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Aflatoxin B_1 effects on the ovarian follicles of White Leghorn laying hens

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ABSTRACT

The objective of this study was to investigate low dose and long duration effects of aflatoxin B_1 on different categories of the ovarian follicles in adult White Leghorn layers. The birds were put in 2 groups, viz. experiment and as controls. Every chicken of experiment group was received AFB1 @ 0.62 ppm/day through oral route for the duration of 120 days. At the end of experiment, all the birds were slaughtered, immediately their ovaries collected and preserved in 10% buffered formaldehyde solution. In both the groups, the macroscopic follicles i.e. SWF, SYF, LYF (including F1, F2, F3, F4, F5), and POF were recorded, and then the specimens were subjected to tissue preparation, for histomorphologic and histomorphometric studies. All types of ovarian follicular distributions (including healthy and atretic) were recorded. There was highly significant ($P<0.001$) decrease in populations of SWF and SYF in test group. The reduction in different types of LYF as well as POF populations in test group were significant. There were high significant reduction in healthy microscopic and raise in atretic microscopic follicles in test group. It may be concluded that aflatoxin B1 in low dose (0.62 ppm/day) for the duration of 120 days causes increase in ovarian follicular atresia and death of different categories of the macroscopic as well as microscopic follicles in Leghorn layers.

Key words: Aflatoxin B_1, Leghorn chicken, Ovarian follicles

Aflatoxins (AFs) B_1, B_2, G_1 and G_2 are mycotoxins that may be produced by 3 fungi of the *Aspergillus* species, *A. flavus, A. parasiticus* and *A. nomius*. The aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of AFB1> AFG1> AFB2> AFG2 (Reddy and Waliyar 2012), thus among the different types of AFs produced, AFB_1 is the most prevalent and potent, often found in high concentrations in cereal grains, peanut meal, maize, groundnuts and their products (Gowda *et al.* 2004, 2005, Cortes 2010). According to Oguz *et al.* (2003) and Aravind *et al.* (2009), aflatoxins influence the metabolism of poultry and the main manifestations of chronic aflatoxicosis in layers are reduced weight, egg production (Siloto *et al.* 2011). These toxins cause considerable economic sufferers for animal industry (Andretta *et al.* 2009). The aflatoxins have nutritional and toxicological effects on reproduction in male and female chicken (Kovacs 2004). In birds, aflatoxins interfere with digestible energy (Applegate *et al*. 2008). In poultry, AFB1 reduces egg output (Coulombe *et al*. 1994).

Chronic consumption of aflatoxin-contaminated foods is a common problem in animals worldwide, especially where there is poor food harvesting, processing and storage of food and food products thus allowing the growth of mold on them. Aflatoxins also can affect almost all the different body systems (Godfrey *et al*. 2013).

The different categories of the ovarian follicles in the domestic hens may differently get affected by noxious impacts of the AFB_1; therefore for the first time we aimed ourselves to investigate this subject matter.

MATERIALS AND METHODS

Animals and housing: Healthy Leghorn pullet layers (50), 60-day old, were procured commercially. The balanced feed and water were given *ad lib*. The chickens were kept for 7 days for adjustment without any experimentation. They were divided into 2 groups— experiment and control 12 h light and 12 h dark, 20–23°C temperature, conditions were observed throughout the experiment.

Administration of aflatoxin B_1: Solution (3,000 ppm) of toxin was prepared. For each bird of experiment group 0.62 ppm/day for the duration of 120 days was fed orally (through gavages). During experiment, each bird received 75 ppm aflatoxin B_1. The birds of control group received same volume of sterile distilled water daily for 120 days.

