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The pathologic effects of intrauterine deposition of pseudorabies virus on the reproductive tract of gilts during early gestation

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ABBREVIATIONS

PrV	pseudorabies virus
CHV	canine herpesvirus
FHV	feline herpesvirus
BHV-1	bovine herpesvirus type 1
EHV-1	equine herpesvirus type 1
EHV-3	equine herpesvirus type 3
HSV-1	herpes simplex virus type 1
HSV-2	herpes simplex virus type 2
MCMV	murine cytomegalovirus
MEM	minimum essential medium
CCID	cell culture infective dose
DPE	days postexposure to virus
DPB	days postbreeding
FA	fluorescent antibody
CL	corpus luteum; corpora lutea
EM	electron microscopy
Ton	tonsil
MLN	mandibular lymph node
RLN	reproductive lymph node
NT	nervous tissue
MT	maternal tissue
Ov	ovary
Ovid	oviduct
Ut	uterus
Vag	vagina
Spl	spleen

INTRODUCTION

Pseudorabies causes considerable economic loss to the swine industry throughout the world (Baskerville et al. 1973). In addition to losses due to mortality, pseudorabies virus (PrV) infection may cause reproductive failure which severely reduces the productivity of infected herds (Baskerville 1981). Pseudorabies has been shown to cross the placenta, resulting in abortion, fetal death and mummification, or stillbirths (Gordon and Luke 1955; Csontos et al. 1962; Saunders et al. 1963; Gustafson and Mitchell 1970; Kluge and Maré 1974; Hsu et al. 1980; Hall 1982). Anecdotal information and numerous authors (Saunders et al. 1963; Rasbech 1969; Glock and Dillman 1975; Maré and Hogg 1977) suggest that early embryonic death and resorbtion also occurs in infected herds. Experimental evidence demonstrating that PrV is a cause of early embryonic death is not available.

Previous studies have shown that PrV interacts with, but does not penetrate, the porcine zona pellucida _in vitro_ (Bolin 1982; Bolin et al. 1983). It is not known whether the early porcine embryo, _in vivo_, is susceptible to infection before or after the zona pellucida is shed. In a recent investigation, gilts inseminated with PrV contaminated semen farrowed litters with normal numbers of pigs (Hall 1982), which suggests that embryonic death did not occur in these gilts. However, the number of animals used in that study was small and a sequential study of the effect of the virus on the reproductive tract and the embryos was not done.

This study was designed to determine the effects of exposure to PrV at the time of breeding on early gestation in swine. Specifically, the distribution of virus, lesion development in the reproductive tract, and the effects of the infection on the embryo were investigated. An intrauterine route of exposure to the virus was chosen to control the time of infection of the reproductive tract so that it coincided with the events of early embryonic migration and development.

LITERATURE REVIEW

Pseudorabies

Introduction

Pseudorabies virus (PrV, porcine herpesvirus type 1) was first recognized as a cause of disease of cattle, dogs, and cats by Aladár Aujeszky in 1902 (Aujeszky 1902). Aujeszky's disease was subsequently referred to in the U.S. as pseudorabies as a result of certain clinical similarities with rabies. The disease was first reported in swine in 1914 (von Rátz 1914) and Shope concluded in 1931 that the virus which caused Aujeszky's disease or "mad itch" in cattle was the same as that which caused pseudorabies in swine (Shope 1931).

In Europe, it has been recognized for many years that pseudorabies can cause significant death loss and abortion in swine. The disease gained economic importance in the U.S. in 1973-1974 when outbreaks of pseudorabies, which had been sporadic, became more frequent and severe (Dillman and Andrews 1974; Glock and Dillman 1975; Maré and Hogg 1977; Gustafson 1981a). Currently, economic losses due to pseudorabies are estimated to exceed 100 million dollars annually (Gustafson 1981b).

Pseudorabies virus is composed of a double stranded DNA core; an icosehadral capsid surrounding the core; and an outer membrane or envelope. The size of the virion, as determined by negative staining techniques and electron microscopy, is approximately 150 to 180 nm. Extensive reviews of the physicochemical properties, the molecular

biology, and replication of PrV are available (Ben-Porat and Kaplan 1973; Roizman 1978).

