

## Journal of the Hellenic Veterinary Medical Society

Vol 66, No 4 (2015)



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doi: [10.12681/jhvms.15863](https://doi.org/10.12681/jhvms.15863)

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### To cite this article:

KOUROUSEKOS (Γ.Δ. ΚΟΥΡΟΥΣΕΚΟΣ) G. D., & THEODOSIADOU (ΑΙΚ. Κ ΘΕΟΔΟΣΙΑΔΟΥ) E. K. (2018). Effects of aflatoxins on male reproductive system: A review. *Journal of the Hellenic Veterinary Medical Society*, 66(4), 201–210. <https://doi.org/10.12681/jhvms.15863>

## **Effects of aflatoxins on male reproductive system: A review**

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## **Επιδράσεις των αφλατοξινών στο αναπαραγωγικό σύστημα του αρσενικού: Ανασκόπηση**

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**ABSTRACT.** Mycotoxins constitute toxic substances produced by certain species of fungi. Among other mycotoxins, aflatoxins are considered to be really dangerous, since they are characterized as carcinogenic for animals and humans. The consumption of aflatoxins through feeds or foods could lead to deleterious effects on animals' or humans' health. Research on animals has shown that the general body condition as well as some of the blood parameters, mainly those of the liver could be negatively affected with aflatoxin administration. Regarding the reproductive system, although not extensively studied, some investigators support the negative effects of aflatoxins either on females or on males. More specifically, in male, the size and weight of the genital organs, the spermatogenesis, the number, the motility and the morphology of sperm cells as well as hormones' concentrations could be affected after exposure of the animals to aflatoxins, making infertility problems more frequent. Most studies refer to laboratory and less to productive animals, while only two studies refer to the possible problems of infertility on men due to aflatoxins. Since reproduction consists one of the most important sectors of animal husbandry, special attention should be paid to nutrition so that the possibility of the aflatoxin consumption by animals would be eliminated, the animal health especially regarding the reproductive system would be protected and economic losses would be ameliorated.

**Keywords:** *mycotoxins, aflatoxins, reproduction, sperm, male animals, spermatotoxic effects*

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*Date of initial submission:* 05.04.2014  
*Date of revised submission:* 18.06.2014  
*Date of acceptance:* 27.07.2014

**ΠΕΡΙΛΗΨΗ.** Οι μυκοτοξίνες αποτελούν τοξικές ουσίες, οι οποίες παράγονται από συγκεκριμένα είδη μυκήτων. Μεταξύ των μυκοτοξινών, οι αφλατοξίνες θεωρούνται ιδιαίτερα επικίνδυνες, καθώς έχουν ενοχοποιηθεί ως καρκινογόνες ουσίες για τα ζώα και τον άνθρωπο. Η κατανάλωση αφλατοξινών θα μπορούσε να οδηγήσει σε αρνητικές επιπτώσεις στην υγεία ζώων και ανθρώπου. Από έρευνες, οι οποίες πραγματοποιήθηκαν σε ζώα, φαίνεται ότι η γενική κατάσταση, όπως και κάποιες βιοχημικές παράμετροι, κυρίως εκείνες του ήπατος, θα μπορούσαν να επηρεαστούν αρνητικά από τη χορήγηση αφλατοξινών. Σε ό,τι αφορά το αναπαραγωγικό σύστημα, αν και δεν έχει μελετηθεί εκτενώς, αρκετοί ερευνητές κάνουν λόγο για αρνητικές επιπτώσεις των αφλατοξινών τόσο στα θηλυκά όσο και στα αρσενικά ζώα. Ειδικότερα, στα αρσενικά ζώα, το μέγεθος και το βάρος των γεννητικών οργάνων, η σπερματογένεση, ο αριθμός, η κινητικότητα και η μορφολογία των σπερματοζωαρίων, καθώς και οι συγκεντρώσεις των ορμονών επηρεάζονται αρνητικά από τη χορήγηση ή την κατανάλωση αφλατοξινών, προκαλώντας, συχνά, προβλήματα υπογονιμότητας. Οι περισσότερες, μέχρι στιγμής έρευνες, αναφέρονται σε ζώα εργαστηρίου και λιγότερο σε παραγωγικά ζώα, ενώ δύο μόνο έρευνες αναφέρονται σε πιθανά προβλήματα υπογονιμότητας λόγω της κατανάλωσης αφλατοξινών στους άνδρες. Δεδομένου ότι η αναπαραγωγή αποτελεί έναν από τους σημαντικότερους τομείς της εκτροφής των ζώων, ιδιαίτερη προσοχή θα πρέπει να δίδεται στη διατροφή, με σκοπό να ελαχιστοποιηθεί η πιθανότητα κατανάλωσης αφλατοξινών από τα ζώα, να προστατευθεί η υγεία των ζώων και ειδικότερα εκείνη του αναπαραγωγικού συστήματος και να περιοριστούν οι οικονομικές απώλειες των εκτροφών.

