doi: 10.17221/8130-CJAS

Effect of prolonged aflatoxin B_1 administration on blood serum oestradiol-17β and progesterone concentrations of goats during the anoestrus period

G.D. Kourousekos[1], E.K. Theodosiadou[2], A.G. Lymberopoulos[3], S. Belibasaki[4], C. Boscos[1]

[1]Faculty of Veterinary Medicine, Aristotle University, Thessaloniki, Greece
[2]Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece
[3]Department of Animal Production, Alexander Technological Educational Institute, Thessaloniki, Greece
[4]Veterinary Research Institute, Hellenic Agricultural Organization Demeter, Thessaloniki, Greece

ABSTRACT: The effect of prolonged aflatoxin B_1 (AFB_1) administration on blood serum oestradiol-17β and progesterone concentrations in goats during the anoestrus period was investigated. Thirty Greek indigenous goats were used; 10 goats received, *per os*, 50 μg (treated group T50) and 10 goats received 100 μg (treated group T100) AFB_1/day/head, respectively, for 31 days, while 10 goats served as controls (C). Blood samples were collected from each goat twice a week to determine serum oestradiol-17β and progesterone concentrations by radioimmunoassay. Multiple linear regression analysis revealed a significant ($P < 0.05$) positive dependence of blood serum oestradiol-17β concentration over group (C = 0, T50 = 50, T100 = 100), in a dose-dependent manner, and a significant ($P < 0.05$) negative dependence over time (0–31 days, from the onset to the end of AFB_1 administration). Linear regression analysis revealed a significant ($P < 0.05$) positive dependence of blood serum progesterone concentration over group (C = 0, T50 = 50, T100 = 100), in a dose-dependent manner. Pearson's correlation coefficient revealed a significant ($P < 0.05$) positive correlation between blood serum oestradiol-17β and progesterone concentration, but only in the goats of the control group. In conclusion, the prolonged administration of 100 or even of 50 μg AFB_1/day/head during the anoestrus period, increased blood serum oestradiol-17β and progesterone concentrations of goats, in a dose-dependent manner, and in the treated groups disturbed the positive correlation between oestradiol-17β and progesterone concentration shown in the goats of the control group.

Keywords: aflatoxins; mycotoxins; ovarian steroids; small ruminants; anoestrus

INTRODUCTION

Aflatoxins (B_1, B_2, G_1, and G_2) are toxic metabolites, with carcinogenic potency, of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi invade the forages and under suitable conditions produce aflatoxins (Thieu et al. 2008). The consumption of aflatoxins by animals or human results in various pathological conditions (aflatoxicoses), with symptoms that vary depending on the species, age, degree of forage invasion by the fungi, and quantity of the consumed feeds or food (Diekman and Green 1992). The regions more affected by aflatoxins are those with tropic or

Supported by the General Secretariat of Research and Technology of the Greek Ministry of Development (PENED 2001-7/4/2003, 01ED 282).

doi: 10.17221/8130-CJAS

subtropic climate, since the levels of humidity and temperature play a positive role in fungal growth (Galvano et al. 1996; Abdulrazzaq et al. 2003). The consumption of AFB_1 results in the excretion of aflatoxin M_1 into the milk. Aflatoxin M_1 is the major of AFB_1 metabolites, mainly produced in the liver, with carcinogenic potency as well, and in some cases it is detected at concentrations higher than the maximum permissible levels fixed by each country, making the milk inappropriate for consumption (El-Nezami et al. 1995; Abdulrazzaq et al. 2003; Kourousekos et al. 2012).

Regarding the reproductive system, the mechanisms through which it is affected by AFB_1 remain unexplained, since the effects of the toxin have not been studied extensively (Shuaib et al. 2010). Concerning the male reproductive system, most reports are related with the negative effect of AFB_1 on the size and weight of genital organs, on spermatogenesis (Gopal et al. 1980), on the number and morphology of spermatozoa (Hafez et al. 1982; Ortatatli et al. 2002; Agnes and Akbarsha 2003), and on blood oestrogens and testosterone concentrations (Gopal et al. 1980; Verma and Nair 2002).

