environ Epidemiol* 2014;**24**:459–66.
- Schettler T. Human exposure to phthalates via consumer products. *Int J Androl* 2006;**29**:134–9; discussion 181–135.
- Ferguson KK, Colacino JA, Lewis RC, Meeker JD. Personal care product use among adults in NHANES: associations between urinary phthalate metabolites and phenols and use of mouthwash and sunscreen. *J Expo Sci Environ Epidemiol* 2017;**27**:326–32.
- Mervish N, McGovern KJ, Teitelbaum SL, Pinney SM, Windham GC, Biro FM, Kushi LH, Silva MJ, Ye X, Calafat AM et al. Dietary predictors of urinary environmental biomarkers in young girls, BCFP, 2004–7. *Environ Res* 2014;**133**:12–9.
- Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: toxicology and exposure. *Int J Hyg Environ Health* 2007;**210**:623–34.
- Larsson K, Ljung Bjorklund K, Palm B, Wennberg M, Kaj L, Lindh CH, Jonsson BA, Berglund M. Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children. *Environ Int* 2014;**73**: 323–33.
- Marie C, Hamlaoui S, Bernard L, Bourdeaux D, Sautou V, Lemery D, Vendittelli F, Sauviant-Rochat MP. Exposure of hospitalised pregnant women to plasticizers contained in medical devices. *BMC Womens Health* 2017;**17**:45.
- Cirillo T, Latini G, Castaldi MA, Dipaola L, Fasano E, Esposito F, Scognamiglio G, Francesco FD, Cobellis L. Exposure to di-2-ethylhexyl phthalate, di-n-butyl phthalate and bisphenol A through infant formulas. *J Agric Food Chem* 2015;**63**: 3303–10.
- Ehrlich S, Calafat AM, Humblet O, Smith T, Hauser R. Handling of thermal receipts as a source of exposure to bisphenol A. *JAMA* 2014;**311**:859–60.
- Andaluri G, Manickavachagam M, Suri R. Plastic toys as a source of exposure to bisphenol-A and phthalates at child-care facilities. *Environ Monit Assess* 2018;**190**:65.
- Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: nHANES 2003–2004. *Environ Health Perspect* 2011;**119**:878–85.
- Wittassek M, Koch HM, Angerer J, Bruning T. Assessing exposure to phthalates – the human biomonitoring approach. *Mol Nutr Food Res* 2011;**55**:7–31.
- Frederiksen H, Skakkebaek NE, Andersson AM. Metabolism of phthalates in humans. *Mol Nutr Food Res* 2007;**51**:899–911.
- Mortensen ME, Calafat AM, Ye X, Wong LY, Wright DJ, Pirkle JL, Merrill LS, Moye J. Urinary concentrations of environmental phenols in pregnant women in a pilot study of the National Children’s Study. *Environ Res* 2014;**129**:32–8.
- Marie C, Vendittelli F, Sauviant-Rochat MP. Obstetrical outcomes and biomarkers to assess exposure to phthalates: a review. *Environ Int* 2015;**83**:116–36.
- Toft G, Jonsson BA, Lindh CH, Jensen TK, Hjollund NH, Vested A, Bonde JP. Association between pregnancy loss and urinary phthalate levels around the time of conception. *Environ Health Perspect* 2011;**120**:458–63.
- Adibi JJ, Hauser R, Williams PL, Whyatt RM, Calafat AM, Nelson H, Herrick R, Swan SH. Maternal urinary metabolites of di-(2-ethylhexyl) phthalate in relation to the timing of labor in a US multicenter pregnancy cohort study. *Am J Epidemiol* 2009;**169**:1015–24.
- Ferguson KK, McElrath TF, Ko YA, Mukherjee B, Meeker JD. Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth. *Environ Int* 2014;**70**:118–24.
