**THE ASSOCIATION BETWEEN PHTHALATE EXPOSURE AND NEWBORN ANOGENITAL DISTANCE: A REVIEW**

by

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University of Pittsburgh, 2018

**ABSTRACT**

AGD is an androgen-sensitive biomarker affected by both maternal phthalate exposure and hormone concentrations. Based on current studies, some phthalates (class of endocrine disrupting chemicals, many used in plastic production) and their metabolites concentration are associated with shorter AGD for the male infant. Moreover, the length of AGD relates to several adverse health outcomes that might originate during the fetal period such as infertility, hypogonadism and prostate cancer. The public health significance for the study is to the pathway from phthalate exposure, maternal hormone level to newborn AGD. The result can be the foundation of further research in public health.

In general, testosterone concentration is positively associated with AGD for both male and females. Longer AGD is an indicator of greater masculinization and shorter AGD is an indicator of hormonal disruption in males and reduced masculinization. Studies focusing on other types of reproductive hormones are rare, and their conclusions are inconsistent. As an example, one study in Spain did not observe a significant association between AGD and follicle-stimulating hormone (FSH), estradiol, and sex hormone-binding globulin (SHBG). In contrast, another study in China reported a negative correlation between AGD and estradiol or SHBG. One study in the US reported the positive relationship between AGD and the hCG in female neonates. Thus, to clarify the inconsistent conclusion, further research to explore the real relationship between AGD and hormone is needed.

While there is a need to further explore how all reproductive hormones affect the length of AGD, there also is a need to address several additional questions. These include research on epigenetic or gene polymorphism to AGD, and the epigenetic studies would be an essential issue. For example, the different association between hCG and AGD among male and female newborns. The sex difference is an important factor for both AGD and phthalate exposure. Besides, there is only one study has identified a SNP on the endocrine receptor related to phthalate exposure and AGD. More studies should be done to investigate how epigenetic or gene affect AGD. Another gap in the literature is the lack of mediation analysis. Through mediation analysis, we can further clarify the pathway from phthalate exposure to hormone levels and then to the anogenital distance, rather than adjusting for hormone concentration in the regression model.

**TABLE OF CONTENTS**

[1.0 INTRODUCTION 1](#_Toc512503690)

[2.0 REVIEW OF ANOGENITAL DISTANCE 3](#_Toc512503691)

[2.1 INTRODUCTION 3](#_Toc512503692)

[**2.1.1 THREE METHODS TO MEASURE ANOGENITAL DISTANCE 3**](#_Toc512503693)

[**2.1.2 RELIABILITY FOR ANOGENITAL DISTANCE 5**](#_Toc512503694)

[2.2 NEGATIVE OUTCOMES RELATED TO THE ANOGENITAL DISTANCE 9](#_Toc512503695)

[**2.2.1 IRREGULAR MENSTRUAL CYCLE 9**](#_Toc512503696)

[**2.2.2 POLYCYSTIC OVARY SYNDROME (PCOS) 9**](#_Toc512503697)

[**2.2.3 INFERTILITY 10**](#_Toc512503698)

[**2.2.4 UNDESCENDED TESTIS 11**](#_Toc512503699)

[2.3 RISK FACTORS FOR LENGTH OF ANOGENITAL DISTANCE 14](#_Toc512503700)

[**2.3.1 BIRTH WEIGHT AND BIRTH LENGTH 14**](#_Toc512503701)

[**2.3.2 MEDICINE USE DURING PREGNANCY 15**](#_Toc512503703)

[**2.3.3 ANOGENITAL DISTANCE APPLICATION IN PUBLIC HEALTH 16**](#_Toc512503704)

[3.0 PHTHALATE EXPOSURE IN PREGNANCY 18](#_Toc512503705)

[3.1 PHTHALATE EXPOSURE IN DAILY LIFE 18](#_Toc512503706)

[3.2 ADVERSE OUTCOMES FOR PHTHALATE EXPOSURE DURING PREGNANCY 20](#_Toc512503707)

[3.3 RISK FACTORS FOR MATERNAL PHTHALATE EXPOSURE 21](#_Toc512503708)

[3.4 ASSOCIATION BETWEEN PHTHALATE EXPOSURE AND INFANT ANOGENITAL DISTANCE 22](#_Toc512503709)

[4.0 OTHER FACTORS AFFECTING THE ASSOCIATION BETWEEN PHTHALATE EXPOSURE AND ANOGENITAL DISTANCE 26](#_Toc512503710)

[4.1 GENDER 26](#_Toc512503711)

[4.2 HORMONE LEVEL 27](#_Toc512503712)

[**4.2.1 MECHANISM 27**](#_Toc512503713)

[**4.2.2 REPRODUCTIVE HORMONES 27**](#_Toc512503714)

[4.3 OTHER FACTORS 30](#_Toc512503715)

[5.0 CONCLUSION 34](#_Toc512503716)

[5.1 OVERVIEW 34](#_Toc512503717)

[5.2 STRENGTHS AND WEAKNESSES 35](#_Toc512503718)

[5.3 THE DIRECTION OF FUTURE STUDIES 36](#_Toc512503719)

[**5.3.1 OTHER HORMONES 36**](#_Toc512503720)

[**5.3.2 GENE RESEARCH 36**](#_Toc512503721)

[**5.3.3 MEDIATION ANALYSIS 37**](#_Toc512503722)

[5.4 CONTRIBUTION TO PUBLIC HEALTH 38](#_Toc512503723)

[BIBLIOGRAPHY 40](#_Toc512503724)

**List of tables**

[Table 1 The distribution of AGD for different countries 8](#_Toc513548231)

[Table 2 The association between length of AGD and adverse health outcome 12](#_Toc513548232)

[Table 3 Risk factors related to short AGD 17](#_Toc513548233)

[Table 4 The association between phthalate exposure during pregnancy and pre-term birth or longer gestational age 21](#_Toc513548234)

[Table 5 Articles relate to the association between phthalate exposure and infant AGD 24](#_Toc513548235)

[Table 6 Articles relate to the association between hormone level and infant AGD 32](#_Toc513548236)

**List of figures**

[Figure 1 Three main methods to measure the AGD 5](#_Toc513548159)

# Introduction

AGD is the length between the center of the anus to either scrotum or penis for males or to the clitoral hood or fourchette for females. Recent articles have illustrated that AGD is associated with several adverse health outcomes in the male newborn, like undescended testis and hypospadias. Moreover, several studies have represented that the length of AGD is strongly related to fertility. Thus, in the public health field, it is important to explore and identify the risk factors related to AGD, and short AGD length.

Previous studies have identified that AGD, an androgen-sensitive biomarker, is strongly associated with the concentration of reproductive hormones, such as testosterone, whether among animals or human. A shorter AGD is suggested to be an index that reflects the hormone level in the human body, whether from the adult or pregnant women (fetal hormone). Recent studies (Alaee, Gharib, & Fouladinejad, 2014; Papadopoulou et al., 2013; Romano-Riquer, Hernandez-Avila, Gladen, Cupul-Uicab, & Longnecker, 2007) reported that the AGD is more reliable than other indices to test the abnormal genital development for newborns, such as penis width and length. Hence, the measurement of AGD might be more commonly used in clinical assessments in the future.

Phthalates are chemicals used frequently in consumer products because it makes plastic both structurally sound and more flexible. Phthalates are contained in several products used by individuals on a regular basis, like plastic water bottles or cosmetics. However, the health effects of phthalate exposure are not yet fully understood (The Centers for Disease Control and Prevention, 2009). Limited previous articles have observed that phthalate exposures, such as DEHP and MEP, are associated with shorter AGD among newborns.

Phthalates have been identified as an endocrine disrupting chemical (EDC), which interferes in the hormone function of humans. Hence, for AGD, a sensitive marker for the hormone effect, it is reasonable for scientists to consider whether AGD would be affected by the phthalate exposure, especially in infants, the most sensitive population. However, studies in this field are limited. Most research focuses on male infants and specific hormones, like testosterone. Thus, there is still a gap in knowledge of the relationship between phthalate exposure and AGD in female infants, and a gap in knowledge of the relationship between AGD and adverse health outcomes.

# Review of anogenital distance

## Introduction

Previous studies have reported that AGD may be a useful biomarker for investigating the negative consequences of environmental exposures during the fetal period. The definition of AGD is the length between the center anus to the vagina or penis based on the gender. AGD is typically measured by trained professionals in the clinical and research settings.

