MYCOTIC ABORTION IN SMALL RUMINANT INDUCED BY ASPERGILLUS FUMIGATUS IN EGYPT.

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ABSTRACT

Mycotic abortion was induced in pregnant ewes and goats, twelve animals were inoculated intravenously with 10 ml of Aspergillus fumigatus (A. fumigatus) spore suspension (containing 2 x 10⁷ viable spores), and four pregnant animals were kept as a control. All experimental animals aborted between 20-30 to 19-29 days postinoculation in ewes and goats respectively. Pregnant ewes and goats appeared to be highly susceptible to A. fumigatus infection. Uteri, maternal and foetal placenta and foetal tissues were examined morphologically and histopathologically. Thrombosis, extensive necrosis, infarctions and invasion with A. fumigatus hyphae were detected in the placenta of aborted ewes and goats. Skin lesions of aborted foeti were detected.

Histopathological examination of the issue organs revealed mycotic granulomatous inflammation to the lung, heart, brain, spleen and kidney.

INTRODUCTION

Bovine mycotic placentitis was first reported by Smith (1920), and since then there have been many reports of the condition from most parts of the world. The disease has become more important recently because of the use of better diagnostic procedures and because of the control of other causes of abortion. On the other hand, wide use of antibiotics, steroid and chemotherapeutic agents has led to a significant increase in the occurrence of systemic mycosis (Chihaya et al., 1992). In Denmark mycotic placentitis and abortion account for 10 to 24 per
cent of the diagnosed bovine abortions and are therefore, an important cause of loss in the production of cattle (Krogh, 1985).

Fungal infections of the reproductive tract of ewes and goats are not well studied and reports of naturally occurring of mycotic abortion in sheep and goats are infrequent (Kirkbridge, 1990). However, Cysewski and Pier (1968) and Pier et al. (1972) studied the pathological changes of experimental mycotic abortion in pregnant ewes produced by A. fumigatus. The pathogenesis of mycotic placentitis and abortion is unclear, moreover, the relationship between mycotic infection and infertility of small ruminant is not well established. Therefore the present work was carried out to clarify the pathological effect of A. fumigatus infection on the reproductive performance of pregnant ewes and goats and study its foetoapathic effects.

MATERIAL AND METHODS

Six pregnant ewes and six pregnant goats (Advanced stage of pregnancy, 3.5 - 4 months), 2 years old of known history and negative for infectious diseases were used for the experiment at Maruit Research Station, Alexandria. Another four pregnant animals were kept as a control.

A. fumigatus was isolated from stomach contents of aborted ovine foetus (field isolate), by El-Naggar (1997) and viable spore suspensions was prepared according to Jensen et al. (1989). The experimental animals were inoculated intravenously with 10 ml of the spore suspension (containing $2 \times 10^7$ viable spore) according to the method of Pier et al. (1972) and Munoz et al. (1989), control animals were given 10 ml of sterile diluents the animal were observed daily and rectal temperature was recorded:

Aborted foeti were examined grossly for any pathological lesions, and autopsied as soon as possible. Tissue samples from skin, lung and liver were fixed in 10% neutral buffer formalin for histopathological examination.

Aborted ewes and goats were sacrificed just after or in the next day of abortion, uterine tissues, placentomes and extra uterine organs were grossly examined, tissue samples were partially examined mycologicoally (Kotb et al., 1986) and partly fixed in 10% formalin for histopathological examination,
then embedded in paraffin wax. Sections at 3-4 μm thickness were prepared and stained with haematoxylin and eosin (H & E), periodic acid-schiff (PAS) and by Grocott’s methenamine silver (GMS) method for fungi (Sheehan and Hrapchak, 1980).

RESULTS

Following intravenous inoculation of the pregnant ewes and pregnant goats with spores of A. fumigatus, there was an increase in the rectal temperature of 2 degrees, anorexia and dullness of the experimental animals within 24 hours of inoculation and the animals returned to normal condition after 3 days of infection. Abortion was detected in ewes after 20-30 days and in goats after 12-24 days post infection.

Gross and Histopathological changes:

a. Uterine and foetal tissues:

The main gross lesion in the placenta included, irregular areas of necrosis with hyperemic borders of the surface of cotyledons (Fig. 1), diffused edema, congestion and hemorrhages of the intercotyledonary chorioallantoic membrane, cut sections of most placentomes showed congestion and in many cases well defined infarctions (Fig. 2). Gross lesion in most aborted foeti were seen in the skin in the form of small multiple foci of congestion.

No characteristic variations could be detected due to species variation between aborted sheep and goats.

