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Mycotoxin exposure and adverse reproductive health outcomes in Africa: a review

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REVIEW ARTICLE

Abstract

It is well established that mycotoxin exposure can have adverse effects on reproductive health resulting to poor reproductive potential. The most studied mycotoxin in relation to poor reproductive health in humans is aflatoxin, although fumonisins, trichothecenes and zearalenone have also been reported to impair reproductive function and cause abnormal foetal development. These potent fungal toxins contaminate many food products making them a prominent agricultural, food safety and public health challenge, especially in Africa due to little or lack of mycotoxin regulation in agricultural products. Neonates can be exposed to aflatoxins *in utero*, as the toxins pass from mother to the foetus through the placenta. This exposure may continue during breast feeding, to the introduction of weaning foods, and then foods taken by adults. The consequences of aflatoxin exposure in mothers, foetus and children are many, including anaemia in pregnancy, low birth weight, interference with nutrient absorption, suppression of immune function, child growth retardation and abnormal liver function. In males, reports have indicated a possible relationship between aflatoxin exposure and poor sperm quality culminating in infertility. Maternal exposure to fumonisin during early pregnancy has been associated with increased risk of neural tube defects among newborns in regions where maize is the common dietary staple with the possibility of chronic fumonisin exposure. Furthermore, zearalenone has been linked to precocious puberty and premature thelarche in girls, correlating with extremely high serum oestrogen levels. This review presents an overview of the several reports linking aflatoxins, fumonisins, trichothecenes, and zearalenone exposure to poor reproductive health outcomes in Africa, with emphasis on birth outcomes, foetal health and infertility.

Keywords: endocrine disrupters, mycotoxins, neural tube defects, maternal and foetal health, infertility

1. Introduction

Mycotoxins contaminate many staple food crops and crops used in animal feed, particularly in sub-Saharan Africa and Asia. Several mycotoxins have been identified, however, those that are of public health significance include aflatoxins (AFs), ochratoxin A (OTA), fumonisins, zearalenone (ZEA) and deoxynivalenol (DON) due to their frequent occurrence in agricultural products and their adverse health effects in humans (Schatzmayer and Streit, 2013). Exposure tends to be highest in low income communities where food may be grown and stored locally, where there is a low diversity in diet and where socioeconomic deprivation contributes to malnutrition (Wild and Gong, 2010). A number of adverse health effects have been reported in various mycotoxins, with varying degrees of evidence. In regions where mycotoxin contamination of food is often high, there are often also high levels of reproductive health problems in

humans, such as high rates of infertility, low birth weight, pre-term delivery, stillbirths and birth abnormalities (Agarwal *et al.*, 2015; Mascarenhas *et al.*, 2012; McClure *et al.*, 2009; Ota *et al.*, 2014). Causes of these reproductive health problems are multifactorial and undernutrition and poverty are major contributors. Nevertheless, the occurrence of chronic mycotoxin exposure in populations affected by high rates of such reproductive health problems has led to research into the possible role of mycotoxins as causes of these conditions. Most human research on the adverse reproductive health effects of mycotoxins has focused on AFs, fumonisins and ZEA, but there are also several animal and *in vitro* studies reporting reproductive toxicity and endocrine disrupting effects of other mycotoxins, including DON, OTA, nivalenol (NIV), and T-2 toxin. However, this review is focused on the evidence that AFs, fumonisins, DON, NIV and ZEA contribute to a range of human reproductive health conditions, with aim of guiding future research and interventions designed to reduce harmful exposures to mycotoxins and reduce the risk of these adverse reproductive health outcomes.

2. Aflatoxins

AFs are major metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* and are frequent contaminants of peanuts, maize, rice, and oil seeds, particularly in Africa and South Asia (IARC, 2002). The naturally occurring classes of AFs are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2), and the hydroxylation forms commonly found in milk and urine (aflatoxins M_1 and M_2 (AFM_1 and AFM_2)) (Kensler *et al.*, 2011). AFB_1 is the most toxic and is a major risk factor for hepatocellular carcinoma, particularly in individuals with chronic hepatitis B virus infection. Acute toxicity causes liver failure and can be fatal. AFB_1 has also been associated with growth impairment in children and modification of immune function (Wild *et al*., 2015). In addition, recent evidence indicates AFs may be involved in adverse reproductive health outcomes (Shuaib *et al.*, 2010a; Smith *et al.*, 2017a).

Prevalence of aflatoxin exposure among pregnant women in Africa

Due to the ubiquitous nature of AFs and their common contamination of food products, biomarkers of aflatoxin exposure have been detected in the blood of pregnant women and infant cord blood in different African countries. For example, Wild *et al.* (1991) assessed umbilical cord blood from neonates of 30 Gambian women, and found that 97% of maternal sera and 70% of cord sera were positive for the aflatoxin-albumin (AF-alb) adducts. AF-alb adducts were also detected in 119 out of 119 (100%) maternal blood (range: 4.8-260.8 pg/mg albumin) and cord blood of 48 out of 99 (48.5%) newborns (range: 5.0-89.6 pg/mg albumin) in The Gambia (Turner *et al.*, 2007). In a subsequent study in Ghana, AF-alb adducts (range: 0.44-268.73 pg/mg albumin) were detected in 100% of 755 pregnant women (Shuaib *et al.*, 2010b). Another cross-sectional study showed that AF-alb adducts were found in 34 of 98 (35%) serum samples (range: 3.0-35.1 pg/mg albumin) and AFM_1 (range: 4.1-408.6 pg/mg creatinine) in 44 of 93 (48%) urine samples taken from pregnant Egyptian women (Piekkola *et al.*, 2012), whilst AF-alb adducts were detected in 100% (99/99) of pregnant Gambian women (range: 4.8-521.6 pg/mg albumin during early pregnancy; 4.4-556.5 pg/mg albumin during later pregnancy) (Castelino *et al.*, 2014). In a more recent study, urinary AFM_1 was reported in 30% of 1,580 pregnant women in Zimbabwe (Smith *et al.*, 2017b). Furthermore, De Vries *et al.*, (1989) detected AFs in 37% of 101 cord bloods in Kenya. AFs in cord blood have also been detected in 54.7% of deliveries in United Arab Emirates, 37.8% in Nigeria, 48% in Thailand, 57% in Taiwan and 34% in Ghana (reviewed in Shuaib *et al.*, 2010a). The high prevalence of AFs and AF-alb adducts in cord blood is an indication that AFB_1 crosses the placental barrier reaching the foetal circulation (Denning *et al.*, 1990; Wild *et al.*, 1991).

Determinants of aflatoxin exposure during pregnancy in Africa

Several authors have reported the major determinants of AF exposure in pregnancy among African women. In a study in Ghana, pregnant women with higher economic status and low parity were 30-40% less likely to have high AF-alb adducts in their blood (Shuaib *et al.*, 2012). Economic status is a major driving factor of AF exposure in Africa as those with low economic status are more likely to consume foods that are prone to AF contamination (e.g. maize and groundnuts) and possibly, take unremoved mouldy foods (Jolly *et al.*, 2006; Leroy *et al.*, 2015; Shuaib *et al.*, 2012). However, geographical location, seasonality and dietary practices were significant determinants of AF exposure among pregnant women in Zimbabwe as determined by urinary AFM_1 (Smith *et al.*, 2017b). In a study in Gambia, we found that seasonal differences affected AF-alb adduct levels in Gambian pregnant women with high levels occurring during the dry season and women in their late pregnancy also had higher AF-alb adducts compared to those in early pregnancy (Castelino *et al.*, 2014). Subsequent study suggest participants' ethnicity, village of residence and the number of individuals in the household as significant predictors of high AF-alb adduct levels in Ghanaian general population (Jolly *et al.*, 2006). The number of individuals in a household in most cases determines the quality of food consumed in Africa and high household numbers could predispose to the consumption of low quality foods which may be contaminated with mycotoxins (Jolly *et al.*, 2006). In contrast, there was a significant correlation between blood AF-lysine adduct in adult Kenyan women and poverty level (Leroy *et al.*, 2015). Notwithstanding the country and exposure biomarker applied, it is generally

accepted that geographical location, season and calendar year, socio-economic status, pre-and post-harvest practices, and household dietary practices are major determinants of AFs exposure in pregnancy (Smith *et al.*, 2017b).

