Effects of Prenatal Exposure to Aflatoxin B1

Subjects: Food Science & Technology

Contributor: Carlos de Oliveira

Aflatoxins are mycotoxins produced as secondary fungal metabolites. Among them, aflatoxin B1 (AFB1) stands out due to its genotoxic and mutagenic potential, being a potent initiator of carcinogenesis. In several animal species, including humans, AFB1 has a teratogenic effect, resulting in bone malformations, visceral anomalies, lesions in several organs, and behavioral and reproductive changes, in addition to low birth weight. The mutagenic capacity of AFB1 in prenatal life is greater than in adults, indicating that when exposure occurs in the womb, the risk of the development of neoplasms is higher.

aflatoxins carcinogenicity mutagenicity prenatal exposure teratogenicity

1. Introduction

Mycotoxins are secondary products of fungal metabolism, causing harmful effects on human and animal health. These substances are commonly found in food, especially when harvest storage or transport practices are inadequate. It is estimated that about 25% of all food worldwide is contaminated with mycotoxins, but some studies suggest that this is an underestimation [1]. As acute intoxications are less common in humans, the effects of chronic exposure to mycotoxins have been more extensively studied and seem to be related to a wide range of health disorders [2].

2. Aflatoxin B1 Biotransformation and Mechanism of Action

After ingestion, AFB1 is rapidly absorbed from the intestine and reaches the liver to be metabolized by mixed-function oxidases. AFB1 goes through a complex process of biotransformation; in the first stage, it may go through different metabolization pathways, yielding several metabolites, followed by the conjugation process for excretion (**Figure 1**). AFB1 may also go through a reversible reduction process in the cytoplasmic reductase system of hepatocytes, yielding to aflatoxicol (AFL), which can be transformed again into AFB1, becoming a source of its own storage. Metabolization of AFB1 culminates with the formation of several metabolites, e.g., aflatoxin P1 (AFP1) and aflatoxin Q1 (AFQ1) [3].

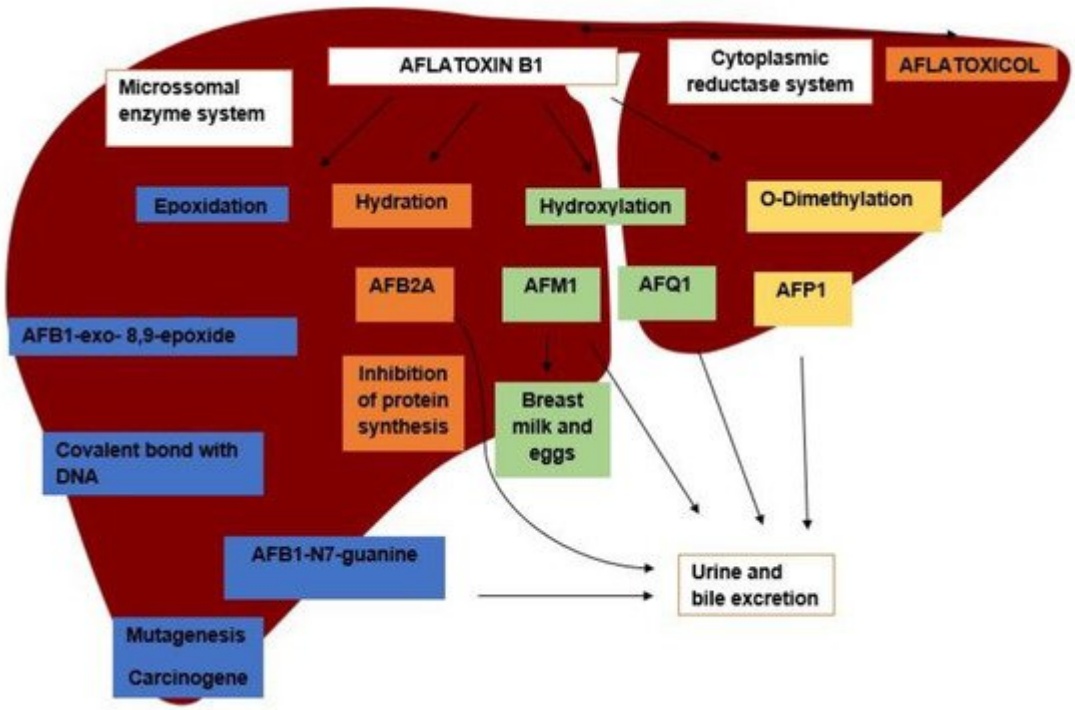

Figure 1. Main metabolization pathways of aflatoxin B1 in the liver, its metabolites, and excretion pathways.