**Λέξεις ευρετηρίασης:** μυκοτοξίνες, αφλατοξίνες, αναπαραγωγή, σπέρμα, αρσενικά ζώα, σπερματοτοξικές επιδράσεις

## INTRODUCTION

**M**ycotoxins constitute secondary metabolites of certain species of fungi and are produced under appropriate environmental conditions. The contamination of animals and/or humans by the mycotoxins could occur through the contaminated feed or food consumption, although recently, some attention has been recently paid to the contamination through inhalation of mycotoxins in indoor air, mainly of mycotoxins found in dust from agricultural environments (Hendry and Cole, 1993). The consumption of mycotoxins through the feeds, mainly by productive even by companion animals (Leung et al., 2006), could lead to the disturbance of the normal function of their organism (Kourousekos and Theodosiadou, 2013). The pathological conditions deriving from mycotoxins are called mycotoxicoses (Whitlow and Hagler, 2005). The symptoms of each mycotoxicosis depend on the type of the mycotoxin; the amount and duration of the exposure; the age, the health condition and the sex of the exposed individual; and many poorly understood synergistic effects involving genetics, dietary status and interactions with other toxic insult (Bennett and Klich, 2003). Food contamination from mycotoxins has

been reported worldwide, mostly in feeds that are susceptible to fungal growth, such as grains and cereals, leading to damage in health and economic losses in agriculture (Heidtmann-Bemvenuti et al., 2011). The feeds most often invaded by fungi are corn, wheat, oat, barley, cottonseed and soy-been. Fungi growth is influenced by the following factors:

- Humidity: fungi usually need high humidity levels, but there are differences even among the same species
- Temperature: most fungi grow in environmental temperatures, but some species could be developed under very low or very high temperatures
- Oxygen: fungi cannot grow without oxygen presence of at least 1-2%
- The sensitivity of certain hybrid plants (Diekman and Green, 1992).

The environmental conditions that could, theoretically, prevent fungi's growth on plants or in feed-stuffs are presented below:

- Temperature < -2.2 oC
- Humidity < 14%
- Relative humidity < 70%
- Oxygen < 0.5% (Diekman and Green, 1992).

Among mycotoxins, aflatoxins consist a special cat-

egory of worldwide spread toxins. Specifically, aflatoxins are produced mainly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. The most known aflatoxins are B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, with aflatoxin B<sub>1</sub> being the most potent among the others (Diekman and Green, 1992). Like other mycotoxins, aflatoxins could occur in feeds or foods contaminating animals or humans through consumption. The usual effects that have been observed in animals after aflatoxin administration are reduction of feed consumption and metabolism, reduction of growth, body weight and production, immunosuppression against microbes and parasites, etc.

After aflatoxin B<sub>1</sub> is consumed by lactating animals another also carcinogenic mycotoxin can be detected in the milk. This is the so-called aflatoxin M<sub>1</sub> or “milk toxin” and consists aflatoxin’s B<sub>1</sub> major metabolite. The consumption of even low concentrations of aflatoxin B<sub>1</sub> by animals could lead to the excretion of aflatoxin M<sub>1</sub> into milk in concentrations exceeding the maximum permissible limit set by the European Union (50 ppt) making the milk liable for public health issues (Kourousekos et al., 2012b).

Furthermore, some years ago, considerable attention has been paid on certain environmental contaminants, called endocrine disruptors that may mimic the action of sex hormones (Nilsson, 2000). It is well stated that such endocrine disruptors may cause effects detrimental to the reproductive system (Fenner-Crisp, 2000). Endocrine disruptors may affect reproduction and may result in alterations in sexual differentiation, reduction of developmental competence in oocytes, arrest of embryonic development, growth and development of certain types of cancer (Brevini et al., 2005; Sanderson, 2006; Crain et al., 2008). Among mycotoxins, zearalenone has been characterized as an endocrine disruptor because it mimics oestrogens’ actions, thus causing hyperoestrogenism (Nilsson, 2000). Zearalenone is produced by the *Fusarium* spp. fungi and can be detected in foods or feeds. Thus has been classified as a phytoestrogen acting as an endocrine disruptor (Humfrey, 1998).

Nowadays, aflatoxin B<sub>1</sub> has also been characterized as a potential endocrine disruptor because of its effect on cytochrome enzymes (CYPs or P<sub>450s</sub>) that involve steroideogenic synthesis, alone or after

being metabolized in another carcinogenic metabolite, called aflatoxicol. Storvik et al. (2011) and Huuskonen et al. (2013) supported that aflatoxin B<sub>1</sub> increases the expression of a cytochrome enzyme (CYP19A1) in human placenta cells. More specifically, Huuskonen et al. (2013) indicated that aflatoxin B<sub>1</sub> affected the placental steroid hormone synthesizing, metabolizing and conjugating enzymes. These alterations may lead to anomalies in the foetoplacental hormonal homeostasis, while Storvik et al. (2011) suggested that aflatoxin B<sub>1</sub>, after being metabolized in aflatoxicol, had effects on genes that were important for endocrine regulation in placental cells.

Before it was well known that aflatoxins could act as endocrine disruptors, some researchers supported the likely effect of aflatoxins on the reproductive system. Specifically, the effects of aflatoxins on the female reproductive system were related to alterations in sexual maturation (Doer and Ottinger, 1980), growth and maturation of the follicles, concentrations of hormones (Ibeh and Saxena, 1997a; b; Kourousekos and Lymberopoulos, 2007; Kourousekos et al., 2008; 2012a), gestation (Ibeh and Saxena, 1997a; b) and growth of the foetus (Schmidt and Panciera, 1980; Arora et al., 1981).

Regarding the male reproductive system, most reports are related to the likely effect of aflatoxins on the size and weight of the genital organs (Sharlin et al., 1980), on spermatogenesis (Egbunike et al., 1980; Gopal et al., 1980), on the number, motility and morphology of sperm cells (Hafez et al., 1982; Agnes and Akbarsha, 2003), and on the concentrations of hormones (Clarke and Ottinger, 1987).

Thus, the aim of this review is to focus on the main effects of aflatoxins on the male reproductive system, showing that such effects could negatively affect the reproductive performance of male animals. There was an effort to refer to the most recent as well as to older literature. Most references in the present study refer to laboratory animals, since the experiments after aflatoxin administration to productive animals are very limited. Regarding men, there are only two studies, which examine the correlation between infertility problems on men and the presence of aflatoxins in their blood and/or semen.