Clinical signs

The clinical signs following exposure of swine to PrV vary. The severity of disease is dependent on the viral strain (Baskerville 1972), age of the animal (Bran et al. 1968), dose of virus (Baskerville 1972), and route of exposure (Sábó et al. 1968; Sabó et al. 1969). The disease in suckling pigs has a high morbidity and mortality and is characterized by fever, anorexia, sneezing, vomition, diarrhea, dyspnea, convulsions, coma, and death. In older pigs, there is less death loss but the clinical signs are similar. Infection of mature swine with PrV may be subclinical or result in fever, anorexia, depression, central nervous system dysfunction (rarely), and death (Maré and Hogg 1977; Gustafson 1981a).

Pseudorabies virus infection of pregnant sows may lead to fetal death and mummification, abortion, stillbirths, or neonatal weakness (Gordon and Luke 1955; Csontos et al. 1962; Saunders et al. 1963; Gustafson et al. 1969; Rasbech 1969; Gustafson and Mitchell 1970; Kluge and Maré 1974; Hsu et al. 1980). Early embryonic death and resorbtion has also been reported to occur with PrV infection but experimental evidence is lacking (Saunders et al. 1963; Rasbech 1969; Glock and Dillman 1975; Maré and Hogg 1977). Experimental evidence indicates that swine infected with PrV during the first third or early second third of pregnancy are more likely to abort than those infected later in gestation. Animals infected at later stages of gestation usually

retain the dead fetuses and farrow at term or beyond (Gustafson et al. 1969; Gustafson and Mitchell 1970).

Reports of clinical signs of pseudorabies in cattle, dogs, and cats are available (Dow and McFerran 1963; Hobson and Ellett 1979; Gustafson 1980).

Epidemiology

Although pseudorabies can affect a wide variety of species of animals, it is most common and least severe in pigs, which are considered the natural host of the virus (Baskerville et al. 1973). It is thought that the usual mode of spread of PrV in swine is lateral and is, for example, frequently associated with the introduction of new stock (Howarth 1969).

Chronic infections of swine with PrV can occur as persistent infections, in which virus can be isolated consistently for a long time, and as latent infections in which virus cannot be recovered by conventional methods. Pseudorabies virus has been isolated from tonsil and trigeminal ganglia of swine, by co-culture or tissue-fragment culture techniques, 6 to 13 months after exposure to PrV (Sabó and Rajcáni 1976; Beran et al. 1980). In addition, the genome of PrV has been detected in tonsil and trigeminal ganglia, by RNA-DNA hydridization techniques, 7 months after vaccinated swine were exposed to PrV. Virus was not recovered from the tissues of these swine by conventional methods (Gutekunst 1979). Dexamethasone or ACTH treatment has induced shedding of PrV in vaccinated swine that had been challenged with virus 3 to 8 months previously (Crandell et al. 1979;

Gustafson 1981a; Wittmann et al. 1983). This suggests that latent infections may be encouraged by vaccination followed by natural infection with PrV (Mock et al. 1981). Spontaneous shedding of PrV in nasal secretions occurred following parturition in a sow infected 19 months earlier (Davies and Beran 1980).

The role of wild animals in the dissemination of PrV is not clear (Gustafson 1981a). Wild rats can become infected, but they probably play little role in the spread of PrV to swine (McFerran and Dow 1970; Maes et al. 1979). Following experimental inoculation with virus, raccoons have transmitted PrV to swine. However, PrV infection of raccoons is usually fatal, hence, they are unlikely to have an epizootiologic role as a long-term, subclinical carrier of the virus (Wright and Thawley 1980). The contamination of swine feed by the saliva of infected wild animals or the consumption of infected carcasses may represent a hazard to susceptible swine. Pseudorabies virus has not been recovered from urine or feces of feral animals exposed to PrV under natural conditions (Gustafson 1981a).

Pathogenesis

The natural routes of infection of swine with PrV are probably intranasal and oral (Sabó et al. 1969). The primary site of viral replication is the mucosal epithelium of the nasopharynx and the tonsil (Rajcáni et al. 1969). Virus gains access to lymphatics, is spread to regional lymph nodes, and may enter the blood and be disseminated hematogenously (Baskerville et al. 1973). Viremia is transient and often difficult to demonstrate but has been detected 1 to 8 days after

infection (Wittmann et al. 1980). There is evidence that the virus may associate with leukocytes in the blood (Baskerville et al. 1973; Wittmann et al. 1980).