Maternal aflatoxin exposure and adverse pregnancy outcomes

AF exposure in pregnant women is mostly due to ingestion of contaminated diets, and has been suggested to contribute to poor maternal, neonatal and child health, especially in Africa and Asia (Shuaib *et al.*, 2010a). The consequences of AF exposure in mothers, neonates and children are many and they include anaemia in pregnancy, intrauterine growth restriction and low birth weight, interference with nutrient absorption, suppression of immune function, child growth retardation and abnormal liver function (Wild *et al.,* 2015).

Aflatoxin exposure during pregnancy, intrauterine growth restriction and low birth weight

Maternal AF exposure during pregnancy has been associated with intrauterine growth restriction and low birth weight, anaemia in pregnancy, neonatal jaundice, and growth faltering in early childhood in Africa (Table 1). Studies evaluating aflatoxin exposure through the presence of AFs or metabolites in serum (AFB_1, AFB_2, AFG_1 and AFG_2) or milk (AFM_1 and AFM_2), instead of validated AF exposure biomarkers (urinary AFM_1 and AFB_1-N7-guanine adduct or serum AF-alb adduct), reported associations between exposure and low birth weight (De Vries *et al.*, 1989), stillbirth (Lamplugh *et al.*, 1988) and neonatal jaundice (Ahmed *et al.*, 1995; Abulu *et al.*, 1997). In The Gambia, AF-alb adduct levels during pregnancy were associated with lower height-for-age (-0.207 *z* scores, P=0.044) and lower weight-for-age (-0.249 *z* scores, P=0.012) in infants during the first year of life (Turner *et al.*, 2007). Shuaib *et al.* (2010c) also reported that of 755 pregnant Ghanaian women, those in the highest quartile of AF-alb (AF-alb: >11.34 pg/mg albumin) were more likely to have babies with low birth weight (odds ratio (OR) 2.09; 95% confidence interval (CI) 1.19-3.68) compared to the lowest quartile (AF-alb: ≤2.67 pg/mg albumin). Mycotoxin exposure biomarkers have advantages over exposure assessments through food and/or foodstuff as they provide more objective data on mycotoxins exposure, and correct for heterogeneous distribution of the mycotoxins in food. Validated biomarkers of AF exposure are biomarkers shown in epidemiological studies to be quantitatively associated with dietary AF intake and include urinary AFM_1 and AFB_1-N7-guanine adduct as well as serum AF-alb adduct (Kensler *et al.*, 2011). Urinary AFM_1 and AFB_1-N7-guanine adduct reflect AF exposure in the previous 24-48 h while serum AF-alb adduct identifies AF exposure over the previous 2-3 months, and are therefore applied to detect short-term and long-term exposure, respectively. Several reports have shown a significant correlation between serum AF-alb adduct and urinary AFM_1 levels (Chen *et al.*, 2018; Jolly *et al.*, 2006; Piekkola *et al.*, 2012) and therefore, reports involving these biomarkers should be accepted with confidence.

One postulated mechanism by which AF exposure could impact on growth is through interference with the insulin-like growth factor (IGF) axis. In Kenyan children exposed to AFs *in utero*, reduced IGF1 mRNA and protein levels were observed (Castelino *et al.*, 2015). *In vitro* exposure of human hepatocyte cell line 16 (HHL-16) to AFB_1 also significantly decreased IGF1 mRNA and protein expression (Castelino *et al.*, 2015). Furthermore, differential DNA methylation of fibroblast growth factor-12 (*FGF12*) and insulin growth factor 1 receptor (*IGF1*R) genes were observed in the white blood cells from Gambian infants whose mothers were exposed to AFs during early pregnancy (Hernandez-Vargas *et al.*, 2015). *IGF1* and *IGF1R* genes are mediators of the growth hormone and plays major roles in embryonic development, foetal and post-natal growth (Baker *et al.*, 1993). It has been reported that serum IGF1 levels are significantly reduced in infants with intrauterine growth restriction compared to controls (Leger *et al.*, 1996). Although these studies provide an insight into the negative effects of AFs on the IGF1 axis leading to perinatal and neonatal growth restriction and low birth weight, these mechanisms remain unclear and needs to be further explored to demonstrate causal association. As AFB_1 is known to cross the placental barrier, it is also possible that foetal cytochrome (CYP) 3A7 bioactivates AFB_1 to the toxic AFB_1-8, 9-epoxide after placental transfer of AFB_1 from maternal blood during pregnancy resulting in foetal toxicity (Partanen *et al.*, 2010).

Aflatoxin exposure in pregnancy and maternal anaemia

Anaemia in pregnancy is one of the leading causes of maternal mortality during child birth in Africa and Asia (Khan *et al.*, 2006; IFPRI, 2016). Recent studies assessing AF-alb adduct levels in pregnant women in Ghana reported significant association between AF exposure and anaemia in pregnancy as well as low birth weight. Shuaib *et al.* (2010b) reported that AF-alb level was associated with anaemia in pregnancy with the odds of being anaemic increasing by 21% (OR, 1.21, P=0.01) and each quartile of AF-alb reaching an 85% increased odds in the highest quartile (AF-alb: >11.34 pg/mg albumin) compared to the low quartile (AF-alb: ≤2.67 pg/mg albumin) group (OR, 1.85; CI, 1.16-2.95).

Experimental studies have shown that AF promotes the haemolysis of erythrocytes, inhibits haematopoiesis, impairs iron absorption, affects haemoglobin levels and induces microcytic hypochromic anaemia in multiple animal species (Andretta *et al.*, 2012; Eisa and Metwally, 2011; Kumar and Balachandran, 2005; Lanza *et al.*, 1978; Verma and Raval, 1991; Yousef *et al.*, 2003). It has also been hypothesised that

Table 1. Human studies on mycotoxins exposure, reproductive and developmental toxicity.[1]

References Study design and location	Mycotoxin levels detected	Outcome	Comments
De Vries *et al.*, 1989 A cross-sectional study of 125 mother-infant pairs in Kenya.	AFs were detected in 53% of maternal blood samples and in 37% of cord samples. Maternal blood: AFB_1 (89-11,574 pg/ml); AFM_1/AFM_2 (12-1,689 pg/ml). Cord blood: AFB_1 (86-6,819 pg/ml); AFM_1/AFM_2 (17-656 pg/ml).	No correlation between AF levels in maternal and cord blood. Female infants born to mothers exposed high levels AF had significantly lower birth weight compared to those from mothers with non-detectable blood AF.	AF occurrence in maternal and cord blood was significantly higher in the rainy season than in the dry season. Stillbirths occurred in mother and cord bloods with high AFs levels.
Turner *et al.*, 2007 Maternal and cord blood was collected from 138 singleton infants followed for 14 months in The Gambia.	AF-alb adducts detected by ELISA and 119 (100%) maternal blood, 48 (48.5%) cord blood and 13 blood samples (11%) from week 16 children had detectable AF-alb adducts. Maternal: 23.3-64.1 pg/mg albumin (mean: 38.9 pg/mg albumin); Cord: 2.5-7.9 pg/mg albumin (mean: 2.5 mg/mg albumin).	Higher AF-alb levels in maternal blood (at 5 and 8 months of pregnancy) were associated with lower HAZ (-0.207 SD; P=0.044) and WAZ (-0.247 SD; P=0.012) scores in children during the first year of life. Maternal AF-alb in pregnancy was not associated with infant weight or length at birth.	There was seasonal variation in maternal AF-alb levels. Maternal AF-alb levels was significantly higher in blood samples collected in December – March compared to samples collected in either April – November (P<0.001).
Shuaib *et al.*, 2010b Blood from 755 pregnant Ghanaian women.	Blood AF-lysine adduct detected by HPLC and all the blood samples had detectable AF-lysine adduct (range: 0.44-268.73 pg/mg albumin; Mean: 10.9±19.00 pg/mg albumin).	Pregnant women with AF-lysine in the highest quartile (>11.34 pg/mg) were 2.09 times more likely to have low birthweight infants (95% CI: 1.19-3.68).	Other parameters showed a trend towards an increased risk (P_{trend}=0.007).
Shuaib *et al.*, 2010c Blood from 755 pregnant Ghanaian women.	Blood AF-lysine adduct detected by HPLC and all the blood samples had detectable AF-lysine adduct (range: 0.44-268.73 pg/mg albumin; Mean: 10.9±19.00 pg/mg albumin). 30.3% had anaemia.	Very high blood AF-lysine adduct was significantly associated with the odds of being anaemic (OR: 1.85; CI: 1.16-2.95).	The odds of being anaemic was stronger in pregnant women after exclusion of those with malaria, intestinal parasitic infection or low folate level. AF exposure in pregnancy was associated with anaemia.
Lamplugh *et al.*, 1988 Blood samples from 188 cord bloods and 264 breast milk samples from Ghana and 77 mother-infants pairs from Nigeria with maternal and cord blood.	AFs detected in 64 (34%) cord bloods and 90 (34%) breast milk from Ghana, and 16 maternal (21%) and 9 (12%) cord blood from Nigeria. Ghana cord: AFB_1 (185-43 ng/l); AFB_2 (11-925 ng/l); AFM_1 (34-7,320 ng/l); AFM_2 (30-572 ng/l); AFG_1 354-1,354 ng/l), AFG_2 (37 ng/l); AFL (117 ng/l). Ghana breast milk: AFB_1 (130-8,218 ng/l); AFB_2 (49-50 ng/l); AFL (64-270 ng/l); AFM_1 (20-1,816 ng/l); AFM_2 (16-2,075 ng/l). Nigeria maternal blood: AFB_1 (540-10,390 ng/l); AFB_2 (28-33 ng/l); AFM_1 (38-483 ng/l); AFM_2 (48-3,480 ng/l). Nigeria cord blood: AFB_2 (0-10 ng/l); AFM_1 (25-8,942 ng/l); AFM_2 (208-378 ng/l)	AF occurrence and concentration was detected more in the rainy season than in dry season. Presence of AF biomeasures in cord blood confirmed AF crosses placental barrier to the foetus.	One stillbirth was recorded from a mother from high AFs exposure level (AFB_1: 553 ng/l).
Maxwell *et al.*, 1994 A cross-sectional study to evaluate the effect of AFs and napthols exposure on birth weight of 625 babies at delivery in Ibadan, Nigeria.	AFs were found in 91 (14.6%) of the cord bloods. The distributions and levels were: AFB_1 (168-69,973 ng/ml); AFB_2 (15-144 ng/ml); AFM_1 (32-11,354 ng/ml); AFM_2 (14-3,644 ng/ml).	*In utero* AF and napthols exposure did not correlate with birth weight at delivery.	