Regarding the female reproductive system, inhibition of sexual maturation (Doerr and Ottinger 1980), libido, growth and maturation of follicles, foetus growth (Schmidt and Panciera 1980; Ibeh and Saxena 1997a, b; Wangikar et al. 2005), reduction of sex organs weight, as well as increased blood progesterone and decreased oestradiol concentrations during oestrus and decrease in maternal oestradiol : progesterone ratio (Ibeh and Saxena 1997a, b) were observed after AFB_1 administration.

Moreover, the high risk of toxicity of AFB_1 to the foetus and placenta due to aflatoxicol production, AFB_1 metabolite with carcinogenic potency as well, was studied in women (Partanen et al. 2010). The risk was found to be more serious in high-risk countries, such as Egypt (Piekkola et al. 2012). Furthermore, Storvik et al. (2011) indicated that chronic exposure to AFB_1 might cause endocrine disruption in the human foetoplacental unit due to its effect on the expression of aromatase enzymes (P450s or CYPs enzymes), categorizing AFB_1 as a potential endocrine disruptor.

Studies dealing with the effects of AFB_1 administration on the reproductive system of farm animals are limited. Moreover, these studies, mainly in laboratory animals, use quite higher concentrations and/or shorter periods of AFB_1 administration.

Thus the aim of the present study was to investigate, for the first time, possible alterations in blood serum oestradiol-17β and progesterone concentrations of goats after prolonged AFB_1 administration (31 days) during the anoestrus period, since anoestrus period in seasonally polyoestrous animals like goats is characterized by the absence of cyclic ovarian activity and oestrous behaviour due to the low control of the hypothalamus–hypophysis axe at the ovaries.

MATERIAL AND METHODS

Animals and experimental protocol. For the aim of the present study, 30 Greek indigenous primiparous goats, 2–3 years old, in anoestrus period, weighing 29.60 ± 3.90 kg, housed in open-fronted covered yard were used. The study was conducted at the Veterinary Research Institute, in northern Greece, from the middle of June to the middle of July (longitude 22°51'37''E and latitude 40°41'19''N; average temperature 25.4°C, average humidity 53.0%; 14.5 h day-light + 9.5 h darkness). All the goats performed parturition about four months ago without their oestrus cycles previously being synchronized. Throughout the experimental period, the goats were not in contact with other animals, they were healthy, without any pharmaceutical treatment. The goats were fed 1 kg/day/head of pelleted concentrate plus grass hay *ad libitum*; water was available for the animals 24 hours a day. The pelleted concentrate feed consisted of corn, barley, gluten, soybean meal, molasses, yeast ranching, sodium chloride, calcium carbonate, dicalcium phosphate, vegetable fat, vitamins, and minerals. The chemical analysis of the pelleted concentrate feed was (in %): dry matter 52.4, total proteins 17.2, fat 3.3, cellulose 3.9, ashes 7.7, moisture 13.0, calcium 1.2, phosphorus 0.7, sodium 0.6, and chlorine 0.04.

To ensure that the goats did not receive any AFB_1 concentrations through their diet, feedstuff samples were analyzed, once a week, for AFB_1 presence using high performance liquid chromatography (HPLC), as described by Akiyama et al. (2001) with the assistance of specific columns (MycoSep® 226 columns for AFB_1). The minimum detection limit for AFB_1 was 0.5 ppb. None of the samples was found positive for AFB_1 presence. Moreover, the body weight of all goats was measured once a week. No significant differences ($P > 0.05$) were observed during the whole experimental period.

