



## Review

## Reproductive health effects of aflatoxins: A review of the literature

Faisal M.B. Shuaib<sup>a,\*</sup>, John Ehiri<sup>b</sup>, Amina Abdullahi<sup>c</sup>, Jonathan H. Williams<sup>d</sup>, Pauline E. Jolly<sup>a</sup><sup>a</sup> Department of Epidemiology, Ryals School of Public Health, University of Alabama at Birmingham, 1665 University Boulevard, Birmingham, AL 35294-0022, USA<sup>b</sup> Division of Health Promotion Sciences, Mel and Enid Zuckerman College of Public Health, University of Arizona, Suite A 256, Drachman Hall, 1295 N. Martin Avenue, P.O. Box 245209, Tucson, AZ 85724-5209, USA<sup>c</sup> Department of Obstetrics and Gynecology, University of Jos Teaching Hospital, Nigeria<sup>d</sup> College of Agricultural and Environmental Services, University of Georgia, Griffin, GA, USA

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## ABSTRACT

**Context:** Although it is known that aflatoxins have many adverse health effects, there is no systematic summary of how it affects the reproductive system or its reproductive health effects.

**Objective:** Summarize evidence on the reproductive health effects of aflatoxins.

**Results:** The search yielded 121 potential studies, of which 25 were retained. One study found a higher concentration of aflatoxins in the semen of infertile men (40% of cases compared to 8% of controls). Six studies found significant associations or correlations between low birth weight and aflatoxins while one study did not find any correlation. One study found maternal serum aflatoxin to be a risk factor for jaundice in infants (OR, 2.68; CI, 1.18–6.10). Overall, maternal breast milk in developing countries had higher rates of aflatoxin contamination than in high income countries.

**Conclusions:** Stakeholders in developing countries need to take steps to reduce exposure of vulnerable populations to the toxic effects of aflatoxins.

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## 1. Introduction

Although it is hypothesized that aflatoxins have adverse effects on birth outcomes [1] there has been no critical summary of the literature on the subject. Reproductive health addresses the reproductive processes, functions and systems at all stages of life [2]. Central to reproductive health is “the right of access to appropriate health care services that will enable women to go safely

through pregnancy and childbirth and provide couples with the best chance of having a *healthy infant*” [2]. Globally, one useful indicator of reproductive health is birth weight. It is estimated that more than 20 million infants worldwide, representing 15.5 percent of all births, are born with low birth weight with 95.6 percent occurring in developing countries [3]. A baby’s low weight at birth (defined as birth weight less than 2500 g) is either the result of preterm birth (before 37 weeks of gestation) or due to restricted fetal (intrauterine) growth. Low birth weight is closely associated with fetal and neonatal morbidity and mortality, inhibited growth, poor cognitive development, and chronic diseases later in life [3].

About 4.5 billion people, mostly in developing countries, are at risk of chronic exposure to aflatoxins from contaminated food crops [4]. Aflatoxins are a family of toxic metabolites which are pro-

\* Corresponding author. Tel.: +1 205 934 1823; fax: +1 205 975 3329.

E-mail addresses: [faisals@uab.edu](mailto:faisals@uab.edu) (F.M.B. Shuaib), [jehiri@email.arizona.edu](mailto:jehiri@email.arizona.edu) (J. Ehiri), [kameenah@yahoo.com](mailto:kameenah@yahoo.com) (A. Abdullahi), [twillia@griffin.uga.edu](mailto:twillia@griffin.uga.edu) (J.H. Williams), [jollyp@uab.edu](mailto:jollyp@uab.edu) (P.E. Jolly).

