MYCOTIC ABORTION IN EWES PRODUCED BY INTRAVENOUS

INOCULATION WITH ASPERGILLUS FUMIGATUS

by

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INTRODUCTION

Infection of the bovine placenta by a fungus was first reported by Theobald Smith in 1920 (20). Bovine mycotic abortion has since been recognized in many parts of the world (1 pp. 53-56, 9, 24). As other causes of abortion are controlled, fungal infections resulting in abortion assume greater importance. In 1961 Austwick and Venn (5), in England, reported that fungi were the etiologic agents in 19.2% of the cases of bovine abortion investigated during a 6-year period. Hillman et al. (14) found fungi to be the cause of 8.8% of the cases of bovine abortion submitted to the New York State Diagnostic Laboratory in 1960-61. However, when considering only cases in which an etiologic diagnosis was made, 36% of the New York cases were due to fungi. These authors state that, "Fungi continue to be the principal diagnosed cause of abortion in New York state cattle." Eighteen species of fungi have been implicated in mycotic abortion; the organism most commonly found is Aspergillus fumigatus, which has been isolated in 64% of the cases (1 pp. 53-56).

Present knowledge of mycotic abortion is largely limited to the fetus and placenta. Except for the act of abortion, this condition is asymptomatic in the dam. Aborting cows are usually retained in the herd with subsequent pregnancies being normal.

The pathogenesis of the disease is not well understood. Mycotic abortion is thought to result from exposure to aerosols with primary pulmonary involvement followed by hematogenous dissemination to the uterus. The lesions in the placenta suggest a hematogenous route of infection of that organ (1 pp. 53-56, 7, 9, 20). The gastrointestinal tract may be the portal of entry in some cases (11) and ascending infection by way of the vagina has also been suggested (18). This latter route, of doubtful significance in the cow, may be important in the mare (15).

Limited investigations of experimentally produced abortion in cattle have been reported (7, 10). These studies were concerned primarily with comparing the placental lesions observed in the experimental disease and those found in naturally occurring cases. Lesions outside of the genital tract have been reported only when a large inoculum was given or when the experimental cows had intercurrent bacterial infection.

Aspergillus fumigatus was used in this study because of its predominance as the etiologic agent in proven cases of mycotic abortion. Since placental infection is believed to be the result of hematogenous dissemination from a primary focus elsewhere in the body the intravenous route of inoculation was chosen. Ewes were chosen for this study instead of cows because of similar placentation, shorter

gestation period, smaller size, and similar nutritional requirements.

The objectives of this investigation were to: (1) produce abortion in ewes by intravenous inoculation with spores of _Aspergillus fumigatus_, (2) observe the course of the disease produced, and (3) study the development of the lesions in the ewe with particular emphasis on those occurring in the placental attachment.

LITERATURE REVIEW

The pathogenesis of mycotic abortion has not been resolved. It has been suggested that placental infection results either from hematogenous dissemination from a primary focus of infection in the lung or gastro-intestinal tract or from an ascending infection of the genital system. Smith [20] believed that the nature of the placental lesions indicated a hematogenous infection of that organ. Bendixen and Plum [7] considered that the primary infection was pulmonary but that the infection was only transient in the lungs and spread hematogenously to the placenta.

Ainsworth and Austwick (1 pp. 53-56) stated that there was every indication that the infection was originally derived from the spores of fungi present in large numbers in mouldy hay, straw and feedstuffs and hence also in the air of cow-sheds. A high incidence of benign nodular lesions was found in the lungs of healthy dairy cows by Austwick [4]. He found asteroid bodies in 66% of 49 pairs of lungs obtained from a slaughter house. One-half of these bodies contained swollen hyphae.

Cordes, Dodd and O'Hara [9] reported mycotic abortions in association with acute mycotic pneumonia. They concluded that the nature of the uterine lesions indicated a hematogenous route of infection but they drew no conclusions as to the route of pulmonary infection.

The gastro-intestinal route of infection was suggested by Gortsevskii (11). He reported a sporadic illness in cows with gastro-intestinal symptoms, lesions in the omasum and abomasum and abortion of cows in late pregnancy caused by A. fumigatus. Rollinson and Haq (18) suggested the ascending route of infection. They isolated Absidia corymbifera from the vaginal discharge of a cow which had aborted and from the prepuce of the bull that had served her.

Limited investigations of experimentally produced abortion in cattle have been reported. Gilman and Birch (10) inoculated 5 cows intravenously with suspensions of Mucor species producing abortion in 1 cow and placental infection without abortion in a second. Bendixen and Plum (7) produced abortion in 5 cows by intravenous inoculation. Three cows were given spores of A. fumigatus, 1 received spores of Absidia ramosa and 1 spores of both organisms. They also attempted to produce abortion by feeding cultures to 4 cows, but were not successful. In both reports the authors concluded that the placental lesions observed in the experimental infection were similar to those found in naturally occurring cases.

Abortions caused by fungi usually occur late in pregnancy; although, they have been observed as early as the third month. Plum (16) found that 75% of 111 cases studied were in the last 3 months of pregnancy. Austwick and Venn

(5) reported the distribution of 140 cases; 73.6% were in the last trimester and 25.7% in the second trimester. Austwick and Venn (5) reported a high winter incidence of mycotic abortion, with 68.8% occurring from November to April inclusive. This high winter incidence did not correspond to the period when most cows were in the 7th to 8th months of pregnancy which occurred in July-August. Van Ulsen (25) noted that the percentage of cases doubled after the wet summer of 1954 when conditions for haymaking were poor.

Ainsworth and Austwick (1 pp. 53-56) in reviewing the literature up to 1957 stated that noticeable symptoms in the dam, either before or after the act of abortion, had not been recorded. Gortsevskii (11), in 1960, reporting a sporadic illness in cows attributed to A. fumigatus infection, noted depression, fever, rumen atony, diarrhea, rapid emaciation, circulatory insufficiency, pulmonary edema and some deaths. Cows in advanced pregnancy aborted. Hemorrhagic infarcts were observed in the abomasal mucosa, folds of the omasum and in the liver. Aspergillus fumigatus was isolated from selected lesions and hyphae were demonstrated in sections. Cordes, Dodd and O'Hara (8) reported 8 cases of mycotic abortions in which the cows died of an acute fungal pneumonia within 4 days after the abortion.

Changes associated with mycotic infection of the

placenta have been described by Smith (20), Bendixen and Plum (7) and Austwick and Venn (5). They found an exudate between the chorion and the uterine wall, necrosis of the caruncular stalk with retention of maternal tissue on the cotyledons and enlarged necrotic cotyledons with a crater-like depressed center. In some cases these changes were not present in all the cotyledons. The intercotyledonary chorio-allantoic membrane (CAM) was edematous and had small raised lesions on the surface. These usually coalesced to give the chorion a thickened leathery appearance. Bendexin and Plum concluded that in most cases the appearance of the placenta was similar to that found in "contagious abortion". The principal microscopic changes described by these authors were hyperemia, hemorrhage, necrosis and neutrophilic exudation. Fungal elements were observed in necrotic cotyledons.

Cordes, Dodd and O'Hara (9) observed similar gross lesion in the placenta as well as lesions in the uterine wall in one of their cases. The latter cow had died of mycotic pneumonia 24 hours after aborting and had irregular, elevated or eroded, dark red patches up to several cm. in diameter on the uterine musosa. These were covered in places by irregular, thin, yellowish grey, firmly adherent pseudomembranes. Similar microscopic changes were found in all tissues. These consisted of necrosis and thrombosis of capillaries, arterioles and small arteries with necrosis,

hemorrhage and suppuration in the surrounding tissues.

Fungi have been recovered from fetal stomach contents, fetal skin lesions and fetal lung. There have been no reports of involvement of other fetal organs. Austwick and Venn (5) determined the incidence of hyphae in the stomach contents of aborted fetuses in which fungal infections of the placenta were established. In 63 such cases they found that 15 (24%) had negative stomach contents but positive placentae.

The most frequent lesions reported in the fetus have been those in the skin. Austwick and Venn (5) reported skin lesions in 49 of 105 fetuses (47%). Different types of skin lesions have been described by various authors (5, 9, 13); (a) discrete, raised, grey foci; (b) diffuse white areas which were not raised or inflammed, and (c) soft red, slightly elevated foci. The histopathologic changes reported (9, 13) were necrosis, hemorrhage and suppuration in the dermis with vasculitis and thrombosis of dermal vessels.

Smith (20) and Gilman and Birch (10) reported the isolation of fungi from fetal lungs. No description of lung lesions was given in either report.

Eighteen species of fungi have been implicated in mycotic abortion; the organism most commonly found is Aspergillus fumigatus, which has been isolated in 64% of the cases. This organism together with other Aspergilli and members of the genera Absidia and Mucor account for 86% of

the fungi associated with abortion (1).

Aspergillus fumigatus, the agent chosen for these experiments, has been described by Raper and Fennell (17). This organism, a cosmopolitan saprophyte, is abundant in soils and in decomposing organic matter, including forage. It is the predominate fungus in moldy hay and straw, baled at 40% moisture content, and in moist plant materials undergoing rapid decomposition with heating. It is known to germinate and grow at temperatures up to 50°C.

Colonies of A. fumigatus growing on Czapek's solution agar vary from velvety to floccose. They are white at first becoming green with development of conidial heads. The reverse of the colony is usually colorless, but maybe yellow, green or even red-brown. Globose echinulate conidia, 2.0 to 3.5 μ's in size, are born in parallel chains in a tightly compact column on a single row of sterigmata. The conidiophores are smooth with a flask shaped vesicle, which is usually fertile only on the upper one-half. Sclerotia or cleistothecia are not found. The hyphae of A. fumigatus are hyaline, septate, have parallel sides and measure approximately 3 μ in diameter.

A knowledge of the anatomy of the ovine placenta is essential for the presentation of the results of this study. The salient features of the placental anatomy of the sheep as described by Wimsatt (26) are presented. In all higher mammals the chorion bears vascular villi which engage the

uterine mucosa. This functional union of maternal and fetal tissues forms the placenta. In sheep the chorionic villi are gathered into tufts, the cotyledons; these cotyledons are separated by extensive areas of avillous chorion, the intercotyledonary CAM. The villi of the cotyledons protrude into rounded elevations of the endometrium, the caruncles. The caruncle plus the cotyledon constitutes the placentome. A cross section of an ovine placentome is depicted (Figure 1). From the base of the concave caruncle (Figure la) maternal septae extend toward the hilus (Figure 1) of the placentome and interdigitate with the fetal villi. The septae are composed of maternal vessels and supporting connective tissue and are covered by a syncytium derived from the binucleate giant cells of the chorionic epithelium. Beginning at about the 70th day of gestation, the tips of the septae undergo hyaline degeneration with rupture of maternal vessels and the formation of hematomata (Figure lc) which are characteristic of the ovine placenta. These are formed in the space between the bases of the fetal villi.

The maternal blood supply to the placentome has been described by Barcroft and Barron (6). Branches of the miduterine artery penetrate the base of the caruncle and send radial branches into the septae. The latter give off few if any branches as they travel toward the tip of the septae. After traversing approximately 3/4 of the distance toward the hilus these arterioles break up abruptly into a

Figure 1. Cross section of an ovine placentome showing the
base of the caruncle (a), the intercotyledonary
CAM, (b) and the hematomata (c). The dotted
line encloses the hilar region. This placentome
was considered to be normal. Hematoxylin and
eosin stain x 6.5.
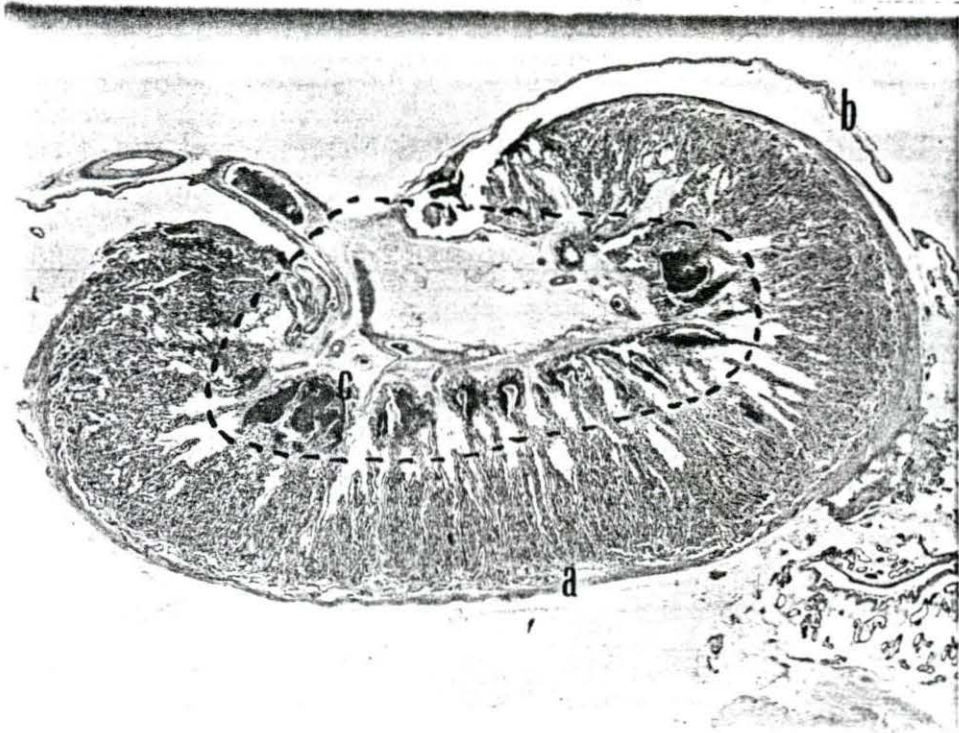
dense capillary network which extends to the end of the septae. Capillaries from this bed, lead back toward the base of the septae approximately parallel to the arteriole and communicate with veins in the base of the caruncle.