doi: 10.17221/8130-CJAS

Goats were randomly divided into 3 groups of 10 animals each (control group (C), treated group (T50), and treated group (T100)). The goats of T50 or T100 treated groups received 50 or 100 μg AFB_1/day/head), *per os*, respectively, for 31 days. These doses were based on the results of a previous study, where the administration of such AFB_1 doses to Greek indigenous goats resulted in the excretion of aflatoxin M_1 into the milk, exceeding the maximum permissible level (50 ppt) set by the European Union (Kourousekos et al. 2012). Ten mg of pure AFB_1 (AFB_1 from *Aspergillus flavus*, A 6636-10 MG, SIGMA; Sigma Chemical Co., St. Louis, USA) were dissolved in 100 ml methanol or in 200 ml methanol and 1 ml of this dilution was received *per os* by each goat of T50 or T100 treated groups, respectively. The goats of the control group received only the solvent of AFB_1 (1 ml methanol/day) in order to be equally handled; according to Battacone et al. (2003) methanol is the best solvent for AFB_1 for *in vivo* treatment, while the administration of methanol at these concentrations has no risk for the animal's health (http://www.epa.gov/chemfact/s_methan.txt). Administration of diluted AFB_1 to goats of T50 or T100 treated groups, as well as of 1 ml methanol to goats of the control group, was achieved using a dosimetric pistollete-like pump in order to be controlled and easily accepted. The administration to all animals was realized the same hour (7:00 h) each morning.

Blood samples were collected from the onset to the end of AFB_1 administration, twice a week, from each goat (always on the same days and the same time at about 8:00 h). Additionally, before the onset of AFB_1 administration, four blood samples had been collected from each goat, in a two-week period, by jugular venipuncture into evacuated blood collecting tubes (Venoject, Terumo, Belgium). After clotting, blood samples were centrifuged (2500 *g*; 20 min; 4°C); serum was aspirated and stored at –20°C until assayed.

Oestradiol-17β and progesterone assays. Oestradiol-17β and progesterone concentrations in blood serum were determined using radioimmunoassay (RIA), after extraction, as described by Martin et al. (1987), following minor modification (Rekkas et al. 1991). The radiolabelled solutions of oestradiol-17β and progesterone were provided by Amersham Pharmacia Biotech (Amersham, UK), while oestradiol-17β and progesterone antiserums were developed by the Institute of Molecular Biology, Iraklion, Greece (Theodosiadou et al. 2004). The intra-assay variability was 3.5–5.5% (n = 6) and 2.4–4.4% (n = 6), while the inter-assay variability was 9.2% (n = 24) and 9.4% (n = 24) for oestradiol-17β and progesterone, respectively. The recovery rate was 93.3 ± 2.8% (mean ± SD; n = 24) for oestradiol-17β and 94.5 ± 2.2% (mean ± SD; n = 24) for progesterone. The sensitivity (lower limit of detection) for oestradiol-17β was 3.90 pg/ml, while for progesterone it was 19 pg/ml (0.019 ng/ml).

Statistical analysis. One-way analysis of variance (one-way ANOVA) was used to compare oestradiol-17β or progesterone concentrations among the three groups studied. Levene's test was used for the control of homogeneity of variances and statistical differences were estimated using Tukey's HSD test. Linear regression analysis was used in order to trace the variability of oestradiol-17β or progesterone concentration over group (C = 0, T50 = 50, T100 = 100) and over time (0–31 days, from the onset to the end of AFB_1 administration) (multiple), or over group, or over time. Pearson's correlation coefficient was used in order to correlate oestradiol-17β and progesterone concentration in each group studied. Linear regression analysis was used in order to describe the significant equation. Statistical analysis was performed using SPSS software (Version 15.0, 2006) for MS Windows and a probability of $P < 0.05$ was the minimum level of significance, in all cases.

RESULTS

Before AFB_1 administration no significant differences ($P > 0.05$) were observed among the three groups studied regarding blood serum oestradiol-17β ((C = 45.08 ± 9.99; T50 = 45.09 ± 9.46; T100 = 48.80 ± 9.52) or progesterone concentration (C = 0.20 ± 0.07; T50 = 0.20 ± 0.09; T100 = 0.21 ± 0.07) (mean ± SD, n = 40)).

