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REPRODUCTIVE PERFORMANCE, CLINICO-PATHOLOGICAL FINDINGS AND CYTOGENETIC ANALYSIS IN RABBITS ACCIDENTALLY EXPOSED TO DIETARY AFLATOXICOSIS

By

Selmi A.*, Abdel - Ghaffar A. E. ** and M. E. Abou -Salem ***

Department of Theriogenology (Zagazig* and Moshtohor**) and Department of Forensic Medicine and Toxicology (Moshtohor)***
Fac. of Vet. Med., Zag. Univ.

ABSTRACT

Chronic aflatoxicosis has been diagnosed in New Zealand White (NZW) Rabbits after consumption of contaminated feed pellets. Total concentration of aflatoxins (AF) in the feed pellets was higher (68.15 µg/kg) than the permissible level (25 µg/kg) . AF was also detected in both liver and kidney specmins taken from the affected rabbits . The rabbits exhibited depression , lethargy , inappetance and growth retardation . Necropsy findings revealed congestion in kidneys , spleen and lungs . The liver was either congested or pale and friable . Clinico-pathological examinations revealed cytotoxic , hepatotoxic and nephrotoxic effects of AF. Chronic aflatoxicosis created a problem of infertility in both males and females . It was manifested by reduced litter size, reduced birth, as well as weaning weights , increased perinatal mortalities , and increased number of unestrus does . Conception rate as well as pregnancey rate were also reduced. Spermatogenic disruption and poor semen quality was recorded in males. Colour mutation and developmental abnormalities were also observed in youngs. Cytogenetic analysis revealed that chromosomes of intoxicated rabbits were inflicted with several types of structural and numerical abnormalities . The deletarious effects of AF on animal health, production and reproduction were also discussed.

INTRODUCTION

The harmful effect of aflatoxins (AF) has been demonstrated by a variety of studies . It causes reproductive impairment in livestock, poultry and laboratory animals (***Sharma et al., 1988)*** . Acute or chronic aflatoxicosis can also impair immune responses and natural defense mechanisms with consequent lack of resistance to infection ***(Pier , 1992)*** . The significance of this fungal metabolite does not rest only on its toxicity , but also on the genetic damage it produces in the biological systems of the individual . The available evidence indicates that AF belongs to a definite class of compounds that causes the three forms of genetic damage; including teratogenicity, mutagenicity, and carcinogenicity ***(Wong and Hsich , 1980)*** . Since mutagenicity and carcinogenicity are highly correlated, testing AF for their cytological effects assumes great importance. Aspergillus

spores when used in the culturing media for human lymphocyte induces chromosomal aberrations ***(Jesul and Aroche , 1977)*** . Moreover, inclusion of AF in the culture media used for human leukocyte ***(Dolimpio et al., 1968 , and Promchainant et al., 1972)*** , and blood lymphocyte of water buffaloe ***(Sharma et al., 1991)*** induces several types of chromosomal abnormalities .

It is pertinent to record that there is paucity of information concerning the cytogenetic changes following dietary aflatoxicosis in rabbit . Therefore , the present study aimed to demonstrate the possible cytogenetic alterations in rabbit after being exposed to naturally occuring aflatoxicosis . Reproductive performance, clinico-pathological changes and necropsy findings were also considered.

MATERIALS AND METHODS

New Zealand White (NZW) rabbits belong to private farm at Menia El-Kamah , Sharkia Governorate were utilized in this study. The farm capacity includes; 87 reproducing females (10 - 16-month-old) that gave bith for 2 - 6 times, 17 bucks (8 -12-month-old) and numerous growing rabbits of different ages. Rabbits were housed in individual cages of commercial type that provided with nest- boxes , feeders , and automatic drinkers . The cages were stabilized in a naturally ventilated building provided with electric fans . A decline in the fertility of rabbits was recorded during the period from March to April, 1996. It was manifested by reduced litter size , increased perinatal mortality and increased number of unestrous does. Conception as well as pregnancy rates also decreased. Necropsy findings beside colour mutation and deformities observed in youngs were strongly suggestive of aflatoxicosis . Thus , ten randum samples of the used pelleted feeds were collected and analyzed for detection of AF . Liver and kidney samples (10 each) were also taken at necropsy and were extracted and prepared for AF determination . Extraction of AF from animal feeds and tissue samples was carried out using multimycotoxin detection method as discribed by ***Patterson and Roberts (1979)*** . AF was determined qualitatively by Thin Layer Chromatography (TLC) and measured quantitatively under long (365 nm) and short waves (254 nm) of ultra violet light against standard AF according to ***FAO (1990)*** .

Heparinized blood for haematological examination was collected from severely affected parents (5 each) . Packed cell volum (PCV), total erythrocytic (RBCs) , leukocytic (WBCs) , and differential leukocytic counts ***(Schalm , 1986)*** were calculated . Sera were also collected from those rabbits for calorimetric determination of serum total proteins ***(Weichselbaum, 1946)*** , albumin ***(Bartholomer and Delancy, 1966)*** , globulin (by substracting albumin from total proteins). Serum immunoglobulins were determined using Kallested Endoplate immunodiffusion method as discribed in the kits (Sanofi Diagnostic Pasteur Inc., Chaska , MN, USA). Liver function was also monitored for those rabbits through calorimetric determination of serum levels of GOT , GPT and alk. phosphatase (ALP) using test kits (Biomerieux, Bains France) after the method adopted by producer. However, renal function was checked through determination of serum

urea levels ***(Fawcett and Scott , 1960)***. Both haematological and biochemical analysis were done twice , at the first visit and repeated 5 weeks later after changing the contaminated feeds.