**Table 1**  
Summary of findings on aflatoxins, infertility and birth outcomes.

Study ID	Objective	Design	Population	Outcomes	Results	Remarks
Ibeh et al. 1994 Benin city, Nigeria [22]	To discover the relationship between aflatoxin levels in serum of infertile men compared to controls.	Cross-sectional	50 infertile men and 50 normal individuals from the same community on the same staple diet.	Mean aflatoxin concentration of semen	40% of semen from infertile men had aflatoxins. 50% of spermatozoa were abnormal. Eight percent of semen from fertile individuals had aflatoxins. 10–15% were abnormal in the fertile men. $1.660 \pm 0.04 \mu\text{g/mL}$ (infertile men) and $1.041 \pm 0.01 \mu\text{g/mL}$ (fertile men)	
Turner et al. 2007 West Kiang region, Gambia [1]	To investigate the effect of in utero aflatoxin exposure on birth weight and infant growth.	Cross-sectional	138 singleton infants	Aflatoxin in maternal blood, cord blood, infant blood, birth weight and height gain in the first year of life	Aflatoxin–albumin in maternal blood predicts birth weight and height gain in the first year of life. $P=0.012$ for birth weight and $P=0.044$ for height gain	Maternal AF-alb level was significantly higher in blood samples collected in December–March, than in April–July or in August–November ( $P<0.001$ )
Maxwell et al. 1994 Ibadan, Nigeria [26]	To determine the extent of fetal exposure to aflatoxins and naphthols and influence on birth weight	Cross-sectional	625 babies	Aflatoxins in serum	14.6% of serum samples were contaminated with aflatoxins. No correlation between the presence of either compound and birth weight.	
Yousef et al. 2002 United Arab Emirates [28]	To determine whether fetuses had been significantly exposed to aflatoxins	Cross-sectional	201 women	Umbilical cord blood levels of aflatoxins.	Aflatoxins were detected in 54.7% of samples. Negative correlation between birth weight and levels of aflatoxins ( $r = -0.63$ ).	$P < 0.01$
Yousef et al. 2004 United Arab Emirates [30]	To assess whether aflatoxin M <sub>1</sub> concentrations in newborn infants correlated with those of their mothers and to determine whether the presence of aflatoxin M <sub>1</sub> in cord blood was associated with an increase in morbidity in the newborn	Cross-sectional	250 samples taken from women admitted to labor wards.	M <sub>1</sub> in maternal and umbilical cord blood	There was a strong correlation between aflatoxin levels and birth weight ( $r = -0.565$ , $P < 0.001$ ) but there was no association between aflatoxin M <sub>1</sub> concentration in maternal or cord blood and rates of jaundice or infection.	
Yousef et al. 2003 United Arab Emirates [29]	To determine whether breast milk of mothers from UAE contained aflatoxins and if there was any correlation with gestational age.	Cross-sectional	140 lactating mothers	Aflatoxin M <sub>1</sub> in breast milk	No significant correlation between aflatoxin M <sub>1</sub> and gestational age, postnatal age, gender, and clinical condition.	
Sedeghi et al., 2009 Tehran, Iran [21]	Exposure of infants to aflatoxin M <sub>1</sub> and of lactating mothers to aflatoxin B <sub>1</sub> , using AFM <sub>1</sub> in breast milk as a biomarker for exposure to AFB <sub>1</sub>	Cross-sectional	Breast milk sample from 160 women	AFM <sub>1</sub> concentration in milk	AFM <sub>1</sub> detection in 157 samples (98.1%) average concentration = $8.2 \pm 5.1 \text{ ng/kg}$ . Range 0.3–26.7 ng/kg.	Significant association between AFM <sub>1</sub> concentration and height at birth ( $P < 0.01$ ). Direction of association unclear
Abulu et al. 1998 Edo, Nigeria [23]	To investigate the presence of aflatoxins in cord blood.	Cross-sectional	164 neonates	Aflatoxin in cord blood and neonatal jaundice.	Neonates with jaundice have a high mean concentration of aflatoxin B <sub>1</sub> . There was significant reduction in birth weight ( $P < 0.05$ ) of jaundiced neonates with aflatoxin.	Rate of detection was higher in wet (81.8%) than dry season (50.0%)

Table 1 (Continued)

Study ID	Objective	Design	Population	Outcomes	Results	Remarks
Ahmed et al. 1995 Zaria, Nigeria [24]	To determine the relationship between perinatal aflatoxin exposure and neonatal jaundice.	Prospective study	77 neonates	Aflatoxins in cord and peripheral blood and neonatal jaundice	Aflatoxins in cord blood of 37.8% of jaundiced neonates and in 22.5% of controls. Mean cord aflatoxins concentration was highest in jaundiced neonates with septicemia but difference not stat. significant. No statistically sig. difference between aflatoxin in peripheral blood of jaundiced and non-jaundiced babies. No correlation between severity of hyperbilirubinemia and serum aflatoxin levels	
De Vries et al. 1989 Embu district, Kenya [25]	To determine foetal and neonatal exposure to aflatoxins	Cross-sectional	125 primigravidae	Aflatoxins in maternal and cord blood	53% of maternal blood contained aflatoxins. 37% of 101 cord blood contained aflatoxins. There was no relationship between aflatoxins in maternal and cord blood. The mean birth weights of females born to aflatoxin positive mothers were significantly lower than those of aflatoxin free mothers. Aflatoxins were detected in 27.4% of jaundiced neonates, 17% of their mothers, 16.6% of controls and 14.4% of control mothers. Serum aflatoxin is a risk factor for neonatal jaundice, OR = 2.68 (CI: 1.18–6.10).	Two stillbirths were recorded in cases with aflatoxins in both maternal and cord blood. The frequency of detection was significantly higher in maternal and cord blood during the 'wet' rainy season than 'dry' months.
Sodeinde et al. 1995 Ibadan, Nigeria [27]	To investigate the prevalence of naphthols and aflatoxins in the sera of babies with neonatal jaundice and their mothers in order to determine whether they contribute to the occurrence of unexplained neonatal jaundice in Ibadan.	Cross-sectional	327 jaundiced neonates and 80 of their mothers, and 60 non-jaundiced controls and seven of their mothers	Aflatoxins in blood.	Aflatoxins were detected in 27.4% of jaundiced neonates, 17% of their mothers, 16.6% of controls and 14.4% of control mothers. Serum aflatoxin is a risk factor for neonatal jaundice, OR = 2.68 (CI: 1.18–6.10).	
Jonsyn et al. 1995 Southern province, Sierra Leone [31]	To examine breast milk for mycotoxin content.	Cross-sectional	113 mothers in two under five clinics.	Aflatoxins, ochratoxins A and other mycotoxins in breast milk.	Eighty-eight percent contained various aflatoxins and 35% contained ochratoxin A; 15% had a single mycotoxin; 32% had two mycotoxins and 40% had three or more.	Girl infants exposed to OTA and aflatoxins have lower birth weights ( $P < 0.05$ )