Collection of ovaries: All the chickens were sacrificed 120 days after commence of experiment, and immediately their abdomens cut opened and their ovaries taken out completely and put in 10% buffered formaldehyde solution for the fixation. The macroscopic follicles, i.e. small white follicles (SWF), small yellow follicles (SYF), large yellow

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follicles (LYF) including F1, F2, F3, F4 and F5 and post ovulatory follicles (POF), were recorded for both groups. After trimming of macroscopic follicles from the ovaries, the tissue preparation procedures were carried out through routine paraffin embedding technique. The specimens were cut at 5–7 µm thickness and stained with hematoxylin – eosin and studied under light microscope. In the histomorphometric study, we used special morphometric lens device, and the distribution of all types of microscopic follicles, including healthy and atretic follicles, in 1mm² of ovarian tissue in 10 different loci of the ovaries were recorded (Gupta *et al*. 1988, Madekurozwa *et al*. 2006, Bharucha and Padate 2010).

Statistical analysis: By the use of the SPSS soft ware (IBM SPSS Inc. 2011) and adopting 2 tailed t- test data were analyzed and the histograms prepared with Excel soft-ware.

RESULTS AND DISCUSSION

Aflatoxins disrupt the reproductive system in both male and female animals, and also cause alterations in the form of the growing and mature ovarian follicles and decrease in number and size of growing follicles with increased number of atretic follicles (El -Azab *et al*. 2009). According to our results, the different aspects of these effects on the ovarian follicles of White Leghorn chicken are discussed here.

Macromorphology: The toxic effects of the AFB_1 could be reflected as reduced weights of ovaries, or populations of macroscopic as well as microscopic ovarian follicles (Coulombe 1994). The gross observations of the ovaries and recording of macroscopic follicles revealed significant differences between the test and control groups. In the test group, some ovaries were small and some exhibited hyperemia. Data analyses on distribution of different types of macroscopic follicles on the surfaces of ovaries in test and control groups revealed significant differences in SWF, SYF as well as LYF (including F1, F2, F3, F4, and F5) between control and test groups and such types of the follicles were greatly reduced in test groups (Fig. 4A).The reason for this more likely is direct (atretogenic effects) or indirect effect (general effect) of AFB_1 on reproductive system, especially the ovaries.

Aflatoxins cause serious acute effects on the gastro-intestinal tract (Gursoy *et al*. 2008). In birds, aflatoxins interfere with intestinal morphology, sialic acid production and apparent digestible energy (Applegate *et al*. 2008). Aflatoxins have serious acute effects on the endocrine (Giambrone *et al*. 1978), respiratory (Jakab *et al.* 1994), cardiovascular, urinary (Sharma *et al.* (2011), immune (WHO 2008) and nervous systems (Thrasher and Crawley 2012), thus, the indirect noxious effects on bird performance (Slwa and Anwer 2009), and the ovarian follicular population should not be neglected.

Interestingly the reduction in population of SWF in comparison to the other follicles in the test group was very outstanding (Fig. 3). Following the reduction in population of SWF, it will be reflected as reduction in populations of the other follicular categories and certainly, the egg production drastically comes down.

Micromorphology: The atretic changes were seen in different ovarian follicular layers, including oocyte, granulosa and theca layers. All the atretic signs were accelerated in the test group (Fig.1). According to Bharucha and Padate (2010) throughout natural physiologic conditions in birds including poultry, beside healthy growing follicles, atretic follicle are present in ovary, but in birds affected by aflatoxin populations of microscopic as well as macroscopic follicles increased (Siloto *et al*. 2011).

In the poultry industry the deleterious effects of mycotoxins should be manifested from different point of views. Aflatoxins are potential agents in the incline of poultry industry to lowering of products and thus economic losses, whilst, the hazards of these agents on health of animals and humans should not be ignored.