PROCEDURES

Animals

Fifteen crossbred western ewes, from one flock, constituted the principal and control animals for the A. fumigatus infection studies. Two Suffolk ewes, from a different flock, were inoculated intra-arterially with latex spheres to determine the effects of non-infectious mechanical blockage of the maternal blood supply to the placenta. All ewes were bred at the National Animal Disease Laboratory and breeding dates were recorded. The animals used for these experiments with duration of pregnancy at the time of inoculation are listed in Table 1.

During the experiments, the ewes were housed in groups in rooms with a filtered air supply and controlled temperature and humidity.

Preparation of Spore Suspensions

A strain of A. fumigatus, originally isolated from the stomach contents of an aborted bovine fetus, was used for these studies. Spores were obtained from 8-day-old cultures, grown at 25°C. on Sabouraud dextrose agar (SDA), by washing the cultures with sterile phosphate buffered saline, 0.15 M, pH 7.4 (PBS) containing 1% Tween 80. These washings were centrifuged at 8,000 x G for 20 minutes, the supernate decanted, and the spores resuspended in 0.01% Tween 80 in PBS.

Table I. Summary of pertinent information for all experimental animals used in this study

Animal number	Duration of pregnancy in days when inoculated	Days PI when killed	Aborted	Placenta infected[a]
16	68	1	no	+
18	100	2	no	+
37	102	2	no	-
67	98	4	no	+
41	100	4	no	+
46	100	8	no	+
17	101	8	no	+
9	101	9	yes	+
10	101	9	yes	+
60	85	11	yes	+
22	97	12	yes	+
69	101	23	yes	+
42	81	26	no[b]	+
38[c]	107	14	no	-
40[c]	98	6	yes	-
74[d]	98	29	no	-
24[d]	105	41	no	-

[a]As determined by microscopic and/or cultural examination

[b]Fetal maceration occurred

[c]Ewes for intra-arterial injection of latex spheres

[d]Diluent controls

The concentrated spore suspension was dispensed into 30 ml. vaccine vials in 5 ml. aliquots, shell frozen in a mixture of dry ice and isopropyl alcohol, and stored at -40°C. Samples of the spore suspension, examined microscopically, were found to be free of mycelial fragments and conidial heads.

The number of viable spores per ml. in this suspension was determined by making plate counts. Serial dilutions of the concentrated spore suspension in PBS were made and pour plates were made in triplicate from appropriate dilutions using SDA. Colony counts were made following 3 days incubation at 25°C.

Inoculation of Ewes

Three days prior to the inoculation of ewes, a vial of frozen spore suspension was thawed and the number of viable spores per ml. determined. On the day of inoculation, the concentrated spore suspension was thawed and diluted with 0.01% Tween 80 in PBS to give an estimated final concentration of 2×10^6 viable spores per ml. based on the previous plate counts.

A plate count was performed on an aliquot of the inoculum in order to determine the actual number of viable spores injected. Each of the principals in this study were given 10 ml. of the inoculum intravenously via the jugular vein; the controls were given 10 ml. of the diluent, 0.01%

Tween 80 in PBS. The intravenous inoculation study was performed as duplicate experiments; 7 principals and 1 control were used in the first experiment and 6 principals and 1 control were used in the second. The spore dose for each principal was approximately 2×10^7 viable spores. These ewes were approximately 3 months pregnant at the time of inoculation (Table I). During the experiments, the ewes were observed and temperatured daily.

Blood Studies

Blood samples for total and differential leucocyte counts, hemoglobin and packed cell volume (PCV) determinations were taken preinoculation and postinoculation (PI) at 2 days, 5 days, and thereafter at weekly intervals or at the time of abortion. The disodium salt of (Ethylenedinitrilio) Tetracetic acid (NaEDTA) was used as an anticoagulant. Smears for the differential leucocyte counts were prepared from this blood within 4 hours after collection. These smears were stained by the Wright-Giemsa technique (19). The PCV was determined by the microhematocrit method[1]. The hemoglobin concentration was determined by the oxyhemoglobin method (21).

Blood samples for culture were taken before inoculation and PI at 5 minutes, 1-1/2 hours, 4 hours, 8 hours, 24 hours

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[1]Hematocrit Centrifuge, Model MB, International Equipment Company, Boston, Massachusetts.

and 48 hours. In the first experiment, blood samples were placed in sterile vials containing dried ammonium and potassium oxalate. This blood was cultured by streaking 0.2 ml. on the surface of a SDA plate and incubating at $25^{\circ}C$. Colonies identified as $\underline{A}$. fumigatus were enumerated as they appeared and plates without growth were held for 2 weeks before discarding.

This procedure was altered in the second experiment in order to enhance the detection of the organism. Two ml. blood samples were taken in syringes containing 0.3 ml. of a 5% solution of sodium citrate. The entire 2.3 ml. was distributed, directly from the syringe, onto 4 SDA plates and streaked on the surface of the agar.

Postmortem Procedures

Ewes were killed by electrocution on the following days PI: 1 (first experiment only), 2, 4, 8, and thereafter at the time of abortion (Table I). The controls were held until all other ewes had been killed.

At postmortem examination, the uterus was removed intact, the uterine wall of the pregnant horn incised, amniotic fluid aspirated with a syringe and needle and then the fetal membranes opened and the fetus removed. Fetal stomach contents and amniotic fluid specimens were centrifuged at 2,900 x G for 10 minutes and the sediment cultured on SDA and examined microscopically for fungal

elements by mounting a drop of sediment in a mixture of
equal parts of 20% KOH and Parker Super-Chrome blue-black
ink (P-51 stain). Fetal tissues routinely taken for
culture and histopathologic examination were lung, liver,
spleen, and kidney. Tissues for culture were placed in
sterile plastic petri dishes and stored at -40°C. Tissues
for histopathologic study were placed in 10% formalin.

After removal of the fetus, the placenta was examined.
Placentomes were sliced and wet mounts prepared of scrapings
from the cut surface using P-51 stain. In cases with more
advanced lesions, some of the cotyledons were separated
manually from the caruncles. Specimens of placentomes
were both frozen for culture and fixed in 10% formalin.
After examination of the placenta was completed, it was
removed from the uterus and the mucosa washed and examined
for visible lesions. In the first experiment, sections of
uterine wall were taken only when gross lesions were present.
In the second, sections were taken routinely for both
culture and histopathologic examination; these included
gross lesions when present.

Tissues outside of the genital tract routinely sampled
culturally were: brain, lung, heart, diaphragm, liver,
spleen, kidney, adrenal; and bronchial, mediastinal, internal
iliac and popliteal lymph nodes. At the time of postmortem
examination, these were frozen. When cultured, weighed

samples of the various tissues were ground in PBS, 9 ml./gm. of tissue, in a Waring blender, or, for small pieces, in a Ten Broeck grinder. In the first experiment, 1 ml. of the resulting suspension was streaked on the surface of a SDA plate; in the second experiment, 3 plates were streaked from each suspension, 1 ml./plate. All plates were incubated at 25°C. and those without growth were held for 2 weeks before discarding.

Tissues for histopathologic study included those taken for culture and, in addition, thyroid, pancreas and pituitary. All tissues, except brain, were fixed in 10% formalin. The brain was fixed in 25% formalin and sections cut from the cerebral cortex, basal ganglia, thalamus, superior colliculus, cerebellum, and medulla oblongata. Paraplast[1] was used as in infiltrating and embedding material. Sections were cut 7 μ thick using a rotary microtome[2]. The stains and staining procedures used are described in the manual of histologic and special staining techniques of the Armed Forces Institute of Pathology (2). Hematoxylin and eosin staining was used routinely and Gridley's Fungus stain for demonstration of the organism.

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[1]Aloe Scientific, 3501 Raleigh Ave., So. Minneapolis 16, Minnesota.

[2]Model 829, American Optical Company, Buffalo 15, New York.

Intra-arterial Injection of Latex Spheres

A suspension of latex spheres[1] was injected into the middle uterine artery of 2 ewes, numbers 40 and 38, which were 98 and 107 days pregnant, respectively, at the time of injection. This suspension contained 4×10^8 particles per ml. as determined by counting in a hemocytometer[2]. Prior to use, Thimersol[3] was added to the suspension to a final concentration of 1:5,000.

A laparotomy was performed using thiamylal[4] sodium intravenously as a general anesthetic, the middle uterine artery exposed, and the suspension injected. For ewe number 38, 1.4 ml. of the suspension was injected into the right middle uterine artery; this was thought to be the pregnant horn at the time of surgery. When the procedure was repeated with ewe number 40, 12 ml. of the suspension were injected, 5 ml. into the left uterine artery and 7 ml. into the right uterine artery.

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[1]Styrene, divenyl benzene, capolymer latex spheres, particle diameter 6-14μ, Run No. EP1358-11, Bio-products Department, Dow Chemical Co., Midland, Michigan.

[2]Spencer Bright-line Hemocytometer, American Optical, Instrument Division, Buffalo 15, New York.

[3]Merthiolate - Eli Lilly Company, 740 So. Alabama St., Indianapolis, Indiana.

[4]Surital, Parke Davis and Company, Joseph Campau Avenue, Detroit 32, Michigan.

Ewe number 38 was killed by electrocution 14 days after injection, and ewe number 40 at 6 days after injection. At postmortem examination, the uterus was removed, opened and examined for gross lesions as described before. Wet mounts in P-51 stain were made of fetal stomach contents and scrapings from canuncular and cotyledonary lesions. These were also cultured on SDA as described above. Sections of uterine wall and placentomes were fixed in 10% formalin, and processed for histologic study as previously described.

RESULTS

Clinical Observations

Following incoulation of the ewes with spores of A.
fumigatus there was a rise in rectal temperature of 2 to 3
degrees and a slight increase in respiratory rate. These
returned to normal values by 4 days after inoculation. No
other abnormalities were observed in these ewes until the
act of abortion had started.

Cultural Examinations

Blood

Cultures of blood samples taken at 5 minutes PI were
positive for A. fumigatus in 5 of the 7 ewes in the first
experiment and in 3 of the 6 ewes in the second experiment.
After this time positive cultures were obtained occasionally
with none being positive after 24 hours in experiment 1 and
after 8 hours in experiment 2. The results of the blood
cultures are tabulated in Tables II and III.

Tissues

Asperigullus fumigatus was isolated most frequently
from placental tissues, fetal stomach contents and amniotic
fluid. Infection in maternal tissues was most pronounced in
the first 4 days PI. Excluding the genital system, A.
fumigatus was isolated most consistently from 5 maternal
organs, i.e., lung, liver, spleen, heart and kidney. In 5

Table II.   Blood cultures

1st Experiment

Ainmal number	Preinoculation blood sample	Time blood taken		Postinoculation			
		5 min.	1-1/2 hr.	4 hr.	8 hr.	24 hr.	48 hr.
16	0**	2**	0	1	0	0***	ND
22	0	2	0	0	0	0	0
42	0	1	0	0	0	0	0
67	0	1	0	0	0	0	0
60	0	0	0	0	0	0	0
46	0	0	0	1	0	4	0
18	0	2	1	0	0	0	0
74*	0	ND	ND	ND	ND	0	0

*Control.

**Number of colonies from 0.2 ml. blood plated.

***20-hour sample for ewe 16; all other samples taken at 24 hours.

ND = Not done.

Table III. Blood cultures

2nd Experiment

Animal number	Preinoculation blood sample	Time after inoculation					
		5 min.	1 hr.	4 hr.	8 hr.	24 hr.	48 hr.
41	0**	0	1**	0	98	0	0
17	0	1	ND	0	0	0	0
9	0	4	0	0	11	0	0
10	0	0	0	0	3	0	0
69	0	0	1	0	0	0	0
37	0	1	0	0	0	0	0
24*	0	ND	ND	0	ND	0	0

*Control.

**Number of colonies from 2 ml. of blood plated.

ND = Not done.

ewes killed during the first 4 days after inoculation the organism was isolated from 15 of the 25 organs; in 8 ewes killed between 8 and 26 days after inoculation, the organism was isolated from only 4 of the 40 organs. In addition, hyphae of the Aspergillus type were present in a lesion in the myocardium of ewe 9 (killed 9 days PI) but cultures were negative for A. fumigatus (Table IV).

Table IV. Culture results

Days PI	Lung	Heart	Liver	Spleen	Kidney	Placenta
1	1/1*	0/1	1/1	1/1	1/1	1/1
2	2/2	0/2	2/2	2/2	1/2	1/2**
4	2/2	1/2	0/2	1/2	0/2	2/2**
8	0/2	0/2	0/2	1/2	0/2	2/2***
9	0/2	2/2***	0/2	1/2	0/2	2/2
11 & 12	0/2	0/2	1/2	0/2	0/2	2/2***
23 & 26	0/2	0/2	0/2	0/2	0/2	2/2
Totals) culture) only)	5/13	3/13	4/13	6/13	2/13	7/13

*Numerator = number of ewes positive; denominator = number of ewes cultured.