**Table 2**  
Summary of findings on contamination of breast milk and body fluids by aflatoxins.

Study ID	Objective	Design	Population	Outcomes	Results	Remarks
Turconi et al. 2004 Lombardy, Italy [40]	To assess the presence of aflatoxins, ochratoxin A, lead and cadmium in human milk.	Cross-sectional	231 puerperal women.	Aflatoxin and ochratoxin A levels in breast milk	Aflatoxin B <sub>1</sub> (11.4ng/l) and aflatoxin M <sub>1</sub> (194ng/l) were found in one sample, while ochratoxin A (6.01 ± 8.31 ng/l) was detected in 198 (85.7%) samples.	Study shows mycotoxins are present in maternal milk
Denning et al. 1990 Songkhla, Thailand [44]	To determine aflatoxin levels in human cord sera at birth and maternal serum immediately after birth.	Cross-sectional	35 mothers and their babies	Aflatoxin (AFB <sub>1</sub> , AFG <sub>1</sub> and AFQ <sub>1</sub> ) levels in human cord sera at birth and maternal serum immediately after birth.	17 out of 35 cord sera (48%) contained aflatoxin, mean 3.1 nmol/ml (range 0.064–13.6 nmol/ml). By comparison, only two (6%) of maternal sera contained aflatoxin (mean 0.62 nmol/ml)	Results demonstrate transplacental transfer and concentration of aflatoxin by the fetoplacental unit.
Polychronaki et al. 2006 Qalyubiyah, Egypt [38]	To assess the level and frequency of breast milk aflatoxin M <sub>1</sub> as a biomarker of maternal exposure to aflatoxins.	Cross-sectional	388 lactating women	AFM <sub>1</sub> levels in breast milk	36% of the 388 mothers tested positive for AFM <sub>1</sub> (median 13.5 pg/ml)	AF contamination of breast milk is frequent, albeit at moderate levels.
Galvano et al. 2008 various sites, Italy [35]	To determine contamination of human milk by aflatoxins and ochratoxin A.	Cross-sectional	82 samples of human mature milk from various Italian hospitals.	Aflatoxin M <sub>1</sub> and ochratoxin A in breast milk.	AFM <sub>1</sub> detected in 4 (5%) of milk samples (mean level: 55.35 ng/L). OTA was detected in 61 (74%) of milk samples (mean level: 30.43 ng/L). OTA levels significantly higher in people who consume lots of bread, bakery products and cured pork meat.	Findings support possibility of dietary recommendations to women during pregnancy, aimed at reducing the OTA in milk.
Hsieh and Hsieh 1993 Taipei Chang Gung, Taiwan [43]	To investigate metabolism, bioactivation and transplacental transfer of procarcinogens through human placental and cord blood.	Cross-sectional	120 placentas and 56 cord bloods from term, uncomplicated pregnancies.	Aflatoxins in placenta and cord blood.	Of the 120 samples of placentas, 57% contained AFB <sub>1</sub> -DNA adducts of range 0.6–6.3 μmol/mol DNA. Of the 56 samples of cord bloods, 5 (8.9%) contained AFB <sub>1</sub> -DNA adducts range 1.4–2.7 μmol/mol DNA.	Results indicate a significant number of individuals in an area of high liver cancer risk have been exposed to AFB <sub>1</sub> , through the transplacental unit.
Lamplugh et al. 1988 Accra, Ghana and Jos, Nigeria [32]	To confirm the presence of aflatoxins in human breast milk and if they cross the human placental membrane.	Cross-sectional	<i>In Ghana</i> : 264 breast milk and 188 cord blood samples. <i>In Nigeria</i> : venous blood from 77 pregnant women and cord blood samples from their infants after delivery.	Aflatoxins in breast milk, cord blood and venous blood samples.	<i>In Ghana</i> : 90 (34%) of the 264 milk samples contained AF. Aflatoxins were detected in 63 (34%) of the cord blood specimens. <i>In Nigeria</i> : blood samples showed AF in 16 (21%) of 77 maternal samples and 9 (12%) of 78 cord blood samples. AF were found in maternal and cord blood in 7 instances.	Frequency of detection of AF was more in the wet season than the dry season. The mean concentration was also higher during the wet "rainy" season. One stillbirth was recorded in the study (maternal blood contained aflatoxin B <sub>1</sub> 553ng/l)