In order to check-up the fertility of bucks used for breeding at that farm , semen samples were collected and examined after ***Zemjanis (1962)***. Males exhibited extensive abnormalities and poor semen quality were replaced by sound ones of good semen quality . However, some of them (10) were subjected for weekly semen analysis to monitor seminal changes after changing the contaminated diet . Meanwhile , bucks showed aspermia were discarded .Some of them (3) were slaughtered and their testes and epididymedes were excised , fixed and prepared for histopathological examinations .

For cytogenetic analysis , heparinized whole blood samples were aseptically collected from 5 infertile bucks (had poor semen quality) and 15 infertile females (exhibiting either reduced litter size, 5; still birth , 5 ; or unestrum for long period ,5). Blood lymphocytes were then cultured and prepared for chromosomal analysis according to the procedures described by ***Badawy (1995)*** . Bone marrow technique ***(Badawy, 1995)*** for cytogenetic analysis was also performed for offsprings showing malformtions (5), colour mutation (5) and stunting (5) . Fertile bucks (2), fetile females (2) and normal offsprings (2) were included in the analysis as controls. Spread metaphase cells (50 per animal) were examined under oil immersion lens. The chromosomal aberrations were scored according to the method described by ***Brusick (1980)*** . Selected metaphase spreads were photographed undr oil immersion lens (X 10 x 100) .

Data were statistically analyzed using Statistical Analysis System"SAS" (1987).

RESULTS

1 - Clinical Observations :

Rabbits at that farm exhibited depression , lethargy and inappetence. The amount of feed consumped was reduced when compared with the previous healthy records (400 gm vs 1115 gm / animal / week) . Water intake , on the other hand, increased (2700 ml vs 1900 ml / animal/ week) . Rectal temperature was nearely normal (39 $\pm$ 0.5°C) and respiration too (130 $\pm$ 10/ min.). Morbidity rate among parents was very high (95%), while mortality was very low. Whereas, perinatal and preweaning mortalities reached 80% among offsprings. Records of monthly parturition were greatly reduced and the number of pregnant rabbits was sharply decreased (25%) with increased number of unestrus does . Litter size ranged from 3 to 5 at birth and 1 to 2 at weaning . Average bunny weight generally lower than the previously recorded values . It was 40 vs 52 gm at birth and 210 vs 420 gm at weaning (5 -week-old) . Moreover, bunnies of the same litter had variable sizes and weights and acquired different abnormalities . Colour mutation (black vs normal white) , incomplete ossification of the cranial bones with or without hydrocephalus, and muscle contracture in the fore limbs of bunnies (Fig.1-3) were

noticed. Nervous manifistation and incoordinated movements were observed in newborns . Those offsprings were unable to reach their mother's breast with consequent high mortality rate (100%) . Keratitis , keratoconjunctivitis , diarrhea and dehydration were frequently noticed among parents , growing rabbits , as well as newborns. However, growth retardation was prominent in growing rabbits .

2 - Necropsy Findings :

Postmortem examinations conducted on rabbit carcases revealed congestion in kidneys , spleen and lungs . The liver was either congested or pale and friable . The heart was filled with dark clotted blood. Mesentric edema was also noticed and the peritoneal cavity contained sangouneous fluids. Mild to severe degree of hydrocephalus with incomplete ossification of the cranial bones were recorded in newborns . Cerebrospinal fluid in those cases was tanged with blood .

3 - Feed and Tissue Analysis :

Types and concentrations of AF in animal feeds (contaminated and replaced fresh feeds) and tissues (liver and kidney) are shown in table 1. Total AF concentration was significantly higher in contaminated feeds than in replaced fresh ones (68.15 ± 14.44 vs 11.46 ± 1.84 µg/kg) . Levels and occurence of AFB1 predominated AFB2 and G1 in both contaminated and fresh feed pellets . Residual amount of AF was significantly high in liver samples when compared with that detected in kidney samples (17.3 ± 2.98 vs 2.40 ± 0.38 ng/ gm) . Residual amounts of AFB_1 was also predominating.

4 - Treatment and Response :

The treatment included regular cleaning and disinfection of the farm . The contaminated diet was changed with sound one's (which chicked also for AF contamination) .The owner was instructed to purchase freshly formulated feed that smells good and not to store too much feed under our climate. The feed store must be dry and well ventilated . Rabbit premix containing vitamines and menirals necessary for normal physiology was added to feeds at 0.3% concentration. Hydrated sodium calcium aluminosilicate was added at 0.5 % concentration in the feed to adsorb the possible AF contamination. Infertile bucks were also replaced with sound one's.

Clinical recovery was acheived by the 7th day of treatment as mentioned by the owner. Feed intake consequently increased (900 gm / animal / week) and percentage of pregnant does, 45 days after treatment , increased (50%) . However, litter size slightly increased (5 - 6) with increased bunny's birth weight (45 gm). Fetal abnormalities and perinatal mortalities were generally reduced . For example , hydrocephalus and limb deformities no longer observed .

5 - Clinico-Pathological Findings :

Total RBCs count's and PCV (Table 2) were reduced indicating that dehydration and haemoconcentration that may be created by aflatoxicosis were

Table (1) : Types and concentrations of AF detected in animal feeds and tissues (Mean ± SEM).