**Positive microscopically but not culturally.

***One ewe positive microscopically but not culturally; the other ewe positive microscopically and culturally.

Aspergillus fumigatus was isolated only from those lymph nodes shown in Table V; all other lymph node cultures were negative.

Table V. Lymph node cultures

Ewe number	Days PI	Lymph node			
		Bronchial	Internal iliac	Mesenteric	Popliteal
16	1	+	+	-	+
18	2	+	-	-	-
17	8	-	-	+	-

Cultures made from the diaphram, adrenal gland and brain were all negative for A. fumigatus.

All cultures of tissues from the control ewes were negative for A. fumigatus.

Cultural examination of the genital tract included specimens from abnormal areas of the uterine wall and all placenta in experiment I. In experiment II uterine wall specimens, excluding placentomes, were cultured routinely. All uterine wall cultures were negative; however, organisms were seen microscopically in wet mount preparations made from 0.5 to 2 mm. purulent foci in the endometium in ewe 69.

Aspergillus fumigatus was isolated from placental tissues of only one of 5 ewes killed between 1 and 4 days PI (ewe 16 at day 1). Aspergillus type hyphae were observed

microscopically in stained wet mounts of abnormal appearing placental tissue in 3 of the 4 remaining ewes. Aspergillus fumigatus was isolated from the placenta of 6 of the 8 ewes killed between 8 and 26 days PI. In 2 ewes which were culturally negative (46 and 60) Aspergillus type hyphae were demonstrated microscopically (Table VI).

The results of cultural examinations of amniotic fluid and fetal stomach contents are given in Table VI. Aspergillus fumigatus was not recovered from or demonstrated in amniotic fluid or fetal stomach contents before 8 days PI. All samples of both amniotic fluid and fetal stomach contents obtained between 8 and 26 days PI were positive either culturally or microscopically with the exception of the stomach contents of the fetus from ewe 10.

Skin lesions were observed in 3 fetuses (from ewes 46, 17 and 9). Aspergillus fumigatus was isolated from or demonstrated in the skin lesions of each of these 3 fetuses. The organism was not cultured from the lung, liver, spleen, or kidney of any of the fetuses. Histopathologic findings are discussed in a later section.

Aspergillus fumigatus was not cultured from or observed microscopically in the amniotic fluid, stomach contents, or tissues from any of the fetuses of the control ewes.

Table VI.   Cultural examination of materials from fetuses

Ewe number	Pays PI	Amniotic fluid	Fetal stomach contents	Placental infection
16	1	-	-	+
18	2	-	-	+*
37	2	-	-	-
67	4	-	-	+*
41	4	-	-	+*
46	8	+*	+	+*
17	8	+	+	+
9	9	ND	+	+
10	9	ND	-	+
60	11	+	+*	+*
22	12	+	+	+
69	23	ND	+	+
42	26	ND**	ND**	+

ND = Not done; in the case of amniotic fluid when abortion occurred this was not available.

*Positive on microscopic examination but not culturally.

**These were not available because of extensive fetal maceration.

Pathology

Gross pathology

Multiple petechial hemorrhages in the lungs were the most consistent abnormality observed outside of the pregnant uterus. These were most prominent in those animals killed within 10 days after inoculation. In ewes 69 and 42, killed 23 and 26 days PI respectively, the lungs appeared normal grossly. Focal, white, caseous lesions approximately 0.5 cm. in diameter, were observed in the myocardium of ewes 9, 10, and 69. No other visible abnormalities were observed in these ewes outside of the genital tract.

The only apparent lesions in the uterus occurred in ewe 69. These consisted of purulent foci 0.5 to 2 mm. in diameter, which were found most frequently in the mucosa of the caruncular stalk.

At one day PI no gross abnormalities were observed in the placenta. At 2 days PI moderate congestion of the cut surface of some placentomes was observed. By the fourth day PI distinct hyperemic zones, 3 to 4 mm. wide, were present on the cut surface of some placentomes. These extended from the hilus out to the base of the maternal tissue. Such zones were present in less that 25% of the placentomes examined, however, the cut surface of all placentomes examined appeared congested.

Necrotic zones, 2 to 8 mm. wide, with a hyperemic border were present in 75% of the placentomes examined at

Figure 2. Cross section of placentomes: (a) normal, (b) 2
         days PI, (c) 4 days PI and (d) 8 days PI. Only
         slight congestion was evident at 2 days PI while
         zones of necrosis (arrow) were evident at 4 and
         8 days PI.

Figure 3. Detached cotyledons in placenta of ewe 69 at 23
         days PI. Marbled appearance (a) was due to
         irregular areas of necrosis with hyperemic borders.
         Uniformly necrotic cotyledons (b) were also
         visible.
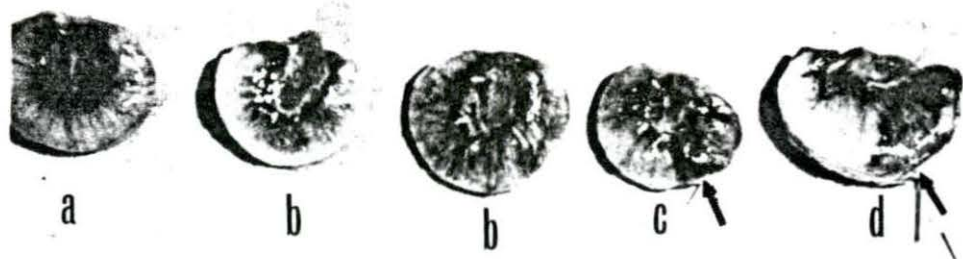
eight days PI. More than one such zone was present in some placentomes (Figure 2).

Visible lesions were present in the intercotyledonary areas of the CAM 8 days after spore inoculation. The membrane around some cotyledons, for a distance of 2 cm., was thickened, up to 0.5 cm., and edematous. A slight amount of reddish brown semifluid exudate was present on the uterine side of the CAM.

All of the six remaining principals, except ewe 42, (Table I) were in the process of aborting when killed. (The cervix was dilated, much of the placental fluids and uterine exudates had been expelled, and some placentomes had parted.)

In placentas examined 9 days or more after inoculation the intercotyledonary CAM was diffusely edematous, up to 0.5 cm. thick and red. Large amounts of thick, tenacious, brown exudate were present on the uterine surface of this membrane. In ewe 69, killed during abortion at 23 days PI, a dry area, several cm. in diameter, with crusted exudate was observed.

The cotyledons of intact placentomes were firmly incarcerated within the caruncles; when the fetal membranes were separated, varying amounts of necrotic caruncular tissue remained attached to the cotyledons. Irregular areas of necrosis with hyperemic borders were present on the surface of separated cotyledons; the intermingling of necrotic tissue, hyperemic border and normal tissue created a marbled appearance (Figure 3). Some placentomes were totally necrotic;

these appeared thickened, dry and tan (Figure 3). The cut
surface revealed that necrosis involved 50% or more of the
tissue of nearly all placentomes so examined.

Ewe number 42 appeared normal when she was killed 26
days after inoculation. The cervix was closed and the uterine
horns measured approximately 6 cm. in diameter. The uterus
was filled with thick, tenacious, dark brown necrotic
material. Distinct cotyledons were present but the inter-
cotyledonary portions of the fetal membranes were a necrotic
mass. The caruncles were small, the largest measuring 1 cm.
in diameter. All were necrotic and many cotyledons had
separated from the caruncles in the anterior 1/2 of the non-
pregnant horn. The fetus was small, with crown-rump distance
of 7 cm., and the skin had a leathery appearance. The
uterine mucosa was normal grossly.

Gross lesions in the fetus were confined to the skin
and lungs. The skin lesions were present in the fetuses of
ewes 46, 17 and 9. These appeared as multiple, erythematous
slightly raised foci up to 1 cm. in diameter in 46 and 17
(Figure 4). In the fetus from ewe 9, they appeared as
multiple yellow foci with erythematous borders. In all 3
fetuses, lesions were present on all areas of their bodies
with the most extensive involvement being on the head, neck
and shoulders where adjacent lesions coalesced.

Figure 4. Fetus from ewe 46 with discrete (a) and confluent
(b) skin lesions. These lesions appeared as
erythematous plaques.
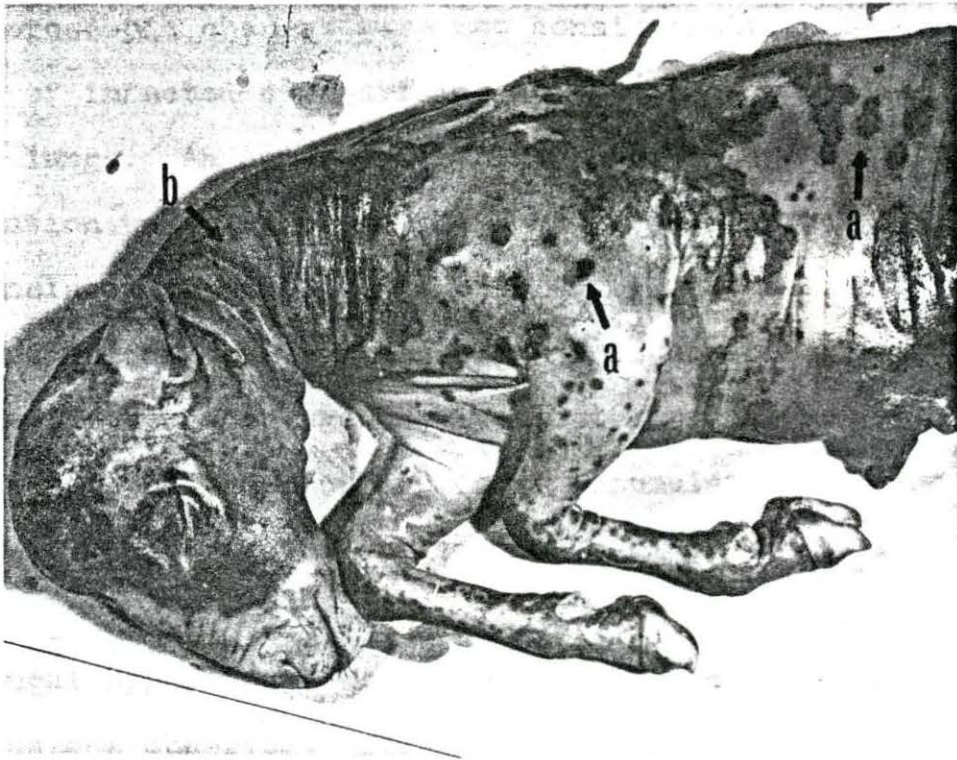
Pulmonary lesions were present in the fetuses from ewes 17 and 9. These consisted of multiple petechial hemorrhages and a single area of hemorrhage, 0.5 x 0.5 cm., at the ventral edge of the diaphragmatic lobe.

Histopathology

Microscopic changes were not consistently found in tissues of infected ewes outside of the genital system except for the lungs. An acute, multiple focal, interstitial inflammation was present in the lungs of all principal ewes. This consisted primarily of a thickening of the alveolar walls due to congestion and edema. In some areas, edema fluid and even hemorrhage were present in adjacent alveoli. Septal cell proliferation and focal accumulations of neutrophils were present occasionally in alveolar walls and in alveoli.

Fungal hyphae were not constantly associated with the focal lesions observed. Through 8 days PI, the average count of fungal elements was 9 per 20 high power fields (hpf's) while after 8 days the average was 2 per 20 hpf's. In ewes 9, 10 and 42 no hyphae were found. Fungal elements observed in lung sections consisted primarily of ungerminated spores. The maximum development of organism in tissue was the production of a germ tube not exceeding 15μ's in length.

In ewes 9, 10 and 22 there was a chronic focal

suppurative myocarditis. A central area of caseous necrosis and neutrophilic exudation was surrounded by a zone of histiocytes which blended with a narrow connective tissue capsule. Neutrophils and lymphocytes were found infiltrating the surrounding myocardium, connective tissue capsule and the histiocytic zone. Narrow branching septate hyphae were present in the necrotic centers of the lesions in ewes 9 and 10 but no fungal elements were found in the lesion in ewe 22. The lesion observed grossly in ewe 69 was not found in microscopic section and the lesion seen microscopically in ewe 22 had not been observed grossly.

Multiple hepatic foci, up to 30μ's in diameter, were observed in 8 ewes, 16, 18, 37, 67, 41, 17, 46, and 9. They consisted of focal accumulations of neutrophils and histiocytes. Fungal elements were observed in liver sections from 4 of these 8 ewes, 16, 18, 37 and 41. These elements were associated with the inflammatory foci and were seen as germinating spores.

In ewe 10 a single hepatic focus of chronic inflammation, 300μ's in diameter, was present. This consisted of histiocytes and giant cells with infiltrating neutrophils and lymphocytes. A narrow connective tissue capsule surrounded the lesion. A fragment of material resembling fungal mycelium was found in a giant cell. No hepatic lesions were observed in the remaining ewes.