Among the metabolization pathways of AFB1, the epoxidation process stands out. In this process, AFB1 is converted into AFB1-8.9-epoxide (AFBO), which is able to bind to macromolecules, such as those of DNA, RNA, and proteins, forming adducts responsible for the toxic potential of the aflatoxins [4]. The binding of AFBO to guanine in the DNA molecules results in the formation of AFB1-N7-Guanine (AFGuan), which is excreted in urine. The production of AFGuan leads to a guanine to thymine substitution in the third base of codon 249 of the host DNA, which is the main basis of the carcinogenic effect of AFB1, as this mutation is responsible for the loss of function of the p53 gene, a trigger for hepatocellular carcinoma (HCC) [5].

Another important route of AFB1 metabolization is hydration, forming AFM1. AFM1 can also go through an epoxidation process and become AFM1-epoxide, which has the same ability to bind to macromolecules as AFBO [6]. AFB1 and AFM1 are classified by the IARC (International Agency for Research on Cancer), respectively, as group I carcinogen and group 2B carcinogen, respectively [7][8]. In addition, AFM1 can be found in human and animal milk, becoming a unique health issue, as animal exposure to AFB1 makes humans vulnerable [9][10][11]. Finally, hydration also yields AFB2A, an important inhibitor of protein synthesis that is related to the effects of acute intoxication after high AFB1 intake [4][12].

3. Effects of Prenatal Exposure to Aflatoxin b1 in Animals

Several harmful effects are associated with exposure to AFB1 during the prenatal period, such as low birth weight, small litters, fetal death and resorption, bone and visceral deformities, reproductive changes, impact on immune capacity, and behavioral changes, in addition to a predisposition to neoplasm development [13].

3.1. Bone Malformations

Bone defects related to intrauterine exposure to AFB1 are the most common problems reported in the literature. In experiments, they are evidenced in several species of animals. Bone defects are most commonly related to ossification failures, changes in bone size and shape, and the absence or alteration of some bone accidents (**Table 1**).

Table 1. Studies showing the effects of exposure to aflatoxin B1 (AFB1) on bone development.

Species	AFB1 Dose/PD/FE	Effects on Bone Development	Reference
Mice	20 mg/Kg PD: 7th or 13th FE: Intraperitoneal	Hypoplasia of the axial skeleton and metacarpal/metatarsal phalanges, cervical and coccygeal vertebrae. Failure in the ossification of the supraoccipital bone, pelvic and thoracic limbs.	[14]
Rabbits	0.05 mg/kg/day PD: 6th–18th FE: Gavage	Sternal and rib malformations. Failure in ossification of the skull, spine, vertebrae and ribs, carpus, tarsus, metatarsus, metacarpus, and phalanges. Decreased bone size in pelvic limb.	[15]
Rats	1 mg/kg PD: 6th–15th FE: Gavage	Failure in ossification of skull, thoracic and pelvic limbs, and spine. Change in shape and size of vertebrae. Absence of or decreased intervertebral disc size, incomplete formation of the pulposal nucleus, alteration and absence of bone accidents in limbs.	[16]

PD = Pregnancy Day. FE = Form of exposure.

3.2. Visceral Changes

Several visceral changes have been reported, among them, the decrease in size and weight of the liver and kidneys [14][15]. Regarding histopathological findings, the liver shows more significant changes, with the presence of fatty degeneration, congestion, and necrosis. Although these changes may also be found in the kidneys, they are less intense (**Table 2**). Other histological alterations are evidenced in the organs of the reproductive tract.

Table 2. Studies showing the effects of exposure to aflatoxin B1 (AFB1) on organs.

Species	AFB1 Dose/PD/FE	Effects on Organs	Reference
Rabbits	0.05 mg/kg/day PD: 6th–18th FE: Gavage	Reduction in weight and absolute size of the viscera. Decreased size of the heart and ventricular lumen. Liver and kidneys containing vacuoles and congestion. Atrophy, glomerular degeneration, and disorganization of hepatocytes.	[15]
Rats	1 mg/kg PD: 6th–15th FE: Gavage	Hepatocyte degeneration and alteration of liver architecture. Congestion of the centrolobular vein and sinusoid capillaries. Kidneys presented tubular degeneration. Thymus presenting lymphoid depletion and reduction in epithelial differentiation.	[17]

Species	AFB1 Dose/PD/FE	Effects on Organs	Reference
Rats	10 µg/kg PD: 12th–19th FE: Intramuscular	Moderate degeneration of the testicles.	[18]
	20 µg/kg	Severe atrophy and reduction of germ cells of seminiferous tubules; reduced liver weight.	
	50 µg/kg	Severe degeneration, cell depletion, and epithelial rupture.	