Tissue	No. of samples	AFB_1	AFB_2	AFG_1	AFG_2	AFM_1	Total AF
A- Animal feed (µg/kg)							
1 - Contaminated feed	10	35.24 ± 7.60^{a} (0.00- 69.22)	8.81 ± 4.76^{bc} (0.00 - 39.53)	24.13 ± 9.59^{ab} (0.00 - 82.17)	0.00 ± 0.00^{d} --	0.00 ± 0.00^{d} --	68.15 ± 14.77^{A} (18.39 - 121.54)
2 - Replaced fresh feed	10	5.52 ± 1.73^{bc} (0.65 - 17.98)	1.97 ± 1.06^{c} (0.00 - 8.63)	3.57 ± 1.71^{c} (0.00 - 13.98	0.00 ± 0.00^{d} --	0.00 ± 0.00^{d} --	11.46 ± 1.84^{B} (1.18 - 22.95)
B - Tissue residues (ng/gm)							
1 - Liver	10	6.25 ± 1.91^{a} (1.42 - 18.82)	2.78 ± 1.04^{ab} (0.68 - 11.48)	3.61 ± 1.43^{ab} (0.66 - 13.49)	0.00 ± 0.00^{d} --	4.49 ± 0.77^{a} (1.46 - 10.18)	17.13 ± 3.58^{A} (6.36 - 32.06)
2 - Kidney	10	0.89 ± 0.29^{bc} (0.12 - 3.08)	0.38 ± 0.16^{c} (0.12 - 1.04)	0.48 ± 0.12^{c} (0.12 - 1.28)	0.00 ± 0.00^{d} --	0.66 ± 0.17^{a} (0.28 - 1.68)	3.40 ± 0.38^{B} (0.98 - 4.42)

Means with different small or capital superscripts in the same category (Animal feed or tissue) significantly (P<0.05 - 0.01) different from each others.
Figures in parenthesis indicate the range values of AF.

Table (2) :Some haematological values (Mean ± SEM) in the affected rabbits at the first observation and 5 weeks after treatment.

Items	First Analysis	Second Analysis
PCV (%)	40 ± 2.55	35 ± 3.50
RBCs (x 10^6/ml)	6.18 ± 0.8	5.5 ± 0.57
WBCs (x 10^3 /ml)	5.2 ± 0.54	6.8 ± 0.60
Heterophil (%)	25.7 ± 1.45	23.45 ± 1.4
Eosinophil (%)	1.85 ± 0.35	1.68 ± 0.35
Basophil (%)	0.87 ± 0.30	0.63 ± 0.25
Monocyte (%)	4.83 ± 0.88	4.40 ± 0.37
Lymphocyte (%)	66.77 ± 0.6	69.85 ± 1.33

Table (3) : Some biochemical values (Mean ±SEM) in the sera from affected rabbits at the first observation and 5 weeks after treatment.

Items	First Analysis	Second Analysis
T. Protein (gm/dl)	5.7 ± 0.10^{a}	6.2 ± 0.20^{a}
Albumin (gm/dl)	2.9 ± 0.09^{a}	3.2 ± 0.17^{a}
Globulin (gm/dl)	2.8 ± 0.05^{a}	3.0 ± 0.14^{a}
IgA (mg/dl)	66.28 ± 0.64^{a}	68.39 ± 0.67^{a}
IgG (mg/dl)	376.11 ± 3.76^{b}	381.36 ± 3.81^{a}
IgM (mg/dl)	49.77 ± 0.89^{a}	51.6 ± 0.53^{a}
Urea (mg/dl)	40.5 ± 1.5^{a}	35.2 ± 1.2^{b}
GOT (iu/L	84.56 ± 3.78^{a}	64.2 ± 5.7^{b}
GPT (iu/L)	42.12 ± 2.12^{a}	33.85 ± 2.30^{b}
ALP (iu/L)	80.4 ± 2.19^{a}	72.6 ± 1.9^{b}

Means in the same row with different superscripts was statistically different (P<0.05).

releaved following treatment. Whereas, total WBCs count's (especially lymphocytes) increased . Moreover, serum total protein, albumin, globulin and immunoglobulins (Table 3) were improved by treatment. However, serum levels of urea , GOT, GPT and ALP (Table 3) were reduced ($P<0.05$) indicating some sort of recovery from aflatoxicosis .

6 - Semen Analysis :

Affected bucks exhibited poor semen quality at the first examination (table 4). However, semen analysis conducted at weekly intervals for 5 times after treatment showed a progressive improvement in semen quality .

Ejaculate volume was consistently higher in the first, compared with the second ejaculate . However, the overall volume of ejaculate significantly increased in the third analysis , but reduced in the next times. Individual motility , on the other hand , in the second ejaculate was generally higher than in the first ejaculate . Whereas , the overall mean of individual motility was significnatly higher at the last examination (5 weeks after treatment) when compared with the previously recorded weekly values. Moreover, live spermatozoa were progressively increased and the highest percentages was recorded at the last examination . Sperm cell concentration was also improved by the fifth week of treatment . Primary sperm cell abnormalities were frequently observed than secondary ones and the middle peice was much affected (Fig. 4). Whileas , total sperm cell abnormalities was much reduced by the fifth week of treatment.