- Veiga-Lopez A, Kannan K, Liao C, Ye W, Domino SE, Padmanabhan V. Gender-specific effects on gestational length and birth weight by early pregnancy BPA exposure. *J Clin Endocrinol Metab* 2015;**100**:E1394–E403.
- Felsenfeld G. A brief history of epigenetics. *Cold Spring Harb Perspect Biol* 2014;**6**:a018200.
- Alegria-Torres JA, Baccarelli A, Bollati V. Epigenetics and lifestyle. *Epigenomics* 2011;**3**:267–77.
- Monk D. Genomic imprinting in the human placenta. *Am J Obstet Gynecol* 2015;**213**:S152–S62.
- Fowden AL, Coan PM, Angiolini E, Burton GJ, Constancia M. Imprinted genes and the epigenetic regulation of placental phenotype. *Prog Biophys Mol Biol* 2011;**106**:281–8.
- Umlauf D, Goto Y, Cao R, Cerqueira F, Wagschal A, Zhang Y, Feil R. Imprinting along the Kcnq1 domain on mouse chromosome 7 involves repressive histone methylation and recruitment of Polycomb group complexes. *Nat Genet* 2004;**36**: 1296–300.

37. Lewis A, Mitsuya K, Umlauf D, Smith P, Dean W, Walter J, Higgins M, Feil R, Reik W. Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. *Nat Genet* 2004;**36**:1291–5.
38. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W et al. Initial sequencing and analysis of the human genome. *Nature* 2001;**409**:860–921.
39. Rusiecki JA, Baccarelli A, Bollati V, Tarantini L, Moore LE, Bonfeld-Jorgensen EC. Global DNA hypomethylation is associated with high serum-persistent organic pollutants in Greenlandic Inuit. *Environ Health Perspect* 2008;**116**:1547–52.
40. Wright RO, Schwartz J, Wright RJ, Bollati V, Tarantini L, Park SK, Hu H, Sparrow D, Vokonas P, Baccarelli A. Biomarkers of lead exposure and DNA methylation within retrotransposons. *Environ Health Perspect* 2010;**118**:790–5.
41. Nelson HH, Marsit CJ, Kelsey KT. Global methylation in exposure biology and translational medical science. *Environ Health Perspect* 2011;**119**:1528–33.
42. Mouillet JF, Ouyang Y, Coyne CB, Sadovsky Y. MicroRNAs in placental health and disease. *Am J Obstet Gynecol* 2015;**213**:S163–S72.
43. Kumar P, Luo Y, Tudela C, Alexander JM, Mendelson CR. The c-Myc-regulated microRNA-17~92 (miR-17~92) and miR-106a~363 clusters target hCYP19A1 and hGCM1 to inhibit human trophoblast differentiation. *Mol Cell Biol* 2013;**33**:1782–96.
44. Fu G, Ye G, Nadeem L, Ji L, Manchanda T, Wang Y, Zhao Y, Qiao J, Wang YL, Lye S et al. MicroRNA-376c impairs transforming growth factor-beta and nodal signaling to promote trophoblast cell proliferation and invasion. *Hypertension* 2013;**61**:864–72.
45. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 2009;**10**:155–9.
46. Troy A, Sharpless NE. Genetic “Inc”-age of noncoding RNAs to human disease. *J Clin Invest* 2012;**122**:3837–40.
47. Wapinski O, Chang HY. Long noncoding RNAs and human disease. *Trends Cell Biol* 2011;**21**:354–61.
48. Adibi JJ, Whyatt RM, Hauser R, Bhat HK, Davis BJ, Calafat AM, Hoepner LA, Perera FP, Tang D, Williams PL. Transcriptional biomarkers of steroidogenesis and trophoblast differentiation in the placenta in relation to prenatal phthalate exposure. *Environ Health Perspect* 2009;**118**:291–6.
49. Xu X, Chiung YM, Lu F, Qiu S, Ji M, Huo X. Associations of cadmium, bisphenol A and polychlorinated biphenyl co-exposure in utero with placental gene expression and neonatal outcomes. *Reprod Toxicol* 2015;**52**:62–70.