PD = Pregnancy Day. FE = Form of exposure.

roductive behavior, sperm production, and testicular and epididymal morphology. However, the most significant finding involves serum hormone levels, marked by a decrease in testosterone [19][20][21]. When exposure occurs in the prenatal period, the effects seem to be even more remarkable.

The mechanisms through which reproductive damage occurs are still being elucidated. AFB1 may act as a potential endocrine disruptor, interfering with the hypothalamus–hypophysis–testicular axis, leading to hormonal dysfunction. The consequences of hormonal disruption may be more severe when exposure occurs in the embryonic phase [18][22]. Another possibility would be the ability of AFB1 to bind to the acute steroidogenic regulatory protein (STAR), thus affecting the transfer of cholesterol to the mitochondria, which has a negative impact on steroidogenesis [20].

3.4. Genotoxicity and Mutagenicity

AFB1 presents high genotoxicity, which is probably its most overwhelming effect, notably leading to several chromosomal aberrations in animals exposed to it [23]. Both DNA damage and mutations can result from the injury caused by AFB1 metabolites (AFBO), and by the oxidative stress that results from the metabolization of this mycotoxin. In the fetuses of rats exposed to 1 mg/kg of AFB1 between the 6th and the 15th day of gestation, several chromosomal aberrations in bone marrow cells were evidenced, mainly gap and breakage lesions, indicators of the genotoxic potential of this mycotoxin in the prenatal period [16].

The mechanisms by which AFB1 causes DNA damage in fetal life seem to be similar to those affecting adult animals, by the metabolization of AFB1 into AFBO in the liver of the fetus and the identification of AFGuan adducts in fetuses [24]. The apurinic site left by AFGuan (after it is released and excreted) is filled by a thymine base, which characterizes the main mutation caused by AFB1, the G:C → T:A transversion in the P53 gene. This change may initiate the carcinogenesis process. These transversions are also evidenced in the fetuses of rats exposed to 6 mg/kg of AFB1 by peritoneal application in a single dose on the 14th day of gestation [13][25].

Another metabolite found in rat fetuses exposed to AFB1 is AFB1-Fapy. This metabolite is the result of a chemical transformation in the aflatoxin molecule, resulting in the opening of the furan ring, and creating a more stable molecule that remains linked longer to DNA. AFB1-Fapy is considered to be more carcinogenic because it prevents repair enzymes from being activated, as it produces less evident structural damage to the DNA helix [3][24][25].

These phenomena are explained by the fact that the fetal liver has the capacity to metabolize AFB1 into AFBO, but has a decreased ability to excrete AFB1 metabolites due to the low number of conjugate enzymes of Phase II of metabolization (e.g., glutathione transferase). Moreover, during fetal life, liver cells are in constant multiplication, which may lead to the expansion of mutations [24][25]. Another important mechanism of genotoxicity is the oxidative stress caused by the metabolization of AFB1, leading to the formation of several adducts that act remotely, causing neoplasms at a distance [3]. Animals exposed to AFB1 have a decrease in the amount of antioxidant enzymes and an increase in markers of oxidative stress [13][16]. Thus, treatment with antioxidant agents may provide protection against oxidative stress and, consequently, reduce the carcinogenic potential of these substances [26][27]. Finally, the importance of epigenetic lesions, mainly DNA methylation and modifications in histones, cannot be discarded in genotoxicity and mutagenicity processes [28].

3.5. Other Changes

Other important changes are low birth weight, both in rats and rabbits, besides the decrease in the number of offspring per litter [14][15][16][17][18]. In rabbits, 0.05 mg/kg/day AFB1 from the 6th to the 18th day leads to increased size of the orbits, microphthalmia, wrinkled skin, and eyelids with fewer hair follicles [15].

4. Prenatal Exposure in Humans

Most unfavorable birth outcomes, such as premature births, miscarriages, low birth weight, and even stillbirths, occur in developing countries [29]. The consumption of foods contaminated with AFB1 is also high in these regions, as exposure to mycotoxin biomarkers during pregnancy was observed in most prenatal exposure studies (**Table 3**). High levels of toxins are sometimes detected, but have not always been associated with deleterious effects on infant health. On the other hand, there are still no data directly linking the presence of this toxin with intrauterine or neonatal deaths in humans. **Table 3** demonstrates the exposure to AFB1 during pregnancy by means of biomarkers.