## MAIN EFFECTS OF AFLATOXINS ON MALE REPRODUCTIVE SYSTEM

### Laboratory animals

The studies on laboratory animals' reproduction after exposure to aflatoxins concern mice and rats. In the study of Sinha and Dharmshila (1994) young weaning Swiss albino mice were orally administered crude aflatoxin B<sub>1</sub> at a dose mimicking human exposure condition (0.05 µg kg<sup>-1</sup> bw per day, for 14 weeks). Decreases in sperm cells count as well as increases in abnormality in the gross morphology of the sperm cells' head were observed upon aflatoxin B<sub>1</sub> treatment.

In another study, Agnes and Akbarsha (2003) administered intraperitoneally aflatoxin B<sub>1</sub> to 90-day-old mice at a daily dose of 50 µg kg<sup>-1</sup> bw for 7, 15, 35 and 45 days. The analysis consisted of fertility testing and counts, motility and abnormalities of the cauda epididymal sperm cells. The fertility of the treated mice was reduced drastically. Sperm cells concentration and motility decreased whereas sperm cells abnormalities increased. In particular, sperm cells abnormalities like two axonemes in a common cytoplasm, sticking together of heads/tails, etc., were noted. The results indicated disruption of the spermatogenic as well as androgenic compartments of the testis by aflatoxin B<sub>1</sub>. The results also revealed an alteration of epididymal function towards the post-testicular sperm maturation process by aflatoxin B<sub>1</sub>.

Faridha et al. (2007), with a view to find if aflatoxins would produce multinucleate giant cells or symplasts in the seminiferous epithelium, treated orally male Swiss mice with 50 µg aflatoxin B<sub>1</sub> kg<sup>-1</sup> bw per day for 35 days and subjected the testis to light and transmission electron microscopic analysis. They found abundant symplastic spermatids in the seminiferous epithelium of the treated mice. The study revealed another manifestation of aflatoxin-induced disruption of spermatogenesis.

Towards finding the cellular targets in spermatogenic compartment for aflatoxin toxicity, Faisal et al. (2008a) administered aflatoxin B<sub>1</sub> to 90-day-old

Swiss mice through intraperitoneal route, at a daily dose of 20 mg kg<sup>-1</sup> bw for 7, 15, 35 and 45 days. The testis and epididymis were subjected to light as well as transmission electron microscopic analysis. One of the newer observations was the occurrence of meiotic micronucleate giant spermatocytes in seminiferous epithelium and epididymal lumen. The origin of these cells could be traced to imminent disruption of spindle apparatus during the meiotic division of spermatocytes, resulting in the lagging of chromosome bivalents or replicated univalents. Such chromosomes appeared to undergo condensation and become micronuclei. Thus, the authors report that aflatoxin B<sub>1</sub> exposure would result in generation of meiotic micronucleate giant spermatocytes.

In another research on albino mice, the effect of aflatoxin-contaminated corn (100 ppb per day for 1-4 weeks) was investigated using the sperm morphology assay (Fapohunda et al., 2008). Sperm cells were early primary spermatocytes and spermatogonia and showed varieties of morphological sperm-head abnormality. Therefore, the authors concluded that abnormal sperm cell induction is concentration-dependent, that is, continuous consumption of aflatoxin-contaminated corn is capable of negatively affecting spermatogenesis by inducing or increasing the frequency of morphologically abnormal sperm cells produced.

Finally, Mathuria and Verma (2008) administered *per os* in young male albino mice 750 and 1500 µg aflatoxin B<sub>1</sub> kg<sup>-1</sup> bw per day for 45 days. On the 46<sup>th</sup> day the animals were killed by cervical dislocation. The cauda epididymis was removed and weighed, then was teased in normal saline to obtain sperm cells. There was a dose-dependent spermicidal effect of aflatoxin B<sub>1</sub>. Sperm cells count, viability and motility were significantly reduced, while different morphologic abnormalities were encountered. Sperm cells from the low-dose aflatoxin-treated group showed coiled tails, swollen heads, and deformities at mid-piece, while sperm cells from the high-dose aflatoxin-treated group showed agglutinations of head-head, head-tail and tail-tail, decapitation, swollen heads, and coiled tails.



Gopal et al. (1980), after injecting rats with aflatoxin B<sub>1</sub> intratesticularly, at daily doses of 5, 10, 25 and 50 µg for 11 days observed that there was a reduction in size and weight of the testes, showing a direct toxic effect on testes, and that spermatogenesis was inhibited. In another study (Sahay, 1993) the effect of oral consumption of 200 ppb of crude aflatoxin (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) showed testicular degeneration and a decrease in the meiotic index in rats. Also, in the study of Ibeh and Saxena (1998) aflatoxin B<sub>1</sub> was given at a dose of 7.5 µg per 200 g bw per day to rats for 14 days by means of oral intubation. Reduced sperm cells quality and marked pathological changes in the testis of rats given aflatoxin B<sub>1</sub> were observed.

Marginal to severe mitochondrial pathologies were observed in spermatozoa and elongated spermatids, when male rats were treated with 20 µg aflatoxin B<sub>1</sub> kg<sup>-1</sup> bw per day, intramuscularly, for 55 days (Faisal et al., 2008b). The authors concluded that the possibility that aflatoxin B<sub>1</sub> treatment would disrupt the cytoskeletal proteins of the flagellum, resulting in the extrusion of outer dense fibres, could not be excluded. Dietary aflatoxins, therefore, could also be contributory factors regarding the deterioration of the reproductive health of men.

