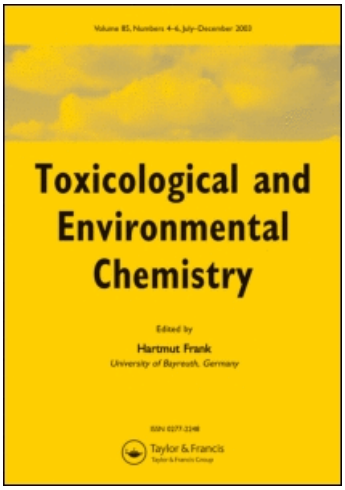

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Study of aflatoxin B_1 as a risk factor that impairs the reproductive performance in females – Egypt

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Infertility among couples in Egypt is causing increasing concern. Investigations showed a relationship between ovulatory disorders and the incidence of female factor-mediated fertility difficulties in couples. However, the factors responsible for the sudden increase in ovulatory disorders are not fully understood. The aim of this study was to assess the presence of aflatoxin in sera of infertile women and to correlate this with ovarian functions and hormonal parameters. Blood samples were collected from 70 adult Egyptian females comprising 50 infertile and 20 fertile control individuals, and screened for the presence of aflatoxin B_1 (AFB_1). Ovarian function was examined by measuring mean ovarian volume, and the umber and size of ovarian follicles. Blood hormonal levels were determined. All blood samples (patients and controls) showed negative results as regards AFB_1 analysis. However, there was a significant enlargement in the mean ovarian volume but a significant decrease in follicular size. In addition, there were significant higher levels of lutinizing hormone (LH) and significant lower levels of mid-luteal progesterone in infertile females. Although blood samples were negative with respect to AFB_1, the role of aflatoxin cannot be excluded as a contributing factor to female infertility, since the toxin was found to produce deleterious effects on the reproductive system in animals. More studies including aflatoxin analysis in ovarian biopsies are recommended to ascertain involvement of this toxin.

Keywords: human female infertility; aflatoxin; ovulatory disorder

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* (Handan and Güleray 2005). The most common form of aflatoxins is aflatoxin B_1 (AFB_1). Aflatoxins constitute a real threat to the health of livestock as

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well as humans by their continuing intermittent occurrence in both feeds and foods (Abdelhamid 1990, 2008; Abdelhamid et al. 1990; Robens and Richard 1992). 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 accumulation of moisture and thus mold 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 are marketed in these countries as well as lack of relevant technology required in monitoring fungi and mycotoxins in grains (Wilkister and Nyaora 2008). AFB_1 was reported to exert deleterious effects on the reproductive capacity of lab and domestic female animals (Ibeh, Saxena, and Uraih 2000; Abdelhamid et al. 2004, 2007). There were decreases in ovarian and uterine size, increases in fetal resorption, implantation loss, and intra-uterine death in aflatoxin-exposed female rats (Ibeh and Saxena, 1997a, b). Histopathological examinations of the ovaries in aflatoxin-treated mature domestic fowls showed follicular atresia, accompanied by cessation of egg production during the whole feeding period (Hafez et al. 1982).

Female factors were thought to be the reason behind all fertility problems. Whereas experts recognize that female infertility accounts for approximately 40% of all infertility cases, ovulatory disorders are a predominant cause for women to be unable to conceive and account for 25% of female infertility (Paul and Lauren 2004). 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 humans (Uriah, Ibeh, and Oluwafemi 2001).

Studies elsewhere showed the presence of aflatoxins in common food items in Egypt, suggesting that exposure of individuals through diet to aflatoxins may be significant (Sayed et al. 2005; Polychronaki et al. 2007; Anwar et al. 2008). The present study was, therefore, initiated to determine whether aflatoxins are present in the blood of infertile females in Egypt and to ascertain whether there is a relationship between the presence of aflatoxins and alterations in human ovulatory functions and hormonal levels.

Subjects and methods

Selection of the patients

Fifty patients (infertile females), mean age (31 ± 3) years with ovulation difficulties referred from the Infertility Unit, Mansoura University Hospital, were enrolled in the study. The underlying ovulation pathology was associated with polycystic ovary syndrome (PCOS) ($n = 43$), and idiopathic anovulation ($n = 7$). The exclusion criteria were patients with acute infections, and occupational pollution. Twenty healthy subjects (fertile females), mean age (30 ± 3) were selected as controls. All participants did not receive any supplements that might affect ovulatory functions within the previous 6 months. Informed consent was obtained from all participants.