Table 3. Biomarkers of aflatoxin B1 (AFB1) exposure found in pregnant women.

Period of Gestation	Biomarker	Country	Main Effects in Babies	Reference
1st Trimester	AFB1-Lisin (Serum)	Uganda	Low birth weight and smaller head circumference	[30]
1st–3rd Trimester	AF-Albumin (Serum)	Gambia	No data	[31]
3rd Trimester	AF-albumin (Serum) AFM1 (Urine)	Egypt	No data	[32]
2nd Trimester	AFM1 (Urine)	Zimbabwe	No data	[33]

Period of Gestation	Biomarker	Country	Main Effects in Babies	Reference
No data	AFM1 (Urine)	China	No data	[34]
1st–2nd Trimester	AF-Albumin (Serum)	Gambia	DNA methylation in white cells	[35]
No data	AFB1-Lisin (Serum)	Ghana	Low birth weight	[36]
1st–2nd Trimester	AFB1-Lisin (Serum)	Tanzania	Small reduction in gestational age at delivery	[37]
1st–2nd Trimester	AFB1-Lisin (Serum)	Nepal	Babies small for gestational age	[38]

ot be the
kins, such
es [39]. In addition, other factors such as maternal malnutrition and lack of prenatal care can have an impact on infant health. However, Lauer et al. [30] and Castelino et al. [31] evidenced an increase in the number of biomarkers in the maternal serum of women in Uganda and Gambia, respectively. This increase can be explained by the higher maternal metabolization during pregnancy, reflected in the greater production of enzymes of the cytochrome P450 family, which are essential in the metabolization of AFB1, and increase its toxicity. These factors, together with the changes evidenced in experimental studies, raise the hypothesis of an increased toxic potential of AFB1 in the prenatal period [14][15][16][17][18][40].

To elucidate the real impacts of the exposure to AFB1 on infant health, especially in the most susceptible populations, more studies must be conducted to determine safe intake during pregnancy and to suggest bases for legislation and awareness campaigns that can ensure acceptable consumption levels of mycotoxins.

References

1. Eskola, M.; Kos, G.; Elliott, C.T.; Hajšlová, J.; Mayar, S.; Krska, R. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited ‘FAO estimate’ of 25. Crit. Rev. Food Sci. Nutr. 2020, 60, 2773–2789.
2. Bennett, J.W.; Klich, M. Mycotoxins. Clin. Microbiol. Rev. 2003, 16, 497–516.
3. Dhakal, A.; Sbar, E. Aflatoxin toxicity. In StatPearls; StatPearls Publishing: Treasure Island, FL, USA, 2020.
4. Mitchell, N.J.; Kumi, J.; Johnson, N.M.; Dotse, E.; Marroquin-Cardona, A.; Wang, J.S.; Jolly, P.E.; Ankrah, N.A.; Phillips, T.D. Reduction in the urinary aflatoxin M1 biomarker as an early indicator of the efficacy of dietary interventions to reduce exposure to Aflatoxins. Biomarkers 2013, 18, 391–398.