### Three methods to measure anogenital distance

There are several different measurements of AGD used in the scientific literature (Figure 1). The first measure was reported by Swan (Swan et al., 2005) used similar methods in the Study of Future Family (SFF) cohort in 2005. They assessed AGD as the extent from the center of the anus to the cephalad base of penis among males, and the extent from the center of the anus to the base of the clitoris for females. Salazar-Martinez in 2004 (Salazar-Martinez, Romano-Riquer, Yanez-Marquez, Longnecker, & Hernandez-Avila, 2004) used the similar method based on Swan’s study. They measured the length between the center of the anus to the junction of the rugated skin of the scrotum with the smooth perineal skin for the male infant. For the female infant, they measured the distance between the center of the anus to the posterior convergence of the fouechette.

In 2015, Sathyanarayana (Sathyanarayana et al., 2015) measure AGD for infants and children based on Swan’s method but with more details. They designed a standard protocol to estimate AGD for participants in The Infant Development and Environment Study (TIDES) cohort. Based on this protocol, trained nurses measure two different distances for either male or female newborns. For boys, nurses measure the length from the center of the anus to the anterior of the penis (AGDAP) or the base of the scrotum (AGDAS). For girls, they measure the extent of the center of the anus to the anterior of the clitoral hood (AGDAC) or the base of the posterior fouechette (AGDAF).

Two other research studies conducted in Mexico (Romano-Riquer et al., 2007) and Ghana (Asafo-Agyei et al., 2017) used another method to estimate AGD. Both used the same method as Sathyanarayana et al. to measure AGD among the female group in TIDES cohort. For males, they utilized the AGDAS, but the researcher designated two other indices to replace the AGDAP. They surveyed the length between the center of the anus to the anterior base of the penis (AGD1), and the distance from the center of the anus to the posterior of the penis (AGD2).

There are two reasons that scientists have considered multiple measurements for AGD. First, according to previous articles, we know that AGD differs between males and females. Thus we can’t apply the same method for both males and females. Second, detailed measurement of AGD can provide more information.

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| Figure 1 Three main methods to measure the AGD  Section I: this method only estimates the distance from central of anus to the junction of the rugated skin of the scortum (male) or from central of anus to the posterior convergence of the fourchette (female) (Salazar-Martinez et al., 2004).  Section II: this method measures two different AGD for each sex. For male population (left), they estimate the AGDAS and AGDAP. For female population (right), they measure the AGDAF and AGDAC (Sathyanarayana et al., 2015).  Section III: this method gauges the AGDAC and AGDAF for female group. Among male group, they measure AGD1 and AGD2 and AGDAP. They gague AGD1 and AGD2 instead AGDAS (Asafo-Agyei et al., 2017; Romano-Riquer et al., 2007). |

### Reliability for anogenital distance

As with all biomarkers, it is important to examine whether AGD has good reliability as a marker before we apply it in research settings. In 2004, Salazer-Martinez conducted a cross-sectional study in Mexico to evaluate the reliability of AGD (Salazar-Martinez et al., 2004). They used the simple definition to estimate the AGD for the newborn. For the male infant, they measured the length between the center of the anus to the junction of the smooth perineal skin with the rugated skin of the scrotum, and for female infant, they measured the length between the center of the anus to the posterior convergence of the fourchette. The result showed that among 20 infants, the reliability of AGD was 0.91.

Before scientists starting use anogenital distance, they usually used penis width or length as the biomarker. The study Romano-Riquer conducted in Mexico indicated that the AGD has better reliability than penis length/width (Romano-Riquer et al., 2007). The result represents that the reliability for penis width was 0.75 and for penis length was 0.78, and the reliability for anogenital distances (AGD1, AGD2, and AGDAS) was from 0.82 to 0.91, respectively. Therefore, it is reasonable that scientists selected AGD as a marker to assess the risk of adverse outcomes instead of penis length or width. Papadopoulou also conducted a study in Europe in 2013 to explore the reliability of AGD (Papadopoulou et al., 2013). They enrolled one to two years old children from Crete, Greece and Barcelona, Spain into the study. They selected Swan’s (Swan et al., 2005) method to measure the AGD for infants. For male newborns, the reliability for AGDAP and AGDAS was 0.89 and 0.96, respectively. For female newborns, the reliability for AGDAC and AGDAF was both 0.91.

However, another study, focused on infants in the US, had a different conclusion (Sathyanarayana, Beard, Zhou, & Grady, 2010). The researchers measured the AGDAS for male and AGDAF for female infants in 1-3 days after birth. The inter-reliability among boys (AGDAS) and girls (AGDAF) was 82% and 18% respectively. The intra-reliability for boys (AGDAS) and girls (AGDAF) was 91% and 76% respectively. Both inter- and intra-reliability is better for male groups than female groups. One possible reason for such different results among the two studies might be the time for measurement. The study in the Us measured AGD in 1-3 days after birth, but the study in Europe estimated AGD during the first to second year for newborns. It might be much more difficult for nurses to measure infants in 1-3 days, and the variance might be greater. In the U.S study, Sathyanarayana et al indicated that two female infants they selected to test the reliability were difficult to measure. Another possible reason is the sample size. Sathyanarayana’s study only recruited two male and three female infant to test the reliability, but the research in the EU enrolled thirteen male and seventeen females to test the reliability. Therefore, the result might be different. To make sure the reliability is acceptable for both male and female, we might need another study in the US with greater sample size.

There is another point that researchers should consider before they use the AGD in their study. First, the type of AGD they select to measure. Like the statement above, there are three types of AGD among the previous studies: 1. A simple measure of AGD, 2. AGD measured from two perspectives (AGDAS, AGDAP in males, and AGDAC and AGDAF in females), and 3. A method with five indices (AGD1, AGD2, AGDAS, AGDAC, and AGDAF). Hence, it is essential to realize the definition of these different measurements and decide which one is more suitable to the purpose of research. Second, the analysis should notice the age distribution of their study population. According to the previous studies, AGD measurements from older infants and children had better reliability than measures in younger populations.

The distribution of AGD is different between countries (Table 1). For example, the mean of AGD1 and AGD2 in Ghana (Asafo-Agyei et al., 2017) was 48.9±5.6mm and 43.7±5.9mm respectively, but the mean for the same index in Japan (Suzuki, Yoshinaga, Mizumoto, Serizawa, & Shiraishi, 2012) was 45.8±6.5mm and 20.3±4.6mm respectively. A similar situation exists when measurements are compared for female infants from Ghana and the US. The mean AGDAF and AGDAC for the study in the US was 16.02±3.22mm and 36.60±3,89mm respectively. Girls in Ghana had shorter measurement for both AGDAF (13.6±2.7mm) and AGDAC (34.2±3.3mm). Although we don't know whether the difference in AGD between countries was significant or not, we can still consider that race/ethnicity could be a factor related to anogenital distance. Moreover, we can also consider whether the difference is made by the day of measurement after delivered.

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| Table 1 The distribution of AGD for different countries | | | | |
| Author (Year) | Location (Country) | Sample size | Result | |
| Male | Female |
| **Adult** | | | | |
| Mira-Escolano et al (2014) | Murica Young Women’s Study. (Spain) | 100 healthy university female student aged 18-23 (from 9th February 2011 to 25th November 2011). | - | Mean AGDAF: 37.7±6.3mm, Mean AGDAC: 80.4±10.5mm. |
| Mendiola et al (2015) | University Hospital in the Murcia Region (Spain) | 91 men aged 25 to 38 and receive infertility services in the hospital. | Mean AGDAS: 46.4±12.9 mm,  Mean AGDAP: 14.0±24.8mm. | - |
| **Newborn** | | | | |
| Suzuki et al (2012) | Central Hospital if the Defense Force (Japan). | 111 pregnant-boy pairs. | Mean AGD1: 45.8±6.5mm,  mean AGD2: 20.3±4.6mm. | - |
| Swan et al (2005) | The Infant Development and Environment Study (TIDES)\*. | 753 mothers visit prenatal clinics from August 2010 to August 2012.  49% of newborns were males (n=366), and 51% were females (n=373) | Mean AGDAS: 24.73±4.55mm,  Mean AGDAP: 49.66±5.91mm. | Mean AGDAF: 16.02±3.22mm,  Mean AGDAC:36.60±3.89mm. |
| Salazar-Martinez et al (2004) | Dr. Ernesto Meana San Roman General Hospital (Mexico) | 87 newborns, 42 were female and 45 were male (in 1999). | Mean AGD: 22mm | Mean AGD: 11mm. |
| Ozkan et al (2011) | Atatürk University Hospital (Turkey) | 135 male newborns and 115 female newborns (from May to August 2009.) | Mean AGD1 : 56±1.0mm,  mean AGD2: 44±0.2mm,  mean AGDAS: 23±0.6mm | Mean AGDAF: 10.3±0.2mm,  Mean AGDAC: 30.0±0.2mm. |
| Jensen et al (2016) | Odense Child Cohort Study (Denmark) | 245 mother – son pairs (from 2010 to 2012). | Median of AGDAS: 39.6mm (19.4-50.6mm),  Median of AGDAP: 70.2mm (49.1-86.2mm) | - |
| Asafo-agyei et al., 2017 | Komfo Anokye Teahing Hospital (KATH) (Ghana) | 1,256 babies (from May 2014 to September 2014),  644 males and 612 females | Mean AGD1 : 48.9±5.6mm,  Mean AGD2: 43.7±5.9mm,  Mean AGDAS: 25.5±5.0mm | Mean AGDAF: 13.6±2.7mm, Mean AGDAC: 34.2±3.3mm. |
| \*: The TIDES study was conducted in 4 different sites in the US: San Francisco, CA (University of California, San Francisco, UCSF), Rochester, NY (University of Rochester Medical Center, URMC), Minneapolis, MN (University of Minnesota, UMN) and Seattle, WA (University of Washington/SeattleChildren’sHospital, UW/SCH) | | | | |