7 - Testicular and Epididymal Histopathology :

Histopathological examinations of the testis and epididymis of the aspermic bucks revealed complete arrest of spermatogenesis . Advanced stages of testicular degeneration was observed. Vaculated seminiferous epithelium and complete absence of spermatozoa were noticed in most tubules (80%). Cellular association characteristic of spermatogenic cell cycle was no longer observed and the seminiferous epithelium consisted of hyperchromatic spermatogonia and few spermatocytes (Fig. 5). Some other tubules were only lined with spermatogonia. Interstitial Leydig's cells showed degenerative changes . Interstitial blood vessels appeared congested and the intertubular connective tissue showing edema (Fig. 6).

Epididymal tubule in the head and tail regions was void of spermatozoa (Fig. 7 & 8). Leukocytic infeltration was observed inside the lumen of the epididymal tubule in the head region . The lining epithelium was thrown into folds in the tail region of the epididymis. Edema was also noticed in the peritubular connective tissue of the epididymis.

8 - Cytogenetic Analysis :

Normal chromosomes of rabbit ($2n = 44$) are shown in Fig. 9 . However, the chromosomes of the examined animals were inflected with several types of aberrations . They included both numerical as well as structural abnormalities (Table 5) . Structural aberrations were isochromatid gap (Fig. 10), centromeric break or attenneuation (Fig. 10),dicenteric chromosome (Fig. 11), end to end asso-

Table (4) : Some semen characteristics (Mean ± SE). of intoxicated bucks examined before and immediately after treatment at weekly intervals for 5 weeks.

Semen characters	1st Analysis	2nd Analysis	3rd Analysis	4th Analysis	5th Analysis	6th Analysis
Ejac. Vol . (ml))						
1 st (10)	0.86 ± 0.03^{b}	0.78 ± 0.02^{c}	0.98 ± 0.03^{a}	0.85 ± 0.02^{b}	0.66 ± 0.02^{d}	0.73 ± 0.03^{cd}
2nd (10)	0.54 ± 0.02^{fg}	0.64 ± 0.03^{de}	0.58 ± 0.01^{ef}	0.49 ± 0.02^{g}	0.42 ± 0.02^{h}	0.58 ± 0.01^{f}
overall (20)	0.70 ± 0.02^{B}	0.71 ± 0.02^{B}	0.78 ± 0.01^{A}	0.67 ± 0.01^{BC}	0.54 ± 0.01^{D}	0.65 ± 0.01^{C}
Ind. motility (%)						
1 st (10)	38.42 ± 0.46^{h}	39.36 ± 0.35^{h}	42.18 ± 0.49^{g}	41.59 ± 0.46^{g}	66.38 ± 0.52^{c}	73.44 ± 0.78^{b}
2nd (10)	43.65 ± 0.38^{fg}	42.52 ± 0.41^{fg}	46.36 ± 0.48^{e}	48.64 ± 0.49^{d}	72.43 ± 0.81^{b}	78.63 ± 0.86^{a}
overall (20)	41.04 ± 0.34^{D}	40.94 ± 0.34^{D}	44.27 ± 0.44^{C}	45.12 ± 0.44^{C}	69.41 ± 0.43^{B}	76.04 ± 0.59^{A}
Live sperm (%)						
1 st (10)	42.86 ± 0.68^{g}	43.98 ± 0.46^{g}	45.48 ± 0.56^{f}	44.63 ± 0.68^{fg}	71.46 ± 0.72^{c}	76.28 ± 0.98^{b}
2nd (10)	46.28 ± 0.62^{ef}	47.88 ± 0.58^{de}	48.18 ± 0.48^{d}	48.82 ± 0.52^{d}	75.82 ± 0.61^{b}	81.46 ± 0.89^{a}
overall (20)	44.57 ± 0.54^{D}	45.93 ± 0.42^{CD}	46.83 ± 0.41^{C}	46.73 ± 0.41^{C}	73.64 ± 0.53^{B}	78.87 ± 0.73^{A}
Sperm cell conc . ($x10^{6}$/ml)						
1 st (10)	287.15 ± 9.11^{gh}	311.41 ± 7.18^{efg}	276.71 ± 11.42^{h}	298.76 ± 8.11^{fgh}	318.52 ± 11.12^{def}	384.68 ± 15.73^{b}
2nd (10)	352.41 ± 8.32^{bc}	344.51 ± 7.96^{cd}	328.62 ± 8.86^{de}	346.48 ± 9.12^{cd}	366.44 ± 8.76^{bc}	446.83 ± 18.22^{a}
overall (20)	319.78 ± 7.98^{CD}	327.96 ± 6.98^{BC}	302.67 ± 8.44^{D}	322.62 ± 7.91^{BCD}	342.48 ± 7.38^{B}	415.76 ± 13.82^{A}
Primary sperm cell abn.(%)						
1 st (10)	16.88 ± 0.38^{b}	18.36 ± 0.46^{a}	14.92 ± 0.58^{cd}	15.82 ± 0.86^{bcd}	9.84 ± 0.24^{e}	8.16 ± 0.29^{f}
2nd (10)	15.56 ± 0.33^{e}	16.98 ± 0.54^{ab}	13.84 ± 0.69^{d}	13.92 ± 0.78^{cd}	7.66 ± 0.26^{f}	6.35 ± 0.21^{g}
overall (20)	16.22 ± 0.26^{B}	17.67 ± 0.38^{A}	14.38 ± 0.48^{C}	14.87 ± 0.62^{BC}	8.75 ± 0.22^{D}	7.26 ± 0.18^{E}
Secondary sperm cell abn . (%)						
1 st (10)	12.68 ± 0.28^{bc}	14.82 ± 0.42^{a}	13.68 ± 0.46^{ab}	12.46 ± 0.48^{bc}	9.18 ± 0.28^{e}	7.86 ± 0.18^{f}
2nd (10)	11.74 ± 0.36^{cd}	12.56 ± 0.32^{bc}	10.98 ± 0.34^{d}	11.18 ± 0.36^{d}	7.28 ± 0.26^{f}	6.12 ± 0.15^{g}
overall (20)	12.21 ± 0.24^{B}	13.69 ± 0.28^{A}	12.33 ± 0.32^{B}	11.82 ± 0.34^{B}	8.23 ± 0.18^{C}	6.99 ± 0.12^{D}
Total sperm cell abn . (%)						
1 st (10)	14.78 ± 0.42^{b}	16.59 ± 0.39^{a}	14.30 ± 0.42^{bc}	14.14 ± 0.46^{bc}	9.51 ± 0.21^{e}	8.01 ± 0.16^{f}
2nd (10)	13.65 ± 0.31^{c}	14.77 ± 0.26^{b}	12.41 ± 0.32^{d}	12.55 ± 0.34^{d}	7.47 ± 0.18^{g}	6.24 ± 0.14^{h}
overall (20)	14.22 ± 0.28^{B}	15.68 ± 0.25^{A}	13.36 ± 0.28^{C}	13.35 ± 0.32^{C}	8.49 ± 0.16^{D}	7.12 ± 0.12^{E}