Sampling for AFB_1 determination

Five milliliter blood sample was obtained 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 Eppley (1968), modified by Abdelhamid (1981). The lab studies were undertaken in the mycotoxin lab of Prof. Dr A.M. Abdelhamid, 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

Five milliliter blood sample was extracted with 24 mL chloroform and 1 mL *n*-phosphoric acid, the chloroform layer was received and transferred into a flask and dried under vacuum on Rota vapor-M (Bűchi-HB-140) at 60°C. Residues were dissolved in 20 mL chloroform for TLC spotting. Development of the plate was done in a closed developing tank for about 40 min, with solution: toluene/acetic acid/formic acid (6/3/1). The plate was dried in air and examined under ultraviolet at wavelength 366. Verification was done through R_f value and the fluorescence color (blue fluorescence) under UV at 366 nm, after comparison with external standard. Confirmatory test was done when the plate was sprayed with 30% methanolic sulfuric acid (30 mL H_2SO_4 + 70 mL CH_3OH) and examined again under the UV. The blue fluorescence of aflatoxin changed to a golden yellow color.

Examination of the ovary

The ovaries for all participants were examined by measuring the mean ovarian volume, 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, USA). The data were expressed as mean ± standard deviation for patients and control separately. Differences in means were analyzed using Student's *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 (control and patient samples) were negative with respect to AFB_1 analysis.

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

Table 3 shows that there were significant higher levels of LH and lower levels of mid-luteal progesterone in infertile female groups compared to controls.

Discussion

The present study was initiated to determine whether aflatoxin is present in the blood of infertile females in Egypt and to correlate this with ovarian functions. All blood samples showed negative results with respect to AFB_1 analysis (Table 1). Despite the negative results, this does not exclude the role of aflatoxin as a contributing factor in causing female

Table 1. Data of AFB_1 determination in human female blood samples under investigation (ppb).

Blood samples	Infertile females	Controls
Aflatoxin B_1	Negative	Negative

Note: ppb: parts per billion.

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

Parameters	Infertile females	Controls
Age (years)	31 ± 3	30 ± 3
Height (cm)	167 ± 2	165 ± 3
Weight (kg)	65 ± 2	67 ± 3
Body mass index ($kg\,m^{-2}$)	23.5 ± 0.5	24.3 ± 0.6
Mean ovarian volume (mL) (TVS)	6.6 ± 0.2	4.9 ± 0.3*
Mean follicular size (mm) (TVS)	8 ± 2	17 ± 2*

Note: TVS: Trans-vaginal scanning, and mean follicular size ≥15 mm indicative of ovulation.
*$p < 0.05$ significant from control.

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

Parameters	Infertile females	Controls
LH ($IU\,L^{-1}$)	21 ± 0.5*	5.6 ± 0.3
FSH ($IU\,L^{-1}$)	5.3 ± 0.2	5.1 ± 0.2
PRL ($ng\,mL^{-1}$)	13 ± 1	11.3 ± 0.3
Testosterone ($ng\,mL^{-1}$)	0.5 ± 0.02	0.4 ± 0.1
Mid-luteal progesterone ($ng\,mL^{-1}$)	0.5 ± 0.1*	30.1 ± 0.2

Note: LH: lutinizing hormone, FSH: follicle stimulating hormone, and PRL: prolactin.
*$p < 0.05$ significant from control.

infertility, as the cumulative effect of feeding low levels of mycotoxins may contribute to a gradual deterioration of organ functions, which in turn may affect fertility (Frank et al. 1994; Abdelhamid 2008). Mycotoxins (including aflatoxins) adversely affect the reproductive systems of various animal species (Abdelhamid 2008), since mycotoxin-contaminated diets led to some significant changes in egg characteristics and composition (Abdelhamid and Dorra 1990). In addition, aflatoxin is found more frequently in females than males (although males are more sensitive for aflatoxin than females) (Abdelhamid et al. 1999).