Foci of reticulo-endothelial hyperplasia 20 to 30 μ's in diameter were observed in sections of spleen from ewes 18, 67 and 41. These foci contained organisms in the form of ungerminated spores or germinating spores with a germ tube 10-15 μ's long. Multiple foci of reticulo-endothelial hyperplasia, up to 300 μ's in diameter, with occasional giant cell formation were present in the section of spleen from ewe 10. Fungal elements were numerous in these foci in the form of fragments of hyphae and germinating spores. Neither lesions nor fungal elements were observed in sections of spleen from the remaining ewes.

Microscopic lesions were found in kidney sections of 3 ewes, 69, 42 and 22. A chronic multiple focal interstitial nephritis was present in ewes 69 and 42. This consisted of focal accumulations of histiocytes, lymphocytes and a proliferation of interstitial connective tissue. Eosinophilic casts containing a granular basophilic material were present in the renal tubules of ewe 69 and homogeneous eosinophilic casts in ewe 42. In ewe 22, there was a chronic multiple focal suppurative interstitial nephritis consisting of focal accumulations of neutrophils with lymphocytes and histiocytes. Within and around these foci there was a proliferation of connective tissue. Fungal elements were not found in Gridley stained sections from any of these three kidneys.

Lesions were not found in sections of bronchial, internal iliac, mesenteric, or popliteal lymph nodes from any ewe. Gridley stained sections of bronchial and internal iliac lymph nodes of all ewes were examined. Two objects, resembling ungerminated spores, were observed in the bronchial lymph node of ewe 37. No fungal elements were found in Gridley stained sections of the popliteal and mesenteric lymph nodes from ewe 16 or the mesenteric lymph node of ewe 17; these nodes had been culturally positive.

Lesions were not found in sections of the brain, pituitary, thyroid, adrenal, pancreas, or diaphragm from any ewe.

Prior to 8 days after inoculation minimal changes were observed in sections of the uterine wall. There was a partial loss of uterine epithelium, a few scattered neutrophils in the mucosa, and an occasional uterine gland contained a few neutrophils. In ewe 37, at 2 days PI, one focal area of suppuration and necrosis was present in the mucosa. The overlying epithelium was gone and in the lumen of the uterus there was as accumulation of necrotic debris and neutrophils. Fungal hyphae of the Aspergillus type were present in this debris and invaded the mucosa.

At 8 days PI in ewe 17, and at 9 days in ewes 9 and 10, there was an acute diffuse suppurative endometritis. Neutrophils were scattered throughout the endometrium and there was edema of the lamina propria. In addition to the diffuse reaction in ewe 17, there was a focal lesion on a

caruncular stalk. The uterine glands were dilated and filled with neutrophils and necrotic debris. The surrounding mucosa was densely infiltrated with neutrophils. There was necrosis of both glandular epithelium and the overlying uterine epithelium. Fungal hyphae were present in the exudate filling the uterine glands.

At 23 days PI, in ewe 69, there were many dilated uterine glands filled with an exudate of neutrophils and necrotic debris (Figure 5). In addition microabscesses were present in the mucosa between the uterine glands. There was a diffuse exudation of neutrophils, edema of the mucosa and focal necrosis of the uterine epithelium. Fungal hyphae were present in the uterine glands containing exudate.

No microscopic lesions were found in the sections of placenta from ewes killed at 24 and 48 hours PI.

Microscopic lesions were present in the placentomes of ewes killed at 4 days PI. These involved the distal half of the maternal septae and the adjacent areas of the fetal villi. The changes in the maternal tissue consisted of congestion and thrombosis of the vessels in the maternal septae and necrosis and neutrophilic exudation in the septae. In the fetal tissues there was a neutrophilic exudation, focal necrosis of chorionic epithelium, and congestion, especially of blood vessels immediately beneath the chorionic epithelium (Figures 6, 7 and 8). In addition there was thrombosis of fetal vessels in the villi and in the arcades

Figure 5. Section of placentome from ewe 69. A dilated
uterine gland in a caruncular stalk filled with
a suppurative exudate is shown. Hematoxylin
and eosin stain. x 40.
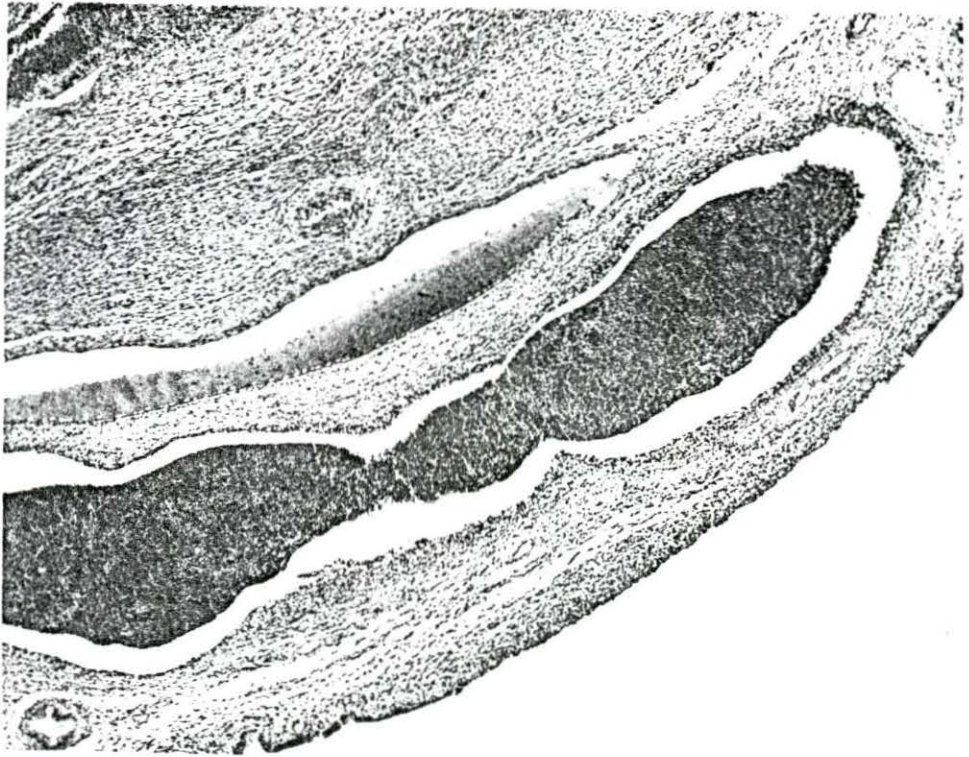
Figure 6. Section of placentome from ewe 41. There was
neutrophilic exudation in fetal villi (a) and a
loss of chorionic epithelium and neutrophilic
exudation, necrosis and thrombosis of vessels
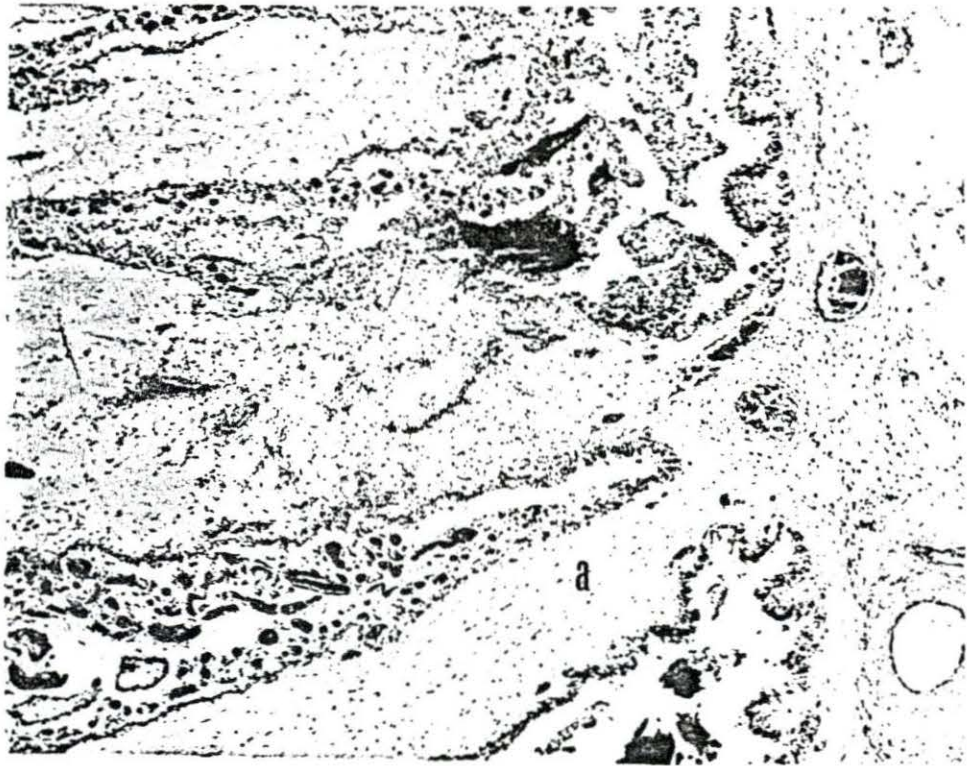in the maternal septum (b). Hematoxylin and
eosin stain. x 125.

Figure 7. Higher magnification of Figure 6. Hematoxylin
and eosin stain. x 300.

Figure 8. Serial section of tissue in Figure 6. Fungal
hyphae of A. fumigatus were visible in the
maternal septum and fetal villus. Gridley fungus
stain. x 300.
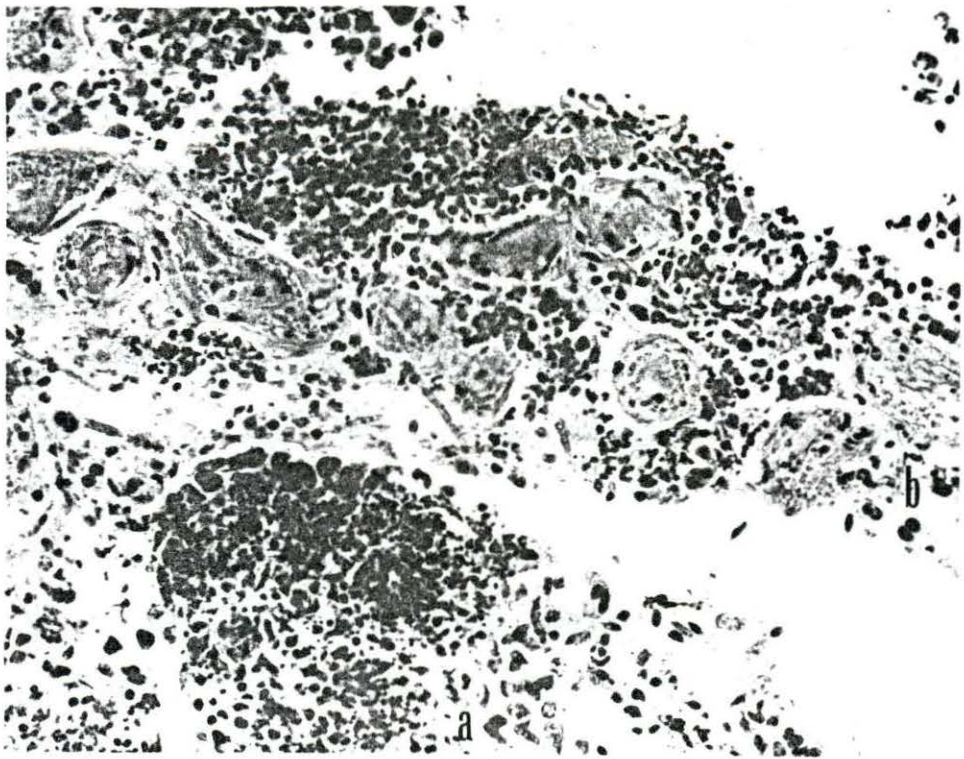
(Figures 9, 10 and 11). Hyphae were present in the maternal
septae and fetal villi and had invaded vessels in the fetal
villi (Figures 8 and 11). Involvement of the hematomata
(Figure 1) was not found. Changes were not observed in the
intercotyledonary CAM at this stage.

Microscopic lesions were present in both placentomes
and the intercotyledonary CAM of all principals killed between
8 and 26 days PI. Placental lesions were similar, differing
primarily in the extent of involvement. Well developed
infarcts were present in the placentomes (Figure 12). These
were triangular in shape with the apex at the hilus of the
placentome. They consisted of central coagulative necrosis
bordered by a zone of hyperemia and neutrophilic exudation.
Along the base of the maternal septae, a zone of necrosis and
suppuration (Figure 13) divided the maternal tissue at the
base of the caruncle. In those placentomes having a diffuse
involvement, this zone extended all the way across the placen-
tome. Vasculitis and thrombosis of the maternal vessels
supplying the placentome were apparent in these areas (Figure
13). In some areas this necrotic process had eroded through
the wall of the caruncle into the uterine cavity. Organized
thrombi were present in some of the largest fetal vessels
supplying the cotyledon. Invasion of the cotyledonary CAM
by fungal hyphae was well advanced. Hyphae were found
penetrating many fetal vessels.

Figure 9. Section of placentome from ewe 67. A developing
thrombus was present in an artery (arrow) in the
CAM near the base of fetal villi (a). Hematoxylin
and eosin stain. x 40.

a

Figure 10. Higher magnification of Figure 9. Hematoxylin
and eosin stain. x 500.