Microscopical examination of placenta of aborted ewes revealed extensive necrotizing placentitis, coagulative necrosis was extended throughout foetal and placental tissues of chorioallantoic villi (Fig. 3, 4). Infiltration of polymorphonuclear cells and lymphocytes throughout the necrotic tissues with small foci of calcification were seen.

A. fumigatus hyphae were massively scattered through the necrotic tissue of the placentome, the hyphae were positive for PAS (Fig. 5) and GMS stain (Fig. 6, 7). It was noticed that A. fumigatus hyphae were concentrated mainly in the maternal placenta then directed to the chorionic villi of the foetal placenta. The hyphae invaded and penetrated the maternal and foetal blood capillaries forming thrombosis and vasculities (Fig. 8).

The endometrium of aborted ewes showed ulceration, focal necrosis and dilatation of uterine gland with neutrophilic infiltration. A. fumigatus hyphae were detected at the upper
surface of the endometrium. The endometrium of aborted goats showed the same changes, however the necrotic foci were extensive and mature reproductive vesicle of A. fumigatus was detected in the center of the necrotic foci (Fig. 9, 10, 11).

Microscopical lesion of the aborted foetuses were restricted to the skin in the form of acute haemorrhagic dermatitis (Fig. 12). Fungal elements of A. fumigatus were distributed throughout the affected skin lesions (Fig. 13). No characteristic lesions could be detected in the lung and liver of the aborted foeti.

Fungal reisolation from stomach contents of foetus and placenta lesion was successful.

b. Extra Uterine Tissues:
The lung tissue presented milliary nodules, microscopical examination of the lesion revealed mycotic granulomatus reaction, (Fig. 14). Colonies of Aspergillus fumigatus were seen in the center of the lesion and infiltrated with dark staining polymorphonuclear cells, lymphocytes, histocytes and sircumscribed by proliferating connective tissue. Oedema and emphysema were detected in the affected tissue. On the other hand, the center of the granuloma contained huge numbers of radiating branching septated hyphae of Aspergillus fumigatus.

The hyphae were highly PAS positive (Fig. 15) and GMS positive (Fig. 16). Also many reproductive vesicles of Aspergillus fumigatus were detected in the lung tissue (Fig. 17). In some cases of diffuse large area of suppurrative inflammation contain colonies of Aspergillus fumigatus was seen specially in the lung of ewes (Fig. 18), also areas of cavitation were detected.

The heart was enlarged and congested, on cut section minute whitish foci were diffused in the myocardium. Microscopical examination of the cardiac tissues revealed diffused nodules of mycotic granuloma. The granuloma were infiltrated with polymorphonuclear cells, histocytes, lymphocytes and surrounded by fibrosis connective tissues. The lesion of mycotic granuloma was detected in both aborted ewes and goats.

The nervous tissues were oedematous and on cut section serous fluid was detected with congestion of the blood vessels.

Microscopic examination revealed focal mycotic granuloma, variable in size and distributed in both cerebrum and cerebellum. Aspergillus fumigatus hyphae were detected in the center of the lesion and infiltrated with
polymorphonuclear cells (Fig. 19, 20).

General perivascular oedema and astrocytic oedema were detected in the brain tissue. Congestion of the blood capillaries with perivascular cuffing were noticed.

Pathological lesions of the brain of aborted ewes were nearly the same as in case of aborted goats, no characteristic species variation could be detected.

The spleen was enlarged with minute focal areas of infarction. Microscopic examination of the spleen revealed focal mycotic granuloma diffused all over the tissue (Fig. 21). The granuloma consisted of center nodules of fungal hyphae surrounded by necrotic tissue elements. (Fig. 22). The granuloma was highly infiltrated with neutrophils and phagocytic cells and spores of Aspergillus fumigatus were detected (Fig. 23).

The kidneys were enlarged, oedematous, irregular areas of infarction were detected when removing the capsules. Histological examination of the kidney of aborted animals revealed severe damage of the renal tissue in the form of interstitial and glomerulonephritis (Fig. 24). Mycotic granuloma were distributed in the cortex and 24).

Aspergillus fumigatus hyphae were detected in the center of granuloma areas. Areas of infarction and necrosis were extended all over the renal tissues. (Fig. 26).

The liver was enlarged and pale in colour. Microscopical examination of the hepatic tissues revealed fatty changes of hepatocytes, focal areas of coagulative necrosis with infiltration of neutrophils and lymphocytes cells, hyperactivity of phagocytic cells were detected. Small focal irregular vaculation could be seen between hepatocytes.