Table 1. Continued.

References Study design and location	Mycotoxin levels detected	Outcome	Comments
Ibeh *et al.*, 1994			
Case-control study to determine the association of AFs exposure and male infertility using 50 infertile males and 50 fertile controls from Benin City, Nigeria.	The mean AF concentrations was 1.660±0.04 µg/ml in infertile men and 1.041±0.01 µg/ml in the fertile control group). Aflatoxins were detected in 40% of the infertile men compared to 8% in Fertile controls.	AF exposure was associated with increased sperm abnormality.	Higher percentage of sperm abnormality (50.0%) was found in infertile males with high exposure to AFs compared to the fertile control group (10.0-15.0%).
Uriah *et al.*, 2001			
A case-control study to examine the association between aflatoxin exposure and human reproduction in Benin City, Nigeria using 30 infertile males and 25 fertile controls.	Serum and semen AFB_1 concentrations ranged from 700-1,392 ng/ml and 60-148 ng/ml, respectively in infertile males with values significantly higher than those in the fertile group. One semen sample with no live spermatozoa (azoospermia) had an AFB_1 levels as high as 1,450 ng/ml. Approximately 37% of semen and blood of infertile men had detectable aflatoxins than the controls.	AF exposure suggested as a contributory factor to male infertility in Nigeria.	AFs exposure caused decreased sperm concentration and increased sperm abnormalities.
Mohammed *et al.*, 2014			
A case-control hospital-based study that examined the AFs concentration in semen samples from 108 (60 infertile men and 48 fertile controls) visiting Sohag University Hospital, Egypt.	AFB_1 was present in 25% of the semen of infertile patients, compared to 2.1% among the controls (P=0.0007).	AFB_1 exposure was suggested as a potential contributor to idiopathic infertility in males.	Severe reduction in sperm count, reduced motility, high percentage of sperm with abnormal morphology, and high viscosity in the semen of the infertile group(P<0.05) compared to the fertile group.
Szuets *et al.*, 1997			
Study on young girls with early thelarche/ mastopathy in Southern Hungary.	ZEA was detected in the serum of 5 out of 36 early patients with concentrations ranging from 18.9-103 µg/ml.	ZEA reported to be associated with early thelarche/ mastopathy.	Childhood ZEA exposure was associated with early breast development in young girls.
Massart *et al.*, 2008			
Study on serum samples of 32 girls with CPP and 31 female healthy female controls from North-West Tuscany, Italy.	ZEA (723.5-1,143.9 pg/ml) and α-ZOL (104.5-108.5 pg/ml) were detected from the serum of 6 of the 32 girls with CPP. All the controls had no detectable mycotoxins.	ZEA suspected of inducing central maturation of the hypothalamic-pituitary-gonadal axis causing CPP in exposed girls.	Mycotoxin positive patients had higher height, weight and height velocity compared to those who had no mycotoxin detected in their serum.
Bandera *et al.*, 2011			
A cross-sectional study to determine the association between the exposure of ZEA and its metabolites on body size and breast development in 163 girls from New Jersey, USA.	78.5% of the girls had detectable levels of ZEA and its metabolites (ZEA: 0.05-33.12 ng/ml; α-ZOL: 0.003-10.69 ng/ml).	ZEA suspected to have anti-oestrogenic effect.	Girls exposed to ZEA and its metabolites presented with shortness and had late-onset of breast development.
Tomaszewski *et al.*, 1998			
Endometrial tissue specimens collected from 49 women (endometrial adenocarcinoma n=27, endometrial hyperplasia n=11, normal proliferative endometrium n=11).	The concentration of ZEA in 3 endometrial hyperplasia samples was 47.8±6.5 ng/ml and 167.0±17.7 ng/ml in 22 adenocarcinoma samples.	ZEA suspected to be associated with hyperplastic and neoplastic endometrium.	Normal endometrial samples had no detectable concentration of ZEA. Also 8 hyperplastic and 5 neoplastic endometrial samples had no detectable concentration of ZEA.

[1] AF = aflatoxin; AF-alb = aflatoxin-albumin; AFL = aflatoxicol; AFs = AFB_1, AFB_2, AFM_1, AFM_2, AFG_1 and AFG_2; CI = confidence interval; CPP = central precocious puberty; ELISA = enzyme-linked immunosorbent assay; HAZ = height-for-age Z-score; HPLC = high performance liquid chromatography; OR = odd ratio; SD = standard deviation; WAZ = weight-for-height Z-score; ZEA = zearalenone; α-ZOL = α-zearalenol.

aflatoxin could cause anaemia by impairing iron absorption and bioavailability as well as inhibition of erythropoiesis (Smith *et al.*, 2017a). To date, no human research has been performed to test this hypothesis.

Aflatoxin exposure and male infertility

Male infertility is increasing in both developed and developing countries (Mascarenhas *et al.*, 2012). The prevalence of male infertility varies between developed countries with USA standing at 6% (Chandra *et al.*, 2013) whereas it is higher in UK at 10-15% (Oakley *et al.*, 2008). In sub-Saharan Africa, infertility prevalence rates are higher and range from 20-35% (Etuk, 2009). The 'infertility belt' stretching from West Africa, through Central to East Africa are known geographical regions with high infertility prevalence in Africa (Etuk, 2009). Our previous case control study has pointed to environmental and food-borne contaminants (e.g. pesticides, phthalates, bisphenol A, mycotoxins, cadmium, lead, arsenic, and caffeine) as possible causes of male infertility in Africa (Okonofua *et al.*, 2005), but the exact contaminants have yet to be identified. Subsequently, we have argued for further research to uncover the role of food contaminants, such as AFs in the rising incidence and prevalence of male infertility in the African region (Eze and Okonofua, 2015) as these toxins are major contaminants of local foods consumed in the region. Although preliminary experiments have identified possible roles for AFs in the causation of infertility in the animal models (Agnes and Akbarsha, 2003; Ahmed *et al.*, 2012; Ortatatli *et al.*, 2002; Supriya *et al.*, 2014), this is yet to be confirmed in humans, where little research is available.