Means with different small superscripts (for both 1st and 2nd ejaculates) or capital superscripts (for the overall) in each category are significantly ($P<0.05$ - 0.01) different from each other .

Figures in parenthesis are the number of examined ejaculates .

Table (5) :Types and frequency (%) of chromosomal aberrations in the examined rabbits .

Examined groups	Total metaph-ase examin-ed	Metaph-ase having chr. aber.(%)	Structural Aberrations (%)									Numerical Aberrations	
			Isochromatid gap	Chromatid gap	Chromatid break	Centromeric break	end to end assoc.	Sticky chrom-osome	Pulver-ization	Dicentric chrom-osome	Ring chromo-some	Hypo-plobly	Poly ploidy
A-Infertile bucks	250	31	9	8	3	10	2	1	-	1	-	-	-
B-Infertile females showing:													
1 - Reduced litter size	250	35	7	9	4	11	1	3	-	1	-	-	1
2 - Still birth	250	28	5	7	3	9	-	1	-	-	-	-	-
3 - Unestrum	250	21	6	4	5	6	1	2	-	-	-	-	-
C -Offsprings showing :													
1 -Malformations *	250	41	11	7	8	12	1	4	2	-	-	1	2
2 - Colour mutation	250	28	4	3	5	13	-	3	1	-	-	1	2
3 - Stunted growth	250	40	5	9	9	11	-	2	4	-	-	3	1
D - Controls**	300	6	-	1	2	3	1	1	-	-	1	-	-

* Malformations included those rabbit showed deformed limbs.
** Belong to rabbit farm of known history at Faculty of Agriculture (Moshtohor), Zag. Univ.

ciation, and contracted sticky chromosomes (Fig.12), chromatid break or delation (Fig.13), pulverization (Fig. 14). Numerical aberrations included hypoploidy (Fig.15) and polyploidy (Fig.16).The frequency of chromosomal aberrations observed in the examined animals showed no consistent pattern , but was different even within the same group.

LEGENDS FOR ILLUSTRATION

Fig.1-3: Photographs showing:1 - A still borne dead litter of different sizes. 2 - Two litters of different ages, the young litter (one- week - old) exhibited muscular incoordination, hydrocephalus and colour mutation , while the older one (6-weeks - old) exhibited different sizes and malformed limbs. 3 - A litter showed limb deformities in two of them .

Fig. 4 : Some sperm cell abnormalities including middle piece abnormalities (a) , abaxially attached middle peice (b) detached head (c) and dead sperm (d) (Eosin & Nigrosin X 10 x 100).

Fig. 5 : Photomicrograph (Photo.) for the testis of aspermic males showing marked depletion and vaculation of the seminiferous epithelium (H&E X 10 x 40).

Fig. 6 : Photo. for the testis showing peritubular edema (H&E X10 x 10).

Fig. 7 : Photo. for the epididymal head showing intratubular leukocytic infeltration and absence of spermatozoa as well as intertubular edema (H & E X 10 x 10).

Fig. 8 : Photo. for the epididymal tail showing absence of spermatozoa. intertubular edema , and the lining epithelium was thrown into folds (H & E X 10 x 10).

Fig. 9 : Photo. for nearly normal metaphase spread (M.S.) showing the diploid number (2n = 44) of chromosomes (Giemsa X 10 x 100).

Fig.10 : Photo. for M.S. showing isochromatid gap (a) and centromeric break (b) (Giemsa X 10 x 100) .

Fig.11 : Photo. for M.S. showing dicentric chromosome (a) and chromatid gap (b) (Giemsa X 10 x 100).