The negative effects of aflatoxin showed on spermatogenesis could influence the quality of the animals' sperm. In the study of Ibeh et al. (2000) *in vitro* fertilization (IVF) medium containing aflatoxin B<sub>1</sub> at concentrations of 2.0, 4.0, 8.0 and 16.0 ppb was cultured with oocytes obtained from superovulated healthy fertile female rats and exposed to sperm cells. Epididymal sperm capacitated in IVF medium, with or without aflatoxin B<sub>1</sub>, were exposed to oocytes, and the rates of fertilization in the two experiments were assessed. Aflatoxin B<sub>1</sub> significantly reduced the mean number of fertilized ova, even at the lowest concentration. Exposure to aflatoxin B<sub>1</sub> caused a significant reduction in the motility of epididymal sperm cells. The authors highlighted the adverse effects of aflatoxin B<sub>1</sub> on oocytes, spermatozoa, and IVF.

Other studies investigated the possible effects of aflatoxin B<sub>1</sub> exposure to hormone concentrations. In the study of Gopal et al. (1980), the intratesticularly daily

injection of rats with aflatoxin B<sub>1</sub>, at doses of 5, 10, 25 and 50 µg for 11 days, lead to a significant reduction of blood plasma oestrogen concentrations compared to controls. The concentrations of blood serum FSH, LH and testosterone in rats receiving daily 0.5 ppm aflatoxin B<sub>1</sub> in feeds for up to six months of age were studied by Hassan et al. (2010). All three hormone concentrations were reduced in animals receiving aflatoxin compared to controls. In this research, also zearalenone, another endocrine disruptor, reduced FSH, LH and testosterone concentrations in blood. In another study, Hasanzadeh et al. (2011) administered aflatoxin B<sub>1</sub> orally in male rats for 48 days at doses of 0.8, 1.6 and 3.2 ppm per day. The concentrations of blood serum LH and testosterone were lower, but conversely the concentrations of blood serum FSH and prolactin were higher in the treated groups. Oestradiol-17β concentration in blood serum was affected by significantly falling, but only in the group receiving the highest dose of aflatoxin B<sub>1</sub>. Finally, Verma and Nair (2002) supported that the reduced blood serum testosterone concentration after oral aflatoxin B<sub>1</sub> administration (25 and 50 µg per animal per day for 45 days) to adult male mice was attributed to mitochondria dysfunction, to inhibition in protein synthesis or enzyme activity or to membrane changes of Leydig cells. These negative effects were ameliorated with vitamin E administration.

### Productive animals

Similar studies have shown effects of aflatoxins on the male reproductive system of productive animals, such as birds, boars, buffaloes, rabbits and rams.

In the study of Sharlin et al. (1980) aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>), at a dose of 20 µg per g of diet per day, was added to the feed of mature White Leghorn males for five weeks and resulted in decreased semen volume and testes' weight and a disruption of the germinal epithelium, although there was no effect on the percent of fertile eggs or that of hatched fertile eggs from hens artificially inseminated with spermatozoa from treated males.

Ortatatli et al. (2002) investigated the effect of aflatoxin exposure on spermatogenesis. Twenty-four

months of age roosters were fed daily a total of 5, 10 and 20 ppm aflatoxin B<sub>1</sub> in their diet for 8 weeks. It was observed that the testes of all aflatoxin-treatment groups' birds were significantly atrophied when compared with those of control birds. Histopathologically, there was no spermatogenesis in the testes of many cocks fed in each group. Furthermore, abnormal sperm cells were observed in some of the aflatoxin-treated groups. It has been shown that aflatoxin might totally or partially (dose-related) suppress spermatogenesis, cause sperm cells abnormalities and atrophy in testes.

In boars, Picha et al. (1986) studied the concentration of aflatoxin B<sub>1</sub> in the seminal plasma for a period of twelve months. The highest aflatoxin B<sub>1</sub> residues in sperm were recorded from March to May and were related with aflatoxin concentration in the feed ration. The group of boars with fertility disorders had higher aflatoxin concentration in their seminal plasma as well as lower concentration, lower survival and a larger proportion of abnormal spermatozoa compared to controls.

In buffaloes, fed daily with rice powder containing 15.6 ppm aflatoxin B and 9.84 ppm aflatoxin G for 2 weeks, the results showed marked decrease in the percentage of alive spermatozoa and a very high increase in spermatozoa abnormalities (Hafez et al., 1982).

In rabbits, Salem et al. (2001) administered two sublethal doses (15 or 30 µg kg<sup>-1</sup> bw; every other day) of aflatoxin B<sub>1</sub>. Results showed that live body weight, dry matter intake and relative testes weight were significantly reduced after treatment with aflatoxin B<sub>1</sub>, in a dose-dependent manner. Aflatoxin B<sub>1</sub> also decreased the volume of the ejaculate, the concentration of spermatozoa, the total sperm output, the sperm motility index and the semen initial fructose concentration. All the negative effects of aflatoxin B<sub>1</sub> on sperm characteristics were dose-dependent. In addition, aflatoxin B<sub>1</sub> treatment, also, increased the numbers of abnormal and dead spermatozoa, in a dose-dependent manner. Also in rabbits, Ahmed et al. (2012) administered 250, 500 and 1000 ppb aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) per kg<sup>-1</sup> of diet per day for 60 days. Peritubular oedema with atrophy of seminiferous tubules was observed. Spermatogonial cells showed degeneration and nuclear pyknosis, with the presence of multinucleated sper-

matic giant cells in the lumen of seminiferous tubules. Moreover, focal hemorrhagia and disturbed process of spermiogenesis due to nuclear pyknosis of most spermatogonial cells were also observed.

