Study Of Aflatoxin B1 As A Risk Factor That Impair The Reproductive Performance In Females- Egypt

S El-Azab, A Abdelhamid, H Shalaby, A Mehrim, A Ibrahim

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Abstract

INTRODUCTION

Aflatoxins are a group of naturally occurring, highly toxic mycotoxins that contain a characteristic dihydrobisfuran moiety in their molecular structures. These fungal metabolites are produced by specific strains of Aspergillus flavus and Aspergillus parasiticus. [1]. The most common form of aflatoxins is aflatoxin B_1 (AFB_1). Aflatoxin constitutes a real threat to the health of livestock as well as humans by their continuing intermittent occurrence in both feeds and foods [2345678]. Several factors may enhance the occurrence of mycotoxin in the human diet in developing countries. These include eating habits, existing marketing problems which encourage long storage periods; the pre and post harvest practices that encourage build up of moisture and thus encourage mould growth, ignorance and poverty. This is aggravated by the fact that there are no strict regulations that impose limits on the concentration of mycotoxins in crops that is marketed in these countries as well as lack of relevant technology required in monitoring fungi and mycotoxins in the grains [9]. Aflatoxin B_1 has been reported to have a deleterious effect on the reproductive capacity of laboratory and domestic female animals [101112]. There were reductions in ovarian and uterine sizes, increases in fetal resorption, implantation loss, and intra-uterine death in the aflatoxin exposed female rats [13]. Histopathological examinations of the ovaries in aflatoxin exposed mature domestic fowls showed follicular atresia, accompanied with cessation of egg production during the whole feeding period [14].

Human infertility due to female factors was thought to be the reason behind all fertility problems. Whereas experts recognize that female infertility accounts for about only 40% of all infertility cases, ovulatory disorders are one of the most common reasons why women are unable to conceive, and account for 25% of women's infertility [15]. Causes of infertility are many, such as sexually transmitted diseases, parasitic diseases, physiological and genetic defects, and toxic agents. One of the least understood among these factors seems to be the impact of toxic agents, including mycotoxins, on the reproductive performance of human beings [16]. Studies elsewhere have shown the presence of aflatoxins in common food items in Egypt, suggesting that exposure of members of this community through diet to aflatoxins may be high [171819]. The present study was, therefore, initiated to determine whether aflatoxins are present in the blood of infertile females in this community and to ascertain whether there is a relationship between the presence of aflatoxin and deviation from normal in human female ovulatory functions, and hormonal parameters.

SUBJECTS AND METHODS

SELECTION OF THE PATIENTS

Fifty patients (infertile females), mean age (31±3) years with an ovulation problems referred from the Infertility Unit, Mansoura University Hospital, were enrolled in the study. The underlying ovulation pathology caused by polycystic ovary syndrome (POCS) (n=43), and idiopathic anovulation (n=7). The exclusion criteria were patients with acute infections, and work place pollution. Twenty healthy subjects (fertile female), mean age (30± 3) were chosen as control. All the participants did not receive any supplements that could increase ovulation function within the previous six months. Informed consent was obtained from all participants.

SAMPLING FOR AFB DETERMINATION

Five ml blood sample was drawn from each participant, all samples were kept frozen until analysis. The samples were tested for the presence of AFB_1. The quantitative

determination of AFB_1 by Thin Layer Chromatography (TLC) was carried out according to the method of Eppley [20], modified by Abdelhamid [21]. The laboratory studies were undertaken in the mycotoxin laboratory of Prof. Dr. Abdelhamid, A. M., Faculty of Agriculture, Mansoura University. All chemicals and solutions used were from United Co. for Chemical and Medical Preparations. Mycotoxin standard used was from Makor Chemicals Co.

METHODS

5ml blood sample was extracted with 24 ml chloroform and 1ml n phosphoric acid, the chloroform layer was received and transferred into a flask and dried under vacuum on Rota vapor-M (B□chi-HB-140) at 60°C. Residues were dissolved in 20ml chloroform for TLC spotting. Development of the plate was done in a closed developing tank for about 40 minute, with solution: Toluene/ acetic acid/ Formic acid 6:3:1. The plate was dried in air and examined under ultraviolet (U.V) at wave length 366. The verification was done through Rf value and the fluorescence colour (blue fluorescence) under U.V at 366 nm, after comparison with external standard . Confirmatory test was done when the plate was sprayed with 30% methanolic sulphuric acid (30 ml H2 so 4 + 70 ml CH3 OH) and examined again under the U.V. The blue fluorescence of aflatoxin converted to golden yellow colour.