After AFB_1 administration, blood serum oestradiol-17β or progesterone concentrations of the goats of the treated groups T50 or T100 presented significantly higher ($P < 0.05$) than those of the goats of group C; the significantly highest ($P < 0.05$) concentration was observed in T100 group. Analytically, the first 3 days after AFB_1 administration, oestradiol-17β or progesterone concentration showed no significant differences ($P > 0.05$) between the three groups studied. From 7 to 14 days

doi: 10.17221/8130-CJAS

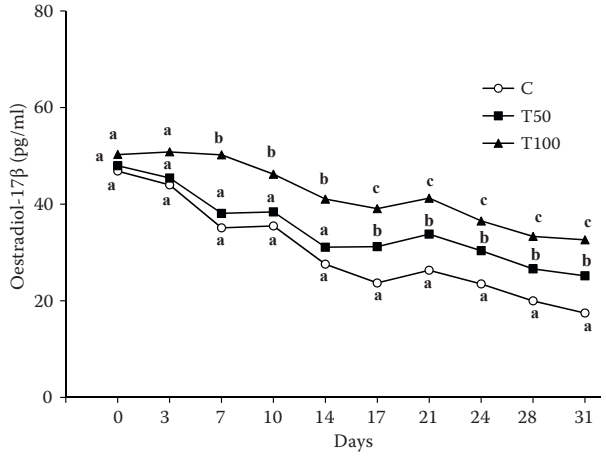

Figure 1. Oestradiol-17β concentration (pg/ml) in blood serum of goats, from the onset to the end of AFB_1 administration (0–31 days), during the anoestrus period (control group (C); treated group T50 (50 μg AFB_1/goat/day); treated group T100 (100 μg AFB_1/goat/day))

[a–c]significant differences between the three groups at each time point studied ($P < 0.05$)

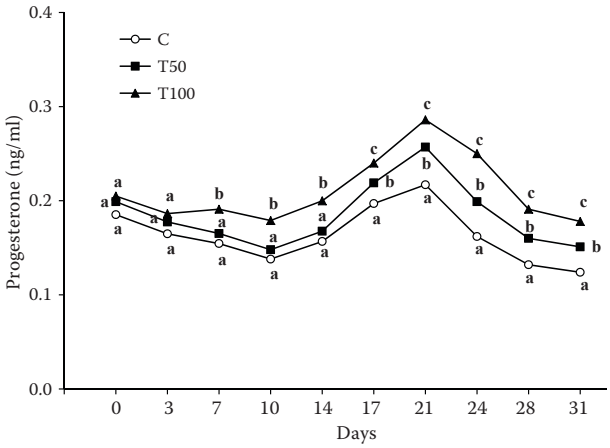

Figure 2. Progesterone concentration (ng/ml) in blood serum of goats, from the onset to the end of AFB_1 administration (0–31 days), during the anoestrus period (control group (C); treated group T50 (50 μg AFB_1/goat/day); treated group T100 (100 μg AFB_1/goat/day))

[a–c]significant differences between the three groups at each time point studied ($P < 0.05$)

after AFB_1 administration group T100 showed significantly ($P < 0.05$) higher oestradiol-17β or progesterone concentration compared to T50 or C group, while no significant difference ($P > 0.05$) was observed between T50 and C group. From day 17 to the end of AFB_1 administration (day 31) either T50 or T100 group showed significantly ($P < 0.05$) higher oestradiol-17β or progesterone concentration than group C, while the significantly ($P < 0.05$) highest concentration was observed in T100 group (Figures 1 and 2).

More specifically, multiple linear regression analysis revealed a significant dose-dependent increase of blood serum oestradiol-17β concentration over group and a significant decrease over time (F = 190.94, df = 297, $P < 0.000$; constant = 41.06 ± 1.01, t = 40.63, P = 7.83E-021; Group = 0.12 ± 0.01, t = 10.85, P = 7.83E-021; Time = –0.74 ± 0.05, t = –16.25, P = 7.83E-021).