50. Li B, Xu X, Zhu Y, Cao J, Zhang Y, Huo X. Neonatal phthalate ester exposure induced placental MTs, FATP1 and HFABP mRNA expression in two districts of Southeast China. *Sci Rep* 2016;**6**:21004.
51. Adibi JJ, Buckley JP, Lee MK, Williams PL, Just AC, Zhao Y, Bhat HK, Whyatt RM. Maternal urinary phthalates and sex-specific placental mRNA levels in an urban birth cohort. *Environ Health* 2017;**16**:35.
52. Nelissen EC, van Montfoort AP, Dumoulin JC, Evers JL. Epigenetics and the placenta. *Hum Reprod Update* 2011;**17**:397–417.
53. Susiarjo M, Sasson I, Mesaros C, Bartolomei MS. Bisphenol A exposure disrupts genomic imprinting in the mouse. *PLoS Genet* 2013;**9**:e1003401.
54. Kang ER, Iqbal K, Tran DA, Rivas GE, Singh P, Pfeifer GP, Szabo PE. Effects of endocrine disruptors on imprinted gene expression in the mouse embryo. *Epigenetics* 2011;**6**:937–50.
55. Avissar-Whiting M, Veiga KR, Uhl KM, Maccani MA, Gagne LA, Moen EL, Marsit CJ. Bisphenol A exposure leads to specific microRNA alterations in placental cells. *Reprod Toxicol* 2010;**29**:401–6.
56. Meruvu S, Zhang J, Bedi YS, Choudhury M. Mono-(2-ethylhexyl) phthalate induces apoptosis through miR-16 in human first trimester placental cell line HTR-8/SVneo. *Toxicol In Vitro* 2015;**31**:35–42.
57. Meruvu S, Zhang J, Choudhury M. Mono-(2-ethylhexyl) phthalate increases oxidative stress responsive miRNAs in first trimester placental cell line HTR8/SVneo. *Chem Res Toxicol* 2016;**29**:430–5.
58. St-Pierre J, Hivert MF, Perron P, Poirier P, Guay SP, Brisson D, Bouchard L. IGF2 DNA methylation is a modulator of newborn’s fetal growth and development. *Epigenetics* 2012;**7**:1125–32.
59. Arbuckle TE, Davis K, Marro L, Fisher M, Legrand M, LeBlanc A, Gaudreau E, Foster WG, Choeurng V, Fraser WD et al. Phthalate and bisphenol A exposure among pregnant women in Canada – results from the MIREC study. *Environ Int* 2014;**68**:55–65.
60. Casas L, Fernández MF, Llop S, Guxens M, Ballester F, Olea N, Irurzun MB, Rodríguez LSM, Riaño I, Tardón A et al. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. *Environ Int* 2011;**37**:858–66.
61. Cantonwine DE, Cordero JF, Rivera-Gonzalez LO, Anzalota Del Toro LV, Ferguson KK, Mukherjee B, Calafat AM, Crespo N, Jimenez-Velez B, Padilla IY et al. Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: distribution, temporal variability, and predictors. *Environ Int* 2014;**62**:1–11.
62. Silva MJ, Samandar E, Preau JL Jr, Needham LL, Calafat AM. Urinary oxidative metabolites of di(2-ethylhexyl) phthalate in humans. *Toxicology* 2006;**219**:22–32.
63. Anderson WA, Castle L, Scotter MJ, Massey RC, Springall C. A biomarker approach to measuring human dietary exposure to certain phthalate diesters. *Food Addit Contam* 2001;**18**:1068–74.
64. Zhao Y, Chen J, Wang X, Song Q, Xu HH, Zhang YH. Third trimester phthalate exposure is associated with DNA methylation of growth-related genes in human placenta. *Sci Rep* 2016;**6**:33449.