There are a few human studies reporting the potential of AF exposure in causing male infertility in Africa (Table 1). In Nigeria, Ibeh *et al.* (1994) examined the presence of AFs in the semen of 100 adult males consisting of 50 infertile men and 50 fertile men to ascertain if there is a relationship between AFs and male infertility. Among the 50 semen samples from the infertile group, 20 (40%) had detectable level of AFs and 50% of the semen samples had sperm with abnormalities in sperm count, morphology and motility whereas only 4 (8%) of the fertile males had AFs in their semen with 10-15% of the semen showing sperm abnormalities. In addition, the concentrations of AFs found in the semen of infertile males were higher than fertile males. When adult male albino rats were fed with animal feed contaminated with 8.5 μg AFB_1/g feed for 14 days, they had decreased sperm count, abnormal morphology, reduced motility and viability (Ibeh *et al.*, 1994). In an *in vitro* study, AFs also significantly reduced the motility of sperm cells (Ibeh *et al.*, 2000). In a case-control study involving 30 infertile and 25 fertile males, Uriah *et al.* (2001) found significantly higher levels of AFB_1 in the blood (700-1,392 ng/ml) and semen (60-48 ng/ml) of the infertile men compared to the fertile men. A case-control hospital-based study that examined the AF concentrations in semen samples from 108 men (60 infertile men and 48 fertile controls) visiting a University Hospital in Egypt has also been reported (Mohammed *et al.*, 2014). It was observed that AFB_1 was present in 25% of the semen of infertile patients, compared to 2.1% found in fertile controls (P=0.0007). Among the infertile patients with AFB_1 exposure, severe reduction in sperm count, reduced sperm motility, high percentage of sperm with abnormal morphology, and high viscosity in the semen were evident compared to the fertile group with low detectable AFB_1 (Mohammed *et al.*, 2014).

The negative effects of AFB_1 on sperm parameters could be attributed to its ability to interfere with the biosynthesis of androgens and alteration of testicular function resulting to the inhibition of spermatogenesis and sperm maturation giving rise to the production of abnormal sperm cells (Adedara *et al.*, 2014; Agnes and Akbarsha, 2003; Ahmed *et al.*, 2012; Picha *et al.*, 1986; Supriya *et al.*, 2014). For instance, in adult male roosters fed with diets containing AFB_1 (5, 10 and 20 mg/l) for 8 weeks, a suppression of spermatogenesis, abnormalities in sperm morphology and count, reduced plasma testosterone and testicular atrophy were observed compared to the control adult roosters (Ortatatli *et al.*, 2002). In a more recent study, exposure of bull semen to AFB_1 (0.1, 1, 10 and 100 μM) *in vitro* for 4 h decreased sperm viability, caused hyper-polarisation of the sperm mitochondrial membrane and negatively affected sperm DNA integrity (Komsky-Elbaz *et al.*, 2018).

3. Fumonisins

Fumonisins are primarily produced by *Fusarium verticillioides* (previously known as *Fusarium moniliforme*) and *Fusarium proliferatum* which contaminate maize and maize-based food products, although their presence in other agricultural products (e.g. sorghum, cowpea, asparagus, rice and farro) has been occasionally reported (Bulder *et al.*, 2012; Rheeder *et al.*, 2016; Voss *et al.*, 2006). Human exposure to fumonisin B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3) is high in regions where home-grown maize are used as dietary staples (Gong *et al.*, 2008; Van der Westhuizen *et al.*, 2011). FB_1 is the most common of the numerous fumonisin analogues and the most studied fumonisins, and has been classified as a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC, 2002).

Consumption of FB_1-contaminated maize and maize-based foods has been associated with high occurrence of oesophageal cancer in the former Transkei region of South Africa (Misihairabgwi *et al.*, in press; Rheeder *et al.*, 1992) and Linxian region of China (Sun *et al.*, 2007). Similarly, in Mazandaran Province of Iran where there is also a high incidence of oesophageal cancer, increased concentrations

of fumonisins have been detected in maize grown in the area (Alizadeh *et al.*, 2012; Shephard *et al.*, 2002; Yazdanapanah *et al.*, 2006). In animals, FB_1 has been linked to spontaneous equine leukoencephalomalacia (Marasas *et al.*, 1988; Smith *et al.*, 2002), porcine pulmonary oedema (Harrison *et al.*, 1990; Hascheck *et al.*, 2001), liver and kidney toxicity in different animal species (Bolger *et al.*, 2001; Voss *et al.*, 2001), and liver and kidney cancer in rodents (Gelderblom *et al.*, 1991; Howard *et al.*, 2001a,b).

Fumonisin exposure, reproductive toxicity and birth defects

Growth retardation, delayed or incomplete ossifications, cleft palate or hydrocephalus, and foetal death have also been found in pregnant animals fed with FB_1-contaminated feeds (Voss and Riley, 2013). Studies evaluating the reproductive and developmental toxicity of FB_1 in humans are very rare. However, consumption of FB_1 contaminated feeds have been reported to impair the reproductive potential of Syrian hamsters (Floss *et al.*, 1994), rabbits (Ewuola and Egbunike, 2010), rats (Flynn *et al.*, 1996; Gbore *et al.*, 2012) and chicken embryos (Bacon *et al.*, 1995; Javed *et al.*, 1993; Zacharias *et al.*, 1996). Gbore and colleagues have performed several studies to examine how dietary FB_1 exposure in piglets and boars affect their reproductive function and they observed that such exposure caused delay in puberty in piglets and impaired sperm production and semen quality of boars (Gbore and Egbunike, 2008; Gbore, 2009a,b).

In humans, fumonisins are reported to be environmental risk factor for birth defects, notably neural tube defects (NTDs). NTDs (spina bifida, exencephaly and craniorhachischisis or meningio-myeloceole) are foetal malformations occurring as a result of failure of the embryonic neural tubes to close properly during the early period of gestation (Voss and Riley, 2013; Voss *et al.*, 2006). Maternal exposure to high concentrations of FB_1 through the ingestion of contaminated maize and maize-based foods during early pregnancy has been associated with increased risk of NTDs among newborns in regions known to consume maize as staple foods (Hendricks, 1999; Marasas *et al.*, 2004; Melnick and Marazita, 1998; Missmer *et al.*, 2006). In the Transkei region of South Africa and Cameron County, Texas (USA) along the Texas-Mexico border, where maize is a dietary staple and where there is high chronic fumonisin exposure, there have been reports of high incidence of NTDs (Hendricks, 1999; Marasas *et al.*, 2004; Missmer *et al.*, 2006). The incidence of NTDs in these regions is known to be 6-10 times higher compared to the average NTDs incidence rate found globally (≈10/10,000 live births) (Gelineau-Van Waes *et al.*, 2009; Hendricks, 1999; Marasas *et al.*, 2004).

A high incidence of NTDs (27 per 10,000 live births) was observed in the newborns of Mexican-American women living along the Texas-Mexico border who conceived between 1990 and 1991 (Hendricks, 1999). As a result, Missmer *et al.* (2006) conducted a population-based case-control study among Mexican-American women who delivered newborns affected by NTDs between 1995 and 2000 to evaluate if maternal fumonisin exposure during pregnancy was associated with NTDs in neonates. In this study, Missmer and colleagues assessed serum sphinganine to sphingosine (Sa:So) ratio of 163 Mexican-American women with NTD-affected pregnancies and 189 Mexican-American women who delivered babies without NTDs within the study period. In addition, fumonisin levels were determined from 146 household corn tortillas and 114 tortillas obtained from grocery stores to estimate pre-conceptional dietary fumonisin exposure. The maternal serum Sa:So ratio ranged from 0.08 to 0.36 whereas fumonisin levels in tortillas ranged from non-detectable to 1,690 ng/g. Moderate consumption of tortilla compared to low consumption during the first trimester of pregnancy was associated with increased OR with NTDs in newborns after adjusting for body mass index, serum B_{12} and folate, and date of conception (OR: 2.4; 95% CI: 1.1-5.3). However, higher intakes of tortillas resulted to either a slight decrease in NTDs incidence or absence of NTDs. With regard to the maternal serum Sa:So ratio, higher levels of fumonisin exposure was associated with higher incidence of NTDs in newborns whereas those with the highest Sa:So ratio (>0.35) had low incidence of NTDs in newborns. It was suggested that at the highest estimated dietary or maternal serum fumonisin exposure, miscarriages or stillbirth might have occurred resulting to low incidence of NTD in this group (Missmer *et al.*, 2006). Worthy of note is that this study estimated fumonisin exposure by measuring FB_1 in foodstuff which has previously failed to provide accurate data on human exposure due to heterogeneous distribution of mycotoxins in food (Shephard *et al.*, 2007). In addition, Missmer *et al.* (2006) applied maternal serum Sa:So ratio as a biomarker of fumonisin exposure. Although serum and urinary Sa:So ratio is suggested as a useful biomarker of fumonisin exposure in animals (Van der Westhuizen *et al.*, 2001), it failed to correlate with human dietary exposure to fumonisins (Van der Westhuizen *et al.*, 2008, 2010). The method of fumonisin exposure assessment applied by Missmer *et al.* (2006) could have inaccurately estimated fumonisin exposure and results should be interpreted with caution. Therefore, future studies should apply urinary FB_1 biomarker which has been shown to correlate well with human dietary fumonisin exposure (Gong *et al.*, 2015; Shirima *et al.*, 2013;Van der Westhuizen *et al.*, 2011).