Also, linear regression analysis revealed a significant dose-dependent increase of blood serum progesterone concentration over group (F = 64.93, df = 298, P = 1.82E-014; constant = 0.16 ± 0.004, t = 42.60, P = 7.72E-021; Group = 4.80E-04 ± 5.90E-05, t = 8.06, P = 1.82E-014).

Finally, a significant positive correlation between blood serum oestradiol-17β and progesterone concentration was revealed, but only in the goats of the control group (Pearson's coefficient = 0.267; n = 100; P = 0.007). The significant positive equation, according to linear regression analysis results, for blood serum oestradiol-17β was (F = 7.52, df = 98, P = 0.007; constant = 16.14 ± 5.18, t = 3.12, P = 0.002; progesterone = 84.96 ± 30.98, t = 2.74, P = 0.007), while for blood serum progesterone was (F = 7.52, df = 98, P = 0.007; constant = 0.14 ± 0.01, t = 13.97, P = 1.10E-018; oestradiol-17β = 8.39E-04 ± 3.10E-04, t = 2.74, P = 0.007).

This correlation disappeared in the goats of T50 group (Pearson's coefficient = 0.136, n = 100, P = 0.177) or T100 group (Pearson's coefficient = –0.123, n = 100, P = 0.223).

DISCUSSION

In the present study, the prolonged administration of 100 or even of 50 μg AFB_1/day/head, during the anoestrus period, increased blood serum oestradiol-17β and progesterone concentrations of the goats, in a dose-dependent manner, and in the treated groups (T50 or T100) disturbed the positive correlation between oestradiol-17β and progesterone concentration shown in the goats of the control group.

Greek indigenous goats are considered to be late-seasoned animals presenting oestrus cycles from the end of August, even middle of September (a strong variability between breeds exists in goats

doi: 10.17221/8130-CJAS

regarding the dates of onset of the breeding season, with a gradient of seasonality from southern to northern latitudes (Chemineau et al. 2010; Fatet et al. 2011)). In the present study progesterone and oestradiol-17β concentrations during the anoestrus period (middle of June until middle of July) were in accordance with those in the study of Goulas (1993) in Greek indigenous goats as well as of Yuthasastrakosol et al. (1975) and Haresign et al. (1975) in ewes, although increased in groups receiving AFB_1, in a dose-dependent manner, compared to control group. The minor fluctuations shown in our study concerning oestradiol-17β or progesterone concentration, as well as the minor peak in progesterone concentration might reflect fluctuations of LH shown close to the end of the anoestrus period that might prepare the ovaries for the oestrus period, as Yuthasastrakosol et al. (1975) supported in ewes.

Other researchers also confirm disturbances on reproductive performance due to AFB_1 administration, in a dose-dependent manner, but during the oestrus period. Ibeh and Saxena (1997a, b) in female rats administered 7.5 mg AFB_1/kg body weight per day for 14 days or 15 mg AFB_1/kg body weight per day for 21 days, respectively, and observed that body, ovaries, and uterus weights were significantly decreased. Inhibition of libido, oocytes growth, and number of ovulations, as well as high percentage of foetal deaths and decrease in maternal oestradiol : progesterone ratio was also observed. Oestradiol and progesterone concentrations appeared significantly lower and higher, respectively, in rats receiving AFB_1. After prolonged AFB_1 administration but also during the oestrus period of the Greek indigenous goats, increased progesterone concentrations during the luteal phase and decreased oestradiol-17β concentrations during oestrous synchronization were also confirmed by Kourousekos et al. (2008). In order to explain these hormones disturbances, the authors proposed either a direct effect of AFB_1 on ovarian secreting cells or on the hypothalamus–hypophysis–ovaries axis.