## negative outcomes related to the anogenital distance

Several negative health implications have been linked to AGD. The following text presents a discussion of the major health issues identified in the literature.

### Irregular menstrual cycle

Since we know that AGD is sensitive to androgen, it is reasonable that scientists have examined the association between AGD and hormone-related disorders (Table 2). Mira-Escolano conducted a study to explore maternal hormone levels and AGD in girls (Mira-Escolano, Mendiola, Minguez-Alarcon, Roca, et al., 2014). The result represents that young girls have longer AGD when their mothers had been diagnosed with irregular menstrual cycles compared to mother did not have been diagnosed with irregular menstrual cycles (β= 3.79, *p*=0.03). This result points out that maternal hormone concentrations may be a significant risk factor for AGD, especially with regards to abnormal hormone levels during pregnancy.

### Polycystic ovary syndrome (PCOS)

AGD has also been linked to Polycystic Ovary Syndrom (PCOS). The PCOS is a common symptom among females which cause by elevated androgen concentrations, like testosterone. This symptom is related to several adverse health events, including heart disease, diabetes. The result from Barrett’s article noted that mothers who have PCOS have female offspring with longer AGD (AGDAF) (β= 1.21 mm, *p*=0.05) (Barrett et al., 2018). Two other articles also reported that the association between PCOS and anogenital distance not only occurs in offspring but also in adults. Sanchez-Ferrer’s research classified AGD into tertiles and make the lowest section as the reference group. The result indicates that compared to women in the lowest tertile group (AGDAC median=67.4mm) Mediterranean women in the highest tertial group (AGDAC median=89.7mm) have a 2.9 times higher risk for PCOS after adjusted for age, weight and episiotomy (OR 2.9, 95%CI 1.4-5.9) (Sanchez-Ferrer et al., 2017). Another study reported a positive association between PCOS and length of AGD among Chinese women. Females in the highest tertile group for AGDAC (>104.7mm) had 6.7 times higher risk (95%CI 3.7 – 12.1) for PCOS compared to females with the lowest tertile AGDAC (≦97.0mm). Similarly, women in the highest tertile group for AGDAF (>26.0mm) had 18.8 times higher risk (95%CI 9.6 – 36.6) for PCOS compared to females with the lowest tertile AGDAF (≦22.1mm). (Y. Wu et al., 2017). The latest case-control study also noted that mother with PCOS will increase the risk of longer AGD among infants after adjusted age, BMI, primary care services and prior mental health status (OR 1.76, 95%CI 1.27 – 2.46) (Berni, Morgan, Berni, & Rees, 2018).. A consistent association measured in these studies in diverse populations is strong evidence that high androgens in the maternal environment and impact female reproductive system development

### Infertility

Recent research has examined the link between AGD and infertility in males. Eisenberg conducted a case-control study in Texas to explore the association between AGD and infertility among male adults (Eisenberg, Hsieh, Walters, Krasnow, & Lipshultz, 2011). They recruited 117 men with infertility issues and 56 healthy controls into the study. The conclusion was that compared to men with normal fertility, infertile men had significantly shorter AGD (31.8±11.3mm vs. 44.6±14.1mm, *p*<0.01). Moreover, when AGD increase 10mm, the sperm density will increase 4.3 million sperm/mL (β= 4.31, *p*=0.03), and the total motile sperm count will increase 6 million sperm (β= 5.96, *p*=0.01). Another study in southern Spain (Mendiola et al., 2015) also supports this conclusion. In 2015, Mendiola conducted a hospital-based cross-sectional study to investigate the relationship between AGD and infertility. In the study, AGDAS was associated with sperm concentration (β= 0.071, *p*=0.02), total sperm count (β= 0.109, *p*=0.04) and total sperm motily sperm count (β= 0.086, *p*<0.05). All of these factors are used to examine fertility in male adults.

The latest study indicated the association between AGD and fertility among female adult (Mendiola et al., 2015). The study reported that women in the highest tertile AGDAF group have 3.1 times higher risk for at least 6 ovarian follicles in an ovary (β= 3.1, *p*<0.01). Similarly, women in the highest tertile AGDAC group have 4.6 times higher risk for at least 6 ovarian follicles in an ovary (β= 4.6, *p*=0.02). Another study noted the negative association between AGD and women who accept the fertility treatment (β= -1.06, *p*=0.03).

### Undescended testis

AGD is not only related to adverse health events among adults, but also for newborns. In 2013, Singal indicated that AGD was a useful biomarker which was related to the undescended testis (Singal, Jain, Gazali, & Shekhawat, 2016). They collected information for 1154 newborns from February 2011 to August 2011 in a secondary level hospital in India. The measurement of AGD was based on Salazar-Martinez’s method. The result denoted that newborns with undescended testis had significantly shorter AGD than healthy controls (2.21±0.36 cm vs. 2.56±0.31 cm, *p*<0.001). AGD was also inversely related with undescended testis (β= -0.191 95%CI -0.266 - -0.115, p<0.001) in multivariate models after adjusting for birth weight, birth length, and gestational age.

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| --- | --- | --- | --- | --- |
| Table 2 The association between length of AGD and adverse health outcome | | | | |
| Author (Year) | Location (Country) | Sample size | Result | |
| Male | Female |
| Eisenberg et al (2011) | Baylor College of Medicine (USA) | 117 infertile men and 56 fertile men (from August 2010). | Infertilite men had significantly shorter AGD then fertile men (31.8mm vs 44.6mm, *p*<0.01).  AGD is associated with sperm density (β=4.31, *p*=0.03) and total motile sperm count (β=5.96, *p*=0.01) | - |
| Mira-Escolano et al (2014) | Murica Young Women’s Study. (Spain) | 100 healthy university female student aged 18-23 (from 9th February 2011 to 25th November 2011). |  | Females would have longer AGDAF measurement if their mother had been diagnosed irregular menstrual cycles (β=3.79, *p*=0.03). |
| Mendiola et al (2015) | University Hospital in the Murcia Region (Spain) | 91 men aged 25 to 38 and receive infertility services in the hospital. | AGDAS was significant associated with the sperm concentration (β=0.071, *p*=0.02), the total sperm count (β=0.109, *p*=0.04), and the total sperm motile count (β=0.086, *p*<0.05) |  |
| Singal et al (2016) | MITR Hospital, Kharghar (India). | 354 pre-pubertal boys, 180 of them had hypospadias and 274 were healthy controls. (from July 2012 to July 2015) | Boys with hypospadias had significant shorter AGDAS then healthy control controls (40.6±9.7 mm vs 45.6±9.4 mm, *p*<0.001)  Both AGDAS and AGD2 were related to the severity of hypospadias (*p*<0.05).  After adjusted age, weight, and height, AGDAS was still significant negatively associate with hypospadias (β=0.016, *p*<0.001). |  |
| Sanchez-Ferrer et al (2017) | ‘Virgen de la Arrixaca’ University hospital. (Spain) | 126 pregnant women with newly diagnostic of PCOS, and 159 pregnant women without PCOS as controls (from September 2014 to May 2016). | - | Pregnant with PCOS had longer AGDAF (80.5±11.3mm vs. 76.0±10.4mm) and AGDAC (27.8±5.6mm vs. 26.5±5.1mm) than healthy controls *(p*<0.05).  After adjusted age, weight and episiotomy, The longest AGD*AC* (89.7mm vs. 67.4mm) was associated with PCOS (OR 2.9, 95%CI 1.6 – 5.3). |
| Wu et al (2017) | Sun Yat-Sen Memorial Hospital (China) | 156 not pregnant women with PCOS and 180 not pregnant women without PCOS, (from October 2015 to July 2016) | - | The highest tertile AGDAF group were more likely to have PCOS than reference group (OR 18.8. 95%CI 9.6 – 36.6).  The highest tertile AGDAC group also were more likely to have PCOS than reference group (OR 6.7, 95%CI 3.7 – 12.1). |
| Barrett et al (2018) | The Infant Development and Environment Study (TIDES) (USA) | 300 mother-daughter pairs (from 2010 to 2012) | - | Mother with PCOS was associated with longer AGDAF in daughter. (β=1.21, *p*=0.05) |

**Table 2 Continued**

## risk factorS for length of anogenital distance

Table 3 summarizes the findings from the primary articles that address the risk factors for short AGD.