5. Wild, C.; Turner, P. The toxicology of Aflatoxins as a basis for public health decisions. Mutagenesis 2002, 17, 471–481.
6. Bujons, J.; Hsieh, D.P.; Kado, N.Y.; Messeguer, A. Aflatoxin M1 8,9-epoxide: Preparation and mutagenic activity. Chem. Res. Toxicol. 1995, 8, 328–332.
7. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 56, Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins; IARC: Lyon, France, 1993; pp. 1–599. Available online: https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Some-Naturally-Occurring-Substances-Food-Items-And-Constituents-Heterocyclic-Aromatic-Amines-And-Mycotoxins-1993 (accessed on 1 November 2021).
8. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 82, Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene; IARC: Lyon, France, 2002; pp. 172–299. Available online: https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Some-Traditional-Herbal-Medicines-Some-Mycotoxins-Naphthalene-And-Styrene-2002 (accessed on 1 November 2021).
9. Battacone, G.; Nudda, A.; Palomba, M.; Pascale, M.; Nicolussi, P.; Pulina, G. Transfer of aflatoxin B1 from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates. J. Dairy. Sci. 2005, 88, 3063–3069.
10. Oliveira, C.A.F.; Sebastião, L.S.; Luciana, S.S.; Fagundes, H.; Rosim, R.E.; Fernandes, A.M. Determinação de aflatoxina B1 em rações e aflatoxina M1 no leite de propriedades do Estado de São Paulo. Food Sci. Technol. 2010, 30, 221–225.
11. Marchese, S.; Polo, A.; Ariano, A.; Velotto, S.; Costantini, S.; Severino, L. Aflatoxin B1 and M1: Biological properties and their involvement in cancer development. Toxins 2018, 10, 214.
12. Giovati, L.; Magliani, W.; Ciociola, T.; Santinoli, C.; Conti, S.; Polonelli, L. AFM1 in milk: Physical, biological, and prophylactic methods to mitigate contamination. Toxins 2015, 7, 4330–4349.
13. Benkerroum, N. Chronic and acute toxicities of Aflatoxins: Mechanisms of action. Int. J. Environ. Res. Public Health 2020, 17, 423.
14. Abdulrazzaq, Y.; Padmanabhan, R.; Salim, M.; Kochyil, J.; Shafiullah, M. Teratogenic effects of aflatoxin B1 in mice exposed in early and late gestation. Pediatr. Res. 2011, 70, 405.
15. El-Nahla, S.M.; Imam, H.M.; Moussa, E.; Ibrahim, A.; Ghanam, A. Teratogenic effects of aflatoxin in rabbits (Oryctolagus cuniculus). J. Vet. Anat. 2013, 6, 67–85.
16. Fetaih, H.A.; Dessouki, A.A.; Hassanin, A.A.; Tahan, A.S. Toxopathological and cytogenetic effects of aflatoxin B1 (AFB1) on pregnant rats. Pathol. Res. Pract. 2014, 210, 1079–1089.

17. Fetaih, H.; Dessouki, A.; Tahan, A. Toxopathological effect of AFB1 on pregnant rats. Global Anim. Sci. J. 2015, 2, 145–153.
18. Supriya, C.; Reddy, P.S. Prenatal exposure to aflatoxin B1: Developmental, behavioral, and reproductive alterations in male rats. Naturwissenschaften 2015, 102, 26.
19. Hasanzadeh, S.; Hosseini, E.; Rezazadeh, L. Effects of aflatoxin B1 on profiles of gonadotropic (FSH and LH), steroid (testosterone and 17β-estradiol) and prolactin hormones in adult male rat. Iran J. Vet. Res. 2011, 12, 332–336.
20. Supriya, C.; Girish, B.P.; Reddy, P.S. Aflatoxin B1-induced reproductive toxicity in male rats: Possible mechanism of action. Int. J. Toxicol. 2014, 33, 155–161.
21. Fauzi, A.; Ahmed, S.; Abead, S. Toxicity effect of aflatoxin B1 on reproductive system of albino male rats. Pak. J. Biol. Sci. 2015, 18, 107–114.
22. Hasanzadeh, S.; Amani, S. Aflatoxin B1 effects on ovarian follicular growth and atresia in the rat. Comp. Clin. Pathol. 2012, 22, 563–572.
23. Abdel-Aziem, S.; Saleh, Z.; Farrag, A. Impact of whey protein on the genotoxic effects of aflatoxins in rats. Int. J. Dairy Sci. 2007, 2, 126–137.
24. Woo, L.L.; Egner, P.A.; Belanger, C.L.; Wattanawaraporn, R.; Trudel, L.J.; Croy, R.G.; Groopman, J.D.; Essigmann, J.M.; Wogan, G.N.; Bouhenguel, J.T. Aflatoxin B1-DNA adduct formation and mutagenicity in livers of neonatal male and female B6C3F1 mice. Toxicol. Sci. 2011, 122, 38–44.
25. Chawanthayatham, S.; Thiantanawat, A.; Egner, P.A.; Groopman, J.D.; Wogan, G.N.; Croy, R.G.; Essigmann, J.M. Prenatal exposure of mice to the human liver carcinogen aflatoxin B1 reveals a critical window of susceptibility to genetic change. Int. J. Cancer 2014, 136, 1254–1262.
26. El-Bahr, S.M. Effect of Curcumin on hepatic antioxidant enzymes activities and gene expressions in rats intoxicated with aflatoxin B1. Phytother. Res. 2015, 29, 134–140.
27. Saad-Hussein, A.; Moubarz, G.; Mohgah, S.A.; Wafaa, G.S.; Aya, H.M. Role of antioxidant supplementation in oxidant/antioxidant status and hepatotoxic effects due to aflatoxin B1 in wheat miller workers. J. Complement. Integr. Med. 2019, 16.
28. Dai, Y.; Huang, K.; Zhang, B.; Zhu, L.; Xu, W. Aflatoxin B1-induced epigenetic alterations: An overview. Food Chem. Toxicol. 2017, 109 Pt 1, 683–689.
29. Lawn, J.E.; Blencowe, H.; Waiswa, P.; Amouzou, A.; Mathers, C.; Hogan, D.; Flenady, V.; Frøen, J.F.; Qureshi, Z.U.; Calderwood, C.; et al. Stillbirths: Rates, risk factors, and acceleration towards 2030. Lancet 2016, 387, 587–603.
30. Lauer, J.M.; Duggan, C.P.; Ausman, L.M.; Griffiths, J.K.; Webb, P.; Wang, J.S.; Xue, K.S.; Agaba, E.; Nshakira, N.; Ghosh, S. Maternal Aflatoxin exposure during pregnancy and adverse birth outcomes in Uganda. Matern. Child. Nutr. 2019, 15, e12701.