Figure 11. Serial section of tissue in Figure 9. Fungal
elements were found within the developing
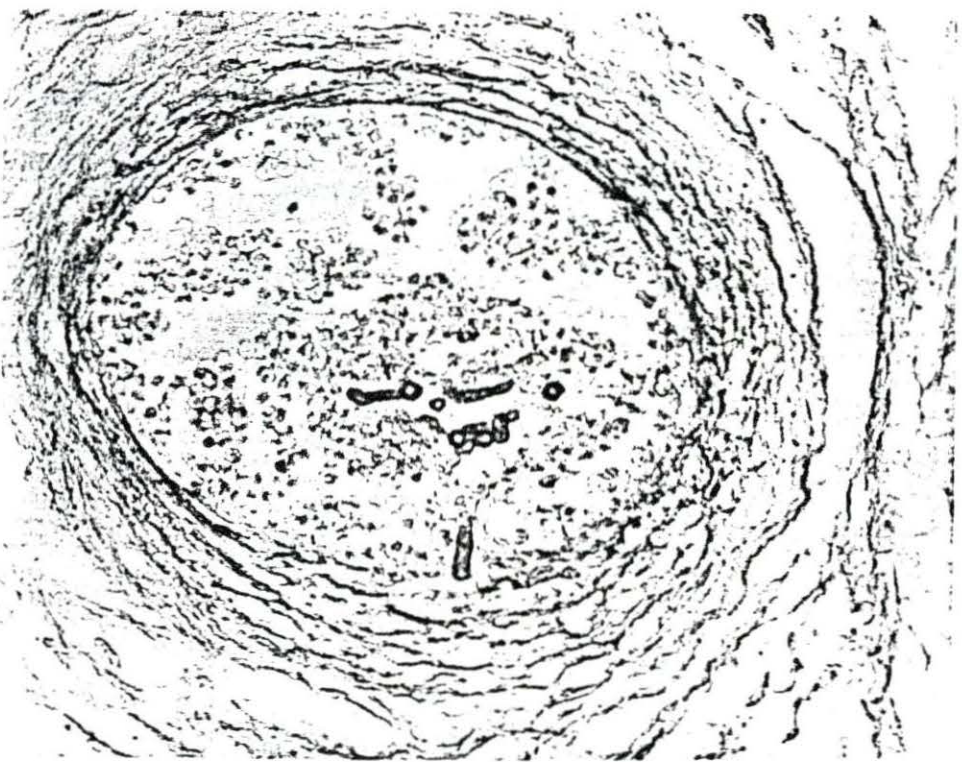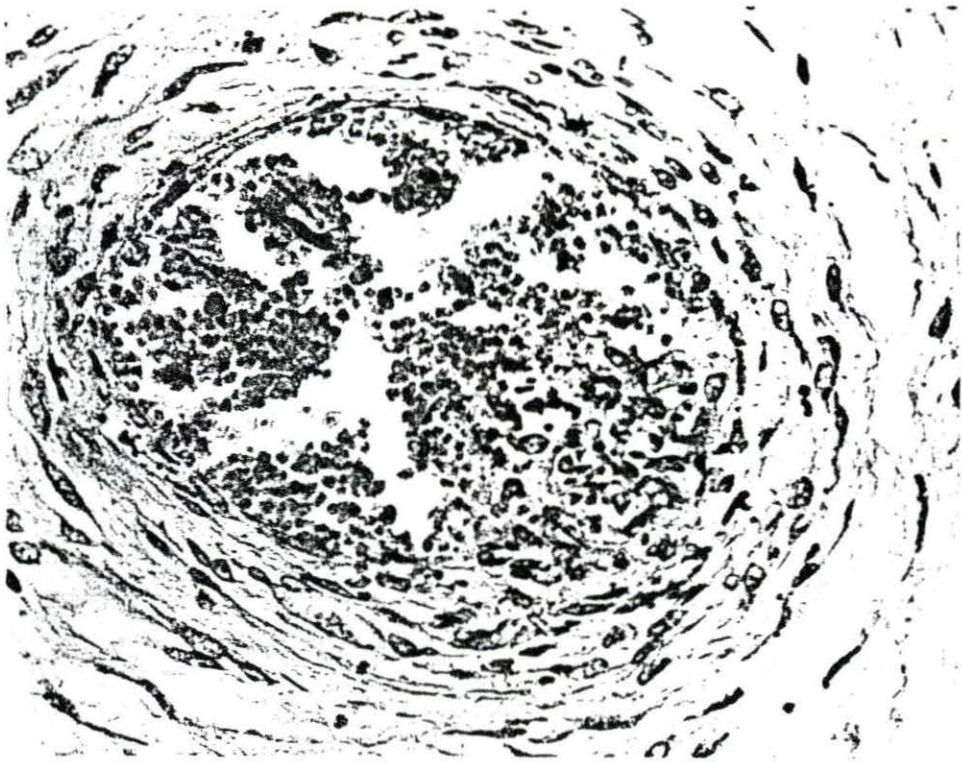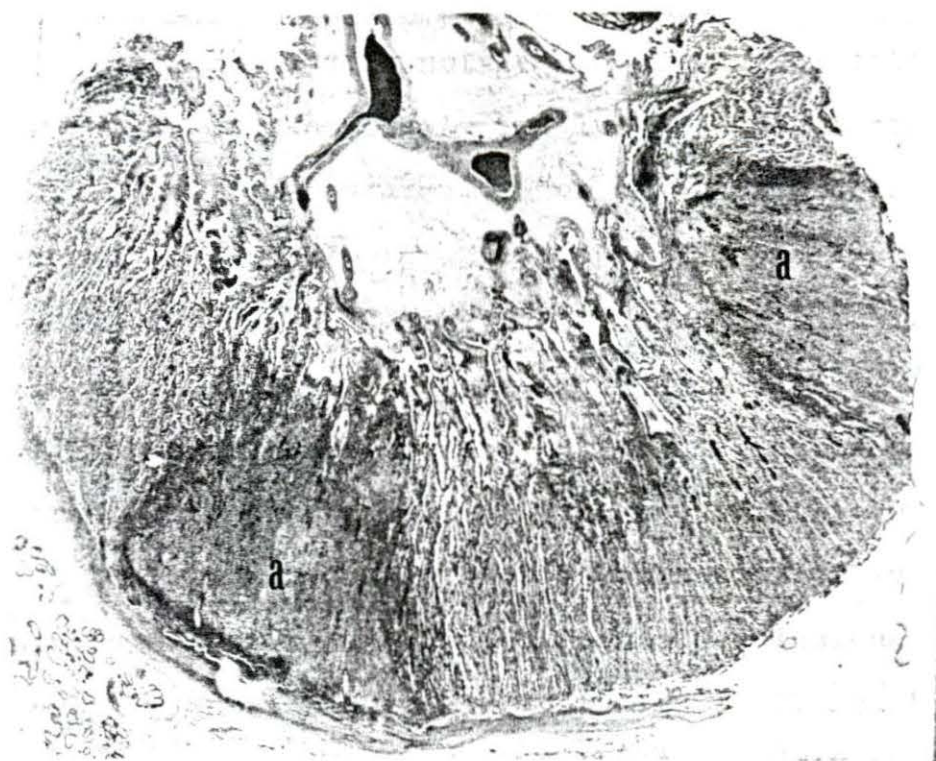thrombus. Gridley fungus stain. x 500.

Figure 12. Section of placentome from ewe 69 with infarcts
(a). Hematoxylin and eosin stain. x 6.3.

Figure 13. Section of placentome from ewe 9. There was
necrosis and suppuration along the base of the
maternal septae and thrombosis and vasculitis
of maternal vessels (arrow). Hematoxylin and
eosin stain. x 50.

Changes observed in the intercotyledonary CAM, of ewes infected for 8 to 26 days, consisted of edema, necrosis, a diffuse exudation of neutrophils and loss of the chorionic epithelium. Dense focal accumulations of neutrophils, which obscured the normal architecture of the tissue, were present in the intercotyledonary CAM on the uterine side (Figures 14 and 15). In these areas the chorionic epithelium was lost. Hyphae were not consistently found in these foci. In other areas there was a mild diffuse neutrophilic exudation into the membrane and a marked congestion of subepithelial vessels with and without necrosis and sloughing of the overlying chorionic epithelium (Figures 16 and 17). On the uterine surface of the intercotyledonary CAM there was an exudate (Figure 16) composed of necrotic debris, neutrophils, and epithelial cells. Fungal hyphae were found in the surface exudate and throughout the intercotyledonary CAM. In some areas the organism had invaded blood vessels (Figure 18) producing vasculitis and thrombosis.

Focal areas of necrosis were found in sections from 4 ewes, 17, 46, 9, and 69. These areas consisted of an amorphous, granular, basophilic material and degenerating fibrocytes and neutrophils (Figure 17). In sections stained with Gridley's stain for fungi the amorphous material appeared purple but definitive fungal forms were not observed.

Figure 14: Section of intercotyledonary CAM from ewe 69. A
loss of chorionic epithelium, dense accumulation
of neutrophils along the margin of the membrane
and diffuse neutrophilic exudation and edema
within the CAM were observed. Hematoxylin and
eosin stain. x 50.

Figure 15. Higher magnification of Figure 14.   Hematoxylin
and eosin stain. x 188.
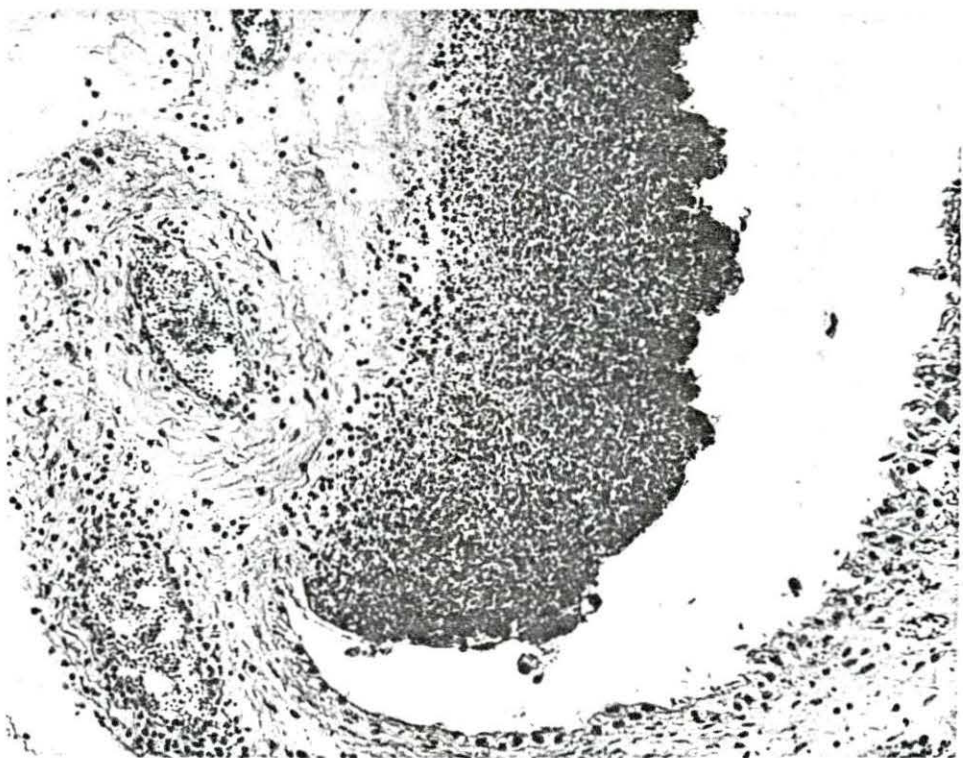
Figure 16. Section of intercotyledonary CAM from ewe 17. A
mass of exudate in the uterine cavity (a),
necrosis of chorionic epithelium, congestion,
edema and a diffuse neutrophilic exudation into
the CAM were present. Hematoxylin and eosin
stain. x 40.
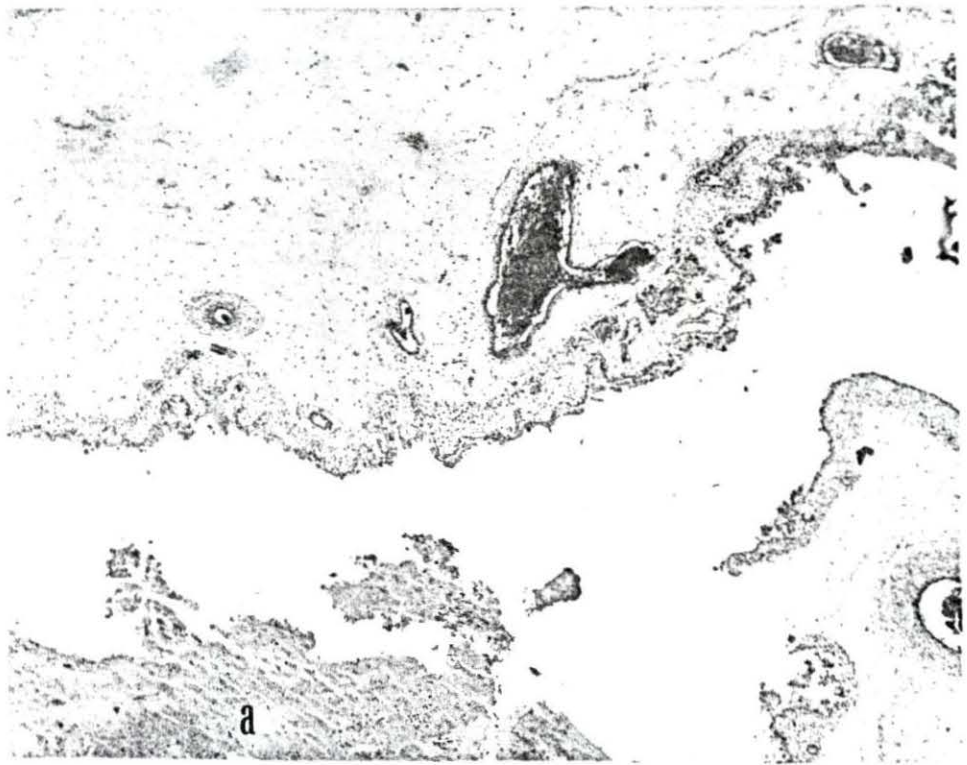
Figure 17. Higher magnification of Figure 16. Focal
          accululations of amorphous basophilic material
          (arrow) were visible. Hematoxylin and eosin
          stain. x 125.