### Birth Weight and Birth Length

Birth weight and birth length have been two factors examined for their relationship with AGD. In 2004, Salazar conducted a hospital-based cross-sectional study to investigate AGD among infants in Mexico (Salazar-Martinez et al., 2004). Forty-two of 87 newborns who participated the study were female infants (48%). The result indicated that both birth weight (β= 0.004, *p*<0.001) and birth length (β= 0.914, *p*=0.001) were positively significantly related with the range of AGD among boys, but only birth weight (β= 0.002, *p*<0.001) related with AGD for girls. The result noted that the female newborns with higher birth weight would have longer anogenital distance. For male newborns both higher birth weight and length would increase the length of AGD.

A similar result was found in a report by Asafo-agyei. In this report, the investigators conducted a hospital-based study in Komfo Anokye Teaching Hospital (KATH), Ghana and enrolled 1,256 babies into the study. The male/female ratio among the total population was 0.51. The result indicated that the birth weight was positively related to all 5 AGD in this study (AGD1: β= 0.306, *p*<0.001; AGD2: β= 0.255, *p*<0.001; AGDAS: β= 0.206, *p*<0.001; AGDAC:β= 0.233, *p*<0.001; and AGDAF: β= 0.123, *p*=0.002). In addition, the birth length also significant related to all 5 AGD measurements in this population (AGD1: β= 0.268, *p*<0.001; AGD2: β= 0.272, *p*<0.001; AGDAS: β= 0.227, *p*<0.001; AGDAC:β= 0.136, *p*=0.001; and AGDAF: β= 0.093, *p*=0.021). Based on the study result, it is obvious that the birth weight and length are two important risk factors for length of AGD, but the strength of effect might be different between ethnics. Moreover, because of the body size is different by ethnics and countries, apply or adjust the growth curve between ethnics might be a better option. The growth curve can reflect the growth of infants and provide us a normalized estimation, and it might be a more precise factor to represent the growth for children.

### Medicine use during pregnancy

Interestingly, three reports have examined the medication or supplements used by women during pregnancy as factors affecting AGD in newborns. One study with the KATH cohort in Ghana indicated that male infants had longer AGD1 (50.7mm vs. 48.6mm, *p*=0.0001), AGD2 (45.7mm vs. 43.4mm, *p*=0.0001) and AGDAS (27.7mm vs. 25.2mm, *p*<0.0001) if their mother had ever taken herbal medicine during pregnancy (Asafo-Agyei et al., 2017). Another hospital-based study in Denmark found that prenatal exposure to antifungal medication affects the length of AGD for male babies (Mogensen et al., 2017). Researchers identified 812 mother-baby pairs from 2010 to 2012 and the results indicated that using particular antifungal medicines (Oral fluconazole) is associated with shorter AGDAS. The most recent study by Fisher, et al., also denoted that pain or fever medications might relate to AGD (Fisher et al., 2016). The Cambridge Baby Growth Study (CBGS) observed that pregnant women taking paracetamol during the 8 to 14 weeks of gestation had effects on AGD in the newborn. Paracetamol use was associated with shorter AGD among male infants, but not for female infants.

Another study in Denmark also reported a similar conclusion (Lind et al., 2017). The researcher recruited 557 boys and 447 girls into the study, and their mothers completed two questionnaires during pregnancy about medicine intake during pregnancy. The results indicated that mothers who ever used paracetamol and other types of analgesics were more likely to have babies with shorter length of AGDAS among male offsprings (β=-4.1, 95%CI -6.4 - -1.7). However, the researchers did not find any significant association among daughters. Possible reasons for this finding are varied. The first explanation is that paracetamol might indeed have a different effect based on the gender of the fetus. Another reason might be the small sample size of paracetamol use may forfeit a reasonable estimate. For Lind’s study, only 20 mothers reported that they had previously used paracetamol and other type of analgesics during pregnancy. In Fisher’s study, only 47 people answered that they had used the paracetamol during the 8 - 14 gestation period. The possible assumption for the medicine use during pregnancy and the AGD is that the pathway these medicines work or metabolites in the human body is close to phthalates, which means they can also disrupt or interfere the human endocrine system. This hypothesis needs further research to verify.

### AGD application in public health

Although AGD has been widely used in child-related research, there are still some scientists who question the value of this marker. The major controversy about applying AGD in the public health field is that we still lack a solid definition for the “normal” value of the distance (McEwen & Renner, 2006; Weiss, 2006). The basic reason for this debate is that we do not have many population-based studies to investigate the distribution of AGD. Most of the published articles have been based upon cross-sectional study designs with a limited study population. Hence, scientists often apply the standardized method to adjust the AGD in their analysis. Besides, based on previous studies, we can realize that the length of AGD is varied by countries. It will make harder for scientists to decide a uniform standard for distinct abnormal AGD. Therefore, to determine the “normal” value of the AGD, population-based studies in different countries are necessary.

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| **Table 3 Risk factors related to short AGD** | | | | |
| Author (year) | Location (country) | Sample size | Result | |
| Male | Female |
| Fisher et al., (2016) | Cambridge Baby Growth Study (SBGS) (UK) | 634 mother-daughter infants and 681 mother-son pairs. (from April 2001 to March 2009) | Mother who used the paracetamol in early pregnancy (8 – 14 weeks) was related to shorter infant AGDAS (β= -0.625, *p*=0.014). | No significant association between pregnant women using paracetamol and AGDAF. |
| Lind et al., (2017) | Odense Child Cohort (Denmark) | 557 mother – son pairs and 447 mother – daughter pairs (from 2010 to 2012) | Mother who ever used paracetamol and other analgesics was related to shorter AGDAS (β=-4.1, 95%CI -6.4 - -1.7) | No significant association observed. |
| Asafo-agyei et al., (2017) | Komfo Anokye Teahing Hospital (KATH) (Ghana) | 1,256 babies, 644 males and 612 females (from May 2014 to September 2014). | The herbal medicine use during pregnancy was related to longer AGD1, AGD2 and AGDAS (*p*<0.001) |  |
| Mogensen et al (2017) | Odense Child Cohort (Denmark) | 812 mother – son pairs (from 2010 to 2012) | Pregnant who use antifungal medication (fluconazole) was associated with shorter AGDAS for male new born (β=-6.4, 95%CI -11.9 - -0.9) |  |

# Phthalate Exposure IN PREGNANCY

## Phthalate exposure in daIly life

Since 2009, the U.S. Environment Protection Agency (USEPA) has sought to control the level of phthalate use in the United States (USEPA, 2012). The Phthalates Action Plan, for example restricts the use of 8 types of phthalates, including butyl benzyl phthalate (BBP), dibutyl phthalate (DBP), di (2-ethylhexyl) phthalate (DEHP), diisobutyl phthalate (DIBP), di-n-pentyl phthalate (DnPP), di-isononyl phthalate (DINP), diisodecyl phthalate (DIDP), and di-n-octyl phthalate (DnOP). However, there are still dozens of phthalates that exist in commercial products that we touch every day.

Except for plastic products, such as plastic toys, there are still many consumer goods that contain phthalate arounds us (National Research Council (US) Committee on the Health Risks of Phthalates., 2008). For instance, people are exposed to diethyl phthalate (DEP) from shampoo, soap, body lotion or cosmetics. The DBP and DIBP types are common materials in cosmetics and adhesives. The DEHP type is one of the raw materials for food packaging. Increasingly, phthalate exposure in the diet has drawn attention.