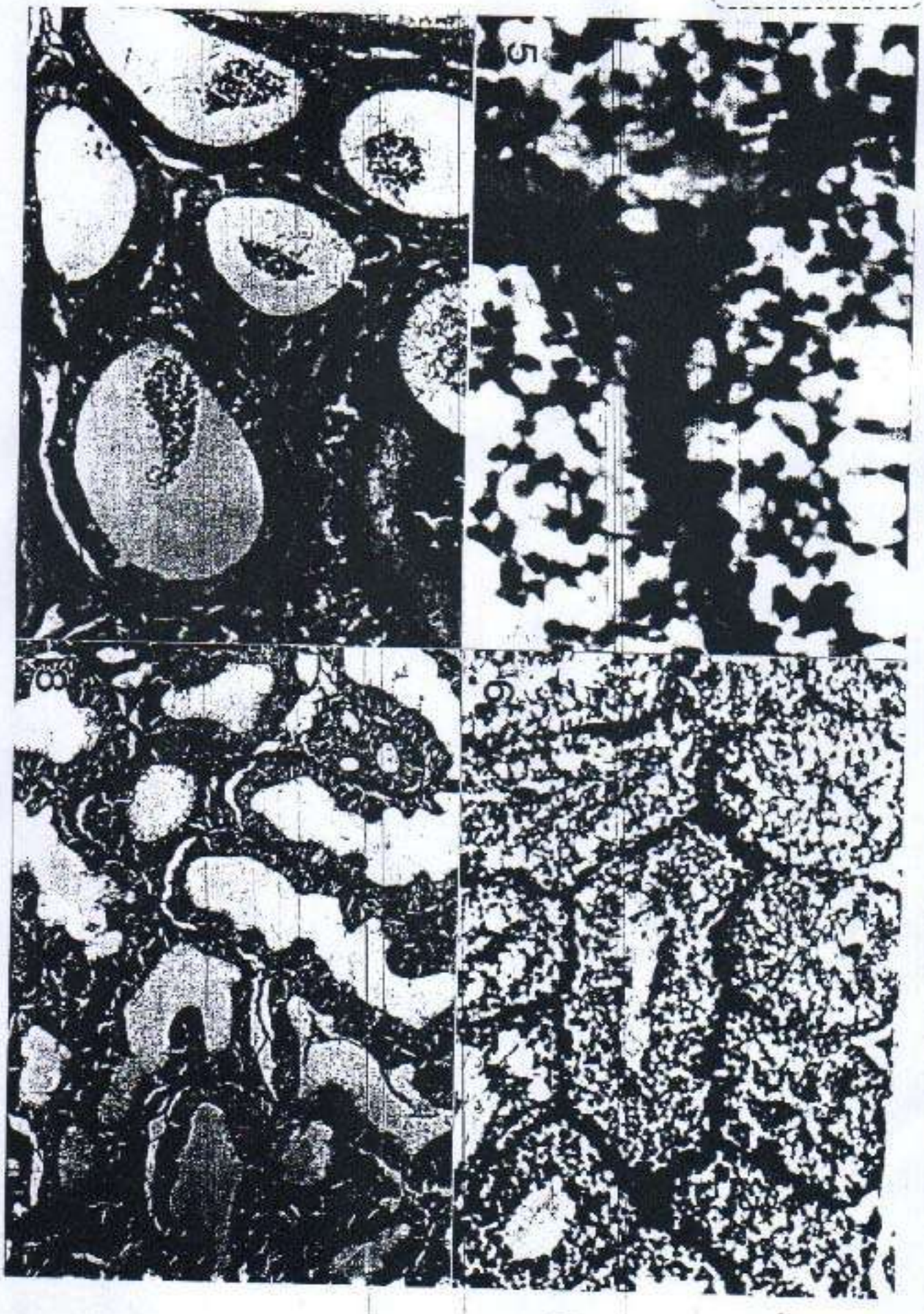
Fig.12 : Photo. for two spread metaphases showing normal spread (a) and sticky chromosomes (b) (Giemsa X 10 x 100).

Fig.13 : Photo. for M.S. showing contracted chromosomes (a) and chromatide break or deletion (b) (Giemsa X 10 x 100).

Fig.14 : Photo. for M.S. showing pulverization (Giemsa X 10 x 100)

Fig.15 : Photo. for M.S. showing hypoploidy "Haploid No. of chromosomes" (Giemsa X 10 x 100) .

Fig.16 : Photo. for M.S. showing polyploidy "Tetraploidy" (Giemsa X 10 x 100)



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DISCUSSION

Acute AF poison causes a distinct overt clinical disease marked by hepatitis , icterus , haemorrhage, and death (***(Pier , 1992)*** . Although acute intoxication can be dramatic, the biological and economic consequences of the more usual chronic form caused by lower levels of mycotoxins are drastic ***(Pier , 1981)*** . Chronic aflatoxicosis was characterized by reduced rate of gain in youngs, immune system depression and the acquired immunity from vaccination programs may be substantially suppressed ***(Pier , 1991)*** . In such cases , the signs of disease observed are those of infectious process rather than those of aflatoxicosis that mainly predispose the animal to infection. Thus , signs of chronic form are so protean that the condition goes undiagnosed for long periods and the economic consequences are often considerable .