In ram, epididymal and ejaculatory sperm cells were added into media containing different concentrations of aflatoxin B<sub>1</sub> (1.96 to 62.5 ppb) (Tajik et al., 2007). Sperm motility patterns for both epididymal and ejaculatory sperm cells were more or less negatively affected after incubation in different concentrations of aflatoxin B<sub>1</sub>, *in vitro*. The results of the study showed that aflatoxin B<sub>1</sub> could decrease the motility of sperm cells obtained after ejaculation or from epididymis, in a dose-dependent manner.

As far as blood hormones' concentrations are concerned, Clarke and Ottinger (1987) administered 10 ppm aflatoxin B<sub>1</sub> per day for 3 weeks and 20 µg LHRH kg<sup>-1</sup> bw to 9-week-old male chickens. Plasma testosterone levels increased soon after the LHRH injection in control males, secondary to elevated LH levels in the peripheral circulation, and continued to increase throughout the experimental period. In contrast, that LH-induced elevation in plasma testosterone was delayed in aflatoxin-treated males, with no substantial increase until the 20 min post-LHRH injection. Also, in the study of Eraslan et al. (2006) 2.5 ppm aflatoxin (78.30% aflatoxin B<sub>1</sub>, 14.60% aflatoxin B<sub>2</sub>, 4.50% aflatoxin G<sub>1</sub> and 2.60% aflatoxin G<sub>2</sub>) was administered daily *per os* to 14-day-old male Japanese breed quails for 21 days and blood plasma testosterone was evaluated. A significant decrease of testosterone was detected in the group receiving aflatoxins. Also, in rabbits, after the administration of two sublethal doses (15 or 30 µg kg<sup>-1</sup> bw; every other day) of aflatoxin B<sub>1</sub>, the concentration of blood serum testosterone was significantly reduced, in a dose-dependent manner (Salem et al., 2001).

### Men

The occurrence of aflatoxins in foods consumed by humans is more frequent in regions with tropical or sub-tropical climate. Thus, it is more possible for the population of these regions to consume aflatoxin-contaminated food and become more susceptible to

the danger of aflatoxins. It is estimated that about 4.5 billion people, mostly in developing countries, are at risk of chronic exposure to aflatoxins from contaminated food crops (Williams et al., 2004). It seems that women more than men are exposed to this risk. In most developing countries, women are engaged in subsistence agriculture. They are actively involved in the full range of farming practices from planting, weeding and pest control to harvesting and storage. They are also invariably responsible for cooking the meals. Consequently, they are primarily exposed to the health hazards of aflatoxins as they may ingest these toxins in high quantities with food in its raw state or during food preparation (Shuaib et al., 2010).

However, in regions where aflatoxins occur in human's food, men cannot be excluded from the possibility of an aflatoxin contamination. Regarding the effects of aflatoxins on men's reproduction only two studies were found, which conclude that men's infertility could be attributed to aflatoxin consumption. In particular, in the research conducted by Ibeh et al. (1994) a random sampling of semen from 100 adult males comprising 50 samples drawn from infertile men and 50 drawn from normal individuals within the same community (Benin City, Nigeria) revealed the presence of aflatoxins in 20 semen samples from the infertile group (40.0%) and four samples from the fertile group (8.0%). Infertile men with aflatoxin in their semen showed a higher percentage of spermatozoa abnormalities (50.0%) than that in fertile men.

Furthermore, in the study of Uriah et al. (2001) blood and semen samples were collected from 55 adult Nigerians comprising 30 infertile and 25 fertile control individuals, and screened for the presence of aflatoxins. Blood and semen aflatoxin levels in infertile men ranged from 700 to 1392 ng/ml and 60 to 148 ng/ml, respectively, values significantly higher than the concentrations of the toxin in fertile men.

Deviation from normal in semen parameters showed a definite pattern in infertile men. Infertile men with high concentrations of aflatoxins in their semen had decreased spermatozoa concentration and increased spermatozoa abnormalities. About 37% of the infertile men had aflatoxin in their blood and semen suggesting that aflatoxins might be a contributory factor to the occurrence of infertility in Nigerians.

The main effects of aflatoxins on male reproductive system in different animal species as well as in men, according to the existing literature, are presented in **Table 1**.

## CONCLUDING REMARKS

In conclusion, it could be supported that aflatoxins could negatively influence the male reproductive system. All stages, from spermatogenesis to spermatozoa fertility after ejaculation, could be negatively affected. Infertility problems due to reduced sperm cells count, viability and motility as well as due to increased sperm cells morphologic abnormalities could derive from aflatoxin consumption. Thus, special attention should be paid to the quality of the feedstuffs so that the consumption of aflatoxins by animals would be eliminated and their reproductive performance would be improved. Finally, although most studies refer to animals, it seems that men do not stay unaffected by exposure to aflatoxins. Further research upon the effects of aflatoxins on men's reproductive system is considered to be essential.

## CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare. ■



**Table 1.** The main effects of aflatoxins (AFs) on male reproductive system in laboratory animals, in productive animals and in men.