Pseudorabies virus may gain access to the central nervous system after establishment of upper respiratory tract infection by ascent along the trigeminal, glossopharyngeal, and olfactory nerves. Virus may also be distributed in nervous tissue during viremia. Studies of the mode of spread of virus by neural routes suggest the principal pathway is the axoplasm (McCracken et al. 1973).

Pseudorabies virus has been isolated from boar semen, the prepuce, and vagina of adult swine and latent infections of the genital tract may occur (Akkermans 1963; Rockborn and Hugoson 1972; Gueguen et al. 1980; Larsen et al. 1980; Baskerville 1981; Medveczky and Szabó 1981). Venereal transmission of virus at breeding can occur, but the importance of this route of infection is not known (Akkermans as cited by Burrows 1966; Stair 1970; Baskerville 1981; Bolin et al. 1982; Hall 1982).

The pathogenesis of reproductive failure associated with maternal PrV infections is uncertain. Severe maternal illness with accompanying pyrexia or transplacental infection of the fetus have been implicated as causes. Prior to 1975, attempts to isolate PrV from the aborted fetuses of naturally infected pregnant swine were usually unsuccessful in the U.S. (Kluge and Maré 1978a). Fetal infection was shown to occur, occasionally, following experimental infection of pregnant sows

(Gustafson et al. 1969; Gustafson and Mitchell 1970; Stair 1970). However, PrV was routinely isolated from aborted fetuses in Europe (Csontos et al. 1962; Gustafson et al. 1969, Kluge and Maré 1974; Kluge and Maré 1976; Maré et al. 1976; Kluge and Maré 1978b). The assumption was made, that in the absence of demonstrable placental or fetal infection, the reproductive failure observed in the U.S. was a consequence of maternal illness and pyrexia. A correlation between the febrile peak in experimentally infected dams and the time of reproductive failure was cited as support of this assumption (Kluge and Maré 1974; Maré et al. 1976). After 1975, however, isolation of PrV from fetuses in naturally occurring cases of abortion became commonplace in the U.S., indicating the presence of viral strains capable of crossing the placental barrier (Kluge and Maré 1976; Kluge and Maré 1978b; Kirkbride and McAdaragh 1978; Wohlgemuth et al. 1978; Mengeling et al. 1981). The pathogenesis of early embryonic death associated with maternal pseudorabies, if it occurs, has not been determined.

Virus can be isolated from many tissues following natural and experimental infection with PrV (Csontos 1966; McFerran and Dow 1965; Sabó et al. 1968; Rajcáni et al. 1969; Sabó 1969; Sabó et al. 1969; Wittmann et al. 1980). Tonsils, lungs, spleen, and brain are the tissues of choice for virus isolation. In cases of reproductive failure, brain and lung of fetal pigs and vaginal and nasal swabs from the dam should be examined for the presence of PrV (Baskerville et al. 1973; Hill et al. 1977).

Lesions

In general, the gross lesions in swine having pseudorabies are variable and slight. Extensive reviews of the gross lesions of pseudorabies in organs outside the reproductive tract are available (Baskerville et al. 1973; Corner 1965, Olander et al. 1966; Baskerville 1971; Alva-Valdes 1981). Briefly, lesions in the central nervous system may include meningeal congestion, an increase in cerebral spinal fluid, cerebellar coning, and petechial hemorrhages in the ependymal lining of the olfactory bulb (Corner 1965; Dow and McFerran 1962). Lesions in the viscera are often composed of minute, pale foci in the liver, spleen, kidneys, and adrenal glands. Petechial hemorrhages in the lung, hydropericardium, and fibrinous pericarditis may be observed. Mucopurulent exudate is often present on the tonsils and in the nasal passages. Lymph nodes may be enlarged, congested, and contain hemorrhages (Olander et al. 1966; Baskerville 1971).

Gross lesions in the male reproductive tract are uncommon, but focal testicular necrosis and subcutaneous scrotal edema have been reported (Corner 1965; Anon. 1977). Gross lesions in the uterus of swine having aborted following PrV infection are limited to uterine mural edema and the presence of a scant yellow-white mucoid endometrial exudate (Kluge and Maré 1978a; Hall 1982). The placenta of aborted fetuses is often autolyzed and may contain areas of hyperemia and hemorrhage (Hall 1982). Aborted fetuses may be autolyzed or mummified. Gross lesions, if present, in aborted fetuses are similar to those seen in suckling pigs and have been described elsewhere (Csontos et al.