Reduced litter size , increased perinatal mortality , growth retardation , colour mutation , and fetal deformities observed at the rabbit farm under investigation led us to postulate that we are facing a problem of chronic aflotoxicosis . This suggestion was confirmed following demonstration of higher level of AF in the feed pellets (68.15 µg/ kg) than the level (25 µg/kg) permitted by ***FDA (1989)*** . AF was also detected in the liver and kidney specimens taken from dead rabbits . Haematological , clinico-pathological , and necropsy findings added another support for our hypothesis . Hepatotoxic effect of chronic aflatoxicosis was manifested by reduced serum albumin (a protein produced mainly by the liver) concentration. However,nephrotoxic effect of aflatoxicosis was evidenced by increased serum urea level . Moreover, the cytotoxic effect of aflatoxicosis , in general, was evident because of reduced leukocytic count (especially lymphocytes) and increased GOT, GPT, and ALP level in the sera of the affected rabbits . These results are consistant with those reported by ***Clark et al. (1980) , Angsubhakorn et al. (1981) , Ray et al. (1986) , and Pier (1992)*** . Whereas, reduced serum levels of IgA, IgG, and IgM in rabbits reflicts the immunosuppressive effect of AF which was demonstrated by ***Pier (1991)***.

Reduced litter size , reduced birth weight , growth retardation , increased perinatal mortality and unestrum recorded in our study coincide with those reported in rats ***(Butler and Wigglesworth , 1966 ; Sharma et al., 1988; and Zaghloul , 1995)*** . They concluded that the rate of intrauterine fetal death and severity of stunting were proportionally related to the dose of AF as well as the extent of maternal liver injury . However, ***Sharma et al., (1988)*** could not explain the manner by which AF reduces the number of corpora lutea and increases the pre and postimplantation losses . Moreover, ***Blankenship et al., (1982)*** do not know the exact mechanism , where by AFB1 and AFM1 inhibit gonadotropin secretion and consequent infertility in bovine . Our explanation , on the other hand , may related to reduced feed intake by intoxicated animals. This explanation was confirmed by the findings of ***Armstrong and Britt (1987)*** . They found that chronic restriction of feed intake had resulted in unestrus and decreased the frequency of LH pulses in gilts. Whereas , flushing of gilts induced a significant increase in insulin , FSH and LH pulses with consequent increase in ovulation rate ***(Flowers***

et al., 1989). Effect of feeding may, therefore , modulate ovulation rate by lessening or increasing follicular atrasia *(Dailey et al., 1975).* Moreover, ***Pond (1973)*** recorded that the birth weight of piglets was reduced and the postnatal growth remains depressed when the mother fed a low energy and protein diffecient diet during pregnancy. Therefore, unestrum , reduced litter size, reduced birth weight , growth retardation and increased perinatal mortality observed in our study may be due to reduced feed intake recorded for rabbits after being exposed to chronic aflatoxicosis . This explanation do not exclude the cytotoxic effect of AF, since the ovaries of hens developed pathological lesions and egg production was ceased after feeding a mouldy ration containing AFB_1 and AFG_1 *(Hafez et al ., 1982).*

The influence of male on litter size was studied by ***Finn (1963)*** . He concluded that litter size was a male trait and not related to male - female interaction . Our study , on the other hand , showed an increase in litter size following replacement of infertile bucks with sound ones. Further improvement in litter size was also noticed after changing the contaminated diet and addition of premix. Thus reduced litter size and rabbit infertility observed in our study may related to male - female interaction . Similar observation was noticed in rats by ***Sharma et al. (1988)*** . They reported that the fertility of female rats was impaired when treated with AF or when sired by male treated with AFB_1 and B_2.

Our results revealed that chronic aflatoxicosis had also resulted in spermatogenic disruption . It was reflected on semen picture and evidenced by histopathological examinations of the testis and epididymis of the affected rabbits . However, semen analysis showed that sperm cell concentration , motility and liveability were improved , while sperm cell abnormalities were reduced with the lapse in time following application of our recommendations. These results are consistant with those recorded in rabbits *(Hafez et al., 1983)* , rats *(Sharma et al., 1988)* , and bufaloe bulls *(Hafez and Megalla , 1982)* following AF administration . Moreover, fertility impairment recorded in male rats was positively correlated to the dose of AF administered *(Sharma et al., 1988).* They found that the number of implantation was decreased and the mortalities of implanted zygots was increased in female rats sired with those treated males during the first week. However, the number of dead implants were relatively lower in females mated with those males in the subsequent weeks . Thus reduced litter size and increased perinatal mortalilies observed in our study may be due to the deletarious effects of AF on both male and female reproduction.

Cytogenetic analysis recorded in our study added further explanation for infertility, reduced litter size, increased perinatal mortalities , mutations and fetal deformities observed at the farm under investigation . Chromosomes of the examined rabbits were inflected with several types of aberrations . They included both numerical as well as structural abnormalities . Our results were in agreement with those reported after AF inclusion in the culturing media for human leukocytes *(Dolimpio et al., 1968 ; Promchainant et al., 1972)* and blood lymphocytes of

water buffalo *(Sharma et al., 1991)*. The last study concluded that the frequency of chromosomal aberrations was positively correlated with the dose of AFB1 as well as its incorporation time in the culturing media prior to harvest.