65. LaRocca J, Binder AM, McElrath TF, Michels KB. The impact of first trimester phthalate and phenol exposure on IGF2/H19 genomic imprinting and birth outcomes. *Environ Res* 2014;**133**:396–406.
66. Yuen RK, Penaherrera MS, von Dadelszen P, McFadden DE, Robinson WP. DNA methylation profiling of human placentas reveals promoter hypomethylation of multiple genes in early-onset preeclampsia. *Eur J Hum Genet* 2010;**18**:1006–12.
67. Chavan-Gautam P, Sundrani D, Pisal H, Nimbargi V, Mehendale S, Joshi S. Gestation-dependent changes in human placental global DNA methylation levels. *Mol Reprod Dev* 2011;**78**:150.
68. Novakovic B, Yuen RK, Gordon L, Penaherrera MS, Sharkey A, Moffett A, Craig JM, Robinson WP, Saffery R. Evidence for widespread changes in promoter methylation profile in human placenta in response to increasing gestational age and environmental/stochastic factors. *BMC Genomics* 2011;**12**:529.
69. Pozharny Y, Lambertini L, Ma Y, Ferrara L, Litton CG, Diplas A, Jacobs AR, Chen J, Stone JL, Wetmur J et al. Genomic loss

- of imprinting in first-trimester human placenta. *Am J Obstet Gynecol* 2010;**202**:e391–e8.
70. Jinno Y, Ikeda Y, Yun K, Maw M, Masuzaki H, Fukuda H, Inuzuka K, Fujishita A, Ohtani Y, Okimoto T. Establishment of functional imprinting of the H19 gene in human developing placentae. *Nat Genet* 1995;**10**:318–24.
 71. Fisher M, Arbuckle TE, Mallick R, LeBlanc A, Hauser R, Feeley M, Koniecki D, Ramsay T, Provencher G, Berube R et al. Bisphenol A and phthalate metabolite urinary concentrations: daily and across pregnancy variability. *J Expo Sci Environ Epidemiol* 2015;**25**:231–9.
 72. Ye X, Wong LY, Bishop AM, Calafat AM. Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. *Environ Health Perspect* 2011;**119**:983–8.
 73. Valvi D, Monfort N, Ventura R, Casas M, Casas L, Sunyer J, Vrijheid M. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. *Int J Hyg Environ Health* 2015;**218**:220–31.
 74. Zhao Y, Shi HJ, Xie CM, Chen J, Laue H, Zhang YH. Prenatal phthalate exposure, infant growth, and global DNA methylation of human placenta. *Environ Mol Mutagen* 2015;**56**:286–92.
 75. Nahar MS, Liao C, Kannan K, Harris C, Dolinoy DC. In utero bisphenol A concentration, metabolism, and global DNA methylation across matched placenta, kidney, and liver in the human fetus. *Chemosphere* 2015;**124**:54–60.
 76. Breton CV, Marsit CJ, Faustman E, Nadeau K, Goodrich JM, Dolinoy DC, Herbstman J, Holland N, LaSalle JM, Schmidt R et al. Small-magnitude effect sizes in epigenetic end points are important in children's environmental health and disease prevention research center's epigenetics working group. *Environ Health Perspect* 2017;**125**:511–26.
 77. Grindler NM, Vanderlinden L, Karthikraj R, Kannan K, Teal S, Polotsky AJ, Powell TL, Yang IV, Jansson T. Exposure to phthalate, an endocrine disrupting chemical, alters the first trimester placental methylome and transcriptome in women. *Sci Rep* 2018;**8**:6086.
 78. De Felice B, Manfellotto F, Palumbo A, Troisi J, Zullo F, Di Carlo C, Di Spiezo Sardo A, De Stefano N, Ferbo U, Guida M et al. Genome-wide microRNA expression profiling in placentas from pregnant women exposed to BPA. *BMC Med Genomics* 2015;**8**:56.
