SYNERGISTIC EFFECT OF ALFATOXIN B1 AND OCHRATOXIN A IN CHICK EMBRYOS

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Veterinary Science

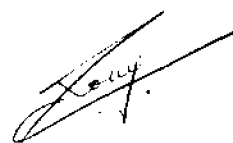Faculty of Veterinary and Animal Sciences
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DECLARATION

I hereby declare that the thesis entitled **"SYNERGISTIC EFFECT OF AFLATOXIN B1 AND OCHRATOXIN A IN CHICK EMBRYOS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"SYNERGISTIC EFFECT OF AFLATOXIN B1 AND OCHRATOXIN A IN CHICK EMBRYOS"** is a record of research work done independently by Ms. Rony Ray John under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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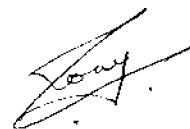
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Rony Ray John

DEDICATED

TO MY

PARENTS

AND TEACHERS

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Introduction

1.INTRODUCTION

The poultry industry in India has grown by leaps and bounds in the past two decades and its position as a high flying industry remains unchallenged. Efficiently managed hatcheries form the backbone of the industry, the measure of success being the number of first quality chicks produced. Embryo mortality, embryo malformations and reduced hatchability rates are the major obstacles faced. Over the years, research has been carried out to consolidate knowledge on the pathogenesis of embryo mortality and to formulate suitable corrective measures after identifying the causative agents.

The avian embryo has an extra uterine existence and hence the incubation period is critical for its development. Any alterations in the form of environmental changes, presence of infections and toxic residues passed on from the hen can lead to malformations and mortality. With the adoption of intensive poultry rearing systems, the main impetus is on the feed supplied and the managemental practices followed. Even with the best quality control systems in

the world, animal producers often find themselves owning mycotoxin contaminated grain or feed. Consumption of such feed by breeder flocks results in embryomortality and reduction in hatchability with the progeny chicks being increasingly susceptible to disease owing to immunosuppression. Toxic metabolites have been found in egg yolks and these observations focus the attention on the safety of food products of animal origin for human consumption.

The earliest recorded effects of mycotoxicosis date back to the 10th century when epidemics of ergotism known as St. Anthony's fire occurred in Europe. The landmark achievement was the discovery of the aflatoxins in 1960. This paved the way for further research on other toxigenic fungi and since then, many other toxic fungal metabolites of different chemical structures and widely differing biological activities have been identified.

The economic impact of reduced production, increased incidence of disease due to immuno suppression and damage to vital organs are hazards to the poultry industry. Besides, the carcinogenic and teratogenic effects of some of the mycotoxins have

added a new dimension in the research on the problem of mycotoxicosis.

Mycotoxins of utmost concern include Aflatoxins, Ocharatoxins and Fumonisins belonging to the Aspergillus, Penicillium and Fusarium genera. Mycotoxins occur together in field conditions and with the presence of one toxin in a feed, the chances are high that other either identified or unidentified toxins are present. Investigations have shown that feed ingredients in Kerala are contaminated with Aflatoxins and Ochratoxins. This co-contamination is not surprising in the light of the ubiquitous nature of the fungi that produces Aflatoxin and Ochratoxin. This association and the extreme toxicity elicited by both mycotoxins prompted this investigation to determine the synergistic toxicity and describe the major effects of these mycotoxins when they are administered simultaneously.

The mechanism of action of these toxins have been studied intensively both individually and in combination and it is proven beyond doubt that toxicity enhancing synergisms do exist between these mycotoxins

and that symptom patterns are altered during multiple mycotoxicosis.

The chick embryo offers a model for understanding the early differentiation of the organ systems and the fundamental process of body formation common to all groups of vertebrates. Unlike in other animals, any agent introduced into the egg system remains there throughout the developmental period and is not lost externally. The effects of the causative agent can be directly seen and assessed and the information can be applied to the development of ameliorative measures.

This experiment has been designed to study the synergistic effects of the mycotoxins Aflatoxin B1 (AFB1) and Ochratoxin A (OA) on chick embryos, the variations in the susceptibility to the toxins both individually and in combination. A better control of mycotoxins will enable producers for greater competitiveness and profitability. In addition, it would reduce the likelihood that mycotoxin residues would appear in animal products destined for human consumption.

Review of Literature

2. REVIEW OF LITERATURE

2.1. Pathogenicity

Romanoff and Romanoff (1972) conducted studies on the teratogenesis in the avian embryo using various agents and observed the mode of occurrence of structural anomalies and their specificity with individual toxic agents.

The chick embryo offered a basis for understanding the early differentiation of the organ systems and fundamental process of body formation common to all groups of vertebrates and has long been a most popular experimental material (Patten, 1976).

Apart from infections, genetic factors, nutritional causes, toxic substances and other physiological causes, it was found that management and incubating conditions were significant factors in the causation of embryomortality (Sunde et al., 1978).

Markaryan (1978) observed the frequency of occurrence of malformations in chick embryos and noted that the malformations of feet and legs were the most

common defects followed by head abnormalities like acrania, exencephaly and microcephaly.

The classification of teratological deformities in chick embryos was put forward by Yoshida *et al.* (1981) in which deformities of the eyes, beak and brain were listed.

2.2. Embryotoxicity of mycotoxins

Wilson and Hayes (1973) observed that mycotoxins were the major cause of feed toxicosis in poultry and livestock. They also posed a serious environmental hazard to human health as well.

The inoculation of embryonated eggs was found to be a useful biological method for detection and estimation of mycotoxins (Lafont and Lafont, 1979).

The embryotoxic effects of numerous compounds including fungal metabolites were tested using the Chick Embryotoxicity Screening Test (CHEST) bioassay, and the effects of mycotoxins including aflatoxin (Cilievici *et al.*, 1980; Vesely *et al.*, 1983; Dietert

et al., 1985) and Ochratoxin (Gilani et al., 1978; Harvey et al., 1987) were studied.

In addition to their adverse effects in adults, many of these toxins were found to produce harmful effects on the growth and development during embryonic life. The deviation of embryogenesis was manifested by one or more characteristics such as late foetal death, complete resorption of the implant, growth inhibition and structural abnormalities or functional defects of the offspring (Arora, 1982; Pichova et al., 1987).

Significant reduction in hatchability due to mycotoxins like Aflatoxin and Ochratoxin indicated the synergistic action of mycotoxins synthesised by individual fungal strains (Vegad and Katiyar, 2001).

2.3. Dose and age of susceptibility

Choudhury and Carlson (1973) experimented on the lethal dose of ochratoxin for chick embryos and found that six day old embryo was the most sensitive one. Investigations carried out by Vesely et al. (1983) on the comparative embryotoxicity of aflatoxins showed that AFB1 was most toxic.

The embryotoxicity dose range was estimated to be between 0.01 to 0.05 µg for ochratoxin A (Vesela *et al.*, 1983) and Prelusky *et al.*(1987) observed that the chick embryo was most sensitive to the effects of toxin and solvents after 1 or 2 days of incubation. As the age of the embryo at dosing increased, a rapid decrease in response was also observed.

Vesely and Vesela (1991) reported that the embryotoxicity of AFB1 and OA ranged from 0.0001 to 0.1 µ g per embryo. Several experiments which included *in vitro* bioassays, however, pointed out a drawback, which was their susceptibility to variation depending on end point, target cell and dosing strategy (Bondy and Armstrong, 1998).

2.4. Effect of Aflatoxin B1 on embryos

Inoculation of 0.05 mg of aflatoxin into the air cell of the fresh egg led to a decrease in size of the embryo at hatching to almost half of the control (Shibko *et al.*, 1968). The decrease in weight of the liver was concurrent with the decrease in weight of the body.

Experiments conducted to study the effect of AFB1 on the chick embryo revealed embryomortality, stunted embryos and gross lesions consisting of haemorrhages on yolk sac and skin (Harish and Mohiyuddin, 1972).

Aflatoxin B1 was found to be ten times more toxic to chick embryos than its degradation metabolite, aflatoxin DELTA (Lafont and Lafont, 1974) and AFB1 produced more toxicity when administered by the air cell route (Hsieh *et al.*, 1974).

Toxic lesions of AFB1 were seen in the heart, liver, skeletal muscle, brain and cartilage. The liver and skeletal muscle of the embryos showed fatty change and toxic myositis respectively. The cardiac damage induced was evidenced as endocardial cushion- like plaques at the base of the atrioventricular valves (Smith *et al.,* 1975).

Hong and Sheu (1977) observed bile duct proliferation in the retarded embryos and extra medullary haematopoiesis in the kidney and liver of the dead embryos treated with AFB1

Egbunike (1978) demonstrated that micro doses of AFB1 in rats did not significantly affect the ovulation rate or conception rate. However, it led to smaller litter size and higher embryo mortality (Egbunike *et al.*, 1980).

The teratogenic effects of AFB1 on chick embryo development were manifested as malformations like spinabifida, anophthalmia, maxillary retrognathism, distorted legs and evisceration (Cilievici *et al.*, 1980).

Vesely *et al.* (1983) established that the general cytotoxic character of AFB1 on the embryonic morphogenetic systems required neither a specific metabolic activation nor any specific target. The development of erythroid anaemia following embryonic exposure to AFB1 was related to the perinatal carcinogenic effect of AFB1 (Dietert *et al.*, 1983).

Studies on the embryotoxicity of various aflatoxins indicated that AFB1 was the most toxic one (Vesely *et al.*, 1983). The acute hepatotoxicity was linked to the liver microsomal enzyme system (Terao *et al.*, 1987).

Ultrastructural studies of chick embryo fibroblasts cell culture inoculated with AFB1 showed the presence of pyknotic or bizarre shaped nuclei with pronounced degenerative changes in the rough endoplasmic reticulum, mitochondria and presence of multiple vacuoles in the cytoplasm (Somvanshi *et al.*, 1992).

Espada *et al.* (1992) noted a significant reduction in the body weight and absolute weights of liver, Bursa of fabricius, spleen and thyroid following an experimental intoxication with AFB1.

Qureshi *et al.* (1998) reported that broiler breeder hens when fed diets amended with aflatoxin resulted in embryonic mortality and reduction in hatchability with the progeny chicks being increasingly susceptible to disease owing to suppression of humoral and cellular immunity.

Aflatoxin intoxication in poultry revealed microscopical lesions in the liver ranging from vacuolation of hepatocytes, karyomegaly, proliferation of bile ducts and extra medullary haematopoiesis (Vegad and Katiyar, 2001).

2.5. Effect of Ochratoxin A on embryos

Choudhury and Carlson (1973) experimented on the lethal dose of ochratoxin for chick embryos and found that the embryonic sensitivity decreased with the increase in the age of the embryo.

Feeding of OA contaminated diets to day old chicks led to kidney damage characterised by degeneration of the tubular epithelium accompanied by regeneration (Krogh et al., 1976).

Following embryonic exposure to OA, malformations like short and twisted limbs, short and twisted neck, microphthalmia, exencephaly, everted viscera, reduced body size and cardiac anomalies like ventricular septal defects and malformations of valves were observed (Gilani et al., 1978). Trials conducted to study the effect of OA on fertility and embryoviability of Japanese quails by Prior et al. (1979) revealed a significant higher percentage of early embryonic death.