EXAMINATION OF THE OVARY

The ovaries for all participants were examined by measuring the mean ovarian volume; and number and size of ovarian follicles. Blood hormonal levels were determined calorimetrically.

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS Software version 10.0 (SPSS, Chicago, IL, U.S.A.). The data were expressed as mean ± standard deviation for patients and control separately. Differences in means were analyzed using student t-test for comparison between two groups. The P- value was considered significant if less than 0.05.

RESULTS

Table (1) shows that all the blood samples (the control and patients' samples) were negative as regard to AFB_1 analysis.

Figure 1

Table 1: Data of AFB determination in human female blood samples under investigation (ppb).

Blood Samples	Infertile females	Controls
Aflatoxin B_1	Negative	Negative

ppb: part per billion

Table (2) shows that there was a significant enlargement in the mean ovarian volume in infertile females compared to controls, whilst, there was a significant increase in the mean follicular size in controls compared to infertile females group (using Trans-Vaginal Scanning).

Figure 2

Table 2: The individual and ultrasound parameters of infertile females compared to controls (means ± SD).

Parameters	Infertile females	Controls	P value
Age (years)	31 ± 3	30 ± 3	P=0.052
Height (cm)	167 ± 2	165 ± 3	P=0.072
Weight (kg)	65 ± 2	67 ± 3	P=0.075
Body mass index (kg/m2)	23.5 ± 0.5	24.3 ± 0.6	P=0.064
Mean ovarian volume (ml) (TVS)	6.6± 0.2	4.9±0.3	P<0.001***
Mean follicular size (mm) (TVS)	8±2	17±2	P<0.001***

P***: highly significant, TVS: Trans-Vaginal Scanning, and Mean follicular size ≥15 mm indicative of ovulation.

Table (3) shows that there were a significant higher levels of LH and a significant lower levels of mid luteal progesterone in infertile females group compared to controls.

Figure 3

Table 3: Hormonal profile in infertile females compared to controls (means ± SD).

Parameters	Infertile females	Controls	P- value
LH (IU/L)	21± 0.5	5.6± 0.3	P<0.001***
FSH (IU/L)	5.3 ± 0.2	5.1± 0.2	P=0.080
PRL (ng /ml)	13 ± 1	11.3± 0.3	P=0.081
Testosterone (ng/ml)	0.5 ± 0.02	0.4 ± 0.1	P=0.062
Mid luteal progesterone (ng/ml)	0.5 ± 0.1	30.1±0.2	P<0.001***

P***: highly significant, LH: leutinizing hormone, FSH: follicle stimulating hormone, and PRL: prolactin.

DISCUSSION

Toxicity of dietary aflatoxin in mammals has been extensively studied and proved to be inducing tissue toxicity [13]. The present study was initiated to determine whether aflatoxin is present in the blood of infertile females in Egypt and its correlation with women ovarian functions. All the

blood samples (the control and patients samples) showed negative results as regard to AFB_1 analysis (Table 1). In spite of these negative results, it does not exclude the role of aflatoxin as a contributing factor may causing female infertility, as the cumulative effect of feeding low levels of mycotoxins (outspreading under the local conditions) may contribute to a gradual deterioration of organ functions, this in turn may affect fertility and overall health [224]. This negative result may be an indicator of a chronic aflatoxicosis, through which the toxin could be deposited in its target organ (in the case of AFB_1 is the liver) and not in the blood pool as in the acute toxicoses. In addition, mycotoxins (including aflatoxins) negatively affect reproductive system of various animal species [323], since mycotoxins-contaminated diets led to some significant changes in egg characteristics and composition [24]. Moreover, aflatoxin is found normally in humans more frequently in females than in males (although males are more sensitive for aflatoxin than females) [625].

Furthermore, there are many reports about the deleterious effects of aflatoxin on the reproduction system, i.e. sexual maturation, growth and maturation of the follicles, levels of hormones, gestation, and growth of foetus [26]. Therefore, aflatoxin lowered the fertility to 13 % and increased the mortality of embryos [27]. However, Kihara et al. [28] indicated that prenatal exposure to AFB_1 produced a delay of early response development, impaired locomotor coordination, and impaired learning ability in the offspring of rats exposed to AFB_1 during middle pregnancy, and the early gestational exposure appears to produce more effects than latter exposure. On the other hand, although, detection of AFB_1 in blood samples have been confirmed by TLC [6], HPLC, as well as by mass spectral analysis with standard errors [29], aflatoxin-serum albumin adduct detection may be more accurate as it has a longer half-life in animals and possibly in humans than low molecular weight aflatoxin derivatives which may be rapidly excreted. A large portion of aflatoxins found in human serum probably exist in adduct forms. Among them the AFB_1-albumin adduct which is considered as a useful biomarker reflecting long- term exposure to aflatoxins in different populations [30]. The previous findings may explain why aflatoxin B_1 was negative in blood samples of infertile females although it may be accumulate in organ tissues including the ovaries. On the other hand, susceptibility to aflatoxin mainly depends on its liver detoxification systems, genetic make up, age and other nutritional factors [31].