It has been shown that AF increases the activity of thiamidine kinase and subsequently depressed protein and DNA synthesis *(Childs and Legator, 1966)*. Mutagens produce some sort of physico-chemical stress which possibly breaks the chromosome at vulnerable points *(Manna and Bardhan, 1973)*. Recently, AF binds to both RNA and DNA and blocks transcription thereby interfere and inhibit their synthesis *(Pier, 1992)*. Based on these reports, chromosomal protein deficiency was suggested upon aflatoxicosis and the protein-deficient weak regions may consequently developed structural aberrations. However, numerical aberrations (aneuploidy) observed in our study may be attributed to abnormality in sequential centromere separation or centromeric atteneuation recorded in the present study.This explanation was consistant with that reported by *Baldev (1991)*. He stated that failure of centromere to separate at late metaphase as well as premature separation of a centromere lead to the failure of chromatids to segregated properly with consequent hypo or polyploidy. Meanwhile, tetraploidy recorded here may be due to arrested mitosis in metaphase with consequent duplication of the chromosomal set inside the nondividing cells. These gaint cells was also observed by *Legator et al., (1965)* in cultures of human lung cells exposed to AFB1 (1 µg/ml). They postulated that gaint cell formation could be due to enlargement of nondividing cells which might be associated with arrested mitosis.

Chromosomal abnormalities have been described in a significant percentage of embryos from normal sows as a cause of infertility or reduced litter size *(Long, 1991)*. Aneuploid in two related boars were associated with reduced litter size in their offsprings as well as some of their daughters *(Vogt et al., 1974)*. In the mouse crosses, the presence of seven translocation chromosomes in the heterozygous state leads to the formation of aneuploid gametes which can fertilize, but resulted in the formation of unbalanced embryos which die *(Tettenborn and Gropp, 1970)*. Robertsonian 1/29 translocation was associated with infertility problem in Swedish cattle that was characterized by early embryonic deaths *(Gustavsson, 1969 and Dain et al., 1985)*. Moreover, *Badawy (1995)* recorded several types of chromosomal aberrations in association with some reproductive trouples in rabbits.

Based on the forementioned studies, it could be concluded that chronic aflatoxicosis would be the immediate cause of chromosomal aberrations observed in the present study. Chromosomal abnormalities, in turn, may account for reduced litter size, colour mutation, fetal deformities, perinatal losses, and infertility recorded in our study. However, growth retardation may be due to the adverse toxic effects of AF on the living individuals.

Mycotoxins, in general, are considered unavoidable contaminants in foods and feeds because the agronomic technology could not absolutely eliminate

mycotic infection from susceptable crops. Thus , AF promise to be a continuing problem. and other adverse effects on both animal and human health may be explored in the future as investigations continue . In order to minimize the adverse effects of AF, the following measures showed be considered : 1 - Regular cleaning and disinfection of feed store , buildings and cages . 2 - Animal feeds should be regularly checked for fungal contamination and the contaminated feeds must be promptly changed with freshly formulated sound one's. 3 - Addition of hydrated sodium calcium aluminosilicate at 0.5% in the animal feed to adsorb the possible AF contaminants. 4 - Addition of premix containing necessary vitamins and menirals at 0.3% in the animal feeds to restore and improve the animal performance .

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الملخص العربى

الأداء التناسلى ، المشاهدات الأكلينيكية والباثولوجية ودراسة الوراثات الخلوية (الكروموسومات) فى الأرانب بعد تغذيتها بطريق الخطاء على عليقة ملوثة بالسموم الفطرية

عاطف عبدالحى خليل سلمى * ، علاء عبدالغفار * * ، محمد أبوسالم ***

قسم الولادة بالزقازيق * ومشتهر ** وقسم الطب الشرعى والسموم بمشتهر ***

كلية الطب البيطرى – جامعة الزقازيق

تم تشخيص حالة تسمم مزمن فى مزرعة للأرانب النيوزيلاندى الأبيض بعد أستخدام عليقة ملوثة بالسموم الفطرية (بطريق الخطاء) . تركيز السموم الفطرية فى العليقة الملوثة كان أعلى (١٥٫٦٨ ميكروجرام / كجم عليقة) من التركيز المسموح به (٣٥ ميكروجرام / كجم عليقة). كما أن عينات الكبد والكلى المأخوذة من الحيوانات المسممة كانت تحتوى أيضاً على السموم الفطرية . الصفة التشريحية فى الحيوانات المصابة أظهرت وجود إحتقان فى الكلى والطحال والرئة . الكبد كان أيضاً محتقن أو أصفر باهت وهش . التحاليل الإكلينيكية والباثولوجية أظهرت التأثير السام على خلايا الجسم والكبد والكلى . كما أن الخصوبة فى الأرانب قد أنخفضت وظهر ذلك فى إنخفاض عدد الأجنة فى كل ولادة ، إنخفاض وزن الأجنة المولودة ، إنخفاض وزن الفطام وزيادة عدد الولادات الناقة . كما أن عدد الأمهات التى لم تظهر علامات الشبق زادت مع إنخفاض معدلات الخصوبة والحمل فى الأمهات.

نوعية السائل المنوى فى الذكور المسممة كانت سيئة للغاية . كما أن الفحوص الهستوباثولوجية للخصية والبربخ فى بعض الأرانب أظهرت إستحالات وتنكرز في أنسجة الخصية وتوقف فى عملية تكوين الحيامن . أما الولادات (الصغار) فقد ظهرت طفرة فى اللون وتشوهات خلقية عديدة . تحليل الوراثات الخلوية (الكروموسومات) أظهر وجود عديد من التشوهات الكروموسومية سواءاً في التركيب أو فى العدد.

التأثير الضار للسموم الفطرية على صحة وإنتاجية الحيوان وتناسلة تمت مناقشتها.