Ultrastructurally, the outer mitochondrial membranes, cristae and matrix were affected by OA, with changes more prominent in the proximal and distal

convoluted tubules of the kidney. Abnormally shaped mitochondria in the kidney tubular epithelium, excessive numbers of lipid droplets, along with proliferation of smooth endoplasmic reticulum were seen in the liver cells of broiler chicks (Dwivedi *et al.*, 1984), chick embryos (Lalithakunjamma, 1987), quails (Maxwell *et al.*, 1987) and rats (Nair *et al.*, 1994).

The experiments conducted to study the effect of OA in chick embryos revealed interstitial oedema and degeneration and necrosis of tubular epithelial cells. Many of the tubules contained epithelial debris. Glomeruli appeared hypercellular. The hepatocytes showed fatty change and islands of degeneration and necrosis (Lalithakunjamma, 1987).

The chicken embryo was used as a model by Harvey *et al.* (1987) to study the immunogenic effect of ochratoxin in ovo. They observed a decrease in the number of immunoglobulin bearing cells of the bursa.

Embryomortality and embryonic anomalies, with most changes reflected in the liver and kidney were observed in the progeny embryos of layer hens fed with ochratoxin contaminated feed (Niemieo *et al.*, 1990).

Experiments conducted by Wiger and Stormer (1990) on the effects of OA on pre chondrogenic mesenchymal cells from chick embryo limb buds revealed that the interference of OA with general protein synthesis, both *in vivo* and *in vitro*, was an important mechanism underlying OA induced embryotoxicity.

Degeneration and necrosis of the cells of the liver and kidney and improper differentiation of embryonal mesenchymal cells, along with osteochondro dystrophic changes were observed in embryos administered OA (Lalithakunjamma and Nair, 1993). Oedema of the head and neck was a consistent feature, histologically seen as disruption and degeneration of muscles, along with haemorrhages.

Ramadevi *et al.* (1994) analysed certain biochemical parameters of OA fed broilers and found that there was an increase in alkaline phosphatase and uric acid levels and a decrease in total proteins, albumin and cholesterol levels in the serum.

Ultrastructural changes in the lymphoid organs of Japanese quail embryos ranged from moderate to severe

organellar damage in the lymphoid cells of the Bursa of Fabricius, spleen and thymus (Farshid *et al.*, 1996).

The particular vulnerability of caudal structures to OA in chick embryos were manifested by varying degrees of caudal dysgenesis with sirenomelia in severely affected specimens. Malformations of the brain focussed on the characteristics of the neural crest cell populations which were selectively vulnerable to teratogens. Failure of closure of neural tube led to the development of malformations of the craniofacial region (Wei and Sulik, 1996).

Investigations on embryonic chick brain (Bruinink and Sidler, 1997) and cell cultures of foetal rat telencephalon (Monnet- Tschudi *et al.*, 1997) revealed that OA was a neurotoxicant during prenatal stages.

Bruinink *et al.* (1997) observed that there was no difference in sensitivity of OA between cell cultures of embryonic chick neural retina and brain compared in relation to meningeal cell cultures.

Li *et al.* (1997) concluded from trials that OA had a long half life and was slowly cleared from the body,

while its metabolites were cleared at a much faster rate with much shorter half lives.

The disturbance of pH homeostasis, consequent to impairment of urinary acidification because of inhibition of HCO_3^- reabsorption in the tubules, was related to the nephrotoxicity of OA (Kuramochi *et al.*, 1997).

2.6. Synergistic effects of AFB1 and OA

Kanisawa and Suzuki (1978) experimented on the carcinogenic potency of OA on the liver and found that AFB1 influenced the OA hepatocarcinogenesis synergistically.

Studies revealed a significant interaction between AFB1 and OA. The kidney was found to be the most sensitive organ to the combined toxicity and nephropathy was the most important characteristic of the synergism. The relative weights of the kidneys were significantly higher. The lipid accumulation influenced by AFB1 was inhibited by OA (Huff and Doerr, 1981).

Combined action of ochratoxin and aflatoxin together threatened the immune status of chickens and made them immunologically incompetent (Campbell *et al.*, 1981).

Doerr and Huff (1981) reported that the kidneys were affected at low, intermediate and high dose level combination of aflatoxin and OA. Degenerative and necrotic changes were seen in the cells of the liver and kidney of rats on feeding AFB1 and OA together (Rati *et al.*, 1981).

The combined effect was found to be more severe than the individual effects and interaction represented additive toxicity and not toxic synergy (Huff *et al.*, 1985).

Alterations in symptom patterns during multiple mycotoxicosis indicated the need for toxicological analysis along with pathological investigations in establishing a diagnosis (Tapia and Seawright, 1985).

Evisceration, crossed beak, cranioschisis, herniation of yolk, curled toe, generalised congestion and oedema of embryo were among the various

pathomorphological changes observed by inoculation of ochratoxin A and citrinin individually and in combination. All the embryos showed reduction in growth and the changes were more severe in the combined toxicity (Lalithakunjamma, 1987).

Trials conducted by Micco *et al.* (1988) revealed that the combined treatment resulted in a higher content of OA in the liver, after long term administration of OA and AFB1 in poultry.

Combined effect of AFB1 and OA in chick embryos produced embryonic mortality and various malformations like everted viscera, exencephaly, crossed beak, small head and eyes and crooked limbs, with significant reduction in embryonic weight and length (Edrington *et al.*, 1995).

Materials and methods

3. MATERIALS AND METHODS

3.1. Experimental design

The experimental schedule is given in Table 1.

Table 1.

Schedule of experimental inoculation

Sl No	Materials used	Diluent used	Dose/egg	Route of admini- stration	Age of embryos at the time of inocul- ation	Number of eggs used
1	Aflatoxin B1	Propylene glycol	0.05 μg	Air cell	4 days	50
2	Ochratoxin A	Propylene glycol	0.25 μg	Air cell	4 days	50
3	Aflatoxin B1 +Ochratoxin A	Propylene glycol	0.05 μg + 0.25 μg	Air cell	4 days	50
4	Diluent control	Propylene glycol	Diluent only	Air cell	4 days	50

3.2. Toxin

3.2.1. Aflatoxin B1

Pure AFB1 supplied by Sigma (St. Louis, USA) was used for the experimental studies. For each one microgram of pure AFB1, one ml of propylene glycol (Sarabhai, India) was used as diluent.

3.2.2. Ochratoxin A

Pure OA supplied by Sigma (St. Louis, USA) was used and for every five microgram of pure OA, one ml of propylene glycol (Sarabhai, India) was used as diluent.

3.3. Group I

AFB1 diluted with propylene glycol was administered to 50 chick embryos on the 4th day of incubation, through the air cell route at the dose rate of 0.05 µg/ egg. Prior to inoculation, the eggs were candled and cleaned with rectified spirit. They were drilled and inoculated, sealed by paraffin wax and incubated at 37°C.

3.4. Group II

OA diluted with propylene glycol was administered to 50 chick embryos on the 4th day of incubation, through the air cell route at the dose rate of 0.25 µg/ egg.

3.5. Group III

0.05 µg of AFB1 and 0.25 µg of OA were administered together into 50 chick embryos on the 4th day of incubation, through the air cell route of inoculation.

3.6 Group IV

Fifty chick embryos were administered 0.1 ml of propylene glycol alone through the same route of administration.

3.7 Group V

Fifty uninoculated chick embryos were kept along with the other eggs and incubated at 37°C.

The pre and post inoculation steps followed were similar for all the embryos subjected to inoculation.

3.8. Bacteriological studies

All the embryos were candled daily. The dead in shell embryos removed for routine examination were

subjected to cultural examination to isolate bacteria, if any. All the embryos were collected under sterile conditions and the embryonic fluid was cultured on Mueller Hinton Agar (Hi-Media Pvt. Ltd., Bombay). The plates were observed for 48 hours and the organisms were identified up to the genus level as described by Cowan (1974).

3.9. Pathoanatomical studies

After gross examination for pathological abnormalities, the malformed embryos were classified with detailed description of the pathoanatomy and tissues were taken for histopathological examination.

Five eggs from each group were collected for embryopathic studies on the 9th day, 14th day, 19th day and all the remaining eggs on the 21st day. The embryos were weighed and after gross examination, tissues were taken for histopathological studies.

Fixation was done in 10 per cent neutral formalin and sections were stained with routine Haematoxylin and Eosin staining (Sheehan and Hrapchak, 1980).

Results

4. RESULTS

4.1. Pathoanatomical studies

The mortality pattern of the mycotoxin inoculated embryos is given in the Table 5. Apart from the prescribed five numbers of embryos that were removed from each of the five groups for the routine examination on the 9th, 12th, 19th and 21st day, there was a mortality of sixteen per cent in Group I (AFB1 alone) and twelve per cent in Group II (OA alone) at the end of the experiment or on the 21st day. The combined inoculation of AFB1 and OA in group III resulted in a higher mortality percentage (38%) compared to Group I and Group II. The mortality pattern of Group IV and V was similar and was very less (4%) compared to the experimental groups.

Various structural anomalies were detected at different ages of the embryos (Table 2). The abnormalities were single or multiple. Oedema in the region of the head and neck was a consistent feature of most of the embryos in the experimental groups (Fig. 1) and it constituted 69 % of the total abnormalities.

Table 2.

Gross pathology of the inoculated embryos

	Group I		Group II		Group III	
9th day of incubation	All embryos were severely congested with haemorrhages at the cephalic region	5	Haemorrhage at cephalic · region along with petechiae throughout the body	6	Severe congestion of the entire embryo along with petechiae throughout	7
14th day of incubation	Dwarf embryos Severe congestion	6	Oedema of head and neck Dwarf embryos	6	Generalised oedema along with beak abnormality – 1 Gastroschisis along with beak defect – 1 Oedema of head and neck – 4	6
19th day of incubation	Embryos were reduced in size Eventration of viscera- 1 Oedema of head and neck- 2	5	All embryos small in size Herniation of yolk Sac – 2 Oedema of head and neck in all Beak abnorma-lity- 1	5	All embryos small in size with oedema of head and neck Craniosch-isis- 1 Herniation of yolk sac-4	6
21st day of incubation	All embryos were reduced in size Herniation of yolk sac- 10 Double-Headed embryo- 1 Oedema of head and neck- 6 Eventration of viscera- 2	27	All embryos were reduced in size Herniation of yolk sac - 12 Crossed beak- 1 Oedema of head and neck in all embryos	29	All embryos were reduced in size Beak abnormality with Cranio-schisis 2 Oedema of head and neck- 10 Eventration of yolk sac- 4	16

Oedema and gelatinisation of the subcutaneous tissue involving the dorsal aspect of the cranium extending dorsally and laterally in the neck region were seen. The gelatinous coagulum involved both the subcutis and musculature. In addition, petechiae and streaks of haemorrhages were noticed in the oedematous region in many cases (Fig. 2).

Weights of embryos of different groups at different age intervals are presented in Table 4. Reduction in size and weight and curled appearance was another abnormality (94%) (Fig. 3). The reduction in size of the embryo was reflected in the reduced size of the internal organs also. Many of these dwarf and stunted embryos had a generalised oedema also.