Figure 18. Serial section of tissue in Figure 16. Fungal
          hyphae were present in and around vessels.
          Gridley fungus stain. x 325.
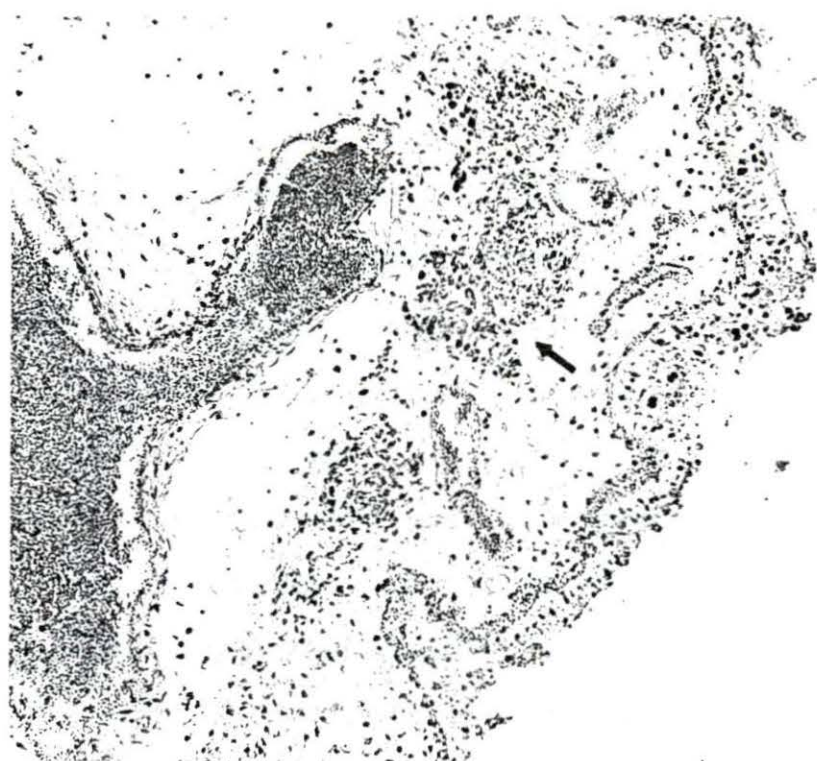
No microscopic alterations were found in sections of placentomes or the intercotyledonary CAM from the 2 control ewes.

Microscopic lesions of the fetus were restricted to the skin and the lung. A focal, acute, suppurative dermatitis, with a variable amount of necrosis and vascular involvement, was found in the skin lesions of fetuses 9 and 17. The more severe lesions occurred in fetus 9 where there was necrosis and vesicle formation in the epidermis, a diffuse neutrophilic exudation in the dermis and congestion and thrombosis of dermal vessels. The vascular alterations were primarily in the vessels around the base of the hair follicles with occasional thrombosis of a vessel in the rete cutaneum. Fungal hyphae were observed in the epidermis, in hair follicles, in the dermis and in thrombosed vessels in the rete cutaneum; the hyphae appeared to be penetrating the epidermis and growing down into hair follicles from the exterior.

Less severe skin lesions were found in fetus 17 (Figure 19). Microabscesses were present in the epidermis and dermis. The inflammatory reaction did not extend below the hair follicles. Hyphae were present on and in the epidermis, in hair follicles (Figure 20) and in the dermis. Occasionally hyphae were seen penetrating small vessels around the base of a hair follicle.

Figure 19. Section of skin from fetus 17. Microabscesses
were found in the epidermis and upper dermis
(arrows). Hematoxylin and eosin stain. x 125.

Figure 20. Serial section of tissue in Figure 19. Fungal
hyphae were present within a hair follicle.
Gridley fungus stain. x 450.
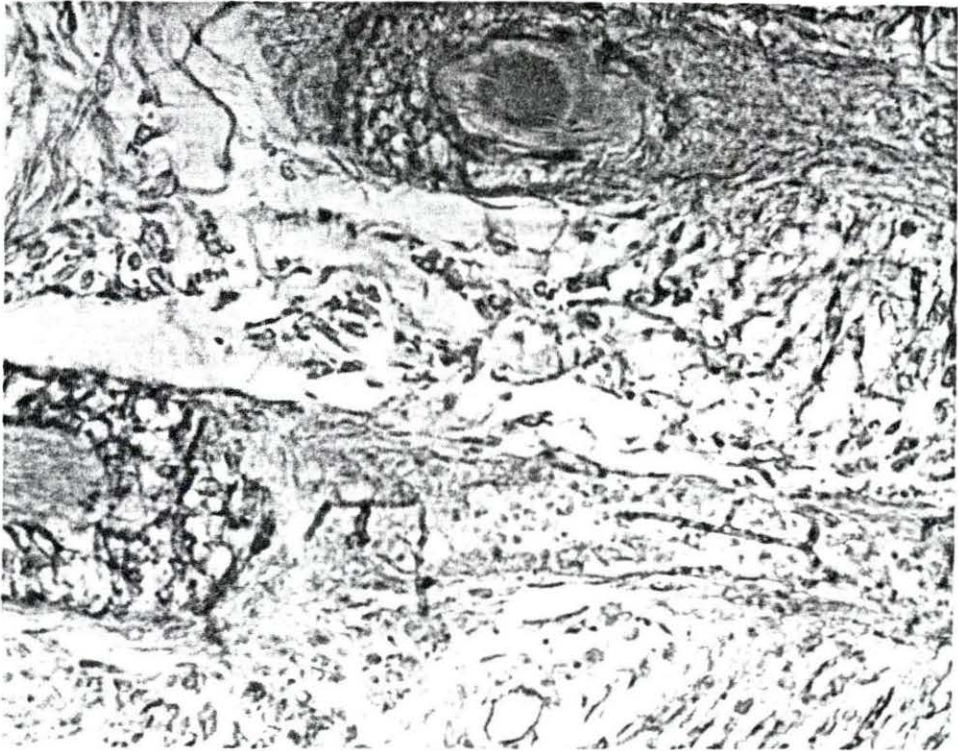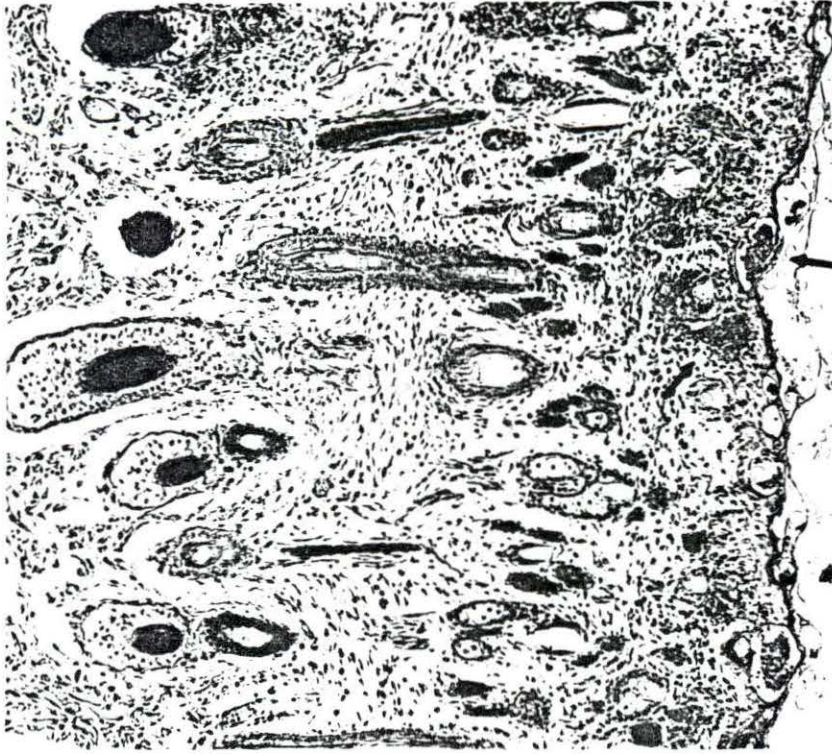
Marked alterations were seen in the skin lesions of fetus 46 (Figure 21). A focal, acute, hemorrhagic dermatitis with hydropic degeneration and necrosis of the epidermis and hemorrhage in the dermis and subcutis was observed. In addition, there was a suppurative vasculitis, with thrombosis, of vessels in the dermis and rete cutaneum. In most areas, fungal hyphae were present in the epidermis, in hair follicles, in the dermis and in thrombosed vessels in the rete cutaneum (Figure 22). In areas adjacent to frank lesions, hyphae were observed occasionally in vessels of the rete cutaneum, but not in surrounding tissues.

A hemorrhagic infarct was observed in the lung of fetus 9 with diffuse hemorrhage, thrombosis of large and small vessels and an exudate of neutrophils and necrotic debris in some of the bronchi. Hyphae were found scattered throughout the parenchyma and in vessels and bronchi in the vicinity of the lesion.

An acute, multiple focal, suppurative bronchitis (Figure 23) was observed in the lung of fetus 17 with neutrophils and necrotic debris within bronchi and a neutrophilic exudation in the surrounding parenchyma. Hyphae were present in bronchi and appeared to be growing out into the surrounding tissue (Figure 24). Penetration of adjacent vessels by hyphae was observed.

Figure 21. Section of skin from fetus 46. There was necrosis
of the epidermis and dermis, diffuse hemorrhage
and suppuration in the dermis and vasculitis of
the rete cutaneum. Hematoxylin and eosin stain.
x 125.

Figure 22. Serial section of tissue in Figure 21. Fungal
elements were found in the epidermis, dermis and
vessels of the rete cutaneum. Gridley fungus
stain. x 125.

Figure 23. Section of lung from fetus 17. Suppurative
exudate was present within and around a bronchus.
Hematoxylin and eosin stain. x 125.

Figure 24. Serial section of tissue in Figure 25. Fungal
elements were found within a bronchus and
appeared to be invading surrounding tissue and
and adjacent vessel. Gridley fungus stain. x 300.
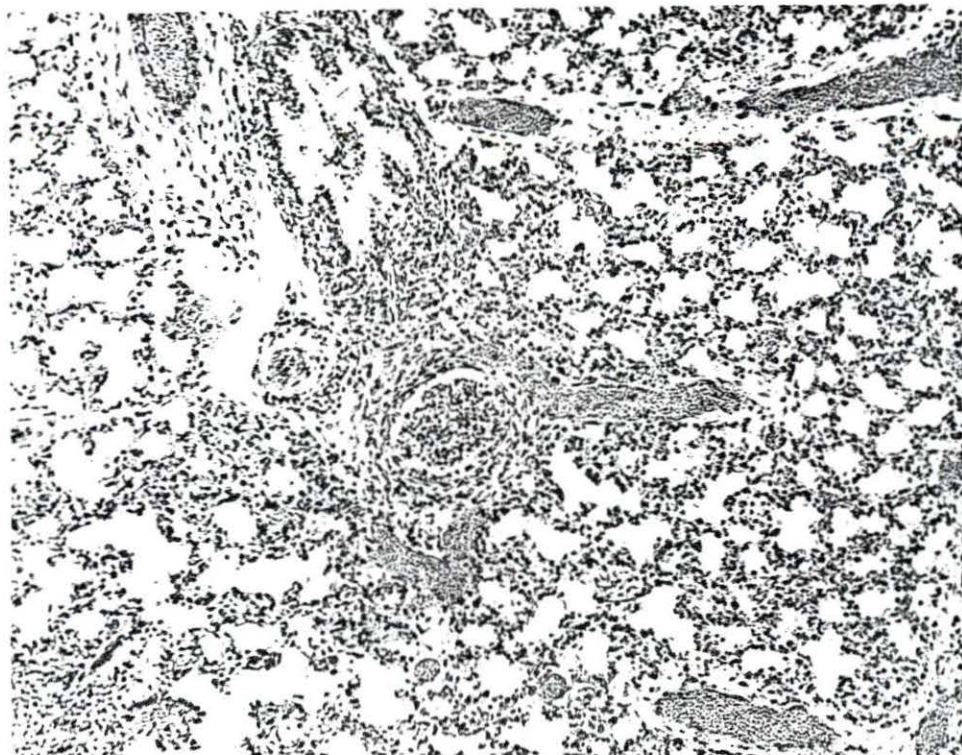
The morphology of fungal elements in various organs

Marked differences were observed in the degree of development of fungal elements in maternal organs and in the placenta. A majority of fungal elements in the maternal lung, liver and spleen were ungerminated spores; however, some spores with a germ tube approximating 15 μ's in length were observed (Figure 25). Two forms of hyphae were found in myocardial lesions: (a) straight, branching, septate hyphae 3 to 4 μ's wide, and (b) irregularly swollen septate hyphae with short lateral and terminal branches (Figure 26). The purulent foci observed in the endometrium of ewe 69 contained septate hyphae 4 to 6 μ's wide; with a hyaline radial deposit (Figure 27); the latter was visible in P-51 stained preparations but not in histological sections stained with either H & E or Bridley's fungus stain.