Species	Doses and Duration	Main Effects	References
Mice	0.05 µg AFB <sub>1</sub> kg <sup>-1</sup> bw/day, orally, for 14 weeks (mimicking human exposure condition)	Decreased sperm cells count; Increased abnormalities in the gross morphology of sperm cells head	Sinha and Dharmshila (1994)
	25 and 50 µg AFB <sub>1</sub> /day, orally, for 45 days	Reduced blood serum testosterone concentration	Verma and Nair (2002)
	50 µg AFB <sub>1</sub> kg <sup>-1</sup> bw/day, intraperitoneally, for 7, 15, 35 and 45 days	Decreased cauda epididymal sperm cells concentration and motility; Increased cauda epididymal sperm cells abnormalities; Reduced fertility; Disrupted testes spermatogenic and androgenic compartments; Altered epididymal function towards the post-testicular sperm maturation process	Agnes and Akbarsha (2003)
	50 µg AFB <sub>1</sub> kg <sup>-1</sup> bw/day, orally, for 35 days	Disrupted spermatogenesis; Abundant symplastic spermatids in the seminiferous epithelium	Faridha et al. (2007)
	20 mg AFB <sub>1</sub> kg <sup>-1</sup> bw/day, intraperitoneally, for 7, 15, 35 and 45 days	Generated meiotic micronucleate giant spermatocytes in the seminiferous epithelium and in the epididymal lumen	Faisal et al. (2008a)
	100 ppb AF/contaminated corn/day, for 1-4 weeks	Increased head abnormalities of early primary spermatocytes and spermatogonia; Disrupted spermatogenesis	Fapohunda et al. (2008)
	750 and 1500 µg AFB <sub>1</sub> kg <sup>-1</sup> bw/day, orally, for 45 days	Reduced cauda epididymal sperm cells count, viability and motility, in a dose-dependent manner; Increased cauda epididymal sperm cells morphologic abnormalities, in a dose-dependent manner	Mathuria and Verma (2008)
Rats	5, 10, 25 and 50 µg AFB <sub>1</sub> /day, intratesticularly, for 11 days	Reduced testes size and weight; Inhibited spermatogenesis; Reduced blood plasma oestrogens concentrations	Gopal et al. (1980)
	200 ppb AFs (B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> ), orally	Degenerated testes; Decreased meiotic index	Sahay (1993)
	7.5 µg AFB <sub>1</sub> per 200 g bw/day, orally, for 14 days	Reduced sperm cells quality; Marked testes pathological changes	Ibeh and Saxena (1998)
	2.0, 4.0, 8.0 and 16.0 ppb AFB <sub>1</sub> in IVF <sup>a</sup> medium	Reduced epididymal sperm cells motility; Reduced fertility rates of oocytes fertilized with epididymal sperm cells cultured in IVF medium containing AFB <sub>1</sub>	Ibeh et al. (2000)
	20 µg AFB <sub>1</sub> kg <sup>-1</sup> bw/day, intramuscularly, for 55 days	Marginal to severe mitochondrial pathologies in spermatozoa and elongated spermatids	Faisal et al. (2008b)
	0.5 ppm AFB <sub>1</sub> /day, in feeds, for up to 6 months of age	Reduced blood serum FSH, LH and testosterone concentrations	Hassan et al. (2010)
Roosters	0.8, 1.6 and 3.2 ppm AFB <sub>1</sub> /day, orally, for 48 days	Reduced blood serum LH and testosterone concentrations; Reduced blood serum oestradiol-17β concentration (only at the highest dose); Increased blood serum FSH and prolactin concentrations	Hasanzadeh et al. (2011)
	20 µg AFs (B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> )/g diet/day, for 5 weeks	Decreased semen volumes; Decreased testes weight; Disrupted germinal epithelium	Sharlin et al. (1980)
	10 ppm AFB <sub>1</sub> /day and 20 µg LHRH kg <sup>-1</sup> bw/day, for 3 weeks	Delayed corresponding increase of blood plasma testosterone concentration at the LH peak	Clarke and Ottinger (1987)
Quails	5, 10 and 20 ppm AFB <sub>1</sub> /day, with feed, for 8 weeks	Atrophied testes; Suppressed spermatogenesis (dose-related); Increased sperm cells abnormalities	Ortatatli et al. (2002)
	2.5 ppm AFs (78.30% B <sub>1</sub> , 14.60% B <sub>2</sub> , 4.50% G <sub>1</sub> and 2.60% G <sub>2</sub> ), orally, for 21 days	Decreased blood plasma testosterone concentration	Eraslan et al. (2006)
	Study on the concentrations of AFB <sub>1</sub> in seminal plasma, for 12 months	Decreased spermatozoa concentration and survival; Increased spermatozoa abnormalities	Picha et al. (1986)
Buffaloes	15.6 ppm AFB and 9.84 ppm AFG/day, with feed, for 2 weeks	Decreased percentage of alive spermatozoa; Increased spermatozoa abnormalities	Hafez et al. (1982)
Rabbits	15 or 30 µg AFB <sub>1</sub> kg <sup>-1</sup> bw, every other day, in 2 sublethal doses	Reduced live bw, dry matter intake, testes weight, ejaculate volume, spermatozoa concentration, total sperm output, sperm motility index and semen initial fructose concentration, in a dose-dependent manner; Increased spermatozoa abnormalities and death, in a dose-dependent manner; Reduced blood serum testosterone concentration, in a dose-dependent manner	Salem et al. (2001)
	250, 500 and 1000 ppb AFs/kg of diet/day, for 60 days	Peritubular oedema; Atrophied seminiferous tubules; Degenerated spermatogonial cells and nuclear pyknosis; Presence of multinucleated spermatid giant cells in the lumen of seminiferous tubules; Disturbed spermiogenesis	Ahmed et al. (2012)
Rams	<i>In vitro</i> study; different concentrations of AFB <sub>1</sub> (1.96 to 62.5 ppb) in the media used for the culture of epididymal and ejaculated sperm cells	Reduced epididymal and ejaculated sperm cells motility, in a dose-dependent manner, <i>in vitro</i>	Tajik et al. (2007)
Men	Study on semen samples of 100 adults	Infertility; Increased spermatozoa abnormalities	Ibeh et al. (1994)
	Study on blood and semen samples of 55 adults	Infertility; Decreased spermatozoa concentration; Increased spermatozoa abnormalities	Uriah et al. (2001)