Omphalitis with herniation of yolk sac (Fig. 4) or enlarged yolk sac was another feature (30%). The yolk was creamy in consistency and had a greenish discolouration (Fig. 5). There was very severe congestion with petechiae in the internal organs. Coelosoma or eventration of viscera was another abnormality noticed (3%). In the extreme cases, the entire viscera was found to be outside the body

(Fig. 6), while in milder cases, only loops of intestine were seen.

Beak abnormalities were noticed in six cases. The main beak abnormalities were crossed beak (Fig. 7), short upper beak and short lower beak.

Cranioschisis due to complete or partial failure of fusion of the cranium resulting in partial herniation of the brain was also observed in three cases (Fig. 8).

Other developmental abnormalities noticed were one diprosopus (embryo with two heads) (Fig. 9) and another had two upper beaks along with cranioschisis (Fig. 10).

4.2. Bacteriological examination

All the 250 eggs were subjected to bacteriological examination. Bacteria could be isolated from 61 cases. More than one type of organism was found to be involved in 15 of the samples. A total of 74 bacterial isolates were obtained during the study, of which 39 were gram positive and 35 were gram negative. The gram positive bacterial isolates included *Staphylococcus* spp. and

Bacillus spp. and the gram negative isolates obtained were *Escherichia* spp., *Pseudomonas* spp, *Aeromonas* spp. and *Alkaligenus* spp. The results are given in the Table 3.

Table 3.

Bacterial isolates from embryos

Total number of embryos examined	Number of positive samples	Number of total bacterial isolates	Number of gram positive bacteria isolated		Number of gram negative bacteria isolated			
			Staphylococcus spp.	*Bacilllus* spp.	*Escherichia* spp	*Pseudomonas* spp.	*Alkaligenus* spp.	*Aeromonas* spp.
			25	14	19	10	4	2
250	61	74	Total - 39		Total - 35			

4.3. Mean embryo weight

The average embryo weights on the 9th, 12th, 14th and 19th day of incubation are shown in Table 4. There was a significant reduction in the embryo weight of group I, II and III ($P<0.05$) when compared to the control Group IV and V.

4.3.1. 9th day of incubation

The mean embryo weights of group I, II and III, which were 1.17 g, 1.36 g and 1.50 g respectively was found to be significantly reduced ($P<0.05$) when compared to the control Group IV (1.96 g) and V (1.97 g). Group I showed a significant difference from Group III whereas Group II did not show a significant alteration from Group III.

4.3.2. 14th day of incubation

The average embryo weights of Group I , II and III which were 8.11 g, 7.45g and 5.61 g respectively, was found to be significantly reduced ($P<0.05$) when compared to the control Group IV (14.64 g) and Group V (13.7 g).

There was a significant difference between group I and Group III whereas group II and III were more homogeneous.

4.3.3. 19th day of incubation

The mean embryo weights of group I, II and III which were 11.93 g, 10.86 g and 9.76 g respectively, was found to be significantly reduced compared to the control Group IV (23.29 g) and Group V (23.88 g).

Group I, II and III were significantly different from each other.

4.3.4. 21st day of incubation

Mean embryo weights of Group I, II and III were 12.7 g, 12.22 g and 10.91 g respectively and was found to be significantly reduced from the control Group IV (28.55g) and Group V (28.84 g).

There was significant difference between Group I and Group III as well as significant difference between group II and group III.

Graph 1. Average weight of embryos

1.1. **9th day of incubation**
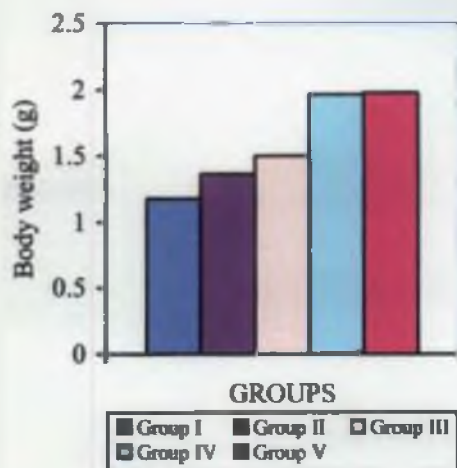
1.2. **14th day of incubation**
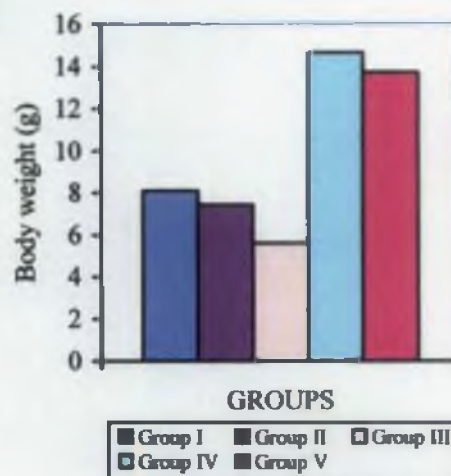
1.3. **19th day of incubation**
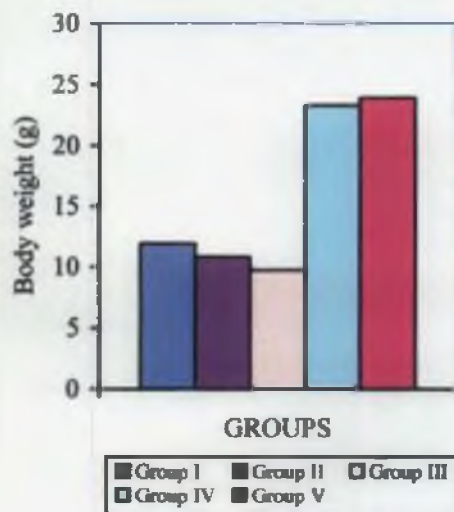
1.4. **21st day of incubation**
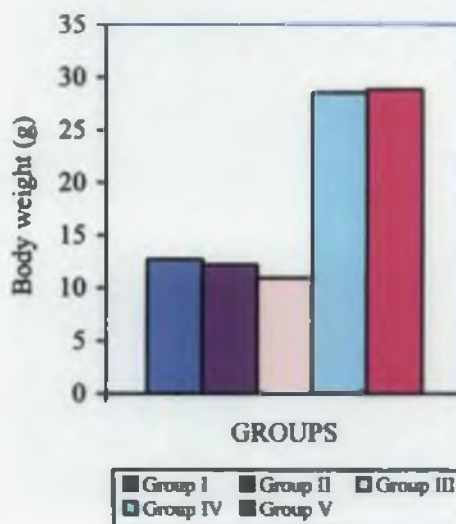
Group I- AFB1 alone

Group III - AFB1 and OA in combination

Group V- Non-injected control

Group II - OA alone

Group IV - Diluent control

Table 4.

Mean embryo weight (values ± SD)

Days of incubation	Group I	Group II	Group III	Group IV	Group V
9th day	1.17 b ± 0.08	1.36 bc ± 0.09	1.50 c ± 0.08	1.96 a ± 0.04	1.97 a ± 0.03
14th day	8.11 b ± 0.21	7.45 bc ± 0.63	5.61 c ± 0.24	14.64 a ± 0.14	13.71 a ± 0.87
19th day	11.93 b ± 0.13	10.86 c ± 0.20	9.76 d ± 0.42	23.29 a ± 0.28	23.88 a ± 0.22
21st day	12.70 b ± 0.30	12.22 b ± 0.10	10.91 c ± 0.23	28.55 a ± 0.52	28.84 a ± 0.61

a-d : Means within a row with no common superscripts

differ significantly (P<0.05)

4.4. Gross pathology

4.4.1. Group I (AFB1 alone)

4.4.1.1. 9th day of incubation

From the day of inoculation (fourth day) upto 8th day of incubation, there was a mortality of four embryos in this group. All the four embryos were severely congested (Fig. 11). On the ninth day, five embryos were sacrificed. All the five embryos showed moderate to severe congestion and petechial haemorrhages all over the body especially at the cephalic region (Fig. 12).

Table 5.

Effect of various treatments on embryomortality

Day	Group I AF B1	Group II OA	Group III AF B1 + OA	Group IV P	Group V Control
D5 -8	4/50	3/50	6/50	1/50	0/50
D9 5 removed from each group	0/41	1/41	2/37	0/44	1/45
D10 -13	3/41	1/40	6/31	0/44	0/44
D14 5 removed from each group	1/33	1/34	1/25	1/38	0/39
D15 - 18	0/32	0/34	3/22	0/38	0/39
D19 5 removed from each group	0/27	0/29	1/16	0/33	1/33
D20	0/27	0/29	0/16	0/33	0/33
D21	0/22	0/24	0/11	0/28	0/28
Total mortality	8/50	6/50	19/50	2/50	2/50
Mortality Percentage	16 %	12 %	38 %	4 %	4 %

4.4.1.2. 14th day of incubation

From the tenth to fourteenth day of incubation there was a mortality of four embryos and five embryos were sacrificed. Out of these, three embryos showed oedema all over the body. All the others were small in size and congested with mild to moderate oedema at the region of the neck which extended over the head region in two embryos. One embryo had multiple abnormality like generalised oedema, beak abnormality (short lower

beak), eventration of viscera and reduction in body size (Fig. 13). The internal organs had congestion and petechial haemorrhages.

4.4.1.3. 19th day of incubation

There was a reduction in size of all the embryos and other abnormalities like herniation of the yolk sac (Fig. 4) were seen in one embryo and oedema of head and neck in two embryos. There was severe congestion of visceral organs in all the embryos, which was more prominent in the liver.

4.4.1.4. 21st day of incubation

All the embryos failed to hatch out. There was marked reduction in size and body weight in all the embryos compared to the controls. Various malformations like herniation of yolk sac (37%), oedema of head and neck (22%), eventration of viscera (7%) and one embryo with two heads were observed (Fig. 9). The herniated yolk sacs were enlarged and contained creamy yolk with greenish discolouration (Fig. 5). The liver and kidneys of these embryos were enlarged and severely congested.

4.4.1.5. Histopathology

In the AFB1 inoculated embryos, there was generalised congestion of all organs irrespective of the age of the embryos. The congestion was more prominent in the liver and kidneys. In the liver, the hepatocytes were radially arranged around the central vein except in few cases where disorganisation of hepatic cells was noticed. There was focal areas of necrosis, widening of the sinusoids, varying degrees of vacuolar changes in the hepatic cells and congestion of the central vein (Fig. 14). The vacuoles in the hepatocytes were single or multiple, which displaced the nucleus to one side. Occasional Kupffer cell hyperplasia was also seen. Extensive haemorrhages into the parenchyma was seen in some cases. Centrilobular necrosis and bile duct hyperplasia (Fig. 15) was another feature seen in the liver.

There were varying degrees of changes in the kidneys. The tubular epithelium showed vacuolations and swelling. There was focal destruction of the epithelium and was separated from the basement membrane. There were haemorrhages in the interstitial spaces which were filled by red blood cells. In focal areas, there was

dilatation of the tubules and in some of the tubules, presence of homogenous pink material was observed in the lumen (Fig. 16).

In some cases, there was mild fibrous tissue proliferation and few inflammatory cells in the interstitial space. Glomeruli were normal looking. Both in the liver and kidney, there were dilated spaces filled with red blood cells indicating haematopoietic zones.

In the lungs, there was congestion and haemorrhages (Fig. 17). Focal areas of haemorrhages in between the muscle bundles of myocardium and separation of muscle fibres could be noticed (Fig. 18). Depletion of lymphocytes and mild congestion in the lymphoid organs like spleen, thymus, bursa and generalised congestion in the gastrointestinal tract were the other lesions in this group.