 79. Mazza A, Piscitelli P, Neglia C, Della Rosa G, Iannuzzi L. Illegal dumping of toxic waste and its effect on human health in Campania, Italy. *Int J Environ Res Public Health* 2015;**12**:6818–31.
 80. Li Q, Kappil MA, Li A, Dassanayake PS, Darrah TH, Friedman AE, Friedman M, Lambertini L, Landrigan P, Stodgell CJ et al. Exploring the associations between microRNA expression profiles and environmental pollutants in human placenta from the National Children's Study (NCS). *Epigenetics* 2015;**10**:793–802.
 81. LaRocca J, Binder AM, McElrath TF, Michels KB. First-trimester urine concentrations of phthalate metabolites and phenols and placenta miRNA expression in a cohort of U.S. women. *Environ Health Perspect* 2016;**124**:380–7.
 82. Takeda Y, Liu X, Sumiyoshi M, Matsushima A, Shimohigashi M, Shimohigashi Y. Placenta expressing the greatest quantity of bisphenol A receptor ERR[gamma] among the human reproductive tissues: predominant expression of type-1 ERRgamma isoform. *J Biochem* 2009;**146**:113–22.
 83. Schonfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I. Parent bisphenol A accumulation in the human maternal–fetal–placental unit. *Environ Health Perspect* 2002;**110**:A703–A7.
 84. Adel S, Mansour A, Louka M, Matboli M, Elmekawi SF, Swelam N. Evaluation of microRNA-210 and protein tyrosine phosphatase, non-receptor type 2 in pre-eclampsia. *Gene* 2017;**596**:105–9.
 85. Sun M, Chen H, Liu J, Tong C, Meng T. MicroRNA-34a inhibits human trophoblast cell invasion by targeting MYC. *BMC Cell Biol* 2015;**16**:21.
 86. Yang W, Wang A, Zhao C, Li Q, Pan Z, Han X, Zhang C, Wang G, Ji C, Wang G et al. miR-125b enhances IL-8 production in early-onset severe preeclampsia by targeting sphingosine-1-phosphate lyase 1. *PLoS One* 2016;**11**:e0166940.
 87. Zhu Y, Lu H, Huo Z, Ma Z, Dang J, Dang W, Pan L, Chen J, Zhong H. MicroRNA-16 inhibits feto-maternal angiogenesis and causes recurrent spontaneous abortion by targeting vascular endothelial growth factor. *Sci Rep* 2016;**6**:35536.
 88. Bortolin-Cavaillat ML, Dance M, Weber M, Cavaillat J. C19MC microRNAs are processed from introns of large Pol-II, non-protein-coding transcripts. *Nucleic Acids Res* 2009;**37**:3464–73.
 89. Noguier-Dance M, Abu-Amero S, Al-Khtib M, Lefevre A, Coullin P, Moore GE, Cavaillat J. The primate-specific microRNA gene cluster (C19MC) is imprinted in the placenta. *Hum Mol Genet* 2010;**19**:3566–82.
 90. Hromadnikova I, Kotlabova K, Ondrackova M, Pirkova P, Kestlerova A, Novotna V, Hympanova L, Krofta L. Expression profile of C19MC microRNAs in placental tissue in pregnancy-related complications. *DNA Cell Biol* 2015;**34**:437–57.
 91. Xie L, Mouillet JF, Chu T, Parks WT, Sadovsky E, Knofler M, Sadovsky Y. C19MC microRNAs regulate the migration of human trophoblasts. *Endocrinology* 2014;**155**:4975–85.
 92. Xie L, Sadovsky Y. The function of miR-519d in cell migration, invasion, and proliferation suggests a role in early placentation. *Placenta* 2016;**48**:34–7.
 93. Zhong J, Baccarelli AA, Mansur A, Adir M, Nahum R, Hauser R, Bollati V, Racowsky C, Machtinger R. Maternal phthalate and personal care products exposure alters extracellular placental miRNA profile in twin pregnancies. *Reprod Sci* 2018;**19**:3719118770550.