Anovulation is the cause of infertility in about a third of couples, polycystic ovary syndrome accounts for 90% of such cases [32]. Polycystic ovary syndrome can be confirmed by the presence of two of the following criteria: biochemical or clinical hyperandrogenism, menstrual irregularity, and polycystic ovaries on ultrasound [33]. Environmental toxins may play a role in the pathogenesis of anovulatory infertility especially polycystic ovary syndrome [34]. In the present results, there was a significant enlargement in the mean ovarian volume in infertile females compared to controls (common criteria in POCS); whilst there was a significant increase in the mean follicular size in controls compared to infertile females group (Table2). These findings may be referred in part to the toxic effects of aflatoxin as proved by Abd El–Wahhab [35], who reported that, microscopic examination of the ovaries of female rabbits treated with 0.15mg AF B_1/kg BW showed some pathological alterations in the form of coagulative necrosis which appeared mainly in the growing and mature follicles, and decrease in number and size of Graffian and growing follicles with increased number of atretic follicles and small areas of degenerative changes.

The pronounced high levels of LH concentration in infertile females compared with controls may be attributed to a direct effect of increasing its basal level from anterior pituitary and / or secretion of Gonadotrophic Releasing Hormone (GnRH) from hypothalamus. Also, such increase might be associated with increasing oestradiol to maximum level. On the other hand, the marked increase in progesterone level during mid luteal phase in controls reflected normal Corpus Luteum (CL) formation. While, the opposite which was observed in infertile females (i.e. the lower levels of progesterone) is due to direct effect of reduced CL size, which was indicated from the significant higher levels of LH in infertile females compared to controls. Two unrelated toxic actions have been suggested to explain the AFB_1 effects on female's fertility: An indirect effect mediated by AFB_1 induced hypovitaminosis A; and a direct antagonistic interaction with steroid hormones receptors interfering with gonadal hormones production as estrogen and progesterone, due to structural similarity of AFB_1 and steroid hormones [36]. This results support our results as there was a significant lower levels of mid luteal progesterone, with higher ovarian volume in infertile females group compared to controls. This could be explained by interfering with the production of progesterone from the un-ruptured ovarian follicles. Furthermore, AFB_1 negatively affects hepatic alphafetoprotein (AFP) synthesis, and AFP has shown to

cause genital function blockade which leads to reduced levels of hormonal promoters [37].

It has been proven that, fertility of pregnant rats decreased after aflatoxin and embryonary resorptions, malformations and developmental retardations occurred [38]. Moreover, chronic exposure to aflatoxin decreased reproduction efficiency of ruminants [39]. A deleterious effect on the gonads and embryo-toxicity in the experimental animals were observed. There were increases in fetal resorption, implantation loss, and intra-uterine death. The data showed disturbances in oestrus cycle, significant reductions in the number of oocytes and large follicles, inhibition, and reduction in the conception rates [40]. It can be concluded that, although the results showed negative blood samples as regard to AFB_1, it does not exclude the role of aflatoxin as a contributing factor might causing female infertility, more studies involving ovarian biopsies to analyze aflatoxin levels are recommended to study this relationship.