4.4.2. Group II (OA alone)

4.4.2.1. 9th day of incubation

From the day of inoculation up to the eighth day of incubation, only three embryos were found to be dead on candling. There was mild congestion of the embryos.

In all the five embryos examined, there was oedema and petechial haemorrhages on the entire embryo which was prominent in the occipital region.

4.4.2.2. 14th day old embryo

All the five embryos showed reduction in size and had oedema of the head and neck region. Among them, one embryo showed herniation of yolk sac and another one had short lower beak. Congestion of internal organs was also noticed which was more prominent in the kidneys.

4.4.2.3. 19th day old embryo

All the five embryos were reduced in size and had oedema of head and neck. In addition, one of the embryos had herniation of the yolk sac and another embryo had a short upper beak. The liver was congested and kidneys were enlarged and congested.

4.4.2.4. 21st day old embryo

All the remaining 29 embryos failed to hatch out. All of them showed reduction in size. Oedema of head and neck was a consistent feature in all these embryos.

In addition, twelve embryos had herniation of yolk sac and one embryo had crossed beak (Fig 7).

4.4.2.5. Histopathology

In the group given OA alone, generalised oedema was a consistent feature. Organogenesis was improper in the embryos which were dead in the early days. Degenerative and necrotic changes were prominent in the kidney and liver. In the kidney, interstitial oedema, haemorrhage, degeneration and necrosis of the tubular epithelium were noticed. Vacuolation and swelling of the lining epithelium of the tubules and in focal areas, necrosis of epithelial cells and cellular debris were seen. In some places, interstitial and intertubular haemorrhages were prominent. Separation of the tubular epithelium from the basement membrane and focal areas of calcification (Fig. 19) were also noticed in the kidneys of two embryos. There was cystic dilatation of the tubules and presence of eosinophilic material in the lumen of the tubules. In the glomeruli also, there were changes like hypercellularity and separation of the cells from the basement membrane (Fig. 20). The damage to the renal epithelium was more

severe in the embryos of later stages of development compared to the early embryos.

In the liver, there was severe haemorrhages. Diffuse degeneration of hepatocytes, focal necrotic areas, congestion of central vein (Fig. 21), sinusoidal dilatation and bile duct hyperplasia were seen in the liver. In one case, there was multiple granuloma like lesion in the liver characterised by central necrosis surrounded by inflammatory cells and fibrous tissue encapsulation. In others, there was severe degeneration and single to multiple vacuoles of varying sizes were seen in the hepatocytes.

Haemorrhages were also seen in the lungs (Fig. 22), myocardium with occasional fragmentation of muscle fibres (Fig. 23).

4.4.3. Group III (AFB1 and OA combined)

4.4.3.1. 9th day of incubation

In this group, the damages were more severe. From the day of inoculation to the eighth day of incubation, six embryos died and were removed. Mild to moderate congestion along with petechial haemorrhage all over the body could be noticed. On the 9th day, all the five

embryos examined, showed generalised oedema. Congestion and petechial haemorrhages were seen throughout the body especially at the dorsal aspect of the neck and head region (Fig. 24).

4.4.3.2. 14th day old embryo

There was marked reduction in size and weight of all the five embryos. One embryo had multiple abnormalities like eventration of viscera and beak abnormality (Fig. 6).

Oedema of head and neck were seen in four of the embryos. There was oedema and gelatinisation of the subcutaneous tissues at the dorsal aspect of the cranium extending dorsally and laterally in the neck region. The gelatinisation involved both the subcutaneous tissue as well as the musculature. There were congestion and petechial haemorrhages on all organs which was prominent in the kidney and liver.

4.4.3.3. 19th day of incubation

All the five embryos examined were reduced in size and had various abnormalities like oedema of head and

neck, cranioschisis and herniation of yolk sac (Fig. 25). All the visceral organs were severely congested. There was congestion and enlargement of the kidney and liver. The bursa of Fabricius was smaller when compared to the controls.

4.4.3.4. 21st day of incubation

On the 21st day, all the remaining 17 eggs failed to hatch. Six of the embryos were live sticky and the rest were dead in shell. All the embryos were grossly reduced in size and showed abnormalities. Oedema of the head and neck was seen in ten embryos while herniation of the yolk sac was seen in four embryos. One embryo had cranioschisis along with beak abnormality (short upper beak) (Fig. 8) and another had two upper beaks (Fig. 10) and eventration of viscera. All the visceral organs were congested with a predominance in the kidney. Bursa was smaller when compared to the controls.

4.4.3.5. Histopathology

The histopathological lesions seen in this group was most severe in comparison to Group I and Group II. There was oedema of the neck in almost all the embryos.

The vessels in this zone were enlarged. There was extensive haemorrhage in all the organs. The extramedullary hemopoietic centres were much reduced in the liver. Irrespective of the age of the embryo, there was severe haemorrhage in the liver and kidney. Degeneration and necrosis of the tubular epithelium of the kidneys were observed among the regularly appearing well lined tubules (Fig. 26). The necrotic changes and extravasation of blood into the parenchyma was more pronounced in the embryos that survived upto 21 days. Some of the glomeruli were swollen and some were necrotic (Fig. 27). In focal areas, there were dilated tubules containing eosinophilic material in the lumen. In some tubules, there was vacuolation and swelling of the tubular epithelium and in focal areas desquamated epithelial cells were seen within the lumen and focal areas of calcification were seen in two cases both in the liver and kidney.

In the liver, extensive haemorrhages into the parenchyma made characterisation of the lesions difficult (Fig. 28). There was extensive degeneration and focal areas of necrosis of the hepatic cells. Vacuolation of the hepatic cells were also observed.

The vacuoles were either single or multiple of varying sizes which were displacing the nucleus to one side. The central veins and sinusoids were severely congested. In the myocardium extensive haemorrhages and myolysis were seen.

The lungs were also severely congested and showed areas of extensive haemorrhages (Fig. 29). There was focal destruction of the bronchial mucosa. Congestion and depletion of lymphocytes from the bursa (Fig. 30) and thymus were also noticed.

The brain was oedematous and showed moderate degenerative changes. The developing bones showed imperfect bone formation (Fig. 31) which resulted in the ill developed cranial bones and herniation of the brain. Imperfect ossification was seen in the long bones. There was severe congestion and epithelial damage in the various organs like the alimentary organs.

4.4.4. Group IV and Group V

In the groups IV and V, there were no appreciable lesions except for mild congestion in various organs.

Fig. 1. Embryo showing oedema of the head and neck (AFB1) - 21st day

Fig. 2. Oedema and gelatinisation of subcutaneous tissue of the dorsal aspect of cranium (AFB1) - 21st day

Fig. 3. Reduction in size of the embryo compared to control (AFB1+OA)- 21st day

Fig. 4. Omphalitis with herniation of yolk sac (AFB1)-19th day

Fig. 5. Yolk with greenish discolouration (AFB1) 21st day

Fig. 6. Eventration of viscera (AFB1)- 14th day

Fig. 7. Embryo showing crossed beak (OA) 21st day

Fig. 8. Cranioschsis and partial herniation of brain   (AFB1 + OA) - 21st day

Fig. 9. Diprosopus - embryo with two heads
(AFB1 + OA)- 21st day

Fig. 10. Embryo with two upper beaks (AFB1 + OA)-
21st day

Fig. 11. Severe congestion of embryo treated with
AFB1 -5th day

Fig. 12. Petichiae in the cephalic region of embryo
treated with AFB1 - 9th day

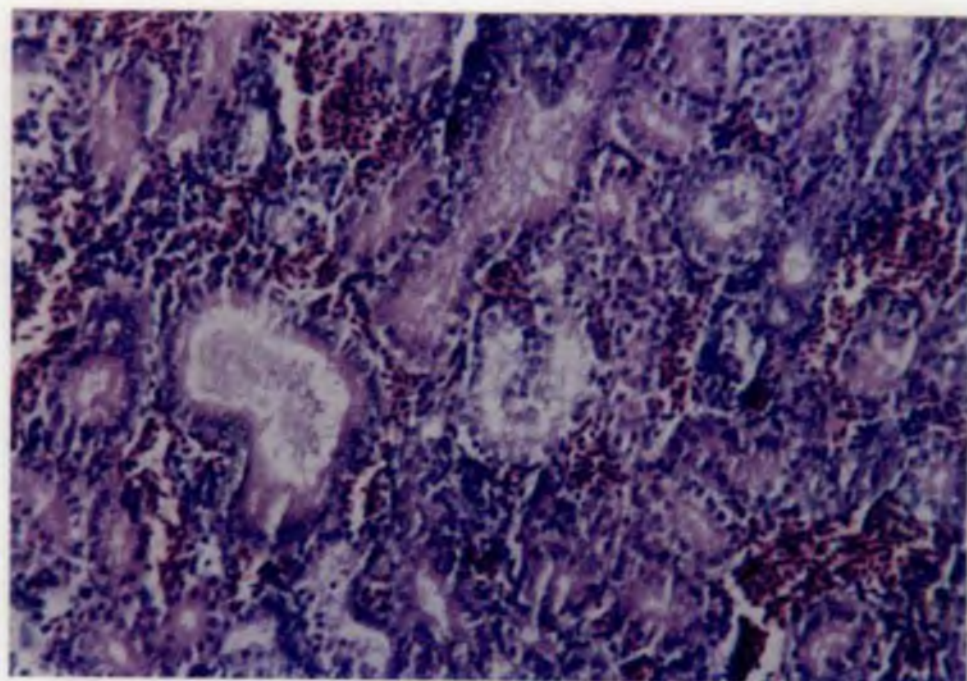Fig. 13. Embryo with short lower beak and eventration of viscera (AFB1 + OA) - 14th day

Fig. 14. Liver: widening of sinusoids, vascular changes in hepatocytes and congestion of central vein (AFB1)
(H&E   X 160)
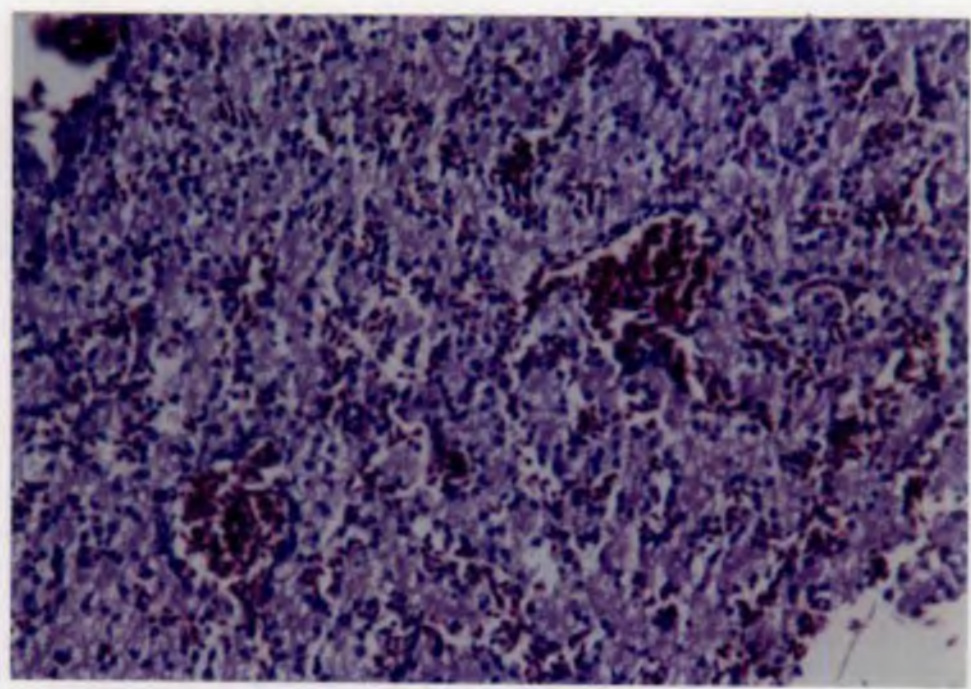
Fig. 15. Liver: bile duct hyperplasia (AFB1)
(H&E X 160)