1962; Kluge and Maré 1974; Kluge and Maré 1976; Wohlgemuth et al. 1978; Kluge and Maré 1978b; Hsu et al. 1979; Hsu et al. 1980).

Microscopic lesions in the central nervous system of pigs with pseudorabies are characterized by a diffuse, nonsuppurative meningoencephalomyelitis and ganglioneuritis. Marked perivascular cuffing with diffuse and focal gliosis associated with neuronal and glial necrosis are also features. There is little correlation between the extent of the lesions in the central nervous system and the severity of the clinical signs. The lesions are more extensive in the cerebrum with lesser involvement of the cerebellum, brainstem, and spinal cord. The relative involvement of grey and white matter varies (Dow and McFerran 1962, Olander et al. 1966).

Randomly distributed foci of coagulative necrosis may be present in a number of sites including liver, kidney, spleen, adrenal cortices and lymph nodes. Intranuclear inclusion bodies may be present in cells at the periphery of the necrotic foci (Dow and McFerran 1962; Corner 1965; Finazzi and Mandelli 1976). Epithelial necrosis and suppuration in tonsilar crypts and in the nasal passages are consistent findings in pigs infected with PrV.

Necrotizing bronchiolitis and alveolitis may be seen in the lungs of affected pigs. With time, a lympho-reticular reaction develops around the necrotic foci and healing occurs by fibrosis. Infected cells in the lung have been described as having thickened nuclear membranes and a ground glass appearance to the nucleus (Baskerville 1971; Ducatelle et al. 1982).

Focal necrosis of the testicle, tunica albuginea, and serosa of the spermatic cord, with intranuclear inclusion bodies, may occur in intramuscularly exposed pigs (Corner 1965). Intrapreputial exposure of boars to PrV resulted in multiple lymphohistiocytic aggregates in the interstitium of the spermatic cord. A similar cellular reaction was present in the preputial sheath, penis, and epididymus of intranasally exposed boars (Hall 1982).

Microscopic lesions in the uterus following PrV infection and abortion are characterized as a necrotic endometritis with degenerate trophoblasts and endometrial lymphoid perivascular cuffing. Placental lesions are characterized by coagulative necrosis of the chorionic fossae with intranuclear inclusions in degenerating trophoblasts and mesenchymal cells (Kluge and Maré 1978b; Hsu et al. 1980). Mononuclear cell aggregates have been observed in the vagina and ovaries of gilts following abortion (Hall 1982). Lesions present in aborted fetuses consist of foci of coagulative necrosis in a number of organs including liver, spleen, lymph nodes, adrenal glands and occasionally the lung. These lesions are characteristic of _in utero_ PrV infection in swine and have been described in detail in a number of reports (Csontos et al. 1962; Kluge and Maré 1974; Kluge and Maré 1976; Wohlgemuth et al. 1978; Kluge and Maré 1978b; Hsu et al. 1979; Hsu et al. 1980).

There are few reports on the effects of PrV on fertilization and early gestation in swine infected at the time of breeding. Ovulation and fertilization were not impaired in gilts given PrV (by intrauterine and/or intranasal exposure) at the time of breeding (Bolin 1982; Bolin

et al. 1982). Transfer of embryos recovered from these gilts resulted in transmission of virus and production of antibodies in the embryo recipients.

Examination of 3-day porcine embryos exposed to PrV *in vitro* has shown that the virus associates with, but does not penetrate the intact zona pellucida (Bolin 1982; Bolin et al. 1983). Gilts inseminated with semen contaminated with PrV became infected, but were shown to carry their litters to term and farrow normal pigs (Hall 1982).

Effects of other Selected Mammalian Herpesviruses on the Reproductive Tract

Canine herpesvirus

Canine herpesvirus (CHV) was isolated in 1964 (Carmichael et al. 1964), and causes fatal disease in neonates, a relatively mild or inapparent respiratory infection in adults, infertility, abortion, and stillbirths (Poste and King 1971; Hashimoto et al. 1979; Hashimoto et al. 1983). In addition, investigators have suggested that CHV causes lymphofollicular conjunctivitis, balanitis, and vaginitis (Jackson and Corstvet 1975; Jackson and Corstvet 1980).