In animal studies, FB_1 has been shown to induce NTDs in cultured mouse embryos (Sadler *et al.*, 2002), and altered embryogenesis and caused NTDs development in foetus of pregnant mouse exposed during the early gestation

(Gelineau-Van Waes *et al.*, 2005). Although FB_1 is suggested as an etiologic agent in the development of NTDs, other factors including genetics and maternal nutrition play an important role in pregnancy outcome and dietary folic acid supplementation prior and during pregnancy is known to reduce the occurrence of NTDs (Greene and Copp, 2005). The potential for FB_1 to cause NTDs is primarily attributed to its structural similarity with sphingamine and sphingosine (Gelineau-Van Waes *et al.*, 2005). FB_1 and other fumonisins competitively interfere with ceramide synthase which is involved in the *de novo* synthesis of complex glycosphingolipids resulting to the accumulation of sphingamine and sphingosine in tissues, serum and urine (Bolger *et al.*, 2001; Merrill *et al.*, 2001; Riley and Voss, 2006; Voss and Riley, 2005; Voss *et al.*, 2006). It has been postulated that NTDs are induced by fumonisins as a result of decreased concentration of complex sphingolipids, disruption of lipid rafts and impairment in the function of the high affinity placental carriers (folate binding protein 1 in mice and folate receptor alpha in humans) involved in folate transport causing folate deficiency in the foetus during early pregnancy and failure of the foetal neural tube to close properly (Gelineau-Van Waes *et al.*, 2005, 2009, 2012; Voss and Riley, 2013; Voss *et al.*, 2001). In addition, the complex glycol-sphingolipids are important in the maintenance of cell membrane integrity, cell growth and migration, cell differentiation, cell morphology and endothelial cell permeability and therefore, disruption of sphingolipid metabolism by fumonisins can negatively affect embryonic morphogenesis and cell apoptosis (Merrill *et al.*, 2001; Yahia and Kamata, 2017). Based on these studies discussed above, the negative impacts of FB_1 on reproductive function in animal and *in vitro* models cannot be ignored as there is a homology in organ systems between animals and humans.

4. Trichothecenes

The trichothecenes are toxic sesquiterpenoid mycotoxins produced by *Fusarium*, *Stachybotrys* and *Myrothecium* species of fungi during their growth in food and/or the environment (Pestka, 2010a,b). The broad family of trichothecenes have been classified into four groups: types A, B, and D based on the substitution pattern of the tricyclic 12, 13 epoxytrichothec-9-ene core structure (Escrivá *et al.*, 2015). Type A trichothecenes include T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol, whereas the type B group are fusarenon-X, NIV and DON and its 3-acetyl and 15-acetyl derivatives. The detailed toxicological effects and molecular mechanisms of trichothecenes toxicity has been reviewed in Pestka (2010a,b) and Escrivá *et al.* (2015).

Reproductive and developmental health effects of deoxynivalenol exposure

DON is one of the type B trichothecenes and is known to cause disruption of gastrointestinal permeability, impair growth hormone signalling, dysregulate the expression of genes involved in inflammatory response, immunotoxicity, haematological disorders, and alteration of the neuroendocrine responses in humans and animals (Pestka, 2010a,b). The molecular mechanisms underlying these pathological effects involve the causation of oxidative damage, induction of apoptosis and autophagy, alteration of membrane integrity, and inhibition of DNA, RNA and protein synthesis (Payros *et al.*, 2016; Pestka, 2010b). The effects of DON on human reproduction and development are scarce, however, there is literature reporting its adverse effects on reproduction and development in animal and *in vitro* models (Yu *et al.*, 2017a).

DON has been implicated in the disruption of oocyte maturation, embryo resorption and foetal malformation in mice (Hou *et al.*, 2014; Khera *et al.*, 1982, 1984; Yu *et al.*, 2017b), rats (Collins *et al.*, 2006), and swine (Alm *et al.*, 2002, 2006). In a study to assess the toxic effects of DON on mouse embryo, Khera *et al.* (1982) exposed pregnant mice in their gestation days (GD: 8-11) to DON (0.5-25 mg/kg/body weight (bw)) through oral gavage. DON at 0.5 mg/kg/bw had no maternal and foetal toxicity, however, at 1, 2.5 and 5 mg/kg/bw DON caused foetal malformation. In addition, there was embryo resorptions in mice treated with 5, 10 and 25 mg/kg/bw whereas significant reduction in live foetuses were observed at >5 mg/kg/bw. In a subsequent study, maternal toxicity, foetal malformation and embryo resorption were reported in mice and rats exposed to DON at >1 mg/kg/bw (Debouck *et al.*, 2001; Khera *et al.*, 1984). Similarly pregnant rabbits fed with DON contaminated diet showed 100% incidence of embryo resorption in the 1.8-and 2.0 mg/kg groups and decreased mean foetal weight in the 1.0 and 1.6 mg/kg group (Khera *et al.*, 1986). However, none of the doses caused foetal malformation or showed teratogenic potential. In accordance with the above results, Hou and colleagues found that exposure of mice with DON-contaminated maize (3.875 mg/kg/diet) for 4 weeks resulted in poor oocyte indices and low developmental competence of the ovaries (Hou *et al.*, 2014). Taken together, these studies indicate that a high exposure to DON during pregnancy could directly induce foetal malformation or indirectly affect embryonic and foetal development as a result of maternal toxicity (Collins *et al.*, 2006). With regard to studies on rats treated orally with DON, the CONTAM Panel (EFSA, 2017a) sets the no-observed-adverse-effect-level as 1.0 mg/kg bw per day for reproductive endpoints, 1.0 mg/kg bw per day for foetal toxicity and 0.5 mg/kg bw per day for maternal toxicity.

The toxicity of DON to male germ cell in animals have also been reported. For instance, exposure of mice to DON induced testicular germ cell degeneration, decreased absolute caudal epididymal sperm numbers, caused reduction in caudal epididymal weights, and reduced serum testosterone levels (Sprando *et al.*, 1999, 2005). This is supported by an *in vitro* study in which exposure of MA-10 murine Leydig cell line to DON increased the release of reactive oxygen species, significantly reduced cell viability and caused significant decline in forskolin-induced progesterone secretion after 24 h of exposure (Savard *et al.*, 2016). In a more recent study, we showed that DON was strongly cytotoxic to MA-10 cells after 48 h of exposure with concentrations as low as 29.63 ng/ml (0.1 μM) and 296.32 ng/ml (1 μM) showing significant cytotoxicity (Eze *et al.*, 2018). Urinary concentrations of 436 ng/ml (1.471 μM) and 1,238 ng/ml (4.178 μM) have been reported for DON and its conjugates in pregnant women (Sarkanj *et al.*, 2013; Wells *et al.*, 2016) and therefore, the concentrations causing cytotoxic effects on MA-10 cells have clinical relevance.

DON can be transferred through the placenta to the foetus in pregnant sows (Goyarts *et al.*, 2007; Dänicke *et al.*, 2007) and it is likely that human foetus will be exposed to DON during pregnancy. In an *in vitro* placental perfusion using BeWo cell line, Nielsen *et al.* (2011) showed that DON was transported across the membrane and caused a dose-dependent decrease in the secretion of beta-human chorionic gonadotrophin (β-hCG). Normal β-hCG level is required for the proper functioning of the placenta and foetal development, therefore the reduction in this important hormone further supports the embryo resorption, foetal death and malformation reported in animal studies. In addition, it has been argued that DON exposure in females causes the accumulation of reactive oxygen species, induction of autophagy and apoptosis, and alteration of epigenetic modification in the female reproductive system resulting to DON-induced reproductive and developmental toxicity (Han *et al.*, 2016; Yu *et al.*, 2017b).

In summary, it is plausible that human exposure to DON either *in utero* or during post-natal period can impair reproduction and development. Therefore, a properly designed *in vitro* and *in vivo* bioassays as well as human epidemiological study is required to provide stronger evidence establishing the association between exposure to DON and adverse reproductive health outcomes in humans.