Fig. 16. Kidney; Dilatation of tubules and presence
of homogeneous pink material in the lumen (AFB1)
(H&E X 250)

Fig. 17. Lung: congestion and haemorrhages (AFB1)
(H&E   X 160)

Fig. 18. Heart: Haemorrhages between the muscle
bundles and separation of muscle fibres (AFB1) (H&E
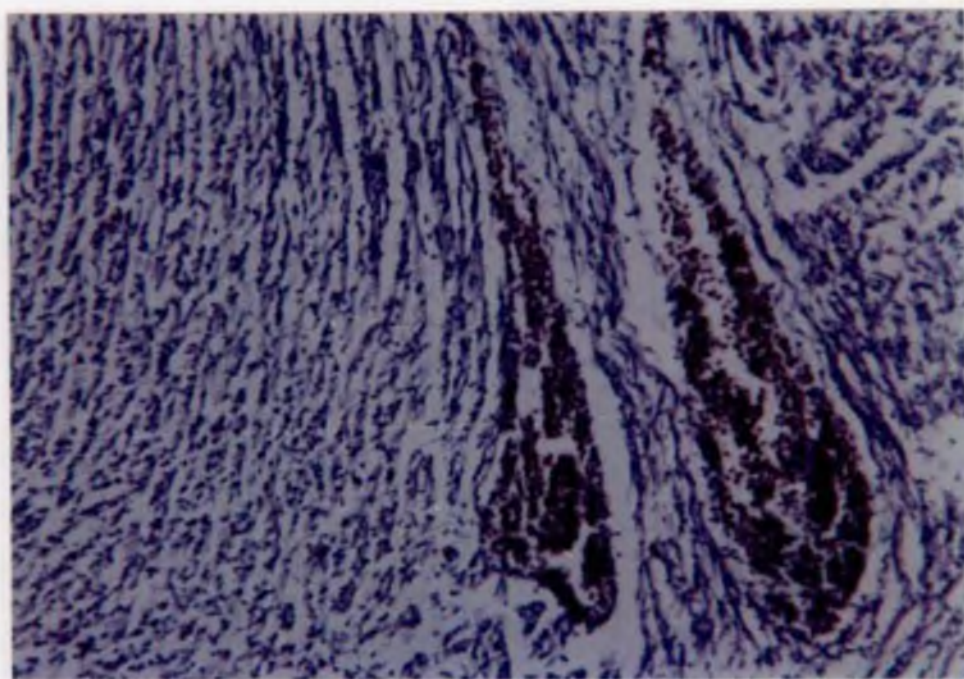X 160)
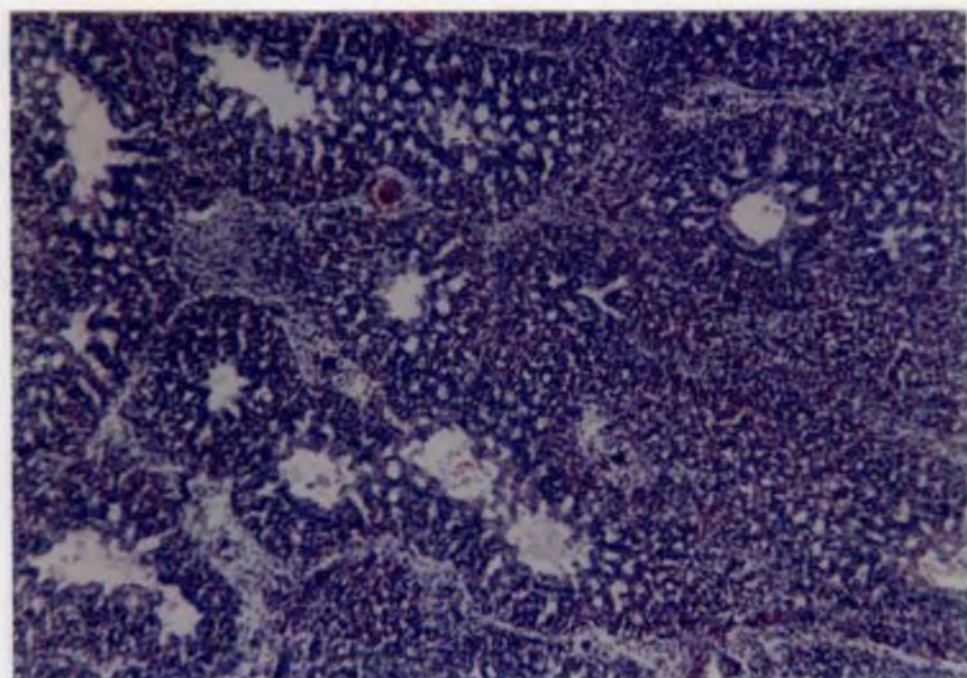
Fig. 19. Focal areas of calcification - kidney (OA)
(H&E X 160)

Fig. 20. Kidney: Hypercellularity of glomeruli (OA)
(H&E X 160)
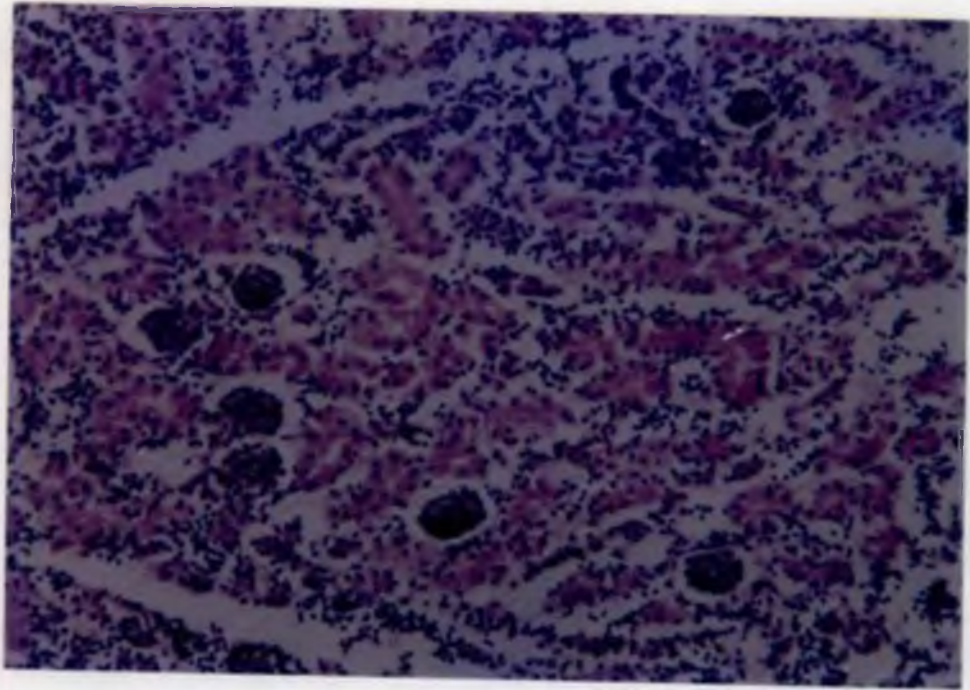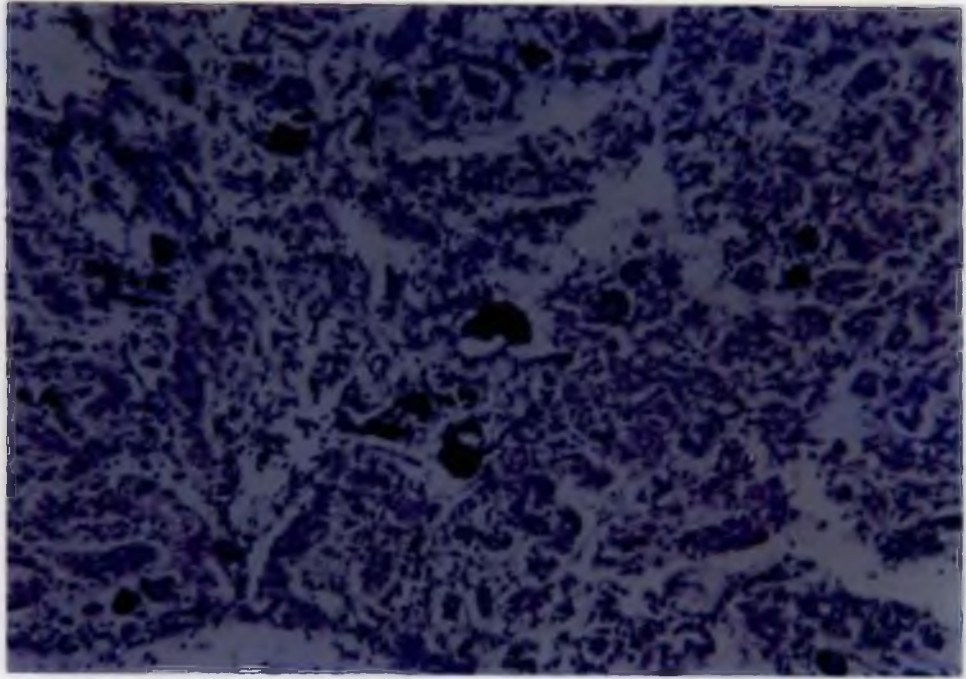
Fig. 21. Liver: Focal necrotic area with congestion of central vein (OA) (H&E X 160)