Intrapreputial and intravaginal inoculation of CHV in adolescent dogs causes focal submucosal hemorrhage and the development of multiple lymphoid nodules on the prepuce, penis, and walls of the vagina (Hill and Maré 1974). Other investigators report the genital lesions to be vesicular, pock-like, and limited to the epithelium of the penis and vagina (Poste and King 1971; Hashimoto et al. 1982).

Transplacental infection of the conceptus leading to fetal death, abortion or stillbirths has been reported in association with natural or experimental CHV infections (Poste and King 1971; Hashimoto et al. 1979; Hashimoto and Hirai 1981; Hashimoto et al. 1982). Placental and fetal lesions are characterized by multifocal necrosis with minimal inflammatory response.

Feline herpesvirus

Feline herpesvirus (FHV, feline viral rhinotracheitis virus) was first isolated in 1957 from a cat with respiratory disease (Crandell and Maurer 1958). Clinical signs of FHV infection include severe upper respiratory disease with pyrexia, anorexia, episodic sneezing, nasal and ocular discharge, and ulceration of the tongue and soft palate (Povey 1979). In addition, the virus causes abortion, keratitis, and encephalitis (Crandell 1971). Clinically normal carriers often result from a primary infection.

Intravenous or intranasal inoculation of queens at 6 weeks of gestation resulted in fetal death and abortion. The pathogenesis appeared to differ with the route of infection. Uterine, placental, and fetal infection occurred when FHV was given intravenously. In animals inoculated intranasally, abortion was probably due to severe maternal illness, as there was no evidence of uterine, placental, or fetal infection (Hoover and Griesemer 1971a; Hoover and Griesemer 1971b). Intravaginal infection late in pregnancy resulted in the birth of congenitally infected kittens (Bittle and Peckham 1971). Focal necrotizing endometritis, vaginitis, and placentitis occur after

intravenous, or intravaginal exposure to FHV (Bittle and Peckham 1971; Hoover and Griesemer 1971b).

Bovine herpesviruses

Bovine herpesvirus type 1 (BHV-1, infectious bovine rhinotracheitis virus) causes a variety of clinical syndromes in cattle. Upper respiratory disease, abortions, pustular vulvovaginitis and balanoposthitis, keratoconjunctivitis, encephalitis, enteritis, mastitis, and metritis have been associated with BHV-1 infection (Weelemans et al. 1974; Kahrs 1977; Reed et al. 1979, Misra et al. 1983). Some investigators have reported that strains of BHV-1 isolated from the genital tract can be differentiated from upper respiratory isolates by restriction endonuclease analysis of DNA and polypeptide analysis (Ludwig 1983). Other investigators find no correlation between the restriction endonuclease digestion pattern of a BHV-1 isolate and the circumstances of its isolation (Misra et al. 1983).

Bovine herpesvirus type 1 infection of the external genitalia occurs at coitus and is characterized by local edema and the appearance of pustules on the prepuce, penis, vulva, and in the vagina. The pustules enlarge and coalesce to form shallow, erythematous ulcers (Gustafson 1981b; Ludwig 1983).

Abortion occurs in 25 to 50% of pregnant cows naturally infected with BHV-1 (McKercher 1973; Gustafson 1981b). Transplacental infection of the fetus is common and results in foci of coagulative necrosis in the liver, kidneys, lymph nodes, and other organs (Kendrick 1973; Kahrs 1977; Gustafson 1981b; Ludwig 1983).

Bovine herpesvirus type 1 is the most commonly reported viral contaminant in bull semen (Kahrs et al. 1980). Virus has been isolated from the semen of bulls with balanoposthitis or following reactivation of a latent infection (Kahrs 1977; Kahrs et al. 1980). The effects of exposure of cows to BHV-1 contaminated semen is dependent on the site of semen deposition. During coitus, the virus contaminated semen is deposited in the vagina and causes pustular vulvovaginitis in the cow, but there is no apparent effect on fertility or conception rates (Parsonson and Snowdon 1975; Allan et al. 1975). However, deposition of virus contaminated semen into the cervix and body of the uterus during artificial insemination can result in endometritis, shortened estrous cycles, and a marked decrease in conception rates (Snowdon 1965; Parsonson and Snowdon 1975).