31. Castelino, J.M.; Dominguez-Salas, P.; Routledge, M.N.; Prentice, A.M.; Moore, S.E.; Hennig, B.J.; Wild, C.P.; Gong, Y.Y. Seasonal and gestation stage associated differences in aflatoxin exposure in pregnant Gambian women. Trop. Med. Int. Health 2014, 19, 348–354.
32. Piekkola, S.; Turner, P.C.; Abdel-Hamid, M.; Ezzat, S.; El-Daly, M.; El-Kafrawy, S.; Savchenko, E.; Poussa, T.; Woo, J.C.; Mykkänen, H.; et al. Characterisation of Aflatoxin and D=deoxynivalenol exposure among pregnant Egyptian women. Food Addit. Contam. 2012, 29, 962–971.
33. Smith, L.E.; Mbuya, M.; Prendergast, A.J.; Turner, P.C.; Ruboko, S.; Humphrey, J.H.; Nelson, R.J.; Chigumira, A.; Kembo, G.; Stoltzfus, R.J. Determinants of recent aflatoxin exposure among pregnant women in rural Zimbabwe. Mol. Nutr. Food Res. 2017, 61, 1601049.
34. Lei, Y.; Fang, L.; Akash, M.S.H.; Rehman, K.; Liu, Z.; Shi, W.; Chen, S. Estimation of urinary concentration of aflatoxin M1 in Chinese pregnant women. J. Food. Sci. 2013, 78, 1835–1838.
35. Hernandez-Vargas, H.; Castelino, J.; Silver, M.J.; Dominguez-Salas, P.; Cros, M.P.; Durand, G.; Calvez-Kelm, F.L.; Prentice, A.M.; Wild, C.P.; Moore, S.E.; et al. Exposure to aflatoxin B1 in utero is associated with DNA methylation in white blood cells of infants in The Gambia. Int. J. Epidemiol. 2015, 44, 1238–1248.
36. Shuaib, F.M.; Jolly, P.E.; Ehiri, J.E.; Yatich, N.; Jiang, Y.; Funkhouser, E.; Person, S.D.; Wilson, C.; Ellis, W.O.; Wang, J.S.; et al. Association between birth outcomes and aflatoxin B1 biomarker blood levels in pregnant women in Kumasi, Ghana. Trop. Med. Int. Health 2010, 15, 160–167.
37. Passarelli, S.; Bromage, S.; Darling, A.M.; Wang, J.S.; Aboud, S.; Mugusi, F.; Griffiths, J.K.; Fawzi, W. Aflatoxin exposure in utero and birth and growth outcomes in Tanzania. Mater. Child. Nutr. 2020, 16, e12917.
38. Andrews-Trevino, J.Y.; Webb, P.; Shively, G.; Rogers, B.; Baral, K.; Davis, D.; Paudel, K.; Pokharel, A.; Shrestha, R.; Wang, J.S.; et al. Dietary determinants of aflatoxin B1-lysine adduct in pregnant women consuming a rice-dominated diet in Nepal. Eur. J. Clin. Nutr. 2020, 74, 732–740.
39. Chen, C.; Riley, R.T.; Wu, F. Dietary fumonisin and growth impairment in children and animals: A review. Food Sci. Food Saf. 2018, 17, 1448–1464.
40. Tracy, T.S.; Venkataramanan, R.; Glover, D.D.; Caritis, S.N. Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. Am. J. Obstet. Gynecol. 2005, 192, 633–639.