At 2 days PI germinating spores with a germ tube up to 150 μ's long were observed in placental tissues. Primary branching was well developed and secondary branching was occasionally observed. At 4 days PI hyphae varied from 600 to 1,000 μ's in length with well developed secondary and occasionally tertiary branching. After 4 days PI multiple branching hyphae (Figure 28), too long for accurate measurement, were observed in all placental preparations from principal ewes.

Figure 25. Wet mount of lung material from ewe 18. Germi-
nating spores were visible. P-51 stain. x 900.

Figure 26. Section of myocardial lesion from ewe 9. Fungal
hyphae approaching the "actinomycetoid form"
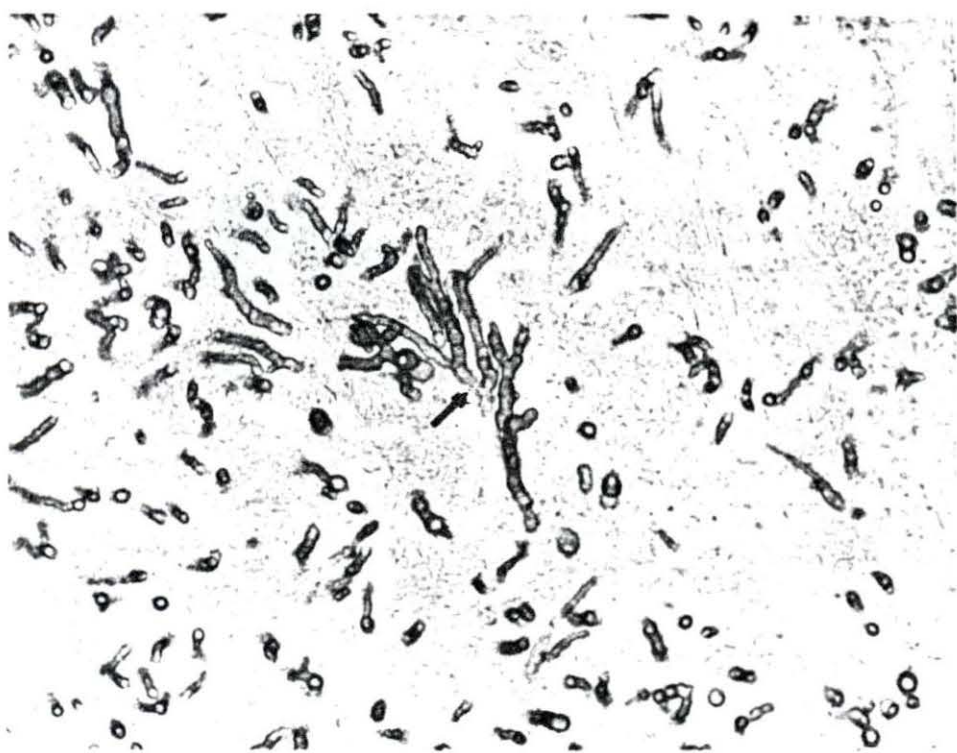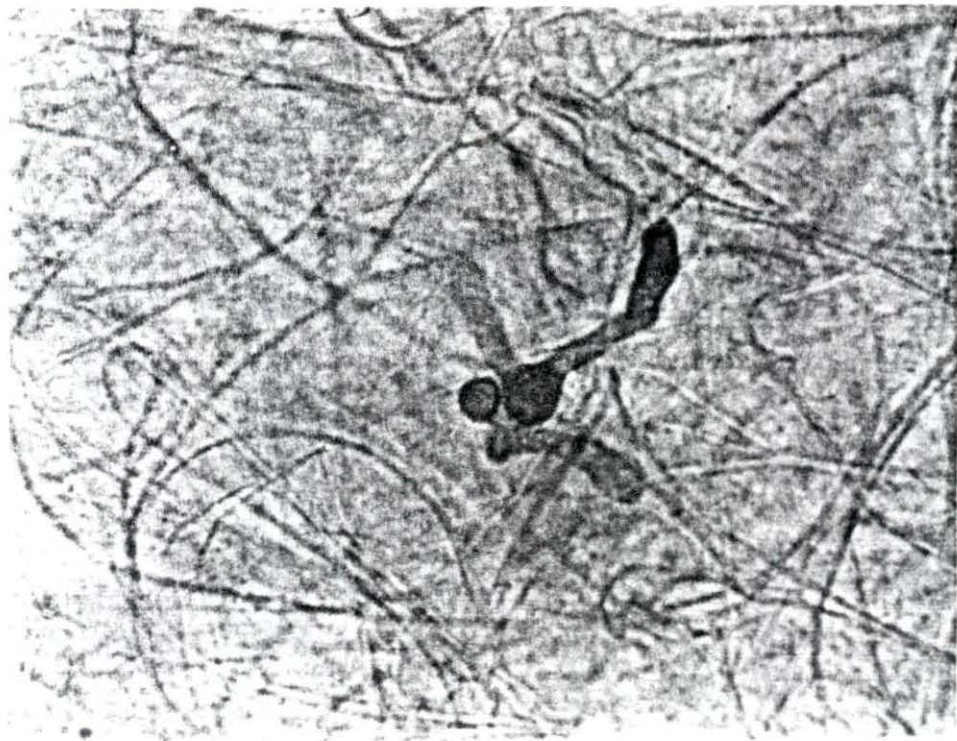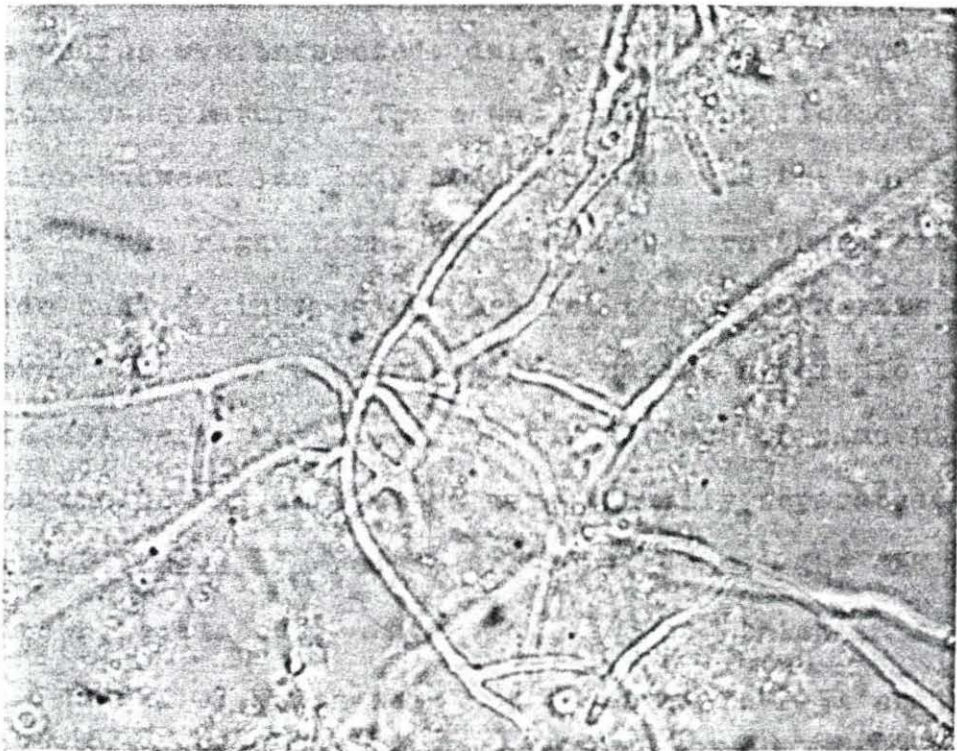(arrow) were present. Gridley fungus stain. x 525.

Figure 27. Wet mount of a uterine pustule from ewe 69.
Fungal hyphae were surrounded by radial deposits
of hyaline material. P-51 stain. x 600.

Figure 28. Wet mounted scraping of a placental infarct.
Numerous hyphae of A. fumigatus in "vegetative
form" were observed. P-51 stain. x 400.

Hemograms

No significant alterations were observed in the total white blood cell counts, differential counts, packed cell volume and hemoglobin concentration. Although these values varied on a daily basis, all determinations were within normal limits.

Intra-arterial injection of latex spheres

Ewe number 38 was killed 14 days after injection of latex spheres. She appeared normal and the pregnancy had not terminated. No gross lesions were observed in the fetus, placenta, uterus, or other tissues of the ewe at postmortem examination.

Ewe 40 aborted 6 days after inoculation. Visible lesions were confined to the genital tract. The right horn of the uterus was infarcted, dark brown in color and had prominent vasculature. There was a distinct line of de-marcation between the body of the uterus and the infarcted portion of the right horn. In the left horn there was a 2 cm. wide band of infarction parallel to the long axis of the horn. Zones of necrosis with a narrow hyperemic border (Figure 29) were present on the cut surface of placentomes. When caruncles and cotyledons were manually separated, caruncular tissue adhered to the cotyledons. The CAM was dark brown, edematous, and up to 1-1/2 cm. thick in some areas. Fungal elements were not observed in P-51 stained

Figure 29. Cross section of a placentome from ewe 40
            following injection of latex beads. Areas of
            infarction were present (arrows) which resembled
            those in placentas infected with A. fumigatus.
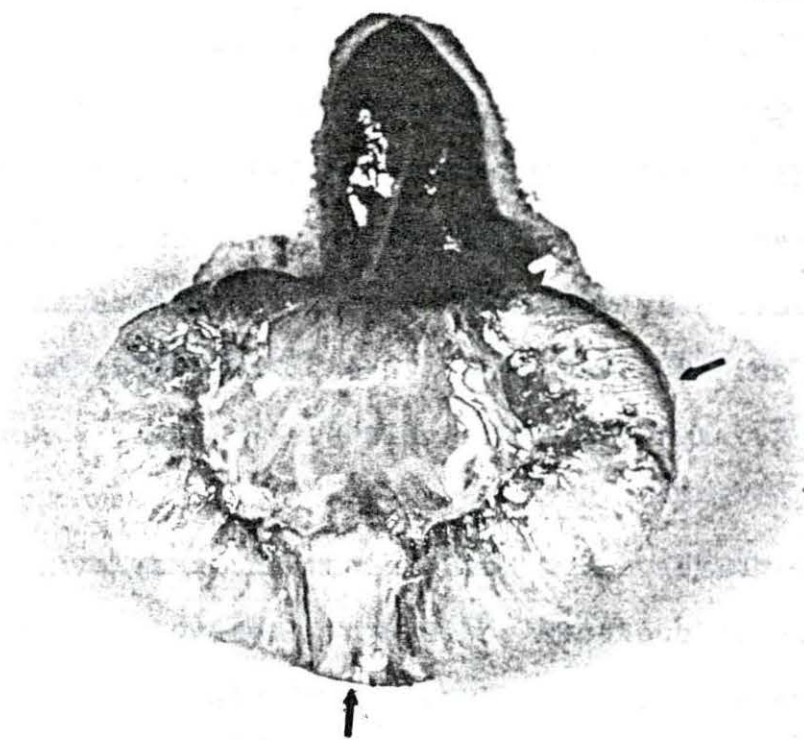
preparations of caruncle and cotyledon. Cultures on Sabouraud dextrose agar, of caruncle, cotyledon and the fetal stomach contents were negative for fungi.

No lesions were observed in sections of placentome from ewe number 38. Infarcts were present in sections of placentomes from ewe number 40. There was coagulative necrosis of fetal and maternal tissues (Figure 30). Within the necrotic area, maternal vessels of varying size were occluded with latex beads (Figure 31). Around the necrotic area was a zone of hyperemia, hemorrhage, necrosis, and neutrophilic exudation. The most extensive exudative changes occurred along the base of the infarct in the maternal tissue. Vessels were plugged with the latex beads and developing thrombi.

Infarcts involving the mucosa, and to a varying degree the myometrium, were present in uterine sections of ewe 40. There was a complete loss of the uterine epithelium and coagulative necrosis of the mucosa in the infarcted area. The epithelium of the uterine glands had sloughed and remained as a basophilic amorphous mass filling the glandular lumen. Coagulative necrosis and hyaline degeneration of muscle fibers were observed in the myometrium. Congestion, hemorrhage and thrombosis of vessels were present throughout the section. Accumulations of latex beads were present in many vessels. There was a diffuse

Figure 30. Section of a placentome from ewe 40. Vessels containing latex spheres (arrow) were present in an area of infarction. Hematoxylin and eosin stain. x 40.