Serrano et al. built a study to survey the level of phthalate exposure in the diets of pregnant women in the US (Serrano et al., 2014). The statistical analysis found that dairy product intake and consumption of homegrown food was inversely associated with DEHP (β= -1.3, 95%CI -2.3–-0.2) and monoisobutyl phthalate (MiBP) (β= -16.6, 95%CI -29.5 – -1.3) respectively. Pregnant women who sometimes consumed frozen fruit or vegetables had higher MBzP concentration than those who rarely used frozen products (β= 21.0, 95%CI 3.3 – 41.7). In May 2011, the Taiwan Food and Drug Administration (TWFDA) surveyed 1,410 products from markets, including foods, beverage, baby food, water, and drugs (C. F. Wu, Chang-Chien, Su, Chen, & Wu, 2014). The results denoted that 234 out of 1,410 products contained phthalates. Another study in Taiwan also denoted that the range of DEHP concentration in food products was from 1.2 to 3,000 ppm, and the range in beverages was between 14 to 224.5 ppm (Chen et al., 2016). However, the USEPA recommended reference-dose (RfD) and the tolerance daily intake (TDI) for DEHP is 0.02ppm, and 0.02-0.048 ppm respectively. Both of these recommendation values are much less than the exposures seen in the study results (Koch, Preuss, & Angerer, 2006; USEPA, 1987). Thus, many scientists hypothesize that this high dose phthalate exposure environment might lead to adverse health effects. Therefore, it is essential to explore the health effect of phthalate exposure, especially for newborns and children.

Several pathways exist for exposure to phthalates, including absorption of phthalate through the skin, inhalation, and digestion through food and drink intake (Braun, Sathyanarayana, & Hauser, 2013; Yan et al., 2009). In general, skin contact and digestion from eating are two essential routes for phthalate exposure for infants.

However, there is another route for phthalate exposure to children, and that is the prenatal exposure from the mother. Pathways we listed above are all after delivery. But it is possible that babies will be affected by phthalate exposure during pregnancy. Phthalate or its metabolites can influence the fetus via blood from the mother through the placenta. Therefore, it is worthwhile to explore how prenatal phthalate exposure affects childrens’ health.

## Adverse outcomes for phthalate exposure during pregnancy

One common and important adverse outcome in pregnancy is the preterm birth. According to World Health Organization (WHO), the definition of a preterm delivery is a delivery where the child is born before 37 weeks of pregnancy. In 2014, Huang’s article pointed out that phthalate concentration is strongly associated with preterm birth in China (Huang, Kuo, Chou, Lin, & Lee, 2009). They collected 207 mother-baby pairs into their study, and found that 33 of them were pre-term births. Further, the results described that 14 types of phthalate exposure were associated with preterm delivery (p<0.05), including DEP and DnOP.

Fergusson built a perspective cohort study to investigate whether the timing of phthalate exposure was related to preterm births (Ferguson, McElrath, Ko, Mukherjee, & Meeker, 2014). They recruited 130 preterm births and 352 random controls from Boston, MA from 2006 to 2008. The results denoted that higher measurements of DEHP at the third trimester were related to spontaneous preterm births (adjusted OR 1.33, 95%CI 1.02 – 1.73). The higher concentration of MECPP, one metabolite of DEHP, in the first trimester was strongly associated with placental preterm birth (adjusted OR 1.46, 95%CI 1.10 – 1.95), and the measurement of DEHP at the first trimester was marginally related to preterm placenta birth (adjusted OR 1.33, 95%CI 0.99 – 1.78).

In addition, the phthalate exposure also related to longer gestational age. Two research had similar conclusion that the MEHP exposure during pregnancy would increase the gestation age. Adibi et al (Adibi et al., 2009) reported that the higher concentration of MEHP (OR 2.0, 95%CI 1.1 – 3.5) and its two metabolites, MEHHP (OR 2.1, 95%CI 1.3 – 3.7) and MEOHP (OR 2.2, 95%CI 1.3 – 4.0) in urine were all relate to longer gestation age (>41 weeks). Whyatt et al (Whyatt et al., 2009) indicated that for each 1 logarithmic specific gravity-adjusted MEHP unit increase, the gestation age would increase 1.1 days (p=0.01).

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| **Table 4 The association between phthalate exposure during pregnancy and pre-term birth or longer gestational age** | | | |
| Author (year) | Location (Country) | Sample size | Result |
| Adibi et al., 2009 | Study of Future Families (SFF) (USA) | 441 pregnant women (from 2003 to 2004) | Increasing one log unit of urine MEHP and its two metabolites, MEHHP and MEHOP, will increase 2 (95%CI 1.1-3.5), 2.1 (95%CI 1.3-3.7) and 2.2 (95%CI 1.3-4.0) times risk for gestation age >41weeks, respectively. |
| Whyatt et al., (2009) | Columbia Center for Children’s Environmental Health (CCCEH) (USA) | 331 African-American or Dominican pregnant women (from January 2000 to July 2006) | For increase 1-logarithmic unit in specific gravity-adjusted MEHP the gestation age will decrease 1.1 days (p=0.01) |
| Fergusson et al., (2014) | Brigham and Women hospital (USA) | 130 cases (women with pre-term birth) and 352 random controls (from 2006 to 2008) | Pregnants who had higher MECPP concentration in gestation week 22.9 to 29.3 had higher risk for pre-term birth (OR 1.04, 95%CI 1.04 – 1.70).  Pregnancies with higher MBP concentration in gestation week 22.9 to 29.3 had higher risk for pre-term birth (OR 1.45, 95%CI 1.08 – 1.96). |
| Huang et al., (2014) | Chongquing Southwest Hospital (China) | 207 volunteer women age 18-35 (from October 2011 to September 2012) | DMP, DEP, DEEP, DPP, BMPP, DNHP, BBP, DNOP, DEHP, DBP, DMEP, DIBP, DBEP were all significant related with higher risk for pre-term birth occur, After adjusted maternal age, BMI, pregnancy history, frequent of pregnant examination, and history of intravenous infusion history. |

## Risk factors for maternal phthalate exposure

Several risk factors for maternal phthalate exposure have been identified in the literature. Age is one common factor found in most reports. Younger age of the females is a significant risk factor for phthalate exposure (Blount et al., 2000). Employment is a frequent risk factor for phthalate exposure. Women who work in hair or nail salons are at higher-risk for phthalate exposure (Braun et al., 2013). The possible reason is that the duration of contact with cosmetics or hair beauty products for these women are much longer than for women in other jobs. Moreover, most of the employees in either nail or hair salons are younger females, and thus, this group might be pregnant with higher phthalate exposure population in the future.

In addition to age and employment, another critical risk factor for phthalate exposure is the general use of cosmetics. Phthalates are widely used to produce cosmetics or personal care products, such as fragrancies and body lotions. Buckley, in a 2012 investigation, found a correlation between consumer product use and urine phthalate levels among pregnant women in the Right From the Start (RFTS) study (Buckley et al., 2012). The statistical results showed that use of makeups, perfumes, eyelash liners, sunscreens, bath oils, or nail nutrients were all positively associated with urine phthalate concentrations, such as MBzP, MBP, MiBP, and MEHP (all *p*<0.05). Interestingly, the use of nail polish affected the level of phthalate exposure in a different direction. Women who used nail polish had significantly higher MBP concentrations then non-users (*p*=0.048). However, MEP and urine phthalate levels had an inverse relationship in the same population (*p*=0.0002). Another study with pregnant women in New York indicated that urine MEP concentration was associated with perfume use (*p*<0.0001) (Just et al., 2010). Several studies also have found confirmatory results (Parlett, Calafat, & Swan, 2013; Romero-Franco et al., 2011).

## Association between phthalate exposure and infant anogenital distance

According to previous studies, it appears that maternal phthalate concentrations are negatively associated with AGD in infants. The first study based on the SFF cohort explored the correlation between phthalate concentration among pregnant women and the AGD in male infants (Swan et al., 2005). The result of this study found that higher amount of MBP (OR 10.2, 95%CI 2.5 – 42.2), MEP (OR 4.7, 95%CI 1.2 – 17.4), MBzP (OR 3.8, 95%CI 1.03 – 13.9), and MiBP (OR 9.1, 95%CI 2.3 – 35.7) were all significantly associated with shorter AGD among male babies. Another study by Swan used the advanced method to estimate AGD in the TIDES cohort. This study also had a similar result (Swan et al., 2015). They concluded that the three metabolites of DEHP, MEHP, MEOHP, and MEHHP were all related with shorter AGDAS (*p*<0.05). Additionally, MEOHP and MEHHP were also associated with shorter AGDAP (*p*<0.05).