Reproductive and developmental toxicity of nivalenol

Similar to DON, the effects of NIV on reproduction and development have also been reported (Pestka, 2010a), although there is no literature on the reproductive health effects in human beings. Ito and colleagues reported that intraperitoneal administration of NIV (0.1-1.5 mg/kg bw/day) to pregnant ICR mice from 7-15 days of gestation caused vaginal bleeding in 60% of the exposed mice, stillbirths and induced 48-88% embryo deaths, especially at 0.5 and 1.5 mg/kg bw (Ito *et al.*, 1986). It was also observed that the injection of 3 mg NIV/kg bw on gestation day 7 was highly toxic to both the embryo and placenta culminating in stillbirths within 48 h. In a similar study, maternal and embryo toxicity which resulted to intrauterine growth retardation was reported when pregnant mice (GD: 7-15) were either fed with 6-30 mg/kg/diet of NIV or 1-20 mg/kg bw of NIV by oral gavage, particularly at the highest doses (Ito *et al.*, 1988). Teratogenic effects were not observed in any of the concentrations of NIV used either in contaminated feed or oral gavage (Ito *et al.*, 1988). When female F344/DuCrj rats were fed diets containing NIV at 6.25, 25 and 100 mg/kg for 90 days, histopathological features showed increase in atretic ovarian follicles and interstitial glands, impaired corpora lutea development, and uterine atrophy with dioestrus endometrial mucosa change in those fed the highest dose (100 mg/kg) (Takahashi *et al.*, 2008; Sugita-Konishi *et al.*, 2008). However, there was no adverse effect on the primary and secondary follicles. On the other hand, there was no effect on the reproductive system in male F344/DuCrj rats fed with same diet containing the same amount of NIV (Takahashi *et al.*, 2008; Sugita-Konishi *et al.*, 2008). In contradiction to the above studies, pregnant sows fed with maize contaminated with NIV did not affect any reproductive parameters assessed (Williams and Blaney, 1994). It can be deduced that female rodents are more susceptible to NIV than their male counterparts and pigs.

5. Zearalenone

Zearalenone (ZEA) produced by *Fusarium species* is also considered as one of the common mycotoxins posing a threat to human and animal reproductive health (Zinedine *et al.*, 2007). ZEA is primarily converted by hydroxysteroid hydrogenases to phase I metabolites, including α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL) and zearalanone (ZAN) (EFSA, 2017b). ZEA and the metabolites are known endocrine disruptors due to their resemblance to 17β-oestradiol, enabling them to disrupt the binding of 17β-oestradiol to oestrogen receptors. Both *in vivo* and *in vitro* studies show that α-ZOL has higher oestrogenic potency compared to ZEA, whereas β-ZOL has lower oestrogenic potency (EFSA, 2017b). It has been reported to cause hyper-oestrogenism with attendant reproductive disorders in farm animals (Zinedine *et al.*, 2007). In animals, ZEA is known to reduce testosterone levels and induce abnormal sperm quality in males (Yang *et al.*, 2007; Zatecka *et al.*, 2014), and cause ovarian follicle atresia, uterine hyperplasia, and degeneration of oocyte meiotic chromatin in females (Alm *et al.*, 2006; Obremski *et al.*, 2003; Skorska-Wyszyńska *et al.*, 2005; Yamini *et al.*, 1997). Despite the evidence that ZEA causes adverse effects on reproduction in animals,

there are currently no published reports of associations of ZEA with infertility or birth defects in human populations.

There have been some reports of the association of ZEA exposure with central precocious puberty (CPP), premature thelarche, and hyperplastic and neoplastic endometrium (Table 1). In a study comprising 32 girls with CPP and 31 healthy female controls, elevated levels of ZEA and α-ZOL were detected in the serum of six of the 32 cases (Massart *et al.*, 2008, 2010). In response to a high incidence of CPP in the Viareggio region of North-West Tuscany in Italy, Massart *et al.* (2008) recruited 32 girls (17 from Viareggio region and 15 from Pisa) with idiopathic CPP visiting the Paediatric Endocrine Centre of Pisa and matched them with 31 healthy female controls. In these groups, ZEA and its metabolites were assessed and matched with height, weight and height velocity, body mass index, bone age, and gonadal secretion. Among the 17 CPP patients from Viareggio region, 6 (35%) had detectable levels of ZEA (mean: 933.7±200.3 pg/ml) and α-ZOL (mean: 106±1.9 pg/ml) whereas none of the 15 CPP patients from Pisa had ZEA or its metabolites detected in their serum. It was also observed that the six girls with ZEA and α-ZOL exposure had higher height, weight and height velocity after 12-month triptorelin (gonadotrophin-releasing hormone agonist) treatment compared to the 26 CPP patients and 31 healthy controls with non-detectable serum ZEA and α-ZOL (Massart *et al.*, 2008). As a result of these parameters, ZEA and α-ZOL exposure were suggested as predisposing factors of idiopathic CPP in prepubertal girls. However, the ZEA and its metabolites could not be detected in the other 26 girls with CPP giving an inconclusive result and therefore, other environmental contaminants may have been predisposing factors of the reported CPP (Massart *et al.*, 2008, 2010). Previously, ZEA exposure was also speculated to be associated with early breast development among young Hungarian girls (Szuets *et al.*, 1997). In this report, five of 36 early telarche/mastopathy patients in the South-East Region of Hungary had high serum level of ZEA (18.9-103.5 µg/ml) and it was speculated that ZEA could be a contributory factor for the development of early telarche/mastopathy (Szuets *et al.*, 1997). However, this study could not clearly state statistically how the concentration of ZEA was related to early telarche/mastopathy development and so, should be interpreted with caution. Other studies also suggest that ZEA may be a contributory factor to premature thelarche and idiopathic CPP in prepubertal girls (Schoental, 1983; Deng *et al.*, 2012; Asci *et al.*, 2014). In contradiction to these studies above, Bandera and colleagues conducted a cross-sectional study comprising 163 girls (age: 9-10 years) from New Jersey in USA to evaluate the effects of exposure to ZEA and its metabolites on body size and breast development (Bandera *et al.*, 2011). In this study, 78.5% of the girls had urinary ZEA (0.05-33.12 ng/ml), α-ZOL (0.003-10.69 ng/ml), β-ZOL (0.05-1.10 ng/ml), α-ZAL (0.02-0.57 ng/ml), β-ZAL (0.04-0.60 ng/ml) and ZAN (0.07-3.31 ng/ml), with those who consumed beef and popcorn the day prior to urine collection having significantly higher concentrations compared to those who did not take these foods. Girls who had detectable levels of urinary ZEA and its metabolites had lower height and delayed onset of breast development after adjusting for age, body mass index, isoflavone intake, and recruitment year, and it was concluded that exposure to ZEA and its metabolites had anti-oestrogenic effects on the girls (Bandera *et al.*, 2011). The difference in the results reported in this study compared to the previous studies could be attributed to the small sample size of ZEA exposure (5 or 6), detection of ZEA and metabolites in serum instead of urine, and having subjects comprising of girls undergoing CPP treatment (Bandera *et al.*, 2011). Therefore, a well-designed longitudinal studies with larger sample size, the inclusion of people with different ethnic background and countries, and the use of valid ZEA exposure biomarkers would certainly provide stronger evidence for establishing a causal association. In addition, reproductive hormones such as oestrogen, progesterone, follicle stimulating hormone, and luteinising hormone should be evaluated and included in the statistical analysis as they can also influence the onset of puberty in girls.

However, biologically relevant doses of ZEA (5-10 mg/kg bw) caused precocious puberty in immature female rats as a result of early induction of the hypothalamic kisspeptin (KISS1)-G-protein coupled receptor-54 (GPR54) signalling pathway which is responsible for the initiation of puberty onset, regulation of the hypothalamo-pituitary-gonad axis and reproductive function (Kriszt *et al.*, 2015; Yang *et al.*, 2016). The precocious puberty in treated rats were evidenced through the induction of *Kiss1*, *GPR54* and *GnRH* (Gonadotropin-releasing hormone) expressions in the hypothalamus at both mRNA and protein levels (Yang *et al.*, 2016). This could be the same mechanism through which ZEA causes precocious puberty in human beings since mutations and/or inactivation of the *GPR54* and/or *KISS1* genes in humans and mice (*gpr54* and *kiss1*) has been linked to reproductive dysfunction, including delayed puberty onset, abnormal oestrus cycles and infertility (Kirilov *et al.*, 2013; Semple *et al.*, 2005).