 94. Machtinger R, Zhong J, Mansur A, Adir M, Racowsky C, Hauser R, Brennan K, Karlsson O, Baccarelli AA. Placental lncRNA expression is associated with prenatal phthalate exposure. *Toxicol Sci* 2018;**163**:116–22.
 95. Perry RB, Ulitsky I. The functions of long noncoding RNAs in development and stem cells. *Development* 2016;**143**:3882–94.
 96. Bouckenheimer J, Assou S, Riquier S, Hou C, Philippe N, Sansac C, Lavabre-Bertrand T, Comtes T, Lemaître J-M, Boureux A et al. Long non-coding RNAs in human early embryonic development and their potential in ART. *Hum Reprod Update* 2016;**23**:19–40.
 97. Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet* 2014;**15**:7–21.
 98. Gonzalez TL, Sun T, Koepfel AF, Lee B, Wang ET, Farber CR, Rich SS, Sundheimer LW, Buttle RA, Chen Y-DI et al. Sex differences in the late first trimester human placenta transcriptome. *Biol Sex Differ* 2018;**9**:4.
 99. Kalisch-Smith JI, Simmons DG, Pantaleon M, Moritz KM. Sex differences in rat placental development: from pre-implantation to late gestation. *Biol Sex Differ* 2017;**8**:17.
 100. Rosenfeld CS. Sex-specific placental responses in fetal development. *Endocrinology* 2015;**156**:3422–34.
 101. Gabory A, Ferry L, Fajardy I, Jouneau L, Gothie JD, Vige A, Fleur C, Mayeur S, Gallou-Kabani C, Gross MS et al. Maternal

- diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta. *PLoS One* 2012;7:e47986.
102. Penailillo R, Guajardo A, Llanos M, Hirsch S, Ronco AM. Folic acid supplementation during pregnancy induces sex-specific changes in methylation and expression of placental 11beta-hydroxysteroid dehydrogenase 2 in rats. *PLoS One* 2015;10:e0121098.
 103. Tarrade A, Panchenko P, Junien C, Gabory A. Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism. *J Exp Biol* 2015;218:50–8.
 104. Kim DW, Young SL, Grattan DR, Jasoni CL. Obesity during pregnancy disrupts placental morphology, cell proliferation, and inflammation in a sex-specific manner across gestation in the mouse. *Biol Reprod* 2014;90:130.
 105. Bale TL. Sex differences in prenatal epigenetic programming of stress pathways. *Stress* 2011;14:348–56.
 106. Pankevich DE, Mueller BR, Brockel B, Bale TL. Prenatal stress programming of offspring feeding behavior and energy balance begins early in pregnancy. *Physiol Behav* 2009;98:94–102.
 107. Mohanty AF, Farin FM, Bammler TK, MacDonald JW, Afsharinejad Z, Burbacher TM, Siscovick DS, Williams MA, Enquobahrie DA. Infant sex-specific placental cadmium and DNA methylation associations. *Environ Res* 2015;138:74–81.
 108. Nielsen CH, Larsen A, Nielsen AL. DNA methylation alterations in response to prenatal exposure of maternal cigarette smoking: a persistent epigenetic impact on health from maternal lifestyle? *Arch Toxicol* 2016;90:231–45.
 109. Lesseur C, Armstrong DA, Murphy MA, Appleton AA, Koestler DC, Paquette AG, Lester BM, Marsit CJ. Sex-specific associations between placental leptin promoter DNA methylation and infant neurobehavior. *Psychoneuroendocrinology* 2014;40:1–9.
 110. Calafat AM, Koch HM, Swan SH, Hauser R, Goldman LR, Lanphear BP, Longnecker MP, Rudel RA, Teitelbaum SL, Whyatt RM et al. Misuse of blood serum to assess exposure to bisphenol A and phthalates. *Breast Cancer Res* 2013;15:403.