Importantly, this inverse association has been found in different populations. In Japan, research has indicated that the log-transformed MEHP was associated with shorter AGD for boys (β= -0.226, p=0.017) (Suzuki et al., 2012). A study in Taiwan found an inverse association between MBP concentration in amniotic fluid and AGD for female infants (Huang et al., 2009). Studies in Mexico and Sweden have also found similar results (Bornehag et al., 2015; Bustamante-Montes et al., 2013).

Interestingly, the latest research from Denmark has markedly different finding (Jensen et al., 2016). The study, based on the Odense Child Cohort Study, recruited 245 mother-son pairs from 2010 to 2012 in Odense, Denmark into the study. The results showed that there was no significant association between AGD and early phthalate exposure, including MEP, MiBP, MnBP, and MBzP. There was also no dose-response relationship for all four phthalate metabolites and AGD. The researchers thought the reason they had a contrasting conclusion is that the population lives in a low phthalate exposure environment. However, the concentration of MEHP in this study is similar to Swan’s research in the US (1.2ng/ml vs. 2.0ng/ml), and MEHP was significantly associated with AGDAP among the US population (Swan et al., 2015). Therefore, further research to explore the association between phthalate exposure and AGD is still needed.

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| **Table 5 Articles relate to the association between phthalate exposure and infant AGD** | | | | |
| Author (year) | Location (country) | Sample size | Result | |
| Male | Female |
| Swan et al (2005) | SFFI and SFFII study (USA). | 134 male new born (from September 1999 to August 2002). | MBP (OR 10.2, 95%CI 2.5 – 42.2), MEP (OR 4.7, 95%CI 1.2 – 17.4), MBzP (OR 3.8, 95%CI 1.03 – 13.9), and MiBP (OR 9.1, 95%CI 2.3 – 35.7) were significantly associated with shorter AGD.  Increasing Phthalate joint Z-score is related to shorter AGD (β=-0.0951, *p*=0.009). | - |
| Huang et al., (2009) | National Cheng Kung University Hospital (Taiwan) | 32 mother with female fetus and 33 mother with male fetus (from 2005 to 2006) | No significant relationship had been observed. | Higher MBP concentration related to shorter AGD (*p*<0.06), AGD/birth weight ratio and AGD/birth length ratio (*p*<0.05). |
| Suzuki et al (2012) | Central Hospital if the Defense Force (Japan). | 111 pregnant-boy pairs. | The log-transformed MEHP was associated with AGD in male newborn (β=-0.226, *p*=0.017). | - |
| Bustamante-Montes et al (2013) | Hospital-based cohort Study (Mexico) | 73 mother – son pairs | Total phthalate exposure (MEHP+MBP+MBzP+MEP, 1ug/l) was associate with AGD1 (β=-0.1914, *p*=0.037), after adjusted creatinine and supine length at birth. | - |
| Bornehag et al (2015) | The Swedish Environmental Longitudinal,  Mother and child, Asthma and allergy  (SELMA) study (Sweden) | 196 Mother-son pairs (from 1st September 2009 to 20th November 2010) | Three DiNP metabolites were associated with reduction for AGDAS: oh-MMeOP (OR =2.61, 95%CI 1.24 – 5.68), oxo-MMeOP (OR=2.99, 95%CI 1.28 – 7.00), and cx-MMeHP (OR=3.11, 95%CI 1.27 – 7.66).  Exposed to MEHP would have higher risk for medium reduction for AGDAP (OR=4.01, 95CI 1.32 – 12.20) | - |
| Swan et al (2015) | The Infant Development and Environment Study (TIDES) (USA) | 753 mothers visit prenatal clinics, and 49% of newborns were males (n=366) (from August 2010 to August 2012) | Three metabolites of DEHP were negatively significant associated with AGDAS: MEHP (β=-1.12, *p*=0.036), MEOHP (β=-1.43, *p*=0.008), and MEHHP (β=-1.28, *p*=0.013).  Two metabolites of DEHP were associated with AGDAP: MEOHP (β=-1.60, *p*=0.011), and MEHHP (β=-1.47, *p*=0.015) | No significant association between phthalate exposure and length of AGD among female group. |
| Jensen et al (2016) | Odense Child Cohort Study (Denmark) | 245 mother – son pairs (from 2010 to 2012) | No significant association between earlier phthalate exposure (MEP, MiBP, MnBP, MBzP) and AGD.  No significant dose-response relationship between phthalate exposure and AGD. | - |

**Table 5 Continued**

# Other factorS affecting the association between phthalate exposure and anogenital distance

## Gender

The majority of the articles related to phthalate exposure and AGD have been focused on male infants. For example, four studies from Denmark, Mexico, Sweden, and the US have only recruited mother-boy pairs, but not mother-girl pairs into the study. One report based on the TIDES cohort found a correlation between AGD and phthalate only in male infants and not in female babies. Adibi’s study showed that the relationship between phthalate exposure and AGD (Z-score for short form) is different for female and male groups (Adibi et al., 2015). Gol et al indicated that pregnant women bearing female fetuses had significantly higher human chorionic gonadotropin (hCG) levels than women carrying male fetuses (Gol et al., 2004). The gender diversity might be due to the biology mechanism, but the study results were inconsistent among female group. Therefore, we should consider gender as a factor for further study.

## hormone level

### Mechanism

A discussion of the association between maternal phthalate exposure and AGD in infants should briefly address the mechanism of how phthalate exposure affects the reproductive system for females. As we know, phthalates are endocrine disrupters (EDC), and EDC interfere with the function of the hormone and endocrine system in humans. EDC can bind to the endocrine receptor to interfere the hormone concentration is the human body (Yang, Kim, Weon, & Seo, 2015). Two animal studies have verified this thought. In 2015, Li et al treated pregnant rats with 100mg/kg/d, 300mg/kg/d and 900mg/kg/d dibutyl phthalate (Li et al., 2015). The result noted that among the 900mh/kg/d DBP group, the development of the testicules and the level of testosterone secretion was decreased. Another study also reported that DBP leads to a negative effect on the reproductive system of male rats, including a decrease in the length of AGD (Ma et al., 2017). Thus, it is reasonable to hypothesize that phthalate exposure will affect the concentration or secretion of hormones in pregnant women. However, Adibi et al. indicated that the mechanism between pregnant rodent and pregnant women might be different (Adibi et al., 2015). The rodent experiment indicated that the phthalate inhibits the testis function and affect the AGD, but such pathway didn’t represent in human population. Therefore, the advanced study to explore the mechanism for phthalate exposure is needed.

### Reproductive hormones

The most well-known hormone related to the length of AGD is testosterone, although the available evidence of this link is still inconsistent. Eisenberg recruited 116 adult men who went to an urology clinic for reproduction issues from August 2010 to August 2011 in Houston, Texas (Eisenberg et al., 2011). They found that testosterone was significantly associated with length of AGD (β= 2.01, *p*=0.03) in a linear regression model. Adults with testosterone levels less than 300 ng/dl had significantly shorter AGD than men who had testosterone levels higher or equal to 300 ng/dl (31.6mm vs.37.3mm, *p*=0.02). Another study in China also reported a significant association between AGD and testosterone, but the relationship was in a different direction (Zhou et al., 2016). Zhou et al reported that AGDAP was significantly correlated with testosterone among male college students (r=-0.131, *p*<0.0001). After adjusted for confounding factors, AGDAP was significantly related to estradiol (β= -0.126, *p*=0.002) and testosterone/estradiol ratio (β= 0.007, *p*=0.001).

Scientists have also observed an association between testosterone and AGD among adult females. A study in Murica, Spain with 100 female college students indicated, after adjusting for BMI and contraception use, that testosterone was positively correlated with AGDAF. (β= 0.006, *p*=0.02) (Mira-Escolano, Mendiola, Minguez-Alarcon, Melgarejo, et al., 2014)

In contrast, some studies have reached different conclusions regarding the relationship of hormone levels and AGD. One study using the SFF cohort did not find any significant association between testosterone and AGD (Sathyanarayana, Barrett, Butts, Wang, & Swan, 2014). Another study in southern Spain with 215 university male students did not observe a substantial relationship among anogenital distances (AGDAS, AGDAP) and testosterone (Parra, Mendiola, Jorgensen, Swan, & Torres-Cantero, 2016). These articles also reported that there was no significant association between AGD and ollicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin-B and sex hormone-binding globulin (SHBG).