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## REFERENCES

- Agnes VF, Akbarsha MA (2003) Spermatotoxic effect of aflatoxin B<sub>1</sub> in the albino mouse. *Food Chem Toxicol* 41:119-130.
- Ahmed KA, El Mahady MM, Badawy SA, Ahmed YF, Aly MA (2012) Pathological consequences of aflatoxins in male rabbit: Cellular, genetic and oxidative damage. *Global Veterinaria* 9:42-52.
- Arora RG, Frolen H, Nilsson A (1981) Interference of mycotoxins with prenatal development of the mouse. I. Influence of aflatoxin B<sub>1</sub>, ochratoxin A and zearalenone. *Acta Vet Scand* 22:524-534.
- Bennett JW, Klich M (2003) Mycotoxins. *Clin Microbiol Rev* 16:497-516.
- Brevini TAL, Cillo F, Antonini S, Gandolfi F (2005) Effects of endocrine disruptors on the oocytes and embryos of farm animals. *Reprod Dom Anim* 40:291-299.
- Clarke RN, Ottinger MA (1987) The response of the anterior pituitary and testes to synthetic luteinizing hormone-releasing hormone (LHRH) and the effect of castration on pituitary responsiveness in the maturing chicken fed aflatoxin. *Biol Reprod* 37:556-563.
- Crain A, Janssen S, Edwards T, Heindel J, Ho S, Hunt P, Iguchi T, Juul A, McLachlan J, Schwartz J, Skakkebaek N, Soto A, Swan S, Walker C, Woodruff T, Woodruff T, Giudice L, Guillette LJr (2008) Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing. *Fertil Steril* 90:911-940.
- Diekman MA, Green ML (1992) Mycotoxins and reproduction in domestic livestock. *J Anim Sci* 70:1615-1627.
- Doerr JA, Ottinger MA (1980) Delayed reproductive development resulting from aflatoxicosis in juvenile Japanese quail. *Poult Sci* 59:1995-2001.
- Egbunike GN, Emerole GO, Aire TA, Ikegwonu FI (1980) Sperm production rates, sperm physiology and fertility in rats chronically treated with sublethal doses of aflatoxin B<sub>1</sub>. *Andrologia* 12:467-475.
- Eraslan G, Akdogan M, Liman BC, Kanbur M, Delibas N (2006) Effects of dietary aflatoxin and hydrate sodium calcium aluminosilicate on triiodothyronine, thyroxine, thyrotrophin and testosterone levels in quails. *Turk J Vet Anim Sci* 30:41-45.
- Faisal K, Faridha A, Akbarsha MA (2008a) Induction of meiotic micronuclei in spermatocytes *in vivo* by aflatoxin B<sub>1</sub>: light and transmission electron microscopic study in Swiss mouse. *Reprod Toxicol* 26:303-309.
- Faisal K, Periasamy VS, Sahabudeen S, Radha A, Anandhi R, Akbarsha MA (2008b) Spermatotoxic effect of aflatoxin B<sub>1</sub> in rat: extrusion of outer dense fibres and associated axonemal microtubule doublets of sperm flagellum. *Reproduction* 135:303-310.
- Fapohunda SO, Ezekiel CN, Alabi OA, Omole A, Chioma SO (2008) Aflatoxin-mediated Sperm and Blood Cell Abnormalities in Mice Fed with Contaminated Corn. *Mycobiology* 36:255-259.
- Faridha A, Faisal K, Akbarsha MA (2007) Aflatoxin treatment brings about generation of multinucleate giant spermatids (sympasts) through opening of cytoplasmic bridges: light and transmission electron microscopic study in Swiss mouse. *Reprod Toxicol* 24:403-408.
- Fenner-Crisp PA (2000) Endocrine modulators: Risk characterization and assessment. *Toxicol Pathol* 28:438-440.
- Gopal T, Oehme FW, Liao TF, Chen CL (1980) Effects of intratesticular aflatoxin B<sub>1</sub> on rat testes and blood estrogens. *Toxicol Lett* 5:263-267.
- Hafez AH, Megalla SE, Mahmed AA (1982) Aflatoxin and aflatoxicosis. III. Effect of dietary aflatoxin on the morphology of buffalo bull spermatozoa. *Mycopathologia* 77:141-144.
- Hasanzadeh Sh, Hosseini E, Rezazadeh L (2011) Effects of aflatoxin B<sub>1</sub> on profiles of gonadotropic (FSH and LH), steroid (testosterone and 17 $\beta$ -estradiol) and prolactin hormones in adult male rat. *Iran J Vet Res* 12:332-336.
- Hassan AA, Rashid MA, Koratum KM (2010) Effect of aflatoxin B<sub>1</sub>, zearalenone and ochratoxin A on some hormones related to fertility in male rats. *Life Sci J* 7:64-72.
- Heidtmann-Bemvenuti R, Mendes GL, Scaglioni PT, Badiale-Furlong E, Souza-Soares LA (2011) Biochemistry and metabolism of mycotoxins: A review. *Afr J Food Sci* 5:861-869.
- Hendry KM, Cole EC (1993) A review of mycotoxins in indoor air. *J Toxicol Environ Health* 38:183-198.
- Humfrey CDN (1998) Phytoestrogens and human health effects: Weighing up the current evidence. *Nat Toxins* 6:51-59.
- Huuskonen P, Myllynen P, Storvik M, Pasanen M (2013) The effects of aflatoxin B<sub>1</sub> on transporters and steroid metabolizing enzymes in JEG-3 cells. *Toxicol Lett* 218:200-206.
- Ibeh IN, Uraih N, Ogonar JI (1994) Dietary exposure to aflatoxin in human male infertility in Benin City, Nigeria. *Int J Fertil Menopausal Stud* 39:208-214.
- Ibeh IN, Saxena DK (1997a) Aflatoxin B<sub>1</sub> and reproduction. I. Reproductive performance in female rats. *Afr J Reprod Health* 1:79-84.
- Ibeh IN, Saxena DK (1997b) Aflatoxin B<sub>1</sub> and reproduction. II. Gametotoxicity in female rats. *Afr J Reprod Health* 1:85-89.
- Ibeh IN, Saxena DK (1998) Effect of alpha-tocopherol supplementation on the impact of aflatoxin B<sub>1</sub> on the testes of rats. *Exp Toxicol Pathol* 50:221-224.
- Ibeh IN, Saxena DK, Uraih N (2000) Toxicity of aflatoxin: effects on spermatozoa, oocytes, and *in vitro* fertilization. *J Environ Pathol Toxicol Oncol* 19:357-361.
- Kourousekos GD, Lymberopoulos A (2007) Occurrence of aflatoxins in milk and their effects on reproduction. *J Hellenic Vet Med Soc* 58:306-312.
- Kourousekos GD, Theodosiadou E, Ververidis H, Belibasaki S, Boscos C, Saratsis P, Lymberopoulos AG (2008) Effect of aflatoxin B<sub>1</sub> administration on serum steroid hormones concentrations of goats. *Reprod Dom Anim* 43:102.
- Kourousekos GD, Theodosiadou E, Belibasaki S, Boscos C, Lymberopoulos AG (2012a) Effect of aflatoxin B<sub>1</sub> on blood serum progesterone and oestradiol-17 $\beta$  concentrations of goats during anoestrus. *Reprod Dom Anim* 47:92.