The possible direct effect of AFB_1 on testes was supported by Gopal et al. (1980) and Verma and Nair (2002). Gopal et al. (1980) injected intratesticularly AFB_1 in male rats at doses of 5, 10, 25, and 50 μg for 11 days. Blood oestrogen concentrations were found significantly lower, while spermatogenesis was inhibited. Moreover, Verma and Nair (2002) supported that the reduced testosterone concentration after oral AFB_1 administration (25 and 50 μg/day/animal for 45 days) in adult male mice was attributed to mitochondria dysfunction, to inhibition in protein synthesis or enzyme activity or to membrane changes of Leydig cells. In the present study, the prolonged AFB_1 administration could have directly affected the ovaries and because of one of the mechanisms above could have increased oestradiol-17β and progesterone concentrations and could have disturbed in both the treated groups the positive correlation between them shown in the goats of the control group.

In order to explain the aflatoxins actions, a lot of years ago some researchers considered the likely binding of the oestrogen receptors by aflatoxins, but this assumption seems not to be in issue. Kyrein (1974) observed that aflatoxins B_1, G_1, and G_2 did not present any binding affinity to oestrogen receptors derived from healthy uterus of calves, although aflatoxin M_1 presented a small extent of receptors binding, but in quite higher concentrations compared with oestradiol-17β. Moreover, no binding affinity was observed for AFB_1 or aflatoxin M_1 to oestrogen or progesterone receptors of cow uterus (Blankenship et al. 1982), while aflatoxicol showed a small binding affinity, but only to oestrogen receptors.

Recently, AFB_1 has been categorized as a potential endocrine disruptor (Storvik et al. 2011). It is known that AFB_1 is metabolized by cytochrome P450 (CYPs) enzymes (Buhler and Wang-Buhler 1998). Furthermore, Storvik et al. (2011) and Huuskonen et al. (2013) supported that AFB_1 increases the expression of CYP19A1 in human placenta cells. More specifically, Huuskonen et al. (2013) indicated that AFB_1 affected the placental steroid hormone synthesizing, metabolizing, and conjugating enzymes and that these alterations may lead to anomalies in the foetoplacental hormonal homeostasis, while Storvik et al. (2011) suggested that AFB_1, after being metabolized in aflatoxicol, had effects on genes important for endocrine regulation in placental cells. Furthermore, since CYPs have been found to take part in steroid hormones synthesis, the increase of the expression of such enzyme by AFB_1 in the placenta could result in increased oestrogen and progesterone production. In our study the increased blood serum oestradiol-17β and progesterone concentration, in a dose-dependent manner, in the goats of either

doi: 10.17221/8130-CJAS

T50 or T100 group might have been a result of such an increased expression of CYPs enzymes on the ovaries due to prolonged administration of AFB_1.

The increased progesterone and oestradiol-17β concentration close to the end of the anoestrus period in goats under the prolonged administration of AFB_1, in a dose-dependent manner, compared to control goats, as well as the disappearance in the treated groups of the positive correlation between oestradiol-17β and progesterone concentration shown in the control group, might reflect disturbances at the reproductive system of the goats coming close to their oestrus period. Further research is needed in order to investigate the effects of AFB_1 prolonged administration on the onset and duration of the oestrus period or on the onset and duration of the first oestrus.

CONCLUSION

Conclusively, the long-lasting administration (31 days) of 100 or even of 50 μg AFB_1/day/head increases, in a dose-dependent manner, blood serum oestradiol-17β and progesterone concentrations in Greek indigenous goats and in the treated groups disturbs the positive correlation between oestradiol-17β and progesterone concentration shown in the goats of the control group during the anoestrus period. These results that might be produced by disturbances in the synthesis and production of ovarian steroids due to the direct effect of AFB_1 at the ovaries might reflect disturbances in the reproductive system of goats coming close to their oestrus period.

REFERENCES

Abdulrazzaq Y.M., Osman N., Yousif Z.M., Al-Falahi S. (2003): Aflatoxin M_1 in breast-milk of U.A.E. women. Annals of Tropical Paediatrics, 23, 173–179.

Agnes V.F., Akbarsha M.A. (2003): Spermatotoxic effect of aflatoxin B_1 in the albino mouse. Food and Chemical Toxicology, 41, 119–130.