Histomorphometry: Analyses of data revealed that in all the 10 loci studied at 1mm² of ovarian tissues, in the test group high significant differences existed between

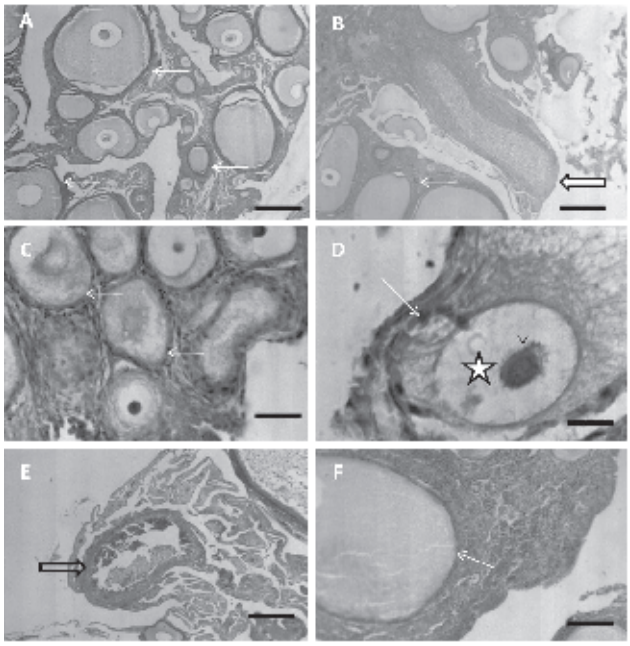


Fig. 1.**A**. Cortex of an ovary belonging to the control group in which different sizes of the growing follicles are seen (arrows). **B**. Cortex of an ovary belonging to the test group in which different sizes of growing (white thin arrow) and a large atretic (white thick arrow) follicles are seen. **C.** Growing and degenerating follicles in an ovary of test group (Arrows indicating the granulosa cell layers). **D**. A degenerating follicle in an ovary of test group in which white arrow indicating the granulosa cell layer, asterisk indicating the oocyte and small black arrow indicating its nucleus. **E.** Cortex of an ovary belonging to the test group in which numerous small and large atretic (arrow) follicles are seen. **F**. Cortex of an ovary belonging to the test group in which a growing (white thin arrow) follicle which is surrounded with hypertrophied theca cells is seen. H&E. Bar = 1mm.

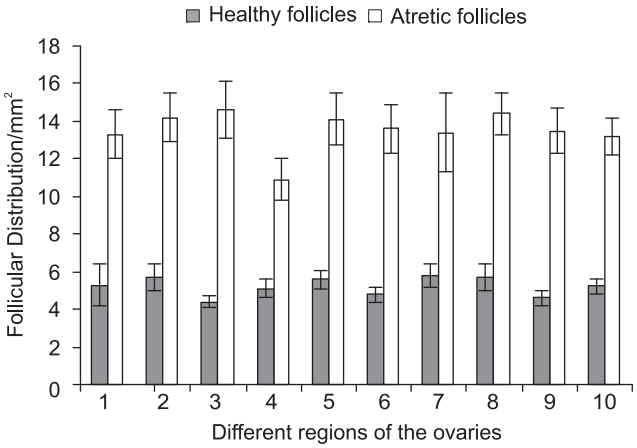


Fig. 2. Distributions of the healthy as well as atretic follicles in 10 different loci of ovaries belonging to test groups.

distribution of healthy and atretic follicles (Fig. 2). The mean distribution of healthy follicles revealed highly significant differences with the distribution of atretic follicles in the test group (Fig. 1).

The analyses of data on micromorphometric observations revealed that in all hens of the experiment group in 10 different sites of their ovarian tissues at 1 mm², significant differences existed between atretic and healthy follicles. The differences in distribution of atretic follicles at 1 mm² ovarian tissue of test and control group were significant (Fig. 4B). According to Silito *et al.* (2011) aflatoxin is toxic for laying hens @ 1ppm/ day but our results revealed that 0.62 ppm caused significant atretic changes in the chicken's ovaries (Fig. 4B). Very high concentrations of aflatoxins are most often found in seeds of maize, nuts and cereal grains in Africa and rice in China and Southeast Asia (Bennett and Klich 2003, Agag 2004, Kitya *et al*. 2009, Thrasher 2012). Aflatoxins are also responsible for the suppression of both the humoral and cell-mediated immunity and thus making individuals susceptible to infectious diseases (Godfrey *et al*. 2013), hence the commercial poultry breeders all over the world should strictly look into this crisis and create policies to avoid the problem.