Table 2 (Continued)

Study ID	Objective	Design	Population	Outcomes	Results	Remarks
Coulter et al. 1984 Sudan [33]	To determine the occurrence of aflatoxins in breast milk, maternal serum and the blood and urine of their infants	Cross-sectional	Breast milk from 99 Sudanese mothers. 80 children	Aflatoxin in breast milk, blood and urine.	Aflatoxin M <sub>1</sub> and/or M <sub>2</sub> were detected in 37 of the milk samples. M <sub>1</sub> occurred in 13 of the milk samples (mean 19.0 pg/ml), while M <sub>2</sub> was detected in 11 of the milk samples (mean 12.2 pg/ml). Aflatoxin was detected in the blood of three children while only urine of two children contained aflatoxin.	There appears to be a linear relationship between M <sub>1</sub> and M <sub>2</sub> where both were excreted. No correlation with the presence of aflatoxin in mothers' blood or the infant's blood and urine.
Saad and Moss 1995 Abu Dhabi, UAE [39]	To determine the occurrence of AFM <sub>1</sub> in donated breast milk	Cross-sectional	445 breast milk donations from women attending 2 hospitals.	Aflatoxins in milk	99.5% of the samples contained aflatoxins at concentrations ranging between 2 pg/ml and 3 ng/ml	
El-nezami et al., 1995 Victoria, Australia and Thailand [34]	To examine the exposure of infants to aflatoxin M <sub>1</sub> (AFM <sub>1</sub> ) and the lactating mothers to aflatoxin B <sub>1</sub> (AFB <sub>1</sub> ), using AFM <sub>1</sub> in breast milk as a biomarker for exposure to AFB <sub>1</sub>	Cross-sectional	73 women from Victoria, Australia and 11 women from Thailand	Aflatoxins in milk	AFM <sub>1</sub> was detected in 11 samples from Victoria and five samples from Thailand at median concentrations of 0.071 ng/ml (range 0.028–1.031 ng/ml) and 0.664 ng/ml (range 0.039–1.736 ng/ml), respectively.	AFM <sub>1</sub> in Thai milk samples significantly higher than in milk samples from Victoria.
Navas et al., 2005 Sao Paulo, Brazil [36]	To determine aflatoxin M <sub>1</sub> and ochratoxin A in milk from the Human Milk Bank of the Southern Regional Hospital, São Paulo, Brazil	Cross-sectional	Total of 50 samples analysed.	Aflatoxins and Ochratoxin A in stored human milk.	Only one was contaminated with AFM <sub>1</sub> , at 0.024 ng/ml, and two with OTA, at 0.011 and 0.024 ng/ml.	Although the incidence observed was low, it is recommended that the study be extended to other milk banks of the city of São Paulo
Wild et al., 1987 Zimbabwe and France [41]	Detection of AF in human breast milk	Cross-sectional	54 samples from Zimbabwe rural women, and 42 women from France	Aflatoxins in breast milk using ELISA	6 breast milk samples from rural villages in Zimbabwe were found to be positive (11%) with levels up to 50 pg AF per ml. No positive samples were detected out of 42 milk samples obtained from women in France.	–
Zarba et al. 1992 Gambia, West Africa [42]	To explore the relationships between dietary intake of aflatoxins and a number of aflatoxin biomarkers including aflatoxin metabolite excretion into breast milk.	Cross-sectional	5 breast milk samples	Aflatoxin M <sub>1</sub> in breast milk by a preparative monoclonal antibody immunoaffinity column/HPLC method	3 out of the 5 breast milk samples contained aflatoxins M <sub>1</sub>	The proportion of aflatoxin in the diet excreted as AFM <sub>1</sub> in milk ranged from 0.09% to 0.43%.
Nyathi et al., 1989 Zimbabwe [37]	Human exposure to aflatoxins in Zimbabwe	Cross-sectional	54 breast milk samples	Aflatoxins in breast milk and urine	AFM <sub>1</sub> detected in 11% of samples	–