References

1. Handan, U. and Güleray, A. (2005): Selenium protective activity against aflatoxin B1 adverse affects on Drosophila melanogaster. Braz. Arch. Biol. Technol., 48: 2.
2. Abdelhamid, A. M. (1990): Occurrence of some mycotoxins (aflatoxin ,ochratoxin-A, citrinin, zearalenone and vomitoxin) in various Egyptian feeds. Arch. Anim. Nutr. Berlin, 40: 647 . 664.
3. Abdelhamid, A. M. (1998): Short Notes in Food's Injuries. 1st Ed., Dar Alnil for Printing and Publication, Mansoura, Egypt, Deposit No. 7106/1998, ISBN: 19-6107-1.
4. Abdelhamid, A. M. (2008): Thirty years (1978-2008) of mycotoxins research at faculty of agriculture, Almansoura University, Egypt. Engormix.com, Mycotoxins Technical Articles, 11.
5. Abdelhamid, A. M.; El-Shawaf, I.; Elayoty, S. A.; Ali, M. M. and Gamil, I. (1990): Effect of low level of dietary aflatoxins on baladi rabbits. Arch. Anim. Nutr. Berlin, 40: 517-537.
6. Abdelhamid, A.M.; El-Mansoury, A.M.; Osman, A.I. and El-Azab, S.M. (1999): Mycotoxins as causative for human food poisoning under Egyptian conditions. J. Agric. Sci., Mansoura Univ., 24: 2751 – 2757.
7. Abdelhamid, A. M and Saleh, M. R. M. (1996): Are aflatoxin and ochratoxin endemic mycotoxins in Egypt? Proc. Food Borne Contamination and Egyptian's Health Conference, Mansoura Univ., 26 . 27 Nov.: 51-59.
8. Robens, J.F. and Richard, J.L. (1992): Aflatoxins in animal and human health. Rev. Environ. Contam. Toxicol., 127:69-94.
9. Wilkister, K. and Nyaora, Moturi. (2008): Factors Likely To Enhance Mycotoxin
10. African Journal of Food Agriculture Nutrition and Development, 8 (3): 265-277.
11. Ibeh, I.N.; Saxena, D.K. and Uraih, N. (2000): Toxicity of aflatoxin: effects on spermatozoa, oocytes, and in vitro fertilization. J. Environ. Pathol. Toxicol. Oncol., 19(4):357-361.
12. Abdelhamid, A.M.; Abdel. Khalek, A.E. ; Mehrm, A.I. and Khalil, F.F. (2004): An attempt to alleviate aflatoxicosis on Nile tilapia fish by dietary supplementation with chicken-hatchery by-products(egg shells)and shrimp processing wastes(shrimp shells) on : 2 – Clinical, blood and histological parameters. J. Agric. Sci. Mansoura Univ., 29: 6175 – 6196.
13. Abdelhamid, A.M.; Mehrim, A.I. and Khalil, F.F. (2007): Detoxification of aflatoxin-contaminated diet of tilapia fish using dietary supplementation with egg shell, Betafin®, clay or silica. Engormix.com, Mycotoxins Technical Articles, 29.
14. Ibeh, I.N. and Saxena, D.K. (1997a): Aflatoxin B1 and reproduction. I. Reproductive performance in female rats. Afr. J. Reprod. Health, 1(2):79-84.
15. Hafez, A.H; Megalla, S.E; Abdel-Fattah, H.M and Kamel, Y.Y. (1982): Aflatoxin and aflatoxicosis. II. Effects of aflatoxin on ovaries and testicles in mature domestic fowls. Mycopathologia, 77 (3): 137-139.
16. Paul, D.S. and Lauren, A. R.(2004): Management of ovulatory dysfunction in the infertile couple. Gunderson Lutheran Medical Journal, 3(1).
17. Uriah, N.; Ibeh, I.N.; and Oluwafemi,. F. (2001): A study of the impact of
18. Afr. J. Reprod. Health 5: 106-110.
19. Sayed, H.A.; El Ayyat A.; El Dusoki, H.; Zoheiry, M.; Mohamed, S.; Hassan, M.; El Assaly, N.; Awad, A.; El Ansary, M.; Saad, A. and El Karim, A.A. (2005): A cross sectional study of hepatitis B, C, some trace elements, heavy metals, aflatoxin B1 and schistosomiasis in a rural population, Egypt. J. Egypt Public Health Assoc., 80(3-4):355-88.
20. Polychronaki, N.; West, R.M.; Turner, P.C., Amra, H.; Abdel-Wahhab, M.; Mykkänen, H. and El-Nezami, H. (2007): A longitudinal assessment of aflatoxin M1 excretion in breast milk of selected Egyptian mothers. Food Chem. Toxicol.1215.-1210:(7)45
21. Anwar, W.A.; Khaled, H.M.; Amra, H.A.; El-Nezami, H. and Loffredo, C.A. (2008): Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt: Possibilities for prevention. Mutat. Res., [Epub ahead of print].
22. Eppley, R. M. (1968): Screening method for zearalenone, aflatoxin and ochratoxin. J. AOAC, 51 (1): 74-78.
23. Abdelhamid, A. M. (1981): Mycotoxine in österreichischen Futtermitteln. Diss. Boku. Univ., Wien.
24. Frank , t. J.; Mary B. G.; Winston M. H.; Jeff A. H. (1994): Understanding and coping with effects of mycotoxins in livestock feed and forage. Electronic Publication, North Carolina Cooperative Extension Service.
25. Abdelhamid, A. M. (2000): Fungi and Mycotoxins. 1st Ed., Dar Anashr for Universities, Cairo, Egypt, Deposit No.13738/1997, ISBN: 977-5526180-9.
26. Abdelhamid, A. M. and Dorra, T. M. (1990): Studies on effects of feeding laying hens on separate mycotoxins (aflatoxins, patulin, or citrinin)-contaminated diets on the egg quality and tissue constituents. Arch. Tierernähr., 40(4): 305 – 316.
27. Abdelhamid, A. M. (2003): Harms of Food and Feeding. 2nd Ed., Dar Anashr for Universities, Cairo, Egypt, Deposit No.11828/1999, ISBN: 977-316-025-4.
28. Kourousekos, G. D. and Lymberopoulos, A. G. (2007): Occurrence of aflatoxins in milk and their effects on reproduction. Journal of the Hellenic Veterinary Medical Society, 58(4): 306 – 312.
29. Abdelhamid, A. M. (2005): Carcinogens. 1st Ed., Dar Anashr for Universities, Cairo, Egypt, Deposit No.1949/2005, ISBN: 977-316-149-8.
30. Kihara, T.; Matsuo, T.; Sakamoto, M.; Yasuda, Y.; and