In earlier studies on different effects of AFB_1 on the biological systems of poultry, the doses used were large and durations were short. In this study the dose chosen was very limited (0.62 ppm/chicken/day) but the duration of exposure was relatively long (120 days) and each chicken from test group received 75 ppm AFB1 during this period of time. In the field conditions, if contamination of feed stuffs to aspergillus is severe, in most instances the condition of receiving AF from this fungus is slow, and in such a situation the birds will receive small amount of toxin in long duration. In harmony to bring field and experiment conditions closer, we anticipated to get relatively reliable result from our trial.

The follicular atresia is a natural and physiologic process which occurs in the ovaries of mammals as well as birds, but some issues, accelerating this process and consequently

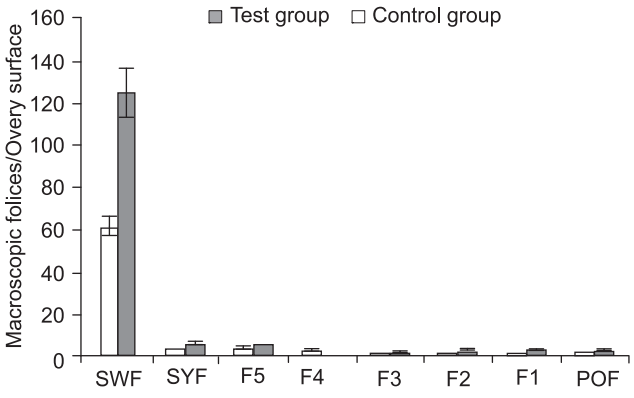


Fig. 3. Different categories of the macroscopic follicles on surfaces of ovaries in the control and test groups are comparatively presented.

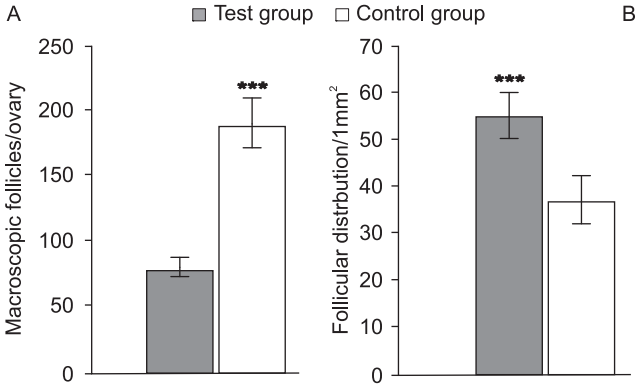


Fig. 4. **A**. Comparative representation of the macroscopic follicles populations on the ovaries surfaces in control and test groups. **B**. Comparative representation of the microscopic atretic follicles distributions in $1mm^2$ ovarian tissues in the control and test groups. ***= P< 0.001.

the follicular reservoir of ovaries reduce and finally the performance of production will be negatively exaggerated by this means. According to Hasanzadeh and Amani (2012) in rats the AFB_1 is toxic for all types of ovarian follicles, including not growing and growing follicles and exerts an atretogenic effect on all types of ovarian follicles. The atretogenic consequence of AFB_1 is dose dependant. Due to its toxic effects (gametotoxicity) the resting pool of ovarian follicles (primordial follicles) drastically decreases. The ovulatory follicular population either decreases or is completely depleted. In ducks, in addition to the reduction in growth and utilization of protein, dietary AF causes liver damages as well and considerable changes in most of blood constituents (Rizzi *et al.* 2003).

AFB_1 in low dose (0.62ppm/day/hen) for the duration of 120 days increased ovarian follicular atresia and death of macroscopic as well as microscopic follicles of Leghorn hens, and thus egg production decreased accordingly.

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