Keys: OTA, ochratoxin A; AF, aflatoxin.

duced by the fungi *Aspergillus parasiticus* and *Aspergillus flavus* [4]. These fungi are ubiquitous in hot and humid environments where mean temperatures are about 27 °C, and relative humidity ranges between 80% and 90% [5]. In these settings, aflatoxins are naturally occurring contaminants of staple foods such as cereals, groundnuts and other oil seeds. Aflatoxins are classified based on immunofluorescent properties. Accordingly, they are classified as blue (B) or Green (G). Of the main aflatoxins B<sub>1</sub> B<sub>2</sub> G<sub>1</sub> and G<sub>2</sub>, aflatoxin B<sub>1</sub> is the most potent and carcinogenic [6]. Aflatoxin M<sub>1</sub> is a metabolite of aflatoxin B<sub>1</sub> that can be found in breast milk and urine. Aflatoxins can be measured by a number of methods: thin layer chromatography (TLC) was one of the earliest methods used to detect aflatoxins. Recently, more sensitive and less cumbersome techniques have evolved: the commonest are, high performance liquid chromatography (HPLC), enzyme linked immuno-absorbent assay (ELISA), and the use of immunoaffinity columns (IAC) [7–9]. Aflatoxin contamination is influenced by high humidity, high temperatures, insect and rodent activity and inadequate drying of crops. This contamination can occur in any stage of food production, from pre-harvest to storage [7].

In most developing countries, women are involved in subsistence agriculture. They are actively involved in the full range of farming practices from planting, weeding, pest control, to harvesting and storage. They invariably also end up cooking the meals. Consequently, they are primarily exposed to the health hazards of aflatoxins as they may ingest these toxins in high quantities with food in its raw state or during food preparation. Studies have shown that a widely eaten staple food in West African countries such as Ghana; “Kenkey” typically contains large amounts of aflatoxin producing *Aspergillus* even after fermentation [10]. Pica (an eating disorder among pregnant women which involves the ingestion of non-nutritive substances such as raw maize, soil, gum, ash and other substances that may be contaminated with *Aspergillus* moulds) is another source of increased aflatoxin consumption [11,12].

While a host of studies on aflatoxins have associated them with hepatic cell carcinoma, malnutrition, impaired growth [13] and immune suppression [14], 25 studies have been conducted to show their relationship with reproductive health outcomes.

Cheap and environmentally sustainable methods that can be applied pre or post-harvest to reduce the contamination of aflatoxins are available. These methods include proper irrigation, choice of genetically resistant crop strains and biopesticide management which involves using a non-aflatoxigenic strain of *Aspergillus* that competitively excludes toxic strains [15,16]. Other methods include sorting and disposal of visibly moldy or damaged seeds, reducing the bioavailability of aflatoxins using clay such as NovaSil [17,18] and chemo-protection with Oltipraz, chlorophyllin or vegetables such as broccoli [19].

Almost a decade after the MDG (Millennium Development Goals) declaration, there has largely been no change in maternal mortality rates and child mortality rates barely decreased by 27% [20]. The continued disparity of health outcomes between nations despite huge investments calls for research on how exposures to toxins like aflatoxins, with their well known health effects, may be contributing to poor health and hampering the attainment of these noble goals.

This review represents an attempt to critically assess and summarize the literature on reproductive health effects of exposure to aflatoxins. It is hoped that the resulting information may be valuable in planning mitigation strategies as part of an overall strategy to promote maternal and child health in resource-poor tropical and sub-tropical countries where aflatoxin contaminated foods abound.

## 2. Methods

We searched online databases, scanned reference lists and hand-searched journals for potentially eligible studies. Specifically, we searched PubMed, OVID, MEDLINE, LILACS, EMBASE, Combined Health Information Database (CHID), National Research Register, PsychINFO, ERIC, Science and Social Science Citation Index, Dissertation Abstracts, Online Computer Library Centre (OCLC) and other bibliographic databases. The search covered articles published in English before May 2009. We restricted our retained studies to those with outcomes relating to reproductive health outcomes as previously defined and aflatoxins in all its naturally existing forms. Among the terms and concepts searched are: aflatoxins and reproduction, aflatoxins and birth, aflatoxins and health, aflatoxins and low birth weight, aflatoxins and newborns, aflatoxins and pregnancy, aflatoxins and preterm babies, aflatoxins and stillbirths, aflatoxins and small for gestational age, aflatoxins and infertility, aflatoxins and reproductive organs. To identify studies published in the “gray” literature, we systematically reviewed the bibliographies of all relevant publications, searched the System for Information on Gray Literature in Europe database (SIGLE), the Grey Literature Database of the New York Academy of Medicine, and Grey Literature Network Service (GreyNet) which covers information produced on all levels of government, academia, business and industry in electronic and print formats not controlled by commercial publishing. We also explored online resources (Google and Google Scholar) extensively.