Yamamoto, Y. (2000): Effect of prenatal aflatoxin B1 exposure on behaviors of rat offspring. Toxicological Sciences, 53: 392 – 399.
31. Tsuboi, S.; Nakagawa, T.; Tomita, M.; Seo, T.; Ono, H.; Kawamura, K. and Iwamura, I. (1984): Detection of Aflatoxin B1 in Serum Samples of Male Japanese Subjects by Radioimmunoassay and High-Performance Liquid Chromatography. Cancer Research 44, 1231-1234.
32. Gan, L.S.; Skipper, P.L.; Peng, X., John D.; Groopman ; Jun-shi Chen, J.; Gerald N. Wogan G. L. and Tannenbaum, S.R (1988): Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis: correlation with aflatoxin B1 intake and urinary excretion of aflatoxin M1. Oxford Journals Life sciences Carcinogenesis, 9(7): 1323-1325.
33. Howard, S.; Ramdell, D. and Eaton, L. (1990): Species susceptibility to Aflatoxin B1 carcinogenesis. Cancer Research, 50: 615-620.
34. Balen, A.H. and Michelmore, K. (2002): What is polycystic ovary syndrome? Are national views important?. Human Reprod., 17: 2219-2227.
35. Fauser, B. ; Tarlatzis, B. and Chang, J. (2004) : The Rotterdam ESHRE/ ASRM. sponsored POCS workshop group . Hum. Reprod., 19: 41-47.
36. Kandarakis, E.D; Piperi, C. and Spina, J. (2006): Polycystic ovary syndrome: The influence of environmental and genetic factors, Hormones, 5(1):17-34.
37. Abd El-Wahhab, M.A. (1996): Effect of aflatoxin B treatment on pregnancy, newborn and quality of milk produced from mammals, Ph.D. Thesis, Ain Shams University, Faculty of Agriculture, Cairo, Egypt.
38. Cheeke, P.R. and Shull, L.R. (1985): Natural Toxicants in Feeds and Poisonous Plants. Avi. Publishing Company, ING. Westport, Connecticut.
39. Castelli, D.; Seralini, G. E.; Lafaurie, M.; Krebs, B. and Stora, C. (1986): Ovarian function during aflatoxin B1-induced hepatocarcinogenesis in the rat. Res. Commun. Chem. Pathol. Pharmacol., 53(2): 183 – 194.
40. Cilievici, O.; Moldovan, A.; and Ghidus, E. (1980). Action of aflatoxin B1 on pregnant rats. Morphol. Embryol., 26(2): 125 . 131.
41. Diekman, M. A. and Green, M. L. (1992): Mycotoxins and reproduction in domestic livestock. J. Anim. Sci., 71: 1615 – 1627.
42. Ibeh, I.N. and Saxena, D.K. (1997b): Aflatoxin B1 and reproduction. II. Gametoxicity in female rats. Afr. J. Reprod. Health, 1(2):85-89.

Author Information

Somaia M. El-Azab
Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Mansoura University

A.M. Abdelhamid
Animal Production Department, Faculty of Agriculture, Mansoura University

Hend A. Shalaby
Obstetric & Gynaecology Department, Faculty of Medicine, Mansoura University

A.I. Mehrim
Animal Production Department, Faculty of Agriculture, Mansoura University

Ayman H. Ibrahim
Obstetric & Gynaecology Department, Mid Staffordshire General Hospital