- Kourousekos GD, Theodosiadou E, Belibasaki S, Deligiannis K, Koukoulas Th, Zoulfos K, Lymberopoulos AG (2012b) Effects of aflatoxin B<sub>1</sub> administration on Greek indigenous goats' milk. *Int Dairy J* 24:123-129.
- Kourousekos GD, Theodosiadou E (2013) Milk composition disturbance and animal organism dysfunction caused by aflatoxins: A review. In: (ed: Rudd LB) *Milk Fat: Composition, Nutritional Value and Health Implications*, Nova Science Publishers Inc., USA, 2013, pp. 1-26.
- Leung MCK, Diaz-Llano G, Smith TK (2006) Mycotoxins in Pet Food: A review on worldwide prevalence and preventative strategies. *J Agric Food Chem* 54:9623-9635.
- Mathuria N, Verma RJ (2008) Curcumin ameliorates aflatoxin-induced toxicity in mice spermatozoa. *Fertil Steril* 90:775-780.
- Nilsson R. (2000) Endocrine modulators in the food chain and environment. *Toxicol Pathol* 28:420-431.
- Ortatatli M, Ciftci MK, Tuzcu M, Kaya A (2002) The effects of aflatoxin on the reproductive system of roosters. *Res Vet Sci* 72:29-36.
- Picha J, Cerovsky J, Pichova D (1986) Fluctuation in the concentration of sex steroids and aflatoxin B<sub>1</sub> in the seminal plasma of boars and its relation to sperm production. *Vet Med* 31:347-357.
- Sahay M (1993) Aflatoxin induced testicular degeneration and decreased meiotic index in *Rattus norvegicus*. *Cytobios* 75:191-195.
- Salem MH, Kamel KI, Yousef MI, Hassan GA, EL-Nouty FD (2001) Protective role of ascorbic acid to enhance semen quality of rabbits treated with sublethal doses of aflatoxin B<sub>1</sub>. *Toxicology* 162:209-218.
- Sanderson T (2006) The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals. *Toxicol Sci* 94:3-21.
- Schmidt RE, Panciera RJ (1980) Effects of aflatoxins on pregnant hamsters and hamster fetuses. *J Comp Pathol* 90:339-347.
- Sharlin JS, Howarth BJr, Wyatt RD (1980) Effect of dietary aflatoxin on reproductive performance of mature White Leghorn males. *Poult Sci* 59:1311-1315.
- Shuaib FMB, Ehiri J, Abdullahi A, Williams JH, Jolly PE (2010) Reproductive health effects of aflatoxins: A review of the literature. *Reprod Toxicol* 29:262-270.
- Sinha SP, Dharmshila K (1994) Vitamin A ameliorates the genotoxicity in mice of aflatoxin B<sub>1</sub>-containing *Aspergillus flavus* infested food. *Cytobios* 79:85-95.
- Storvik M, Huuskonen P, Kyllonen T, Lehtonen S, El-Nezami H, Auriola S, Pasanen M (2011) Aflatoxin B<sub>1</sub> - a potential endocrine disruptor - up-regulates CYP19A1 in JEG-3 cells. *Toxicol Lett* 202:161-167.
- Tajik P, Mirshokraee P, Khosravi A (2007) Effects of different concentrations of aflatoxin B on ram epididymal and ejaculatory sperm viability and motility *in vitro*. *Pak J Biol Sci* 10:4500-4504.
- Uriah N, Ibeh IN, Oluwafemi F (2001) A Study on the Impact of Aflatoxin on Human Reproduction. *Afr J Reprod Health* 5:106-110.
- Verma RJ, Nair A (2002) Effect of aflatoxins on testicular steroidogenesis and amelioration by vitamin E. *Food Chem Toxicol* 40:669-672.
- Whitlow LW, Hagler WM (2005) Mycotoxins: A review of dairy concerns. *Mid-South Ruminant Nutrition Conference*, pp. 47-58.
- Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am J Clin Nutr* 80:1106-1122.