**DISCUSSION**

Intravenous inoculation of pregnant ewes and goats at advanced stage of pregnancy by viable spores suspension of A. fumigatus caused abortion in all experimental animals. It is evident that placental tissues favourable environment for growth of A. fumigatus. In this respect Gorbel and Eades (1973) added that, placenta has been found to contain substances which enhance fungal growth. On the contrary non pregnant uterus is highly resistant to fungal infection (Laing, 1979).

Extensive coagulative necrosis of the placenta of aborted ewes and goats in the present study may be as a result
of vascular lesions due to A. fumigatus hyphae and or their mycotoxins formation (Grealt et al., 1989). In addition fungi in the tissue are potent sources of antigens and cause hypersensitivity responses (Latage & Paris, 1991).

High infiltration with polymorphonuclear and lymphocytic cells in the necrosed area indicated that, A. fumigatus may cause suppurative inflammation to the infected

Diamond et al. (1978) and Diamond and Clark (1982) reported that polymorphonuclear cells play a significant role in host defence against Aspergillosis. On the other hand, genus Aspergillus contains numerous polar lipids as well as neutral lipids, phenolic compound and heterocyclic toxins which have been shown to inhibit phagocytosis and has an immunosuppressive effect (Washburn et al., 1990).

Hyphae of A. fumigatus were massively scattered throughout the necrotic tissue of placentome and concentrated mainly in the maternal placenta directed to the chorionic villi of the foetal placenta. The presence of A. fumigatus hyphae within the pathological lesion of placenta is considered a final confirmatory diagnosis of mycotic abortion. These results agreed with that reported by Hillman (1969) and Krogh (1985).

The hyphae of A. fumigatus tend to penetrate the vascular wall of blood capillaries causing vasculities, emboli or thrombi leading to ischemia and infarctions of the placental tissues. Similar findings were reported by Cysewski and Pier (1968). There are many factors which illustrate the affinity of A. fumigatus to invade blood vessels of the host, one of them is iron which is necessary for microbial growth, A. fumigatus produces two major hydroxamate siderophores, these compounds can compete successfully with human iron binding protein to acquire iron to support fungal growth (Rhodes et al., 1992).

The endometrium of aborted ewes and goats showed focal necrosis and A. fumigatus hyphae were detected at the upper service of the endometrium. In one case of aborted goat, A. fumigatus reproductive vesicle was detected. No available literature dealing with formation of reproductive vesicles of A. fumigatus in the endometrium however, Chandler et al. (1980) reported that, at any well aerated cavity, the conidiophores (fruting bodies, vesicles) may be developed.

Skin lesions of aborted foeti in the form of subcutaneous
haemorrhages and infiltration of polymorphonuclear cells with fragments of A. fumigatus hyphae were detected. This result indicated that, the foetal skin lesion resulted from fungal invasion from exterior through foetal fluids.

Foetal death and abortion in the experimental animals resulted most probably from toxic effect and invasion of the A fumigatus hyphae to the vascular system of placenta. This lead to extensive necrosis of all placentomes which interfered with the supply of oxygen and nutrients to the foetus and resulted in abortion.

Extensive suppurative mycotic Pneumonia accompanied with areas of cavitation in the lung tissue, indicated chronic pulmonary lesion. These pathological findings are in agreement with the findings of Ainaworth and Austvick (1973), Chattopadhyay et al. (1987) and Sing et al. (1995).

Pathological examination of the heart of aborted ewes and goats revealed mycotic granuloma, this result is agreed with that reported by Hill et al. (1971) who reported that, microscopic granuloma were seen in the myocardium in case of experimental mycotic abortion in bovine.

Foci of mycotic granuloma were detected in the brain tissue of experimented animals due to angiinovation and dissemination of Aspergillus fumigatus spore through the circulation. In this respect, Khoo et al. (1966) Boon et al. (1990 & 1991) and Bodey et al. (1992) reported that, haematogenous spread of fungi may affected other organs specially the brain and heart.

Macroscopic and histopathological examination of the spleen of experimented animals revealed diffused mycotic granuloma, this result are in agreement with that reported by Hill et al. (1971) in Cattle infected with Aspergillus fumigatus. Spores of Aspergillus fumigatus were seen in the splenic tissue, this indicates that, the lymphatic spread of fungi from aspergillosis lesion can occure. In this respect Jensen (1994) reported that, generally lymphatic spread of fungi from aspergillosis lesion are rarely seen. This may be as a result of clearance mechanisms of the body against spore infection of fungus.