Fig. 22. Lung: Haemorrhage in the parenchyma (OA) (H&E X 250)
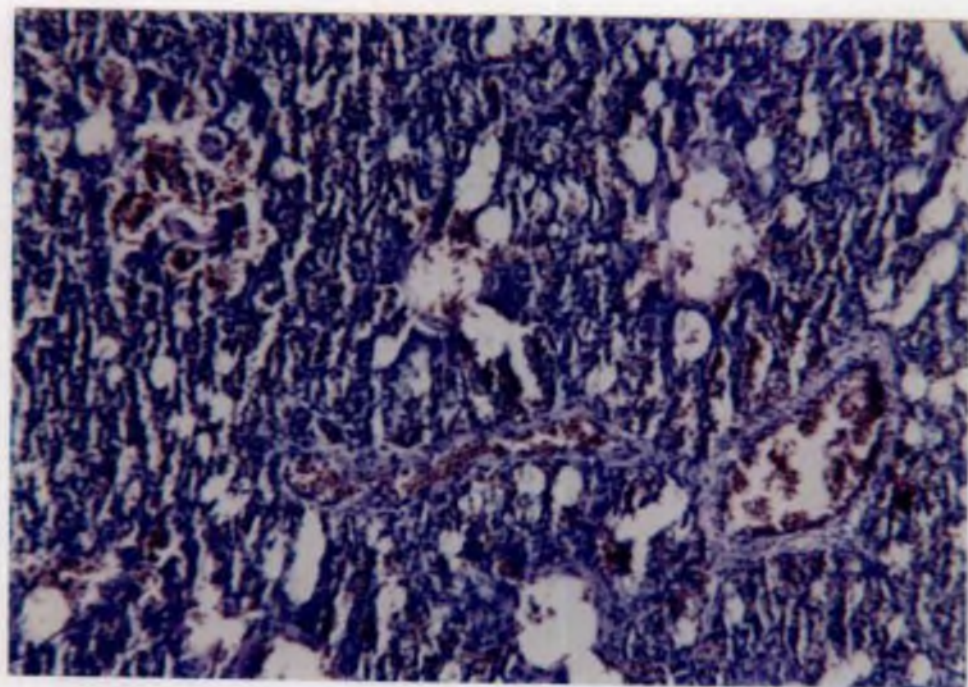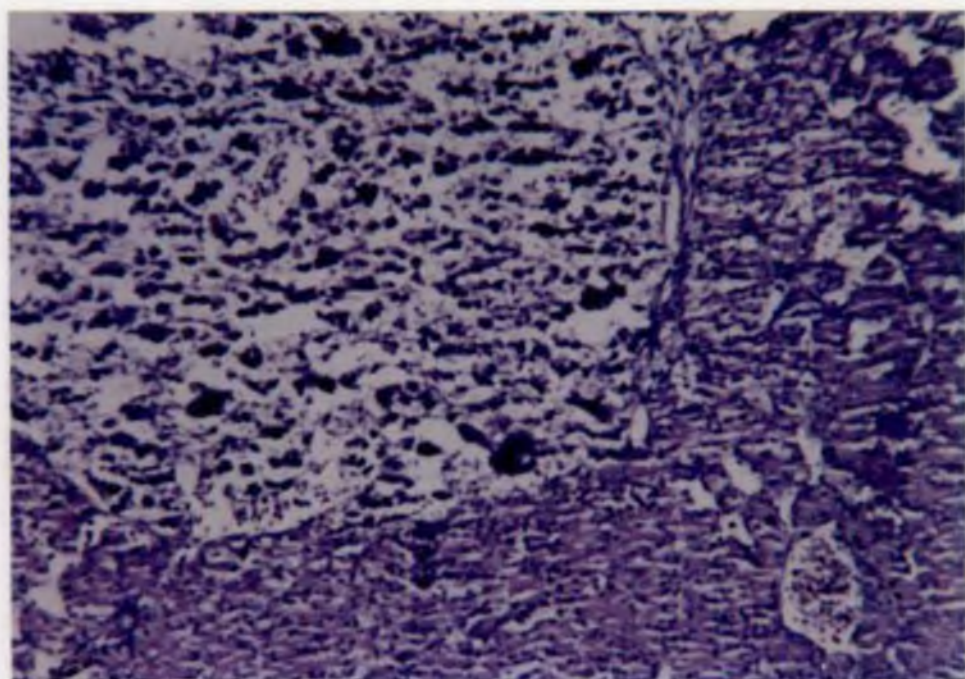
Fig. 23 Heart: Haemorrhage and fragmentation of muscle fibres (OA) (H&E X 250)

Fig. 24. Congestion and petichial haemorrhages in dorsal aspect of head and head region in 9 day old embryo (AFB1+OA)
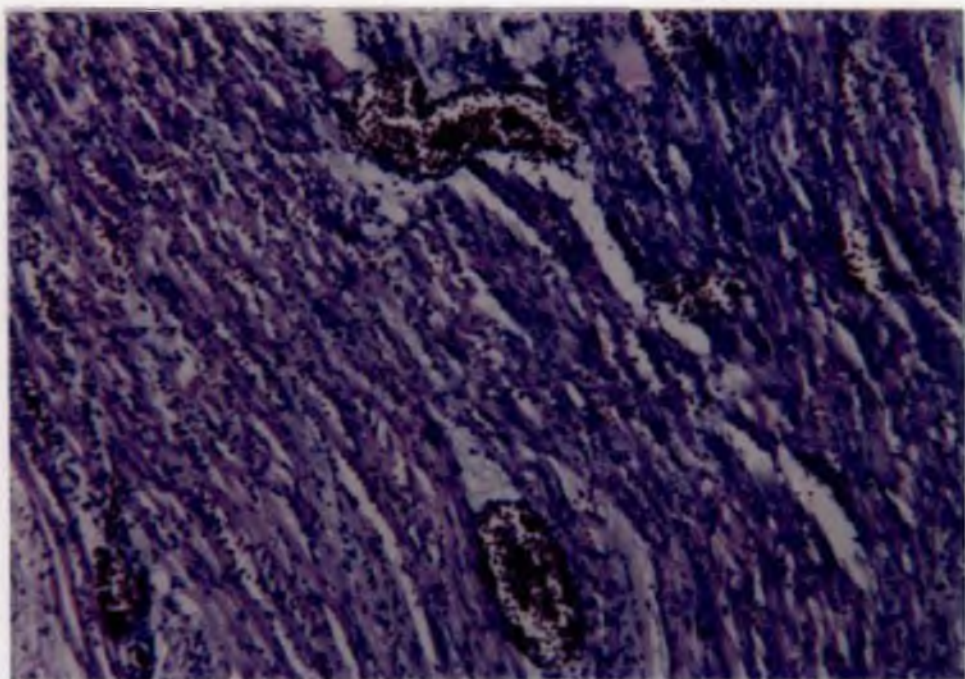
Fig. 25. Herniation of yolk sac: 19 day old embryo
with oedema of head and neck (AFB1+OA)

Fig. 26. Kidney: Degeneration and necrosis of
tubular epithelium(AFB1+OA) (H&E X 250)
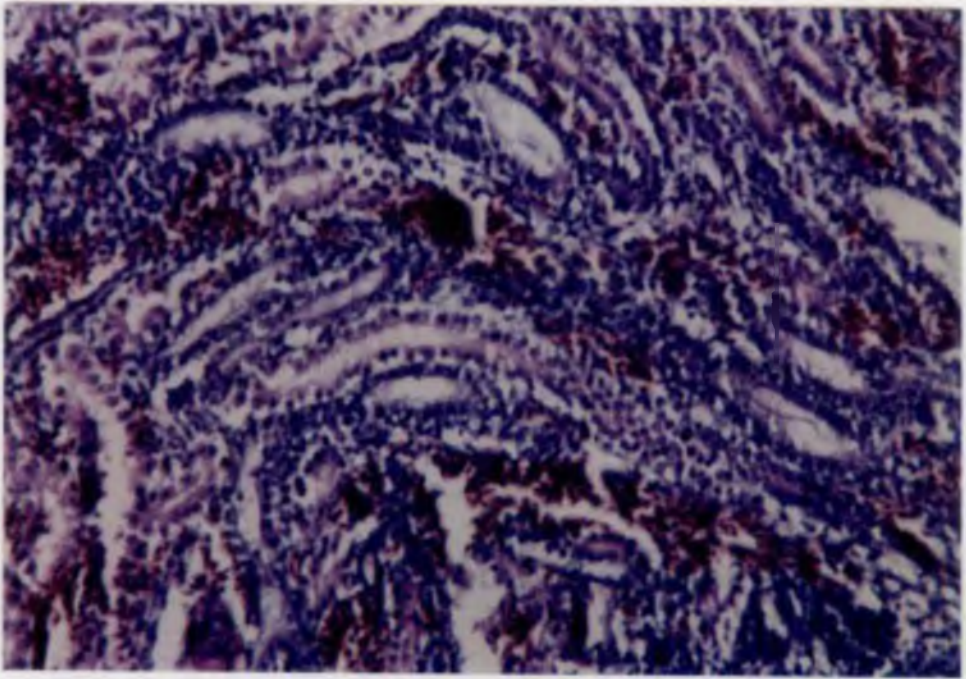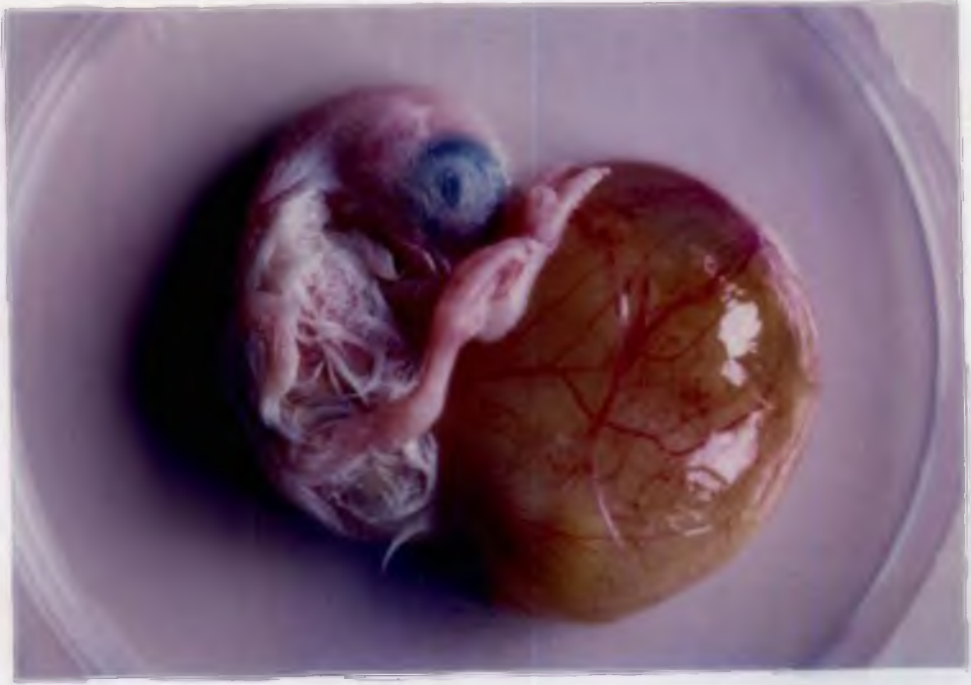
Fig. 27. Kidney: Swollen and necrotic glomeruli
(AFB1+OA) (H&E X 160)