The authors screened titles and abstracts to assess their eligibility for inclusion in the review. Hard copies of studies that were potentially relevant were retrieved for further assessment. The search yielded 121 potential studies. Of these, 25 studies involving 4942 participants qualified for inclusion and were retained for the review. Most studies evaluated were cross-sectional in nature and of varying quality. Some studies did not document how adjustment for known confounders was conducted while others did not provide effect estimates to quantify relationships between outcomes and independent variables.

## 3. Results

Overall, after excluding duplicate studies, we retained 25 studies. In Table 1, we summarize the findings of studies on the effect of aflatoxins on fertility and birth outcomes. In Table 2, we summarize findings relating to the contamination of body fluids by aflatoxins. The tolerable limit of aflatoxin M<sub>1</sub> in breast milk accepted by the European Union and the USA is 25 ng/L while the limit accepted by Australia and Switzerland is 10 ng/L [21].

### 3.1. Aflatoxins and fertility

One study examined the possible effect of aflatoxins on fertility. The relationship between aflatoxin levels in serum of infertile men was compared to controls [22]. The investigators found that semen from 40% of infertile men had aflatoxins compared to 8% of semen from fertile men. The concentrations of aflatoxins detected in the semen were consistently higher among infertile compared to the fertile men. Fifty percent of the infertile men with high aflatoxin semen levels also showed abnormalities (sperm count, morphology and motility) of their spermatozoa on semen analysis. On the contrary, 10–15% of the fertile men showed comparative abnormalities of spermatozoa. In the same study, a parallel experiment was conducted in which adult male rats were given aflatoxin contaminated food. Analysis of their semen showed that rats exposed to dietary aflatoxin (cases) showed changes in their semen which were significantly different from that of the control group ( $P < 0.01$ ). The changes were similar to those observed among human semen containing aflatoxins.

### 3.2. Aflatoxins and birth outcomes

All studies were cross-sectional in design. We found twelve (12) studies [1,21–31] that dealt with the effects of aflatoxin contamination of body fluids on birth outcomes. While seven studies [1,23,25,26,28,30,31] reported on relationship between aflatoxins and birth weights, others reported a mixture of other findings such as birth height [21], gestational age [29], and jaundice [23,24,27,30], among these, two studies examined the relationship between aflatoxins in maternal blood and cord blood [25,30]. While one of these



two studies found no relationship [25], the other [30] was not clear about the outcome of this assessment. It is noteworthy that various studies used different body fluids (such as umbilical cord, maternal serum, and breast milk) to measure aflatoxin metabolites such as aflatoxin B<sub>1</sub>, M<sub>1</sub> and their association with birth outcomes. Consequently, the comparability of these outcomes must be interpreted with caution.

There was no consensus on findings regarding the relationship between aflatoxins and birth weight. While four studies [1,23,28,30] reported a negative correlation between birth weight and aflatoxin levels (with *P* values ranging from <0.001 to <0.05), two studies found this relationship only when the sex of the infant was female (*P* < 0.5) [25,31]. One study conducted in Ibadan Nigeria did not find any correlation between the presence of aflatoxins and birth weight [26]. Similarly, De Vries et al., did not find any correlation between aflatoxins in maternal blood and cord blood [25]. Two studies reported the occurrence of stillbirths among mothers who had significantly high levels of maternal serum aflatoxins [32] or both maternal and neonatal serum aflatoxin [25]. One study by Sedeghi et al., in Iran found an association between aflatoxin M<sub>1</sub> concentration in breast milk and height of the infant at birth (*P* < 0.01) [21]. Yousef et al. [29] did not find any significant correlation between aflatoxin M<sub>1</sub> and gestational age, postnatal age, gender or clinical condition.