 111. Huang Y, Garcia JM, Shu W, Rong H, Zhang L, Wang Y, Tan Y, Lin H, Zeng H, Chen JA. Peroxisome proliferator activated receptor gamma in human placenta may mediate the adverse effects of phthalates exposure in pregnancy. *Reprod Toxicol* 2018;75:121–6.
 112. Dedes I. Kisspeptins and the control of gonadotrophin secretion. *Syst Biol Reprod Med* 2012;58:121–8.
 113. Zhu YD, Gao H, Huang K, Zhang YW, Cai XX, Yao HY, Mao LJ, Ge X, Zhou SS, Xu YY et al. Prenatal phthalate exposure and placental size and shape at birth: a birth cohort study. *Environ Res* 2018;160:239–46.
 114. Alwasel SH, Harrath AH, Aljarallah JS, Abotalib Z, Osmond C, Al Omar SY, Thornburg K, Barker DJ. The velocity of fetal growth is associated with the breadth of the placental surface, but not with the length. *Am J Hum Biol* 2013;25:534–7.
 115. Barker DJ, Thornburg KL, Osmond C, Kajantie E, Eriksson JG. The surface area of the placenta and hypertension in the offspring in later life. *Int J Dev Biol* 2010;54:525–30.
 116. Eriksson JG, Kajantie E, Thornburg KL, Osmond C, Barker DJ. Mother's body size and placental size predict coronary heart disease in men. *Eur Heart J* 2011;32:2297–303.
 117. Mandaviya PR, Stolk L, Heil SG. Homocysteine and DNA methylation: a review of animal and human literature. *Mol Genet Metab* 2014;113:243–52.
 118. Salminen A, Kauppinen A, Kaarniranta K. AMPK/Snf1 signaling regulates histone acetylation: impact on gene expression and epigenetic functions. *Cell Signal* 2016;28:887–95.
 119. Zhang T, Kraus WL. SIRT1-dependent regulation of chromatin and transcription: linking NAD(+) metabolism and signaling to the control of cellular functions. *Biochim Biophys Acta* 2010;1804:1666–75.
 120. Su X, Wellen KE, Rabinowitz JD. Metabolic control of methylation and acetylation. *Curr Opin Chem Biol* 2016;30:52–60.
 121. Luo SS, Ishibashi O, Ishikawa G, Ishikawa T, Katayama A, Mishima T, Takizawa T, Shighara T, Goto T, Izumi A et al. Human villous trophoblasts express and secrete placenta-specific microRNAs into maternal circulation via exosomes. *Biol Reprod* 2009;81:717–29.
 122. Jensen TJ, Kim SK, Zhu Z, Chin C, Gebhard C, Lu T, Deciu C, van den Boom D, Ehrich M. Whole genome bisulfite sequencing of cell-free DNA and its cellular contributors uncovers placenta hypomethylated domains. *Genome Biol* 2015;16:78.
 123. Macaulay EC, Weeks RJ, Andrews S, Morison IM. Hypomethylation of functional retrotransposon-derived genes in the human placenta. *Mamm Genome* 2011;22:722–35.
 124. Novakovic B, Wong NC, Sibson M, Ng HK, Morley R, Manuelpillai U, Down T, Rakyan VK, Beck S, Hiendleder S et al. DNA methylation-mediated down-regulation of DNA methyltransferase-1 (DNMT1) is coincident with, but not essential for, global hypomethylation in human placenta. *J Biol Chem* 2010;285:9583–93.
 125. Logan PC, Mitchell MD, Lobie PE. DNA methyltransferases and TETs in the regulation of differentiation and invasiveness of extra-villous trophoblasts. *Front Genet* 2013;4:265.
 126. Court F, Tayama C, Romanelli V, Martin-Trujillo A, Iglesias-Platas I, Okamura K, Sugahara N, Simon C, Moore H, Harness JV et al. Genome-wide parent-of-origin DNA methylation analysis reveals the intricacies of human imprinting and suggests a germline methylation-independent mechanism of establishment. *Genome Res* 2014;24:554–69.