Additional research has indicated that hormone levels are also associated with phthalate levels in humans. Sathyanaryana’s study with the SFF cohort showed that in pregnant women bearing a male fetus, the log-total testosterone (β= 0.09, *p*=0.04) and log-free testosterone (β= 0.10, *p*=0.04) were all related to logMEP level (Sathyanarayana et al., 2014). For women bearing a female fetus, the log-total testosterone (β= -0.20, p=0.04) and log-free testosterone (β= -0.21, *p*=0.05) was related to logMBP. They also observed that log-sumDEHP was associated with log-total testosterone (β= -0.15, *p*=0.01) and log-free testosterone (β= -0.15, *p*=0.01) among women bearing a female fetus. Chang et al. reported a similar finding in 2015 (Chang, Li, Wu, Pan, & Lee, 2015). The result noted that MMP, MiBP, and MEHP were all inversely related to serum testosterone levels among adult males in Taiwan (*p*<0.05). Also, MiBP, MBzP, and MEHP were negatively associated with free-form testosterone for men (*p*<0.05). Another study found that after adjusting for age and birth weight, urine MEOHP (OR 9.99, 95%CI 3.32 – 34.39) and MEHHP (OR 7.79, 95%CI 2.84 – 23.85) levels were positively related to FSH among young girls in Taiwan (Wen et al., 2017).

Based on these current studies, we can understand that hormones are associated with both AGD and with phthalate exposure, but that these relationships are not yet conclusive in terms of the quality of the scientific evidence. Moreover, phthalate exposure is significantly related to AGD for both males and females. Therefore, the role that reproductive hormones play in the association between AGD and phthalate exposure still requires further investigation. Our latest analysis observed that the estriol and Inhibin-A were associated with AGD among male newborn. We selected the study population from the TIDES cohort and applied the z-score transformation to let AGD fit the normal distribution. The first section of our study is to explore the association between phthalates (MnBP, MBzP, MEHP, MEP, and MiBP) and reproductive hormones (PAPP-A, estriol, Inhibin-A, and AFP), and the second section of the study is to investigate the relationship between these four hormones and the AGD. The statistical result indicated that the higher log-transformed MnBP was associated with higher estriol (β= 0.28, 95%CI 0.01 – 0.55) and inhibin-A (β= 0.61, 95%CI 0.09 – 1.12) concentration among pregnant women carrying females. In contrast, we only found the significant association between hormone and AGD from pregnant carry males but not females. The estriol is negatively associated with short-form AGD (β= -0.35, 95%CI -0.65 – -0.04), and the Inhibin-A is positively related to both short-form (β= 0.25, 95%CI 0.05 – 0.44) and long-form AGD (β= 0.28, 95%CI 0.07 – 0.49).

## Other factors

Except for gender and reproductive hormone levels, scientists have sought to identify other risk factors that might affect the association between AGD and phthalate exposure, although the number of studies is limited. In 2016, Barrett reported that maternal stress might be a modifier for this relationship (Barrett et al., 2016). In this research, pregnant women were classified into low or high-stress groups based on their answers on stress recorded from a questionnaire. In high-stress group, 41 of 45 (91%) women reported they had 2 or more prenatal life stress events, and 40 out of 45 (89%) noted they had 2 or more maternal life stressors. For low-stress group, 133 of 238 (59%) noted they didn’t have prenatal life stressors and 125 of 238 (56%) answered they don’t have maternal life stress events. Among individuals in the low-stress group, the investigators observed that maternal natural log transformed DEHP levels in the first trimester was inversely associated with AGDAS (β= -1.78 mm one change in stressful life events, 95%CI -2.97 - -0.15) and AGDAP (β= -1.61 mm one change in stressful life events, 95%CI -3.01 - -0.22) in male infants. However, a similar result was not observed in the high-stress group.

Another possible factor to consider in this evaluation is the timing of phthalate exposure. Martino-Andrade et al. noted that MEHHP levels in a 1st-trimester urine sample from pregnant women were marginally and negatively associated with AGDAP among male infants (β=-1.73, 95%CI -3.45 – 0.0004) (Martino-Andrade et al., 2016). In addition, several recent studies illustrated the association between the hCG or thyroid hormone to AGD and phthalate. Adibi et al (Adibi et al., 2015) reported the negatively association between MCOP and z-score hCG among pregnant women carrying female fetus (β= 0.38, 95%CI 0.15-0.61). They also observed that the higher maternal hCG z-score was related to longer short-form AGD among female newborn (β= 0.13, 95%CI 0.01-0.26). Liu et al (Liu et al., 2016) denoted that the longer AGD among male newborn was related to maternal free triiodothyronine (FT3) (β= 1.36mm for 1pmol/L FT3, 95%CI 0.58-2.13), free thyroxine (FT4) (β= 0.12 mm for 1pmol/L FT4, 95%CI 0.00-0.25), and thyroid stimulates hormones (TSH) (β= 3.14 mm for 10 folds TSH increase, 95%CI 0.65-5.63). However, they didn’t found significant relationship in female newborn group. Except the research above, there is no other phthalate and metabolite measurements from the 2nd or 3rd-trimester urine samples were significantly associated with anogenital distance.

The latest research also reports that ethnic might be another factor that could affect the relationship between phthalate exposure and AGD (Wenzel et al., 2018). One study recruited 193 white and 187 African American pregnant women to examine the possible implications of race on this relationship. The results represented that MEHP concentration levels in the second trimester were associated with shorter AGDAP, and that this relationship was stronger in African American (β=-2.07, *p*=0.04) than in White women (β=-1.73, *p*=0.22). In contrast, the association between DBP and AGDAS was significant among White women (β=1.30, *p*=0.04) but not among African American women (β=0.39, *p*=0.59). Although the ethnic interaction term did not reach statistical significance, we can still observe that phthalate exposure had a different level of effect within each race. Thus, we can still consider that race/ethnicity may be an essential issue in the association between phthalate exposure and AGD.

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| --- | --- | --- | --- | --- |
| **Table 6 Articles relate to the association between hormone level and infant AGD** | | | | |
| Author (year) | Location (country) | Sample size | Result | |
| Male | Female |
| Eisenberg et al., (2012) | Baylor College of Medicine (USA) | 116 men recruited from the urology clinic in reproductive medicine (from August 2010 to November 2011) | The AGD was significant associate with serum testosterone level (β= 2.01, *p*=0.03) but not FSH or LH.  Men with less than 300ng/dl testosterone had significant shorter AGD (*p*=0.02) | - |
| Mira-Escolano et al., (2014) | The Murcia Young Men’s Study (MYMS) (Spain) | 100 female college students (from 9th February to 25th November 2011) | - | Higher testosterone level is associated with AGDAF (β= 0.006, *p*=0.02). |
| Parra et al., (2014) | The Murcia Young Women’s Study (MYWS) (Spain) | 215 Male college students (from October 2010 to November 2011) | No significant association between wither AGDAS or AGDAP to hormones (FSH, inhibin b, LH, testosterone, SHBG, estradiol, and cFT) | - |
| Sathyanarayana et al (2014) | The Study of Future Families phase I (SFFI) (USA) | 180 pregnant mothers (from September 1999 to August 2002). | Testosterone, free form testosterone and estradiol weren’t associated with AGDAS (p>0.05). | Testosterone, free form testosterone and estradiol weren’t associated with AGDAF (p>0.05). |
| Adibi et al., (2015) | The Infant Development and Environment Study (TIDES) (USA) | 541 mothers with available genital and phthalate exposure and accept the prenatal serum screen in first and second trimester (from August 2010 to August 2012) | No significant result had been reported. | The MCOP concentration was positively associated with hCG among women carrying female fetus (β= 0.38, 95%CI 0.15-0.61).  Short-form AGD was positively relate to higher hCG z-score among women carrying female fetus (β= 0.13, 95%CI 0.01-0.26). |
| Liu et al., 2016 | Two local hospitals in Guiyu and Haojiang (China)  **Table 6 Continued** | 616 mother and newborns (from May 2011 to May 2012) | Longer AGD was associate with free triiodothyronine (FT3) (β= 1.36mm for 1pmol/L FT3, 95%CI 0.58-2.13), free thyroxine (FT4) (β= 0.12 mm for 1pmol/L FT4, 95%CI 0.00-0.25), and thyroid stimulates hormones (TSH) (β= 3.14 mm for 10 folds TSH increase, 95%CI 0.65-5.63) | No significant result had been reported. |
| Zhou et al., (2016) | The Male Reproductive Health in Chongquin College Students (MARCHS) study (China). | 656 male college students (June 2013 to June 2014) | AGDAP positively related to with sperm progressive motility (r=0.084, *p*=0.032).  AGDAP was inversely correlated with Estradiol (r=-0.034), testosterone (r=-0.131) and SHBG (r=-0.142) (all *p*<0.05)  AGDAP was significant related with estradiol (β=-0.126, *p*=0.002). | - |
| Lai et al., (process) | The Infant Development and Environment Study (TIDES) (USA) | 326 mothers with available genital and phthalate exposure and accept the prenatal serum screen in first and second trimester (from August 2010 to August 2012) | Maternal Estriol level was negatively associate with short-form AGD (β= -0.35, 95%CI -0.65- -0.04).  Maternal Inhibin-A concentration was related to longer AGD for both short-form (β= 0.25, 95%CI 0.05-0.44) and long-form (β= 0.28, 95%CI 0.07-0.49). | MnBP concentration was positively related to PAPP-A (β= 0.28, 95%CI 0.01-0.55) and Inhibin-A (β= 0.61, 95%CI 0.09-1.12) in pregnant women carrying female fetus.  MEHP concentration was inversely associated with Inhibin-A level (β= -0.46, 95%CI -0.84- -0.09) in pregnant women carrying female fetus. |