Akiyama H., Goda Y., Tanaka T., Toyoda M. (2001): Determination of aflatoxins B_1, B_2, G_1 and G_2 in spices using a multifunctional column clean-up. Journal of Chromatography A, 932, 153–157.

Battacone G., Nudda A., Cannas A., Cappio Borlino A., Bomboi G., Pulina G. (2003): Excretion of aflatoxin M1 in milk of dairy ewes treated with different doses of aflatoxin B_1. Journal of Dairy Science, 86, 2667–2675.

Blankenship L.T., Dickey J.F., Bodine A.B. (1982): *In vitro* mycotoxin binding to bovine uterine steroid hormone receptors. Theriogenology, 17, 325–329.

Buhler D.R., Wang-Buhler J.L. (1998): Rainbow trout cytochrome P450s: purification, molecular aspects, metabolic activity, induction and role in environmental monitoring. Comparative Biochemistry and Physiology, 121, 107–137.

Chemineau P., Bodin L., Migaud M., Thiery J.C., Malpaux B. (2010): Neuroendocrine and genetic control of seasonal reproduction in sheep and goats. Reproduction in Domestic Animals, 45, 42–49.

Diekman M.A., Green M.L. (1992): Mycotoxins and reproduction in domestic livestock. Journal of Animal Science, 70, 1615–1627.

Doerr J.A., Ottinger M.A. (1980): Delayed reproductive development resulting from aflatoxicosis in juvenile Japanese quail. Poultry Science, 59, 1995–2001.

El-Nezami H.S., Nicoletti G., Neal G.E., Donohue D.C., Ahokas J.T. (1995): Aflatoxin M_1 in human breast milk samples from Victoria, Australia and Thailand. Food and Chemical Toxicology, 33, 173–179.

Fatet A., Pellicer-Rubio M.T., Leboeuf B. (2011): Reproductive cycle of goats. Animal Reproduction Science, 124, 211–219.

Galvano F., Galofaro V., Galvano G. (1996): Occurrence and stability of aflatoxim M_1 in milk and milk products: a worldwide review. Journal of Food Protection, 59, 1079–1090.

Gopal T., Oehme F.W., Liao T.F., Chen C.L. (1980): Effects of intratesticular aflatoxin B_1 on rat testes and blood estrogens. Toxicology Letters, 5, 263–267.

Goulas P. (1993): Breeding patterns and production of the indigenous goats (*Capra prisca*), kept under extensive and semi-intensive conditions in comparison with the Saanen goats. Ph.D. Thesis, Aristotle University, Thessaloniki.

Hafez A.H., Megalla S.E., Mahmed A.A. (1982): Aflatoxin and aflatoxicosis. III. Effect of dietary aflatoxin on the morphology of buffalo bull spermatozoa. Mycopathologia, 77, 141–144.

Haresign W., Foster J.P., Haynes N.B., Crighton D.B., Lamming G.E. (1975): Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone. Journal of Reproduction and Fertility, 43, 269–279.

Huuskonen P., Myllynen P., Storvik M., Pasanen M. (2013): The effects of aflatoxin B_1 on transporters and steroid metabolizing enzymes in JEG-3 cells. Toxicology Letters, 218, 200–206.

Ibeh I.N., Saxena D.K. (1997a): Aflatoxin B_1 and reproduction. I. Reproductive performance in female rats. African Journal of Reproductive Health, 1, 79–84.

doi: 10.17221/8130-CJAS

Ibeh I.N., Saxena D.K. (1997b): Aflatoxin B_1 and reproduction. II. Gametotoxicity in female rats. African Journal of Reproductive Health, 1, 85–89.

Kourousekos G.D., Theodosiadou E., Ververidis H., Belibasaki S., Boscos C., Saratsis P., Lymberopoulos A.G. (2008): Effect of aflatoxin B_1 administration on serum steroid hormones concentrations of goats. Reproduction in Domestic Animals, 43, 102.