Moderate to severe necrotizing and purulent endometritis and lymphocytic vaginitis have been seen following insemination with BHV-1 contaminated semen. The endometritis resolves leaving aggregates of lymphocytes and perivascular cuffing in the endometrium (Kendrick and McEntee 1967). In a recent report, heifers received intrauterine injections of virus 1 day after mating. The principal lesion was a diffuse lymphohistiocytic endometritis with necrosis, edema, and hemorrhage. Focal necrosis and lymphoid proliferation were often seen in the corpora lutea of infected heifers (Miller and Van Der Maaten 1984; Van Der Maaten and Miller 1984).

The effects of BHV-1 on the bovine preimplantation embryo were studied by exposing 5- to 7-day embryos to virus *in vitro* and culturing

them for 24 to 48 hrs. The virus associated with, but did not appear to penetrate the zona pellucida of the exposed embryos. Trypsin treatment of the BHV-1 exposed embryos removed the virus without damaging the embryos (Singh et al. 1982).

Bovine herpesvirus type 3 (malignant catarrhal fever virus), a cytomegalovirus, and Movar strains of herpesvirus have been isolated from cases of abortion in cattle (Reed et al. 1979). Bovine herpesvirus type 6 (caprine herpesvirus) causes pustular vulvovaginitis in female goats (Horner et al. 1982, Ludwig 1983).

Equine herpesviruses

Originally, equine herpesvirus type 1 (EHV-1) was associated with cases of equine abortion and the disease was named equine viral abortion. Subsequently, the virus was identified as a cause of respiratory disease in foals and the disease became known as equine rhinopneumonitis (O'Callaghan et al. 1983). A neurologic syndrome has recently been associated with EHV-1 infection (Bryans 1980). Restriction endonuclease analysis of the DNA of respiratory and abortogenic strains of EHV-1 indicate significant differences between the two types. These differences may lead to the designation of the fetal strains as EHV-1 and the respiratory strains as EHV-4 (Studdert et al. 1981; Studdert 1983).

Abortion occurs in approximately 25% of mares infected with EHV-1 during pregnancy (Gleeson and Coggins 1980). Although cases of abortion have been observed during the third month of gestation (Prickett 1970), there appears to be an enhanced susceptibility to abortion from

8 months of gestation to term (McGee 1970; O'Callaghan et al. 1983). It is assumed that the virus infects the placenta and fetus as a result of a leukocyte-associated viremia (Bryans 1980; Gleeson and Coggins 1980).

The effects of EHV-1 on the reproductive tract of pregnant mares has been examined. Mares were necropsied and their tissues examined after abortion had occurred or after fetal infection but prior to abortion (Prickett 1970; Bryans and Prickett 1970). After abortion, the most consistent uterine lesion was an intense perivascular lymphoplasmacytic infiltrate just beneath the glandular layer of the endometrium. Lesions in the uterus of mares sacrificed prior to abortion consisted of complete separation of the allantochorion and endometrium, a mononuclear perivascular infiltrate, and flattening of the uterine epithelium. The regional lymph nodes of all mares examined were markedly hyperplastic.

The placentas of aborted fetuses contain multifocal areas of necrosis. The presence or absence of an inflammatory reaction in the placenta is dependent on the competence of the fetal immune system at the time of infection (Prickett 1970; Bryans and Prickett 1970).

Equine herpesvirus type 3 (EHV-3) causes coital exanthema, a fairly innocuous genital disease of both sexes. Virus is transmitted at coitus and the incubation period is up to 10 days. The lesions on the external genitalia appear initially as small vesicles which progress rapidly to pustules as a result of secondary bacterial infection. Ulcerations with erythematous borders develop within days.

Microscopically the lesions are characterized by ballooning degeneration, lysis, and sloughing of epithelium leaving ulcers with a fibrino-purulent exudate on their surface (O'Callaghan et al. 1983).

Infection with EHV-3 during pregnancy has been reported to cause abortions (Gleeson et al. 1976). In another study, however, mares bred to stallions undergoing a clinical EHV-3 infection had normal conception rates and no abortions were noted (Pascoe 1981).

Herpes simplex viruses

Herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) are common pathogens of people. They cause severe, and often fatal disease in newborns and immunocompromised hosts, and usually mild disease in others. Herpes simplex type 1 infection is most commonly associated with recurrent herpes labialis and HSV-2 with herpes genitalis. The tissue tropism of these viruses is not complete and HSV-1 has been associated with genital disease and HSV-2 has been isolated from nongenital lesions (Adam 1982).