Fig. 28. Liver: Extensive areas of haemorrhages in
the parenchyma (AFB1+OA) (H&E X 160)
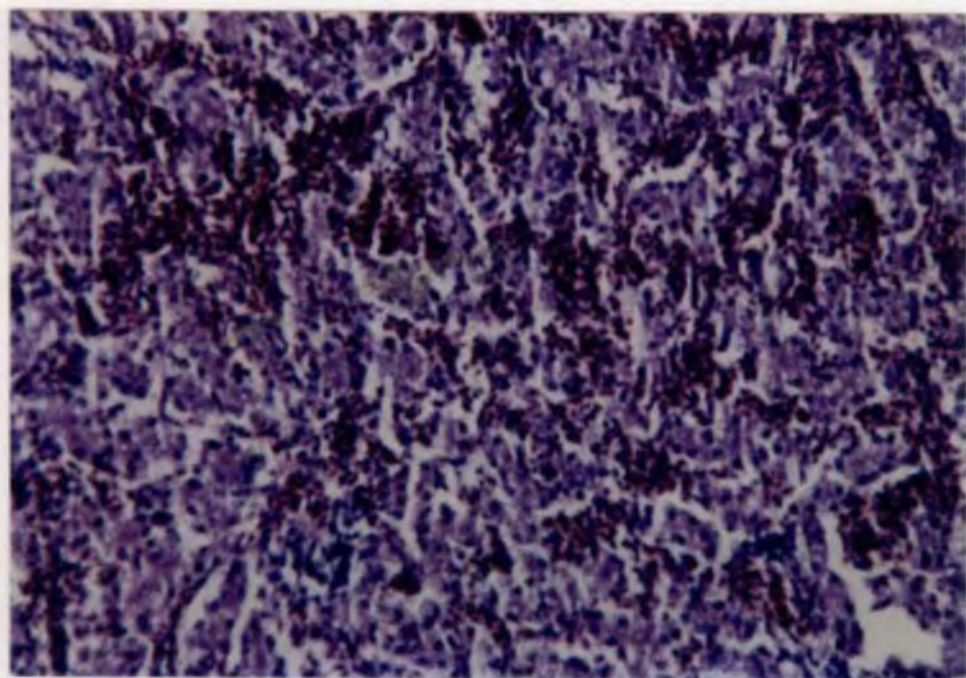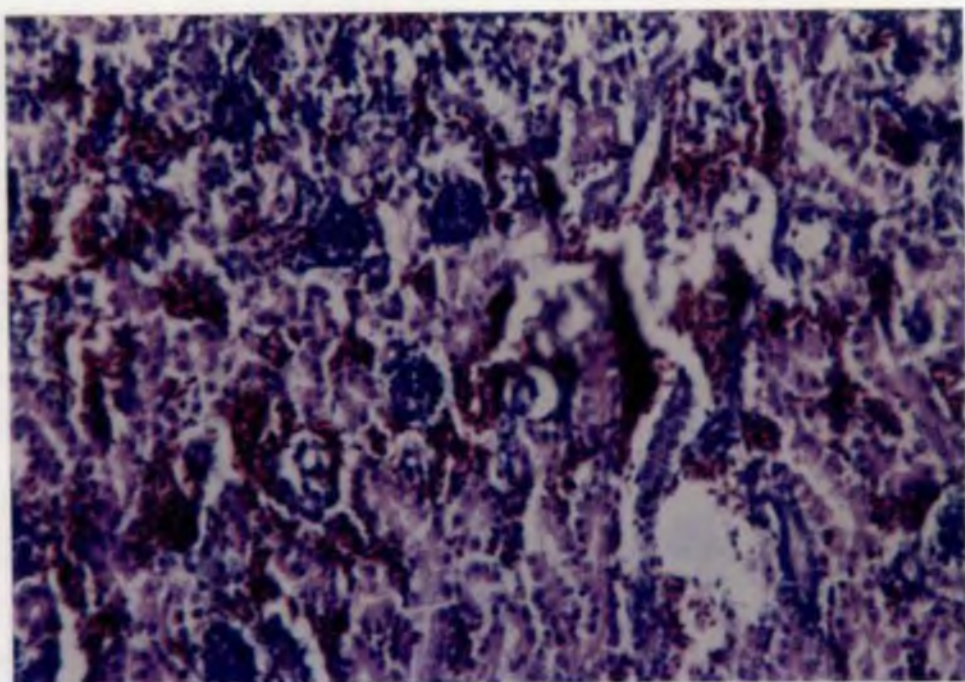
Fig. 29. Lung: Congestion and extensive areas of
haemorrhages (AFB1+OA) (H&E X 250)

Fig. 30. Bursa: Congestion and depletion of
lymphocytes (AFB1+OA) (H&E X 160)
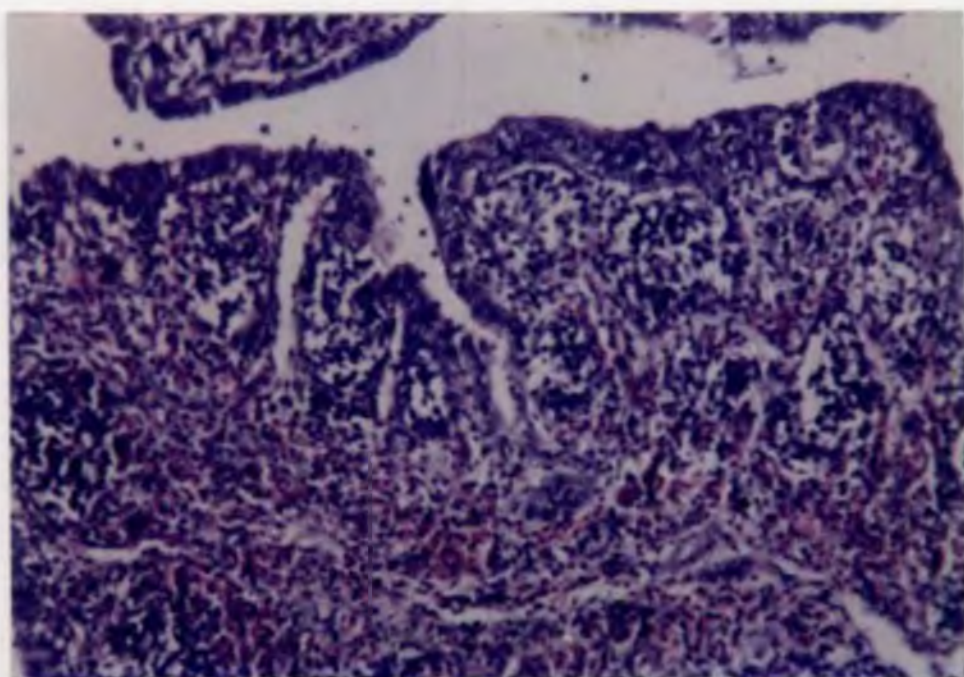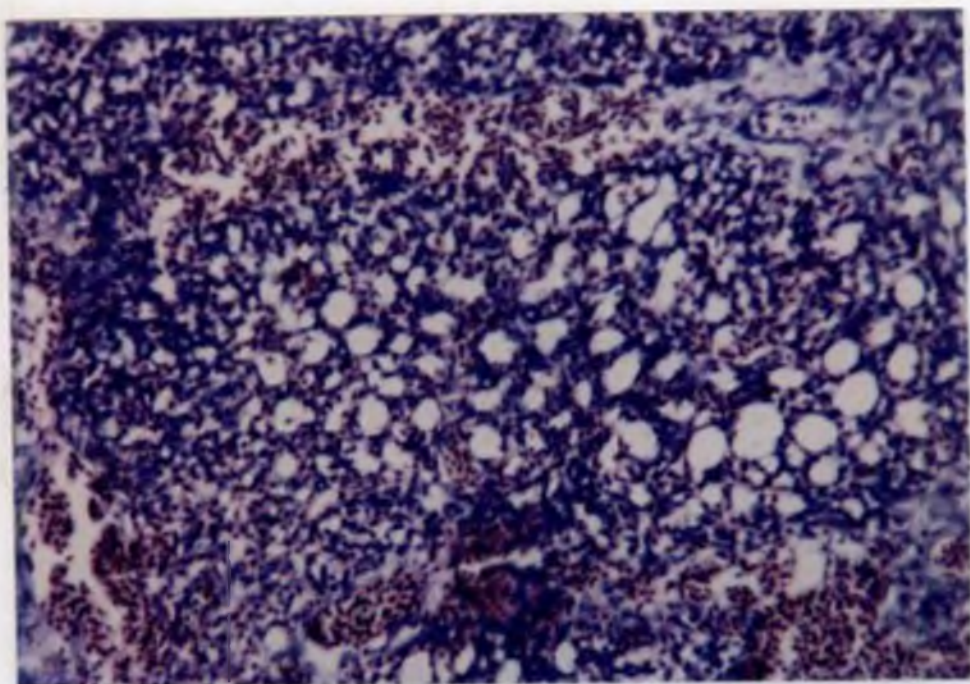
Fig. 31. Bone: Imperfect ossification of bone
(AFB1+OA) (H&E   X 250)
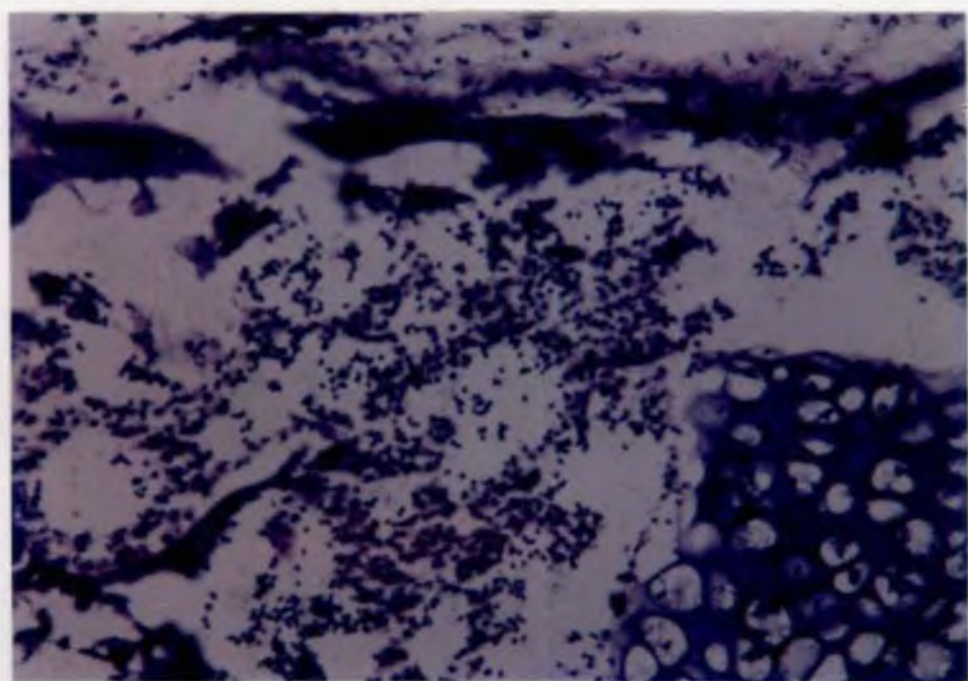
Discussion

5. DISCUSSION

A number of studies have demonstrated that different mycotoxins occur simultaneously in field situations causing toxic changes and resulting in a profound impact on the turnover of the poultry industry.

The significance of mycotoxins in causing lesions in the embryos and their likely residual effects in the yolk focuses the need for the identification as causative factors for various embryopathies. It has been proved that OA and AFB1 are teratogenic in the mammalian embryo and several studies have shown that the presence of these mycotoxins either individually or in combination have a direct implication on the embryo mortality and reduction in hatchability (Arora, 1982). The feed given to the hens may contain varying quantities of mycotoxins like AFB1 and OA simultaneously.

The combination of low levels of mycotoxins with the stress associated with commercial production situations and exposure to disease causing organisms

produced effects in poultry which were subtle, indirect and sometimes ill-defined. These harmful effects were seen in the flocks exposed to the contamination as well as in the progeny of such flocks (Qureshi et al., 1998).