Kourousekos G.D., Theodosiadou E., Belibasaki S., Deligiannis K., Koukoulas Th., Zoulfos K., Lymberopoulos A.G. (2012): Effects of aflatoxin B_1 administration on Greek indigenous goats' milk. International Dairy Journal, 24, 123–129.

Kyrein H.J. (1974): The binding affinity of aflatoxins on the uterine oestrogen receptor. Zeitschrift für Lebensmittel, Untersuchung und Forschung, 154, 285–287.

Martin G.B., Sutherland S.R.D., Lindsay D.R. (1987): Effects of nutritional supplements on testicular size and the secretion of LH and testosterone in Merino and Booroola rams. Animal Reproduction Science, 12, 267–281.

Ortatatli M., Ciftci M.K., Tuzcu M., Kaya A. (2002): The effects of aflatoxin on the reproductive system of roosters. Research in Veterinary Science, 72, 29–36.

Partanen H.A., El-Nezami H.S., Leppanen J.M., Myllynen P.K., Woodhouse H.J., Vahakangas K.H. (2010): Aflatoxin B_1 transfer and metabolism in human placenta. Toxicological Sciences, 113, 216–225.

Piekkola S., Turner P.C., Abdel-Hamid M., Ezzat S., El-Daly M., El-Kafrawy S., Savchenko E., Poussa T., Woo J.C.S., Mykkanen H., El-Nezami H. (2012): Characterisation of aflatoxin and deoxynivalenol exposure among pregnant Egyptian women. Food Additives and Contaminants, 29, 962–971.

Rekkas C., Bellibasaki S., Taitzoglou I., Kokolis N., Smokovitis A. (1991): Increased plasminogen activator activity and plasminogen activator inhibition in spermatozoa and seminal plasma of the ram after serum gonadotrophin (PMSG) administration. Correlation with the increased level of testosterone in the blood. Andrologia, 23, 273–278.

Schmidt R.E., Panciera R.J. (1980): Effects of aflatoxins on pregnant hamsters and hamster foetuses. Journal of Comparative Pathology, 90, 339–347.

Shuaib F.M., Ehiri J., Abdullahi A., Williams J.H., Jolly P.E. (2010): Reproductive health effects of aflatoxins: a review of the literature. Reproductive Toxicology, 9, 262–270.

Storvik M., Huuskonen P., Kyllonen T., Lehtonen S., El-Nezami H., Auriola S., Pasanen M. (2011): Aflatoxin B_1 – a potential endocrine disruptor – up-regulates CYP19A1 in JEG-3 cells. Toxicology Letters, 202, 161–167.

Theodosiadou E., Goulas P., Kouskoura T., Smokovitis A. (2004): Oestrogen and progesterone concentrations in plasma and oviductal tissue of ewes exhibiting a natural or induced oestrus. Animal Reproduction Science, 80, 59–67.

Thieu N.Q., Ogle B., Pettersson H. (2008): Screening of aflatoxins and zearalenone in feedstuffs and complete feeds for pigs in southern Vietnam. Tropical Animal Health and Production, 40, 77–83.

Verma R.J., Nair A. (2002): Effect of aflatoxins on testicular steroidogenesis and amelioration by vitamin E. Food and Chemical Toxicology, 40, 669–672.

Wangikar P.B., Dwivedi P., Sinha N., Sharma A.K., Telang A.G. (2005): Effects of AFB_1 on embryo fetal development in rabbits. Food and Chemical Toxicology, 43, 607–615.

Yuthasastrakosol P., Palmer W.M., Howland B.E. (1975): Luteinizing hormone, oestrogen and progesterone levels in peripheral serum of anoestrous and cyclic ewes as determined by radioimmunoassay. Journal of Reproduction and Fertility, 43, 57–65.

Received: 2014–05–06
Accepted after corrections: 2014–10–23

Corresponding Author

Dr. Georgios D. Kourousekos, Aristotle University, Faculty of Veterinary Medicine, Vyzantos 9, 54633, Thessaloniki, Greece

Phone: +30 6972 268 002, e-mail: kourousekos@gmail.com