Figure 31. Higher magnification of tissue in Figure 30. A vessel (arrow) containing latex spheres. Hematoxylin and eosin stain. x 300.
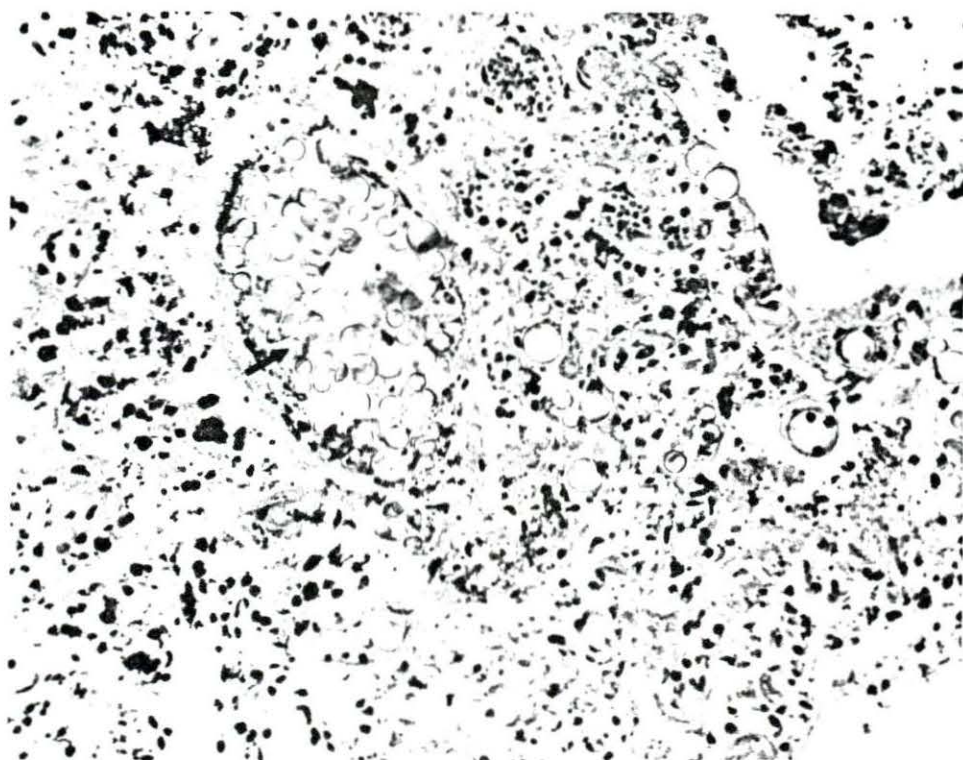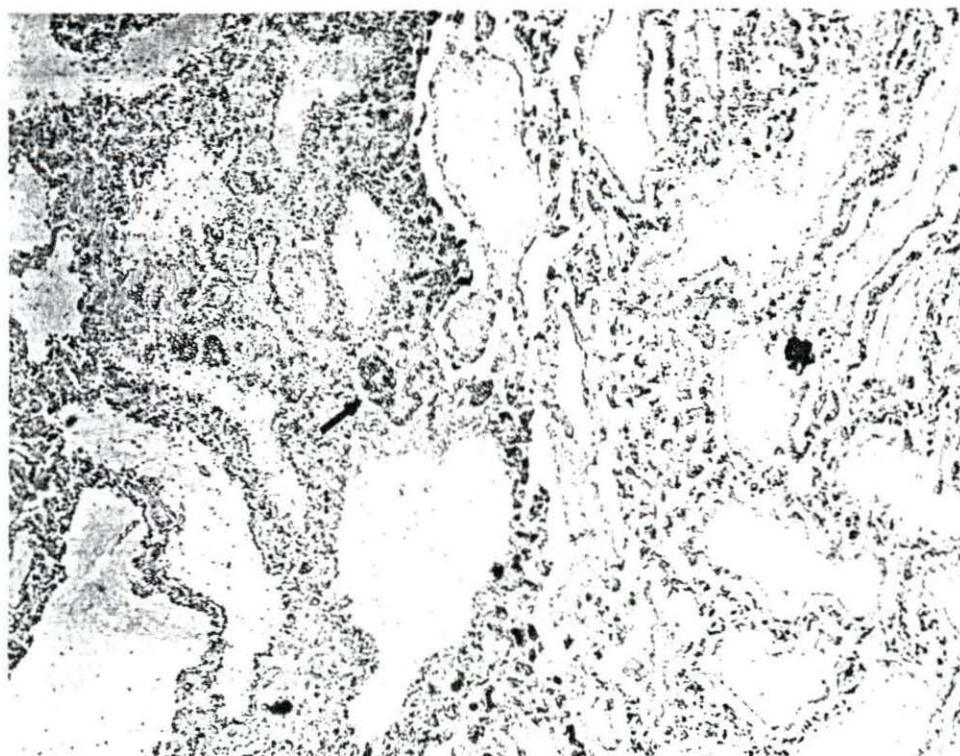
neutrophilic exudation in the infarcts and in adjacent normal tissue. A neutrophilic exudate filled some of the uterine glands outside of the zone of infarction.

DISCUSSION

Bovine mycotic abortion has been reported as being asymptomatic in the dam except for the act of abortion (1 pp. 53-56). Similarly, ewes in the present study showed no abnormal signs, except for a transient rise in body temperature, until abortion occurred.

In addition to the paucity of clinical signs in infected ewes, there was an apparent resistance to the development of progressive infection in maternal tissues. This resistance was evidenced by the nature of the gross and microscopic pathology, the morphology of fungal elements in maternal tissues and the clearance of the organism from those tissues. A large number of spores, 1 to 2 x 10^7, was injected intra-venously into each ewe; however, with few exceptions, maternal lesions were limited to a mild acute inflammatory response. The only exceptions were the myocardial lesions observed in 3 ewes and myocardial, hepatic and splenic lesions of ewe 10 where small foci of chronic inflammation surrounded fungal elements of limited development.

Fungal morphology in maternal tissues was also con-sistent with resistance to progressive infection. Austwick (3) has described 4 morphological stages of A. fumigatus in tissue. (1) Globose or oval-celled hyphae averaging 4 to 10 μ wide which represent the germinated conidium and its primary hypha and which are associated with the initial stage of an

acute infection; (2) straight or spiral, unbranched, septate or non-septate hyphae 2 to 3 μ's in diameter which may indicate that the initial resistance to infection has been overcome; (3) densely branching, septate hyphae 2 to 4 μ's in diameter (the "actinomycetoid" hyphae) associated with chronic lesions; and (4) normal vegetative hyphae 2 to 3.5 μ's in diameter which are found in the bovine placenta and which rapidly invade dead or weakened tissue. Fungal elements in ovine maternal tissue during the present experiment were usually ungerminated spores or spores with a short primary hypha and rarely proceeded beyond this form which has been associated with the initial stage of infection. The only exceptions noted were in the myocardial lesions in which fungal development approached the actinomycetoid form.

Further evidence of resistance by the ewe to progressive infection was found in the results of culturing various organs. After 4 days PI there was a marked decrease in the number of tissues, excluding the placenta and fetus, from which A. fumigatus was isolated; only 1 positive culture was obtained from 1 of 4 ewes killed between 11 and 26 days PI.

In comparison to the apparent resistance of maternal tissues to the development of a progressive infection, there was an apparent lack of resistance in placental tissue. The initial placental lesions observed were foci of necrosis and suppuration near the hilus of the placentome. These pro-

gressed to infarcts and finally to a diffuse necrosis of many placentomes. The morphology of fungal elements observed in placental tissue at 2 days PI exceeded the maximum development found at any time in maternal tissue. Fungal elements observed in placental tissue were all of the vegetative type. Cultural and microscopic methods revealed the presence of the organism in the placenta of 12 of the 13 principals. Cultural demonstration of the organism grew more consistent as the time PI increased. While fetal lesions were relatively infrequent, the morphology of the invading fungus resembled that found in infected placentas, indicating that once infected, fetal tissue resistance was similar to that of the placenta.

The focal lesions in the wall of the uterus appeared to result from invasion of the uterine glands by fungal elements from the exudate in the uterine cavity. Fungal elements were observed only in the gland lumen and the most severe reaction occurred in and around the glands.

A chronic intestitial nephritis was present in 3 ewes, 22, 69 and 42. Fungal elements were not found in sections of these kidneys stained with the Gridley stain. It did not appear that these lesions were associated with the experimental infection produced in these ewes.

The possible routes of infection of fetal tissues presented several interesting possibilities. Fungal elements

were found in 3 locations in the fetus; the stomach contents,
lung, and skin lesions. Of these the stomach content was most
frequently involved, apparently as a result of swallowing
contaminated amniotic fluid. Amniotic fluids were avail-
able in only 4 cases with positive stomach contents. In all
4 cases the amniotic fluid was also positive. The pulmonary
infection in fetus 17 appeared to be the result of aspiration
of contaminated amniotic fluid; the principal lesion was with-
in bronchi where fungal elements apparently invaded the
surrounding tissue and adjacent veesels. A more severe
lesion was present in the lung of fetus 9; fungal elements
were present in both thrombosed vessels and within bronchi.
The marked vascular involvement probably followed infection
by aspiration because of the close proximity of vessels to
bronchi and the propensity of A. fumigatus to invade blood
vessels. The absence of infection in other organs of this
fetus diminish the probability of a hematogenous route.

A superficial involvement of the skin was present in
fetus 17. Fungal elements were observed in the epidermis,
papillary dermis and in hair follicles. There was no
involvement of the deep vascular supply. More extensive
lesions with considerable vasculitis and thrombosis of deep
vessels of the skin were present in fetuses 9 and 46. Fungal
elements were visible at all levels. It appears more
probable that skin invasion in all 3 fetuses was from the

exterior. If it had been by a hematogenous route some involvement of other organs such as the liver, spleen, or kidney would be expected.

The location and configuration of the early lesions in the placentomes may be explained on the basis of the blood supply.

As described earlier (in the review of literature), the maternal vasculature of the placentome does not ramify into a capillary bed until it reaches the distal portion of the maternal septae. Thus fungal emboli would tend to lodge in the capillary bed in the distal portion of the septae. An obstruction at this point would hinder the flow of maternal blood through the placentome and lead to hemostasis, engorgement and infarction. Due to the concave nature of ovine caruncle the septae are closer together at the hilus than at the base. Obstruction at the capillary bed thus produces a triangular lesion with the apex toward the hilus.

Fetal death and abortion apparently resulted from the destruction of the placental attachment with the resulting interference with the supply of oxygen and nutrients to the fetus. Extensive necrosis was present in all placentomes in ewes killed after 8 days PI. In addition there was marked vasculitis and thrombosis of maternal vessels in the caruncles at the base of the septae. Latex spheres, approximating the size of A. fumigatus spores, injected into the uterine artery of 2 pregnant ewes produced gross and microscopic

lesions in placentomes of one ewe which resembled the lesions resulting from infection by A. fumigatus. This mechanical interference with the maternal blood supply to the placenta resulted in abortion. A possible contributing factor is the hemolytic and nephrotoxic endotoxin produced by A. fumigatus (23). It has been postulated that disintegrating hyphae within a lesion could release sufficient toxin to cause tissue destruction (12, 22). The exact role that this toxin may have in mycotic abortion is not known; however, no evidence of tubular necrosis was seen in sections of fetal kidneys. Despite possible contributions of A. fumigatus endotoxin, vascular invasion by hyphae of A. fumigatus appeared sufficient to cause most of the placental lesions observed.

SUMMARY

Seventeen pregnant ewes constituted the principal and control animals for these experiments. Thirteen ewes were inoculated intravenously with a suspension of Aspergillus fumigatus spores and 2 were held as uninoculated controls. A suspension of latex spheres, 6 to 14 μ's in diameter, was injected into the middle uterine artery of the 2 remaining ewes.

Ewes receiving A. fumigatus spores were killed at 1, 2, 4 and 8 days postinoculation (PI) and thereafter when abortion occurred. The 2 controls were killed at the end of the experiment. The 2 ewes receiving latex spheres were killed at 6 and 14 days PI. Maternal, placental and fetal tissues were examined mycologically and histopathologically.

Cultural and histopathologic evidence of infection was found most consistently in placental tissues. Placental infections were detected in 12 of 13 ewes inoculated with A. fumigatus. The fungus was cultured from 19 of 60 maternal organs (5 ewes) examined between 1 and 4 days PI and from only 5 of 96 organs (8 ewes) examined between 8 and 26 days PI. Abortions were observed only after 8 days PI; 5 of 6 ewes examined between 9 and 26 days PI had aborted and fetal maceration had occurred in the remaining ewe.

The predominance of placental infection, the morphology of the invading fungal elements and the progressive nature

of placental lesions suggested a high susceptibility of placental tissues to A. fumigatus infection. In contrast, the sparcity of detected infection of maternal tissues, the retarded development of the infecting fungus and the restricted nature of the maternal lesions suggested that maternal tissues were more resistant to A. fumigatus infection.

Fungal elements were found in 3 locations in the fetus: the stomach contents, skin lesions and the lung. Of these, the stomach content was most frequently involved. The limited involvement of fetal organs and the histopathological findings suggested that infection of the fetus resulted from invasion by fungal elements in the amniotic fluid rather than by a hematogenous dissemination from the placenta.

The injection of latex spheres into the middle uterine artery resulted in infarction of the uterus and placentomes of one of two ewes. The gross and microscopic lesions resembled those resulting from infection by A. fumigatus. This mechanical interference with the maternal blood supply to the placenta resulted in abortion.

There was no evidence of fungal infection of maternal, placental or fetal tissues in the control ewes and no abnormalities of pregnancy were noted.

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