In order to assess the pathological alterations the embryos were experimentally inoculated with AFB1 and OA individually and in combination. The mortality data showed a numerical increase with singly OA or AFB1 and with the combination of both. From the day of inoculation, mortality was observed to be more within 14 days of incubation in all the toxin treated groups. Similar studies showed characteristic embryomortality pattern in which the embryos were increasingly susceptible to the toxins after 1 to 2 days of incubation and being less susceptible as the embryo advances in age (Choudhury and Carlson, 1973; Prelusky et al, 1987). This was further evident from the fact that no mortality was observed in the single toxin administered groups beyond 14th day of incubation. However, there was a further eight per cent increase in mortality in the combined toxin group upto 21st day of incubation which demonstrated the additive toxicity of AFB1 and OA in embryos (Huff et al., 1985).

Embryo mortality, teratogenicity and suppressed immune system function were some of the deleterious effects observed in chick embryos, which were inoculated with AFB1 and OA, individually or in combination (Gilani *et al.*, 1978; Cilievici *et al.*, 1980; Lalithakunjamma, 1987).

Embryonic mortality has been reported by others following exposure to AFB1 (Cilievici *et al.*, 1980; Vesely *et al.*, 1983; Dietert *et al.*, 1985), OA (Choudhury and Carlson, 1973, Gilani *et al.*1978, Harvey *et al.* 1987) and when AFB1 and OA were administered in combination (Edrington et *al.*, 1995).

Difference in the mortality pattern reported here and by others can be attributed to the variations in the type and volume of the carrier solvent injection, routes of administration and age of the embryos at the time of inoculation. There was a significant reduction in the embryonic weight in all the toxin treated groups when compared to the controls. The mean embryonic weight was least in the combined toxin treated group. Among the individual toxin groups, AFB1 treated embryos had lesser body weights. In this study, there was a significant reduction in embryonic weights between the

groups administered toxins singly and in combination. Various were the abnormalities observed in different groups. This could be due to the variations in embryo sensitivities among batches of eggs. However, no significant difference in embryonic weights were observed in single toxin administered groups, in experiments conducted by other workers (Cilievici *et al.*, 1980; Vesela *et al.*, 1983).

The number of abnormalities observed was greater when both mycotoxins were administered in combination. The individual toxin treated groups also showed abnormalities. The various anomalies observed in the different groups included stunted embryos, evisceration, cranioschisis, herniation of yolk sac, beak abnormalities, and oedema of head and neck. These abnormalities occurred singly or in combination.

Growth retardation and similar abnormalities have been reported in embryos exposed to AFB1 (Cilievici *et al.*, 1980), OA (Gilani *et al.*, 1978; Lalithakunjamma, 1987) and AFB1 and OA combination (Edrington *et al.*, 1995).

Teratological studies on chick embryos have recorded that infections, genetic factors, nutritional causes, toxic substances and other physiological causes had a role in causing structural anomalies and embryo mortality. Contamination of the hatching eggs either by vertical transmission from the hen or by exogenous contamination have been proven to have a direct impact on the embryo mortality and hatchability (Arora, 1982).

A total of 74 bacterial isolates were made and multiple infections were present in 15 samples. *Staphylococcus, Bacillus, Escherichia, Pseudomonas, Alkaligenus* and *Aeromonas* were the organisms isolated. Embryo mortality consequent to yolk sac infection by bacteria present in the alimentary tract have been demonstrated earlier (Romanoff and Romanoff, 1972).

Over the years, research has shown AFB1 as a potent hepatotoxin and OA as a potent nephrotoxin. OA is reported to be an inhibitor of mitochondrial transport and early biochemical effects of AFB1 exposure include inhibition of both DNA and RNA synthesis. A prolonged decrease in protein synthesis was observed and the further interference with ATP production was correlated with their toxicity. Many of

the alterations seen could be related to the metabolic damage or with the direct damage to the membrane systems (Vesely *et al.*, 1983; Terao *et al.*, 1987,; Wiger and Stormer, 1990).

The histological patterns of injury reflect the biochemical manifestations of toxicity. From the spectrum of liver lesions, fatty change was a common histological abnormality observed. The extent of fatty change seemed to be related to the severity of the other accompanying degenerative changes like disorganisation of hepatic cell plates, dilatation of sinusoids and presence of hepatocytes with pyknotic nuclei. The severity was evident most in the combined toxin treated group. In some cases, relatively mild to severe inflammatory infiltration was observed depending on the extent of necrotic changes within and around the cells.

The kidney receives 25 per cent of the cardiac output which exposes the organ to a variety of chemical agents. One event related to the nephrotoxic response to xenobiotics is alteration in active transport systems of the renal tubules. It has been shown that the proximal convoluted tubule is the target segment of

the nephron in OA induced nephropathy (Kuramochi *et al.*, 1997).

Both in AFB1 and OA toxicity the cell damage seen ranged from vacuolation of the cytoplasm, necrosis and desquamation of epithelial cells. There was dilatation of the intertubular spaces along with the presence of eosinophilic material and epithelial debris within the lumen of the tubules. All these changes are in agreement with those of previous studies (Krogh *et al.* 1976) and are characteristic of acute tubular necrosis.

The combined toxicity elicited a more toxic response with respect to all the parameters looked into in this study. Although OA has been implicated as a nephrotoxin and AFB1 as a hepatotoxin, the results of this study showed that both the liver and kidney were equally damaged and it was not possible to pinpoint which was more affected. The variation seen here could be attributed to the fact that embryonic morphogenetic systems are equally susceptible to toxins and no specific targets or metabolic pathways are required. But in OA treated group structural alterations characterised by imperfect ossification, oedema and

degeneration of developing organs were more pronounced. This is in agreement with the type of lesion reported in chick embryos in experimental inoculation of OA (Lalithakunjamma, 1987). Huff and Doerr (1980) however projected nephropathy as the main feature of combined toxicity.

Erythropoiesis in the embryo is mainly carried out by the liver, with other organs like the kidney also playing a lesser role. In the toxin treated groups, the extra medullary haemopoietic zones were very much lesser in both organs with maximum impact being in the combined toxin treated group. Following embryonic exposure to AFB1, development of erythroid anaemia was the feature demonstrated (Dietert et al. 1983) and the results give evidence that such embryos if hatched out, give rise to unthrifty and anaemic chicks.

On and beyond the 19th day of incubation, haemorrhages were seen in all the organs and it was extensive in the group which was treated with the combined toxins. Here the lesions in the underlying parenchyma were not clearly visible. The haemorrhages were mostly in the liver and kidney, with the lungs, bursa and heart also showing haemorrhagic lesions of

lesser intensity. This can be attributed to the direct toxic effect of the mycotoxin on the endothelium of the blood vascular system. Previous reports of impairment of histogenesis of lymphoid organs like bursa of Fabricius and thymus were also seen in the present study. This is a significant change because even if these embryos hatch out, the chicks may be immunodeficient.

There was focal areas of calcification both in the liver and kidneys in the combined toxin treated group. This was seen on the 19th and 21st day old embryos. It was not sure whether this type of calcification is specific. However, this is in agreement with the findings by Lalithakunjamma (1987) where there were similar types of calcification in the liver and kidney in the chick embryos treated with OA and citrinin simultaneously.

Mycotoxins can deleteriously affect a number of parameters associated with embryonic growth and differentiation in a concentration dependent manner (Wiger and Stormer, 1990) and the results of this study produce evidence for the direct action of these on the developing embryo.

The study has thus helped in identifying the toxic effects of AFB1 and OA in chick embryos individually and in combination. This information can, therefore, be applied for the development of ameliorative means and thereby improve the economy of the poultry industry.

Summary

6. SUMMARY

The experiment was designed to study the synergistic effects of the mycotoxins AFB1 and OA on chick embryos, the variations in the susceptibility to the toxins both individually and in combination.

Four day old embryos were inoculated with AFB1 and OA both singly and in combination and experimental studies were conducted on the 9th, 14th, 19th and 21st days of incubation.

The mortality data showed a numerical increase with either AFB1 or OA and with the combination of both. Mortality was found to be more within 14 days of incubation in all the toxin treated groups. Beyond the 14th day of incubation, no mortality was observed in the single toxin administered group, but there was an eight per cent increase in mortality in the combined toxin treated group up to the 21st day of incubation which reflected the additive toxicity of AFB1 and OA in embryos.

There was a significant reduction in the embryonic weight in all the toxin treated groups when compared to the controls. The mean embryonic weight was least in the combined toxin treated group. Among the individual toxin groups, AFB1 treated embryos had lesser body weights.

Teratogenicity of the mycotoxins was demonstrated by the presence of abnormalities in the toxin treated groups, with more anomalies observed in the combined toxin administered group. The various anomalies observed in the different groups included stunted embryos, cranioschisis, herniation of yolk sac, eventration of viscera, beak abnormalities and oedema of head and neck. These abnormalities occurred singly or in combination.

Bacteriological studies revealed a total of 74 bacterial isolates and multiple infections were present in 15 samples. The organisms isolated were *Staphylococcus, Bacillus, Escherichia, Pseudomonas, Alkaligenus* and *Aeromonas.* Embryomortality consequent to yolk sac infection by bacteria present in the alimentary tract can be attributed as the causative factor.

Many of the alterations seen were related to the metabolic damage or with the direct damage to the membrane system. From the spectrum of liver lesions, fatty change was a common histological abnormality observed. The extent of fatty change seemed to be related to the severity of the other accompanying degenerative changes like disorganisation of hepatic cell plates, dilatation of sinusoids and presence of hepatocytes with pyknotic nuclei. The severity was most evident in the combined toxin treated group.

In the kidney, the cell damage ranged from vacuolation of the cytoplasm, necrosis and desquamation of epithelial cells. The combined toxicity elicited a more toxic response with respect to all the parameters looked into in this study.

The results of this study showed that both the liver and kidney were equally damaged and this was attributed to the fact that embryonic morphogenetic systems are equally susceptible to toxins.

The direct toxic effect of the mycotoxins on the endothelium of the blood vascular system was demonstrated by the haemorrhages which were seen in all

the organs and it was extensive in the combined toxin treated group.

Impairment of histogenesis of lymphoid organs which was seen in the present study is significant as these embryos if hatched out will lead to immunodeficient chicks.

The results of the study provide evidence for the direct action of mycotoxins on the developing embryo. This information can be used for the development of ameliorative measures and for the overall improvement of the poultry industry.

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SYNERGISTIC EFFECT OF ALFATOXIN B1 AND OCHRATOXIN A IN CHICK EMBRYOS

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ABSTRACT OF THE THESIS

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ABSTRACT

The present study was taken up to investigate the
synergistic effects of the mycotoxins AFB1 and OA on
chick embryos, the variations in the susceptibility to
the toxins both individually and in combination.

Embryomortality, teratogenicity and reduced
embryonic weight were some of the deleterious effects
observed in the inoculated chick embryos. The
abnormalities seen were more in the combined toxin
treated group and they included stunted embryos,
evisceration, herniation of yolk sac, beak
abnormalities, cranioschisis and oedema of head and
neck.

Both the liver and kidney were equally damaged and
extensive haemorrhage into the parenchyma of all the
organs was the main histological lesion observed in
addition to degenerative and necrotic changes. This
study showed that mycotoxins deleteriously affect a
number of parameters associated with embryonic growth.