# Conclusion

## Overview

This review presents a brief introduction to the literature addressing the association between maternal phthalate exposure and AGD among infants. From the studies reviewed, we can understand that AGD one physical characteristic of the human body, is related to adverse health issues, such as descended testis and infertility. Therefore, it is vital for public health research to realize the cause of the shorter AGD or even to make a standard for classifying what is normal and abnormal AGD.

Phthalates, endocrine disrupting chemicals, are typical chemical products in people’s lives. Individuals can easily be exposed to phthalates under many situations. Therefore, it is an important issue to explore to learn more about what kind of health hazards people will face from phthalate exposure. According to previous research, phthalate exposure during pregnancy is an important risk factor for shorter AGD for male infants but not female infants. The reason for this difference is still unclear, but reproductive hormone concentrations might be a factor for this phenomenon.

However, since reproductive hormone levels are inversely linked to AGD, and also to phthalate concentration in the human body, it is necessary for future studies to clarify the actual role of maternal hormone levels in the association between phthalate exposure and AGD.

## Strengths and weaknesses

One strength of this review is the systematic examination of three important factors: anogenital distance, phthalate exposure, and reproductive hormone levels. Most previous reviews have only considered two of these issues. This discussion reviews these three factors at the same time, and thus the relationship between them will be easier to understand. Another strength of the review is that the scientific papers included in the review are not only from a single country, but from the whole world. Therefore, the generalizability of the discussion for this article should be enhanced. The primary weakness related to this topic is that we did not explore detail biological mechanisms from phthalate exposure to AGD whether for adult or newborns. This review does not address the results from several animal studies that exist investigating the mechanism of how would phthalates and/or hormones affect the length of AGD. However, since we reviewed many human exposure research, the results from these articles should also give scientists many details about the mechanism in human body. This review may also be affected by publication bias. It is possible that the published articles reviewed only reflect significant results. This type of bias is hard to avoid, but we did find some articles what found different relationship directions or had a non-significant conclusion (Huang et al., 2009; Jensen et al., 2016; Swan et al., 2015). Thus, we think the effect of publication bias on this summary should be limited. Another limitation is that most studies were focused on male infants, and hence the result of the review might not be able to generalize to the female population.

## The direction of future studies

### Other hormones

There are several approaches that future research can consider. The first is to explore the effect of more types of hormones on AGD. Most of the previous literature has focused on testosterone, FSH, LH, and hCG. However, there are still many more hormones related to fetal growth that may warrant attention. For example, the Inhibin-A and the pregnancy-associated plasma protein A (PAPP-A) are two important hormones related to Down’s syndrome. These two hormones could reach their highest value in the third trimester just like hCG. Therefore, these hormones might also factors involved in the association between the phthalate exposure and AGD. In fact, our latest analysis indicated that PAPP-A was positively related to MnBP and Inhibin-A was inverse associate with MEHP among women carrying female fetus. We also observed the pregnant women with higher inhibin-A concentration was related to longer AGD, but higher estriol would decrease the short-form AGD among male newborn.

### Gene research

The second focus for future research may lie in the investigation of gene mutations or polymorphisms for the androgen receptor and their contribution to the link between phthalate exposure and AGD. As we know, phthalate is linked to the androgen receptors already. Thus, it is reasonable to explore if gene mutations could also be related to AGD. Sathyanarayana had found that single nucleotide polymorphisms (SNPs) located in the estrogen receptor alpha (ESR1) and the activating transcription factor 3 (ATF3) are significantly related to shorter AGD among adult males (Sathyanarayana et al., 2012). Moreover, two recent articles noted that the sex hormone concentrations differed between ethics (Kim et al., 2012; Rohrmann et al., 2007). Among male adults, non-Hispanic Black had higher estradiol (E2) level then non-Hispanic White. In contrast, non-Hispanic White females had significant higher E2 concentration than African-American. Therefore, it is reasonable for us to assume that the gene mutation could affect the AGD.

### Mediation analysis

Another direction of focus for future research is mediation analysis. As mentioned above, maternal hormone concentration is related to both phthalate exposure and AGD for infants. Thus, the role of maternal hormone concentration may be involved in the link between phthalate exposure and AGD in different facets, including as a potential confounder or potential mediating variable. Further examination of the role of maternal hormones is warranted, including mediation analysis.

As an example, mediation analysis could classify the effect of exposure and mediator to the outcome to three different categories: natural direct effect, natural indirect effect, and direct control effect. Compared to the traditional analysis method, such as adjusted variables in the model or stratified analysis with the specified factor, mediation analysis can better estimate how the mediator could affect the exposure and the outcome of interest. Adibi et al. used mediation analysis in her research with the TIDES cohort to explore the association between maternal phthalate levels, placental hormone concentration and AGD among infants (Adibi et al., 2015). The result denoted that among male newborns, 52% of relationship from MnBP to z-score for AGDAS and 25% of relationship from MEHP to z-score for AGDAS could be blocked by hypothetically blocking hCG hCG level. Similarly, among female babies, the 78% of the association between MBzP exposure to the z-score for the AGDAP could be attenuated by blocking the phthalate effect on hCG hCG. From this analysis, we can potentially better understand the pathway from exposure to the outcome, and increase insight into causality

There are several methods that scientists can use in conducting the mediation analysis, including the inverse probability weight (IPW), the structure transformation, and the G-estimation. The result from each method will differ. Naimi et al. applied different mediation analysis methods to explore whether breastfeeding before the hospital can explain the level of racial disparity in infant mortality (Naimi, Schnitzer, Moodie, & Bodnar, 2016). The statistical result reported that the proportion that are breastfed can explain the relationship between race inequity and infant mortality. Each methodological approach gave a different result (IPW: 33%, structure transformation: 8%, and G-estimation: 31%). Therefore, the essential task for people who want to apply mediation analysis is to understand the characteristics of the data and to select the suitable statistical method for the study.

## Contribution to public health

This review provides a comprehensive overview of the association between maternal phthalate exposure and infant AGD. Several risk factors that may contribute to this association were also reviewed, such as maternal hormone concentration and stress. We also introduced the application of mediation analysis in this field. However, there are still several factors that need to be investigated. For example, other types of hormones likes inhibin-A and AFP, and the role of gene mutation and polymorphisms. If we can understand the mechanisms as to how maternal phthalate exposure affects birth characteristics, we can look to the development of possible interventions to improve health. For example, we can suggest that manufacturers reduce the use of phthalates or select alternative materials to produce consumer goods. If hormones contribute to this association, we can encourage develop prenatal testing in pregnant women to measure their hormone concentration. According to an annual survey in the US (Palomaki, Knight, Ashwood, Best, & Haddow, 2013), only 60 % of pregnant women had second-trimester screening for prenatal test, 19 percent of these women had first-trimester screening, and 21 received both first and second-trimester testing. Therefore, if future research can infer an association between phthalate exposure, hormone concentration, and AGD, it is necessary for public health professionals to encourage pregnant women to uptake a prenatal test. Once we can know the relationship between maternal phthalate levels, hormone concentrations during pregnancy and AGE for newborn, maybe the department of health from government can make recommendation to pregnant women to use hormone supplements to reduce the adverse effect from phthalate exposure.

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