Lesions in the genital tract are characterized by vesicles present on the vulva, walls of the vagina, ectocervix, prepuce, and penis. The vesicles become confluent to form bullous lesions. The vesicles or bullae rupture, become ulcerated, and are often secondarily infected. Microscopically, epithelial cells undergo ballooning degeneration. The underlying layer of basal epithelial cells usually remains intact. Multinucleated cells form, and occasionally intranuclear or intra-cytoplasmic inclusions are observed. Mononuclear leukocytes infiltrate the underlying tissue (Adam 1982). Subsequent to a primary genital

infection, virus can become latent in the spinal sacral ganglia (Walz et al. 1974; Stevens 1975). Reactivation of the infection can occur and is usually associated with stress.

Primary HSV infection in pregnant women is associated with abortions, prematurity, and many congenital anomalies. The neonate often becomes infected during passage through an infected birth canal. Infection of the neonate is often systemic, frequently fatal, and involves the central nervous system (Adam 1982).

Cytomegaloviruses

Prenatal infection of human infants with cytomegalovirus is fairly common. The majority of congenitally infected infants are asymptomatic, but some suffer a severe generalized disease and others have subtle disease recognized as central nervous system dysfunction later in life (Sullivan and Hanshaw 1982).

Murine cytomegalovirus (MCMV) has been studied extensively as a model for the human disease. Cytomegalovirus has been isolated from the testicles and ovaries of parenterally infected mice (Neighbour and Fraser 1978; Mims and Gould 1979). Pregnant mice have been shown to be more susceptible to ovarian infection with MCMV than nonpregnant females. Viral antigens can be detected around areas of necrosis in the corpora lutea and occasionally in the ovarian stroma of infected mice by immunofluorescent staining (Mims and Gould 1979). The genome of MCMV has been detected by hybridization techniques in the ovaries and testes of mice 6 months after primary infection. This suggests

that these tissues serve as a reservoir of latent virus (Brautigam and Oldstone 1980).

Artificial insemination of mice with MCMV contaminated semen has resulted in maternal infection and a reduction in the number of morphologically normal embryos at 14 days of gestation. One 14-day embryo was found to be infected with MCMV (Young et al. 1977). Parenteral inoculation of mice with MCMV 7 days before or 1 day after mating caused uterine inflammation. A consequence of this was a reduced pregnancy rate and retarded growth of embryos (Neighbour 1976). Numerous extracellular virus-like particles were seen below the zona pellucida of mouse embryos fertilized in vitro with MCMV contaminated semen. However, there was no evidence of a productive infection of the blastomeres in these embryos (Neighbour and Fraser 1978).

MATERIALS AND METHODS

Experimental Animals and Husbandry

Twenty postpubertal crossbred gilts and three mature boars were obtained from a commercial source. The animals were free of neutralizing antibody to PrV and the herd had never experienced clinical signs of pseudorabies. Abortion, stillbirth, and infertility rates for the herd of origin were within normal limits.

The gilts were housed in groups so that they had fenceline contact with the boars. Estrus was detected by turning the boars in with the gilts twice daily for 15 minutes. The gilts were then observed for behavioral signs of standing heat. When it was determined that a gilt was in estrus, she was separated from the others and bred by one boar; 12 hours later, the gilt was bred by one of the other boars. After breeding, the gilts were moved to isolation facilities. The day of the second breeding was designated as Day 0.

Viral Propagation and Animal Inoculation

The Funkhouser strain of PrV (tenth cell culture passage) was propagated in monolayer cultures of an established porcine kidney cell line (MVPK)[1] with minimum essential medium (MEM)[2] containing 10% fetal

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[1]Provided by E. C. Pirtle, NADC, Ames, IA.

[2]Gibco, Grand Island, NY.

calf serum and 50 µg/ml gentamycin.[1] Virus was harvested when 90-100% of the cells exhibited cytopathic effect. The cultures were subjected to 3 cycles of freezing and thawing, the cell debris was removed by centrifugation (500 xg for 10 minutes), and the supernatant was frozen at $-70^{o}C$ in 2 ml aliquots. The virus stock described above contained 2.0×10^{8} cell culture infective doses per ml ($CCID_{50}$/ml) as determined by microtiter assay methods (Hill et al. 1977). The gilts were inoculated with 1 ml of a 1:100 dilution of stock virus mixed with 50 ml of MEM (2.0×10^{6} $CCID_{50}$/pig).