Pathological changes of the hepatic tissue revealed fatty changes, focal coagulative necrosis with leukocytic infiltration, no mycotic granuloma could be detected in the examined tissue. Hill et al. (1971) reported mycotic
granuloma in the liver of cattle infected with Aspergillus fumigatus. This variations may be as a result of species variation phagocytic activity of the tissue organs against infection.

Pathological examination of the renal tissue indicated that, kidney is highly sensitive organ for infection with Aspergillus fumigatus. The pathological lesions may be attributed to invasion of infective agent to the blood capillary forming thrombosis and infarction necrosis due to ischemia of the renal tissue. This results are in agreement with that reported by Young et al. (1970).

Angiogenesis and penetration of Aspergillus fumigatus to the different tissues of the body without any regard for structural boundaries illustrate the ability of the fungus to breakdown of local elastic tissue barriers. There are many factors which illustrate the affinity of Aspergillus fumigatus to invade blood vessels of the host. One of them is iron which is necessary for microbial growth, Aspergillus fumigatus produces two major hydroxymate siderophores, these compounds can compete successfully with human iron binding protein to acquire iron to support fungal growth (Rhodes et al., 1992). On the other hand, fungi of the genus Aspergillus contain numerous polar lipids which have been shown to inhibit phagocytosis and also has an immunosuppressive effect (Washburn et al., 1990). Also Aspergillus species produce a proteinase enzyme which plays a direct role in pathogenesis and facilitates the entry of the organism from a colonization site into the parenchyma (Liotta et al., 1979 and Goldfarb, 1983) all of these factors share together to inhibit the host defence.

In conclusion, systemic mycosis due to Aspergillosis in domestic animals under Egyptian environmental conditions needed more studies in regard to the rate and percentage of infection and pathological lesions.

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Fig. 1: Dorsal view of cytoledons in placenta of aborted ewe, showing necrosis and hyperemic borders (arrows).

Fig. 2: Cross section of placentome of aborted ewe showing triangular area of infarction (arrows).

Fig. 3: Placentome of aborted ewe, showing hyphae of A. fumigatus in the necrotic PAS, X 220

Fig. 4: Placentome of aborted goat, showing areas of coagulative necrosis of both foetal and H & E, X 100.

Fig. 5: Placentome of aborted goat, showing areas of coagulative necrosis of both foetal and H & E, X 100

Fig. 6: Placentome of aborted goat, showing highly invasion of A. fumigatus hyphae into necrotic GMS, X 100

Fig. 7: Placentome of aborted ewe showing A. fumigatus hyphae. GMS, X 400.

Fig. 8: Placentome of aborted ewe, hyphae of A. fumigatus penetrating the vascular wall of GMS, X 220.

Fig. 9: Endometrium of aborted goat, showing large focal area of necrosis contain vesicle (arrow) H & E, X 100

Fig. 10: High magnification of the PAS, X 200

Fig. 11: High magnification of the GMS, X 400

Fig. 12: Foetal dermatitis due to A. fumigatus H & E, X 100

Fig. 13: Aspergillus fumigatus hyphae in foetal skin lesion. GMS, X 400

Fig. 14: Lung of aborted ewe, showing large mycotic granulomat H & E., X 50.

Fig. 15: Lung of aborted ewe, showing multiple hyphae of Aspergillus fumigatus and reproductive vesicle (arrow) in the necrotic tissue. PAS, X 200

Fig. 16: Lung of aborted ewes, showing Aspergillus fumigatus hyphae. GMS, X 440

Fig. 17: Lung of aborted ewe, showing reproductive vesicles of the Aspergillus fumigatus (cross...
Fig. 18: Lung of aborted ewe, showing large area of suppurative inflammation and fungal colonies of Aspergillus

PAS, X 100

Fig. 19: Brain of aborted ewe, showing mycotic granuloma and hyphae

PAS, X 400

Fig. 20: Brain of aborted goat, showing mycotic granuloma and polymorphonuclear cells.

PAS, X 440

Fig. 21: Spleen of aborted ewe, showing multiple focal mycotic granuloma

H & E, X 50

Fig. 22: Spleen of aborted ewe, showing Aspergillus fumigatus hyphae.

H & E, X 440

Fig. 23: Spleen of aborted ewe, showing spores of Aspergillus fumigatus (arrows)

PAS, X 1000

Fig. 24: Kidney of aborted ewe, showing intensive glomerulonephritis.

H & E., X 150

Fig. 25: Kidney of aborted ewe, showing multiple focal mycotic granuloma with extensive

H & E X 50.

Fig. 26: Kidney of aborted ewe, showing area of infarction necrosis

H & E.

X 100.