Four studies [23,24,27,30] reported findings relating aflatoxins and jaundice among newborns. Only one found that the serum levels of aflatoxin in the infant is a risk factor for neonatal jaundice (OR, 2.68; CI, 1.18–6.10) [27]. Of the two studies that did not find any statistically significant correlation between aflatoxins and jaundice, one used serum from the neonate [24] while the other used cord blood [30]. The fourth study reported that aflatoxins were associated with jaundice in low birth weight babies but did not state whether any association exists between aflatoxins and jaundice among babies of normal weight [23]. It is noteworthy that the aflatoxin levels in body fluids vary by season as was demonstrated by three studies that noted that the frequency of detection of aflatoxins was higher during the wet than the dry season [23,25,32].

### 3.3. Aflatoxins and contamination of body fluids

Body fluids which were found to be contaminated by aflatoxins include maternal breast milk, cord blood, and maternal blood.

Eleven (11) studies demonstrated the presence of aflatoxins in breast milk [31,33–42]. These were all cross-sectional studies. Fresh breast milk was obtained from puerperal women for the studies except in one study, where the breast milk was obtained from a milk bank in Sao Paulo, Brazil [36]. Three cross-sectional studies investigated aflatoxins in maternal blood, and cord blood [32,43,44]. Of these three, one also examined aflatoxin contamination of breast milk [32]. Methods for detection of aflatoxins varied between studies. Six studies used high performance liquid chromatography (HPLC) while others used enzyme linked immuno-absorbent assay (ELISA). These have different detection limits but the results from both methods are generally comparable [45]. On the whole, there were significant differences in contamination of breast milk between studies that were conducted in developing countries and those conducted in developed countries. While breast milk samples from three studies conducted in developed countries had contamination rates ranging from zero percent in France to five percent in Italy (mean concentration 55.35 ng/L), 34–99.5% of those from developing countries were contaminated with aflatoxins [32,39]. The mean concentrations of aflatoxins found in these samples ranged from 130–8218 ng/L in Accra, Ghana, and 2 pg/ml–3 ng/ml in Abu Dhabi, UAE. In many cases, several types of aflatoxins and other mycotoxins were found to contaminate milk. The predominant mycotoxins detected were aflatoxin M<sub>1</sub>, M<sub>2</sub>, B<sub>1</sub> and ochratoxin

A. Two studies investigated the presence of aflatoxins in maternal blood. One study conducted in Songkhla, Thailand [44], found aflatoxins in two of 35 (6%) maternal sera (mean concentration of 0.62 nmol/mL), while the other in Jos, Nigeria, found aflatoxins in 21% of 77 maternal samples (range 33–10,390 ng/L) [32]. Cord blood examined in the aforementioned Thai study contained between 0.074 and 13.6 nmol/mL of aflatoxins with a mean of 3.1 nmol/L. In two other studies, the presence of aflatoxins in cord blood was demonstrated: Hsieh and Hsieh [43] working in an area of high liver cancer risk (Taipei Chang Gung, Taiwan) found that 57% of 120 placenta samples contained AFB<sub>1</sub>–DNA adducts in the range of 0.6–6.3 μmol/mol DNA. In the same study, 8.9% of 56 cord blood samples contained AFB<sub>1</sub>–DNA adducts (range 1.4–2.7 μmol/mol DNA). In the second study, Lamplugh et al. [32] who studied samples from Ghana and Nigeria found that 34% (63 out of 188) of the Ghanaian cord blood specimens contained aflatoxins, while 12% (9 out of 78) of the Nigerian cord blood samples contained aflatoxins.

## 4. Discussion

This systematic review of the reproductive health effects of aflatoxins indicates that a significant proportion of people living in low income countries are exposed to environmental and food-borne toxins which may compromise reproductive health.

While the study on aflatoxin levels among infertile men is interesting, details were not provided of how the selection of cases and controls was made to avoid bias. Nevertheless, animal studies suggest that aflatoxins are spermatotoxic [46–48] therefore, it is reasonable to theorize on the possible link between sperm cell dysgenesis and aflatoxins in humans. Numerous mechanisms for these effects have been postulated. The toxic effects of aflatoxins on the liver may inhibit hormone synthesis, fatty acid metabolism and production of sex hormones precursor molecules [22]. It has also been suggested that aflatoxins cause a direct lysis of sperm cell membrane, which results in the loss of lysozyme, an enzyme which facilitates the penetration of the ova by spermatozoa [22,49]. There is further evidence that suggests that aflatoxins may cause DNA damage and mutations [50,51]. The pathway for this toxicity is thought to involve epoxide intermediates which bind DNA and RNA. The resulting metabolites interfere with DNA-dependent RNA polymerase, thereby inhibiting RNA and protein synthesis [52]. This could lead to interference with spermatogenesis, maturation of spermatozoa and consequently result in abnormal sperm cells. Further research in this area is necessary to assess these relationships.