 127. Schroeder DI, Jayashankar K, Douglas KC, Thirkill TL, York D, Dickinson PJ, Williams LE, Samollow PB, Ross PJ, Bannasch DL et al. Early developmental and evolutionary origins of gene body DNA methylation patterns in mammalian placentas. *PLoS Genet* 2015;11:e1005442.
 128. Januar V, Desoye G, Novakovic B, Cvitic S, Saffery R. Epigenetic regulation of human placental function and pregnancy outcome: considerations for causal inference. *Am J Obstet Gynecol* 2015;213:S182–S96.
 129. Adibi JJ, Hauser R, Williams PL, Whyatt RM, Thaker HM, Nelson H, Herrick R, Bhat HK. Placental biomarkers of phthalate effects on mRNA transcription: application in epidemiologic research. *Environ Health* 2009;8:20.
 130. Edgar RD, Jones MJ, Robinson WP, Kobor MS. An empirically driven data reduction method on the human 450K methylation array to remove tissue specific non-variable CpGs. *Clin Epigenet* 2017;9:11.
 131. Manokhina I, Wilson SL, Robinson WP. Noninvasive nucleic acid-based approaches to monitor placental health and predict pregnancy-related complications. *Am J Obstet Gynecol* 2015;213:S197–S206.
 132. Manokhina I, Singh TK, Penaherrera MS, Robinson WP. Quantification of cell-free DNA in normal and complicated pregnancies: overcoming biological and technical issues. *PLoS One* 2014;9:e101500.
 133. Price EM, Cotton AM, Penaherrera MS, McFadden DE, Kobor MS, Robinson W. Different measures of “genome-wide” DNA methylation exhibit unique properties in placental and somatic tissues. *Epigenetics* 2012;7:652–63.
 134. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X et al. Characterization of microRNAs in serum:

- a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008;**18**:997–1006.
135. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K. The microRNA spectrum in 12 body fluids. *Clin Chem* 2010;**56**:1733–41.
136. Chim SS, Shing TK, Hung EC, Leung TY, Lau TK, Chiu RW, Lo YM. Detection and characterization of placental microRNAs in maternal plasma. *Clin Chem* 2008;**54**:482–90.
137. Regnault TR, Galan HL, Parker TA, Anthony RV. Placental development in normal and compromised pregnancies – a review. *Placenta* 2002;**23**(Suppl A):S119–S29.
138. Coolman M, Timmermans S, de Groot CJ, Russcher H, Lindemans J, Hofman A, Geurts-Moespot AJ, Sweep FC, Jaddoe VV, Steegers EA. Angiogenic and fibrinolytic factors in blood during the first half of pregnancy and adverse pregnancy outcomes. *Obstet Gynecol* 2012;**119**:1190–200.
139. Ferguson KK, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. Phthalate metabolites and bisphenol-A in association with circulating angiogenic biomarkers across pregnancy. *Placenta* 2015;**36**:699–703.
140. Huhtaniemi IT, Korenbrot CC, Jaffe RB. HCG binding and stimulation of testosterone biosynthesis in the human fetal testis. *J Clin Endocrinol Metab* 1977;**44**:963–7.
141. Adibi JJ, Lee MK, Naimi AI, Barrett E, Nguyen RH, Sathyanarayana S, Zhao Y, Thiet MP, Redmon JB, Swan SH. Human chorionic gonadotropin partially mediates phthalate association with male and female anogenital distance. *J Clin Endocrinol Metab* 2015;**100**:E1216–E24.
142. Dean A, Sharpe RM. Clinical review: anogenital distance or digit length ratio as measures of fetal androgen exposure: relationship to male reproductive development and its disorders. *J Clin Endocrinol Metab* 2013;**98**:2230–8.