6. Mycotoxin co-exposure and implication for human reproductive health

Multiple mycotoxin exposure biomarkers have been reported in several population studies which show that human beings are often simultaneously exposed to mixtures of mycotoxins (Table 2). For instance, in the former Transkei region of South Africa, Shephard and colleagues examined 53 urine samples and demonstrated that mixtures of biomarkers such as DON, ZEA, FB_1 and OTA as well as their metabolites can be detected frequently in the human urine samples (Shephard *et al.*, 2013). Similarly, out of

Table 2. Summary of selected studies on human exposure to mycotoxin mixtures.

References	Country	No.	Individuals examined	No of positive samples (%)	Mycotoxin exposure biomarkers detected[1]	Co-exposure in a single individual[1]
Ahn *et al.*, 2010	Korea[2]	12	11 adults, 1 child	12 (100%)	AFM_1, OTA	AFM_1, OTA
Rubert *et al.*, 2011	Spain[2]	27	adults	not stated	AFG_2, OTA, DON	not stated
Solfrizzo *et al.*, 2011	Italy[2]	10	adults	10 (100%)	OTA, DON	DON, OTA
Warth *et al.*, 2012a	Austria[2]	27	adults	26 (96%)	DON, DON-3-GlcA, DON-15-GlcA	DON, DON-3-GlcA, DON-15-GlcA
Warth *et al.*, 2012b	Cameroon[2]	175	145 HIV-positive adults, 30 HIV-negative adults	110 (63%)	AFM_1, OTA, FB_1, FB_2, DON, DON-3-GlcA, DON-15-GlcA, NIV, ZEA, ZEA-14-GlcA, α-ZOL	AFM_1, OTA, FB_1, FB_2, DON, DON-3-GlcA, DON-15-GlcA, NIV
Ediage *et al.*, 2012	Belgium[2]	40	adults	9 (23%)	DON, OTA, OTα, 4-OH-OTA, ZEA, CIT, β-ZOL	DON, OTA, OTα, ZEA, β-ZOL
Shephard *et al.*, 2013	South Africa[2]	53	adult women	53 (100%)	OTA, FB_1, DON, DON-3-GlcA, DON-15-GlcA, NIV, ZEA, ZEA-14-GlcA, α-ZOL, β-ZOL	OTA, FB_1, DON, DON-3-GlcA, DON-15-GlcA, ZEA, ZEA-14-GlcA, α-ZOL, β-ZOL
Rodriguez-Carrasco *et al.*, 2014	Valencia[2]	54	38 adults, 16 children	37 (68.5%)	HT-2, NIV, DON	DON-HT2, DON-NIV
Ezekiel *et al.*, 2014	Nigeria[2]	120	81 adults, 20 adolescents, 19 children	61 (50.8%)	AFM_1, DON, DON-15-GlcA, FB_1, FB_2, OTA, ZEA, ZEA-14-GlcA	distribution of mycotoxins: 75% (46/61) had a single mycotoxin and 25% (15/61) had more than 1 mycotoxin [8 had 2 different mycotoxins, 5 had 3 different mycotoxins, 2 had 4 different mycotoxins, 7 had more than one mycotoxin/metabolite]
Cao *et al.*, 2013	China[3]	10	6 men, 1 pregnant woman, 3 lactating women	not stated	AFB_1, AFB_2, HT-2, DON, DOM-1, ZEA, α-ZOL, β-ZOL, FB_1, FB_2, AFM_1, OTA, NEO, T-2 Triol	3 samples had AFBs and FBs.
Abia *et al.*, 2013	Cameroon[2]		145 HIV-positive adults (29 male, 116 female), 30 HIV-negative adults	110 (63%)	DON, NIV, ZEA, OTA, FB_1, FB_2, DON-15-GlcA, DON-3-GlcA, ZEA-14-GlcA, α-ZOL, AFM_1	DON, OTA, NIV, FB_1, ZEA, AFM_1, FB_2
Ediage *et al.*, 2013	Cameroon[2]	220	children	160 (73%)	OTA, DON, AFM_1, FB_1, ZEA, α-ZOL, β-ZOL	co-occurrence of 2, 3 and 4 mycotoxins was 35, 5 and 5%, respectively
Solfrizzo *et al.*, 2014	Italy[2]	52	26 males, 26 females	52 (100%)	DON, OTA, AFM_1, FB_1, ZEA, α-ZOL, β-ZOL	distribution of mycotoxin mixtures in samples: 2 (DON, ZEA, FB_1, OTA, AFM_1); 27 (DON, ZEA, FB1, OTA); 20 (DON, ZEA, OTA); 1 (DON, ZEA, OTA, AFM_1); 2 (ZEA and OTA)
Gerding *et al.*, 2014	Germany[2]	101	adult volunteers	87%	DON, ZEA, CIT, T-2, ENNB, DON-3-GlcA, ZEA-14-GlcA, DH-CIT	DON-ENNB-ZEA, DON-CIT-T-2, DON-CIT, DON-ZEA-DON-ENNB
Gerding *et al.*, 2015	Bangladesh[2], Germany[2], Haiti[2]	287	95 adult Bangladeshis, 50 adult Germans, 142 adult Haitians	Bangladesh (87%), Germany (80%), Haiti (68%)	DON, OTA, CIT, ENNB, AFM_1, FB1, α-ZOL, DON-3-GlcA, DH-CIT	DON-CIT-OTA-FB_1, DON-CIT-OTA, DON-OTA-ENNB, CIT-OTA-ENNB, CIT-OTA-FB1, AFM_1-CIT-OTA, AFM_1-CIT-DON, ENNB-OTA, DON-OTA, CIT-OTA, CIT-FB_1, CIT-ENNB, AFM_1-CIT

[1] 4-OH-OTA = 4-hydoxyl-ochratoxin A; AFB_1 = aflatoxin B_1; AFG_2 = aflatoxin G_2; AFM_1 = aflatoxin M_1; CIT = citrinin; DH-CIT = dihydrocitrinone; DOM-1 = de-epoxy-deoxynivalenol; DON = deoxynivalenol; DON-15-GlcA = deoxynivalenol-15-glucuronide; DON-3-GlcA = deoxynivalenol-3-glucuronide; ENNB = enniatin B; FB_1 = fumonisin B_1; FB_2 = fumonisin B_2; HIV = human immunodeficiency virus; NEO = neosolaniol; NIV = nivalenol; OTA = ochratoxin A; OTα = ochratoxin alpha; ZEA = zearalenone; ZEA-14-GlcA = zeralenone-14-O-glucuronide; α- and β-ZOL = α- and β-zearalenol; α-ZOL-14-GlcA = α-zearalenol-14-O-glucuronide; α-ZOL-7-GlcA = α-zearalenol-7-O-glucuronide; β-ZOL-14-GlcA = β-zearalenol-14-O-glucuronide; β-ZOL-16-GlcA = β-zearalenol-16-O-glucuronide.

[2] Only urine samples were analysed.

[3] Urine, faeces, breast milk and amniotic fluid were analysed.

220 urine samples obtained from children in Cameroon, 160 (73%) had biomarkers for OTA, DON, AFM_1, FB_1, ZEA, α-ZOL and β-ZOL. Co-occurrence of 2, 3 and 4 mycotoxins was 35, 5 and 5%, respectively (Ediage *et al.*, 2013). In a recent study in Nigeria, mycotoxins and their metabolites, including AFM_1, DON, OTA, FB_1, FB_2, FB_3, ZEA, zearalenone-14-O-glucuronide and deoxyvalenol-15-O-glucuronide were detected in 51% of urine samples from 120 volunteers involving children, adolescents and adults, and 25% of them indicated multi-mycotoxin exposure (Ezekiel *et al.*, 2014). In another study in Italy (Solfrizzo *et al.*, 2014), biomarkers of ZEA + ZOLs (100%), OTA (100%), DON (96%), FB_1 (56%), and AFM_1 were found in urine samples with 52% exposures involving mixtures of DON, ZEA, FB_1 and OTA. Furthermore, in a three-year survey of the global occurrence of mycotoxins using 7,049 feed and feedstuff samples, it was reported that 48% were contaminated by two or more mycotoxins (Rodrigues and Naehrer, 2012). Globally, the rate of co-occurrence of AFB_1, DON, ZEA, OTA and fumonisins in feeds and feedstuff continues to be high (Schatzmayer and Streit, 2013) and therefore, requires toxicological consideration. Despite the fact that human beings can be exposed to a range of mixtures of mycotoxins, most toxicological studies have only taken into account of the effects of exposure to a single mycotoxin. However, combinations of mycotoxins can be agonistic, additive or antagonistic, and therefore, could pose a significant threat to human reproductive health (Alassane-Kpembi *et al.*, 2013).