We found only five studies that have been conducted on the relationship between birth weight and aflatoxins and all of these were of cross-sectional design with limitations with respect to causality. Though four of the five investigators found a correlation or association between aflatoxins and low birth weight, the results must be interpreted with caution in view of the obvious paucity of studies and issues with the quality of the studies. It is noteworthy that some studies did not adjust for other possible causes of low birth weight such as malaria, and other infectious diseases. At best, findings from these studies ought to lay the groundwork for further studies with more rigorous study designs. By the same token, the inconclusive evidence on the association between aflatoxins and neonatal jaundice would benefit from research which employs more rigorous study designs. However, these studies should also be reviewed in the light of a growing body of evidence that indicate that conditions in utero set the stage for how the offsprings develop throughout their lifetime [53]. Furthermore, only two studies have commented on the possible link between birth outcomes such as stillbirths and aflatoxins. These two studies had too few events and no statistical analysis was conducted to draw any conclusions on the association between aflatoxins and stillbirths [25,32]. One study found no relationship with gestational and postnatal age, but the equivocal

pattern of outcomes seen in other studies suggest that further work needs to be done to clarify these relationships.

Table 1 indicates that apart from the highly industrialized countries that have taken steps to curb the contamination of food-stuff by aflatoxins, these mycotoxins are widely consumed in developing countries in amounts that exceed the maximum allowable limits (10–25 ng/ml) by several factors. The discovery of aflatoxins in maternal blood and cord blood further attests to the ubiquitous nature of these toxins. Selected studies indicate a pathway that may be characterized by ingestion of food contaminated by aflatoxins, absorption into the systemic circulation via the digestive system and sequestration in the mammary glands and placenta, so that these aflatoxins become evident in breast milk and cord blood respectively [38,39]. The disparity in the concentrations of aflatoxins between maternal serum and cord blood has been shown by various authors. Denning et al. showed higher levels of aflatoxins in cord blood compared to maternal sera, which indicates not only the transfer of the toxins but also their concentration by the fetoplacental unit [44]. This may be the pathway for deleterious health effects such as low birth weight and stillbirth, which has been reported by other investigators.

While the importance of breast milk for the nutrition, and indeed survival, of the infant in developing countries cannot be overemphasized, its potential for negative health outcomes is indicated by these studies. In recent times, UNICEF and other international organizations have been in the fore-front of campaigns to promote exclusive breast feeding. In rural settings, where infant formula are not available, breast feeding for not only 6 months but almost 24 months is the norm [54]. Given the well known effects of aflatoxins in causing immune suppression, chronic liver disease and malnutrition [4], it is perhaps not surprising that children in these areas are often caught up in the vicious web of illness, poor education and poverty. The reproductive effects that result from growth retardation are well documented [55].

The finding that aflatoxin levels are higher during the wet season is not surprising since this is when crops that have been stored for long periods under hot and humid conditions (with increased potential for contamination by the aflatoxin producing fungi) are eaten. This is the pattern that has been documented in other studies conducted in West Africa [56]. However, it may also indicate that freshly harvested crops are contaminated very rapidly. The seasonality (i.e. wet vs. dry season) in the contamination rate of food-stuff presents a window of opportunity for policy makers and program managers, to plan interventions that will decrease exposure to these toxins during periods when communities are most at risk. Such interventions may benefit the most vulnerable population of women and children and thus contribute towards achieving millennium development goals 4 and 5 which are targeted at reducing maternal and child morbidity and mortality.

The studies reviewed were limited by the fact that they were cross-sectional in design. These have their obvious drawback of being unable to link causality to observed associations. Most of the studies were conducted in developing countries where the equipment and personnel to conduct experiments requiring highly skilled expertise may be in short supply. As aforementioned, there were also variations in measurement methods of the aflatoxins. Thus, it is difficult to comment on the accuracy of results obtained from some of the measurements on aflatoxins. Although some studies adjusted for social class, a well known confounder of birth weight, it is possible that residual confounding due to measurement error persisted.

In sum, we found that few studies have been conducted to investigate the relationship between reproductive health and aflatoxins. The available studies have largely focused on birth outcomes such as low birth weight and contamination of breast milk by aflatoxins. Even so, the lack of rigorous study designs limits the drawing of

conclusions about causality. Our findings show a higher rate of contamination of breast milk in developing countries by aflatoxins, at levels beyond the acceptable limits. Although the reviewed studies were unable to draw definitive conclusions about the effects of aflatoxins on reproductive health, the high contamination rate of breast milk by aflatoxins and the known adverse effects of aflatoxins on other organ systems require stakeholders in affected countries, to take urgent steps to reduce exposure of vulnerable populations to these toxins.

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### Conflict of interest statement

There are no competing interests.

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