There are reports on 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 fetus (Kourousekos and Lymberopoulos 2007). Abdelhamid (2005) found that aflatoxin lowered the fertility to 13% and increased the mortality of embryos. Kihara et al. (2000) 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 mid-pregnancy, and early gestational exposure appeared to produce greater effects than later exposure. Although detection of AFB_1 in blood samples was confirmed by TLC (Abdelhamid et al. 1999), HPLC, as well as by mass spectral analysis with standard errors (Tsuboi et al. 1984), aflatoxin-serum albumin adducts have a longer half-life than low molecular weight aflatoxin derivatives which may be excreted rapidly. A large portion of aflatoxins found in human serum probably exists in adduct forms. Among them the AFB_1-albumin adduct is considered as a useful biomarker reflecting chronic exposure to aflatoxins in different populations (Gan et al. 1988). The previous findings may explain why AFB_1 was negative in blood samples of infertile females although it may be cumulative in tissues including the ovaries. On the other hand, susceptibility to aflatoxin mainly depends on liver detoxification systems, genetics, age, and other nutritional factors (Howard, Ramdell, and Eaton 1990).

Anovulation is the cause of infertility in 30% of couples, PCOS accounts for 90% of such cases (Balen and Michelmore 2002). Polycystic ovary syndrome is confirmed by the presence of two of the following criteria: biochemical or clinical hyperandrogenism, menstrual irregularity, and polycystic ovaries on ultrasound (Fauser, Tarlatzis, and Chang 2004). Environmental toxins may play a role in the pathogenesis of anovulatory infertility, especially PCOS (Kandarakis, Piperi, and Spina 2006). In the present study, there was a significant enlargement in the mean ovarian volume in infertile females (common criteria in POCS), and a significant decrease in mean follicular size (Table 2). These findings may be in part due to the adverse effects of aflatoxin as shown by Abd El–Wahhab (1996), who noted from microscopic examination of ovaries of female rabbits treated with 0.15 mg AFB_1/kg BW that there were some pathological alterations in the form of (1) coagulative necrosis which appeared mainly in the growing and mature follicles and (2) decrease in number and size of Graffian and growing follicles with increased number of atretic follicles and small areas of degenerative changes.

The significant high levels of LH concentration in infertile females may be attributed to a direct effect of increasing basal levels from anterior pituitary and/or secretion of gonadotrophic releasing hormone (GnRH) from hypothalamus. This increase might also be associated with increasing estradiol concentrations to a maximal level. On the other hand, the marked rise in progesterone levels during mid-luteal phase in controls reflected normal corpus luteal (CL) formation. However, in infertile females (i.e., the lower levels of progesterone) may be due to direct effect of reduced CL size, as evidenced from the significantly higher levels of LH in infertile females.

Two unrelated adverse actions have been suggested to explain the AFB_1 effects on female fertility: An indirect effect mediated by AFB_1 induced hypovitaminosis A; and a direct antagonistic interaction with steroid hormone receptors interfering with gonadal hormone production of estrogen and progesterone, due to structural similarity of AFB_1 and steroid hormones (Cheeke and Shull 1985). Data support our results as there were significantly lower levels of mid-luteal progesterone, with higher ovarian volume in infertile females. This may be explained by interference with the production of progesterone from the unruptured ovarian follicles. Furthermore, AFB_1 negatively affects hepatic alphafetoprotein (AFP) synthesis, and AFP is known to produce genital function blockade which leads to reduced levels of hormonal promoters (Castelli et al. 1986).

Fertility of pregnant rats decreased after aflatoxin and embryonic resorptions, malformations, and developmental retardations occurred (Cilievici, Moldovan, and Ghidus 1980). Moreover, chronic exposure to aflatoxin decreased reproductive efficiency

of ruminants (Diekman and Green 1992). Data showed disturbances in estrus cycle, significant reductions in the number of oocytes and large follicles, as well as inhibition and reduction in conception rates (Ibeh and Saxena 1997b). It may be concluded that although the results showed negative blood samples with respect to AFB_1, it does not exclude the role of aflatoxin as a contributing factor that might produce female infertility. More studies involving ovarian biopsies to analyze aflatoxin levels are recommended to study this relationship.

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