Epidemiological research evaluating the effects of mycotoxin mixtures on human reproduction is currently scarce, however, there are few *in vitro* studies evaluating their effects on the female and male reproductive system. Pizzo and colleagues examined the negative effects of single and combinations of DON, α-ZOL and β-ZOL on cell proliferation, steroidogenesis and gene expression of bovine small-follicle granulosa cells after 48 h exposure *in vitro* (Pizzo *et al.*, 2016). The presence of α-ZOL (3.1 μM) and β-ZOL at 31 μM significantly inhibited the growth of the bovine small-follicle granulosa cells whereas DON (0.1-3.3 μM) had no significant effects on the graulosa cell proliferation. Worthy of note is that the exposure of the granulosa cells to binary combinations of α-ZOL (3.1 μM) and DON (3.3 μM) significantly increased cell proliferation, indicating synergistic effects. In addition, combination of α-ZOL with DON or β-ZOL inhibited oestradiol secretion higher than the effects obtained in single treatment of each of the toxins, but had no significant effect on progesterone production. In the same study (Pizzo *et al.*, 2016), co-exposure of DON and β-ZOL to the granulosa cells decreased cell proliferation, and strongly inhibited oestradiol production. Similarly, DON with α-ZOL or β-ZOL caused a significant up-regulation in the expression of *CYP11A1* in IGF1 stimulated granuolsa cells. In a different study by Pizzo *et al.* (2015), the individual and combined effects of DON and α-ZOL on cell proliferation and steroidogenesis of follicle-stimulating hormone (FSH) plus IGF1 stimulated bovine large follicle granulosa cells was examined after 48 h of exposure. It was observed that DON (3.3 μM) significantly reduced cell proliferation by 22% whereas α-ZOL (0.09 and 0.31 μM) stimulated large granulosa cell growth. Interestingly, binary combination of DON (0.01 μM) and α-ZOL (0.09 μM) stimulated cell proliferation by 45% when compared to vehicle control, although this was not significantly different from the stimulatory effect of single treatment with α-ZOL (0.09 μM). It was also reported that that DON (0.33 and 3.3 μM) significantly impaired both oestradiol and progesterone production compared to controls. Interestingly, co-exposure of DON and α-ZOL to the granulosa cells in the presence of FSH inhibited progesterone secretion, but induced oestradiol production in the granulosa cells (Pizzo *et al.*, 2015). Taken together, it can be deduced that co-exposure of DON with α-ZOL or β-ZOL at concentrations relevant to human exposure can impair bovine ovarian cell proliferation and steroidogenesis which may negatively affect normal follicle development and oocyte function. Therefore, human research should be taken to elucidate the molecular mechanisms through which co-exposure of DON with α-ZOL or β-ZOL affect ovarian function and morphology.

In a subsequent study, Albonico *et al.* (2016) evaluated the effects FB_1 in single and combined with DON, α-ZOL or β-ZOL on bovine granulosa cells in the presence of IGF1 *in vitro*. FB_1 (30 ng/ml – 5 μg/ml), α-ZOL (5 μg/ml) and β-ZOL (30 ng/ml) had no significant effect on the bovine granulosa cell growth. However, combination of FB_1 (30 ng/ml) with same concentration of β-ZOL significantly stimulated the cell growth. At higher concentrations, FB_1 (5 μg/ml) strongly reduced cell numbers, and co-exposure of FB_1 (5 μg/ml) with β-ZOL (5 μg/ml) also inhibited cell numbers. It was also reported that co-treatment of FB_1 with α-ZOL caused a significant decline in granulosa cell numbers, indicating either additive or synergistic effects as none of the mycotoxins had significant effect on cell numbers in a single exposure. On hormone production, FB_1 (30 and 100 ng/ml) had no effect on granulosa oestradiol secretion, but significantly stimulated its production at 5 μg/ml. In contrast, α-ZOL (5 μg/ml) and β-ZOL (5 μg/ml) strongly caused a decline in oestradiol production. In addition, combination of FB_1 (5 μg/ml) with α-ZOL (5 μg/ml) had no effect, but a co-exposure with β-ZOL (5 μg/ml) caused a reduction in oestradiol secretion. Surprisingly, co-treatment of FB_1 alone or in combination with DON, α-ZOL or β-ZOL did not affect bovine granulosa cell progesterone release. In contradiction with the work of Albonico *et al.* (2016), Cortinovis and colleagues reported that co-treatment of FB_1 with α-ZOL amplified the level of progesterone produced by porcine small granulosa cells in single toxin exposure (Cortinovis *et al.*, 2014), indicating additive effects. The

difference in the response of porcine and bovine granuolsa cells to co-exposure of FB_1 with α-ZOL could be as a result of the species sensitivity of these mycotoxins. For instance, it is well known that FB_1 is more toxic to horses and pigs than to cattle (Albonico *et al.*, 2016). In an *in vitro* study, Tatay *et al.* (2014) studied the cytotoxic and interactive effects of ZEA, α-ZOL and β-ZOL in ovarian (CHO-K1) cells. It was observed that binary or ternary combinations of these mycotoxins showed higher cytotoxicity than the individual toxins. In addition, co-exposure of ZEA with α-ZOL or β-ZOL generally showed additive effects either at 24, 48 or 72 h of exposure. Interestingly, ternary mixtures of ZEA, α-ZOL and β-ZOL exhibited synergistic effects at lower concentrations (Tatay *et al.*, 2014), further emphasising the need for the inclusion of mycotoxin mixtures in toxicological assessment. In summary, the co-occurrence of mycotoxins can cause perturbation in cell proliferation and dysregulation of endocrine function of ovarian cells, highlighting the significance of co-occurrence of mycotoxins to female reproductive health. Future research should also examine the role of co-occurrence of mycotoxins in placental morphology and function, embryogenesis and endometrial function.

The mechanisms through which mycotoxin mixtures can impact on testicular morphology and steroidogenesis also deserves attention. In a previous study, we have shown that DON, α-ZOL and OTA were strongly cytotoxic to MA-10 Leydig cells *in vitro* after 48 h of exposure (Eze *et al.*, 2018). In the same study, OTA did not induce much cytotoxicity up to 16 μM when cells were treated with only this compound, but co-exposure of OTA with DON increased the cytotoxicity at 8-32 μM compared to DON or OTA alone. In addition, OTA had a synergistic effect on the cytotoxicity induced by ZEA, α-ZOL and β-ZOL. We also reported that combinations of DON/OTA, DON/ZEA, DON/α-ZOL, DON/β-ZOL, OTA/ZEA, OTA/α-ZOL, and OTA/β-ZOL were generally additive and synergistic at low concentrations (Eze *et al.*, 2018), raising the possibility that co-exposure to these mycotoxins could contribute to adverse male reproductive health in exposed populations.

7. Concluding remarks

Mycotoxins are contaminants of many staple food crops for human consumption, particularly in sub-Saharan Africa and South Asia making these toxins a major public health, food safety and economic problem. There is scientific evidence on the adverse reproductive health effects of human exposure to mycotoxins in Africa, including low birth weight, stillbirth, intrauterine growth restriction, maternal anaemia, NTDs and male infertility. However, this review identified papers that were mainly cross-sectional studies and most of the studies did not adjust for other factors that could affect low birth weight, intrauterine growth restriction, male infertility, stillbirth, and anaemia, such as malaria, hormonal pathology, malnutrition, poor food quality, and other infectious disease conditions.

At present, the molecular pathways through which mycotoxins induce reproductive and developmental toxicity are still not well understood. Future research should employ validated biomarkers of mycotoxin exposure in human epidemiological studies, and reproductive cell lines or primary cells representing the reproductive system of both genders to investigate possible molecular pathways in which mycotoxins affect the reproductive systems, the sex gametes, placental and endometrial function, and embryogenesis. In addition, randomised control trials evaluating the effects of mycotoxins and poor reproductive health outcomes in humans using validated exposure biomarkers are urgently needed. Lastly, intervention strategies should be put in place in Africa to mitigate mycotoxin contamination of food crops, especially those consumed by vulnerable groups such as pregnant women, infants and young children.

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