Sixteen of the gilts were exposed to virus within 6 hours after the final breeding (designated Day 0). The virus was inoculated into the uterus through an insemination tube passed into the lumen of the cervix. The remaining four gilts were similarly inoculated with 50 ml of MEM and served as controls.

Necropsy and Sample Collection

Three or four gilts were necropsied at 3, 6, 10, 14, and 28 days postexposure (DPE), respectively. Each gilt exposed to virus was assigned an arabic numeral for identification. One or two control gilts were necropsied at 6, 10, or 14 days postbreeding (DPB). Rectal temperatures of the gilts were taken immediately before euthanasia. A blood sample was collected from each animal at necropsy. An

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[1]Gentocin, Shering Corp., Kenilworth, NJ.

examination of all major organ systems was conducted at necropsy and gross lesions were recorded. The reproductive tract was excised from each gilt and saved for further examination.

Samples for histopathology and fluorescent antibody (FA) staining were collected from the following sites: uterine and periaortic lymph nodes, liver, spleen, kidney, adrenal gland, lung, tonsil, mandibular lymph nodes, brain, and spinal cord (cervical, thoracic, and lumbar).

Tissues for viral isolation were collected from the following sites: uterine and periaortic lymph nodes, tonsil, mandibular lymph nodes, brain, spinal cord (cervical, thoracic, and lumbar), and a pool of maternal tissue (liver, kidney, spleen, adrenal gland, lung, and mesenteric lymph nodes). A nasal swab was obtained from each gilt and eluted into 1 ml of MEM.

Examination of Reproductive Tract and Embryo Recovery

The serosal surface of the reproductive tract of each gilt was examined grossly and the number of corpora lutea (CL) on the ovaries was recorded.

Embryos were recovered from gilts necropsied 3 DPE by flushing the oviduct and proximal portion of the uterus with medium. Details of the procedures used are described elsewhere (Bolin 1979). Briefly, a glass canula was placed in the lumen 10 to 15 cm distal to the uterotubal junction of each uterine horn. Forty to 50 ml of MEM were flushed through the oviduct, into the uterus, out through the canula, and into a collection vessel. Embryos were identified in the flush fluid with

the aid of a stereoscopic dissecting microscope. The embryos were saved for electron microscopic (EM) examination. An aliquot of the uterine flush fluid from each gilt was saved for viral isolation.

At 6 DPB (control and virus exposed gilts), canulas were placed in each uterine horn near the bifurcation and medium was flushed the length of the uterine horn. The embryos were identified as described above and saved for EM. Uterine flush fluid was saved for viral isolation.

At 10 DPB, each uterine horn was severed near the bifurcation and media was flushed the length of the uterine horns into a collection vessel. The embryos were easily identified in the flush fluid with the unaided eye. Flush fluid was saved for viral isolation. A portion of the embryos recovered from gilts necropsied 10 DPB were saved for EM, a portion for FA staining, and a portion for viral isolation.

After the embryos were recovered, the uteri of gilts (control and exposed to virus) necropsied 3, 6, or 10 DPB were opened along most of their length and the endometrium examined for gross lesions. Tissue samples of the ovary (3 sections), oviduct (proximal, middle, and distal), uterus (5 sections) and vagina were collected for histopathology and FA staining. In addition, a sample of each tissue was saved for viral isolation.

The uteri of gilts (control and exposed to virus) necropsied 14 or 28 DPB were opened along their length and the endometrium examined for the presence of implantation sites or embryos. Embryos were removed from the endometrium and saved for FA staining, viral isolation or

histopathology. Samples of the tissues of the reproductive tract were collected for FA staining, viral isolation, and histopathology as described above.

Viral Isolation

The tissue samples and 28-day embryos saved for viral isolation were minced with scissors, ground in sea sand with mortar and pestle, and resuspended to 10% (weight/volume) in MEM. The suspensions were clarified by centrifugation at 1500 xg for 15 minutes. The supernatant was decanted and used to inoculate cell cultures.

MVPK cells were grown to 90 to 100% confluence on coverslips in Leighton tubes[1] with MEM supplemented to 10% with fetal calf serum and 50 µg/ml gentamycin. Medium was decanted from the tubes and a 0.2 ml aliquot of each ground tissue sample and the nasal swab eluant was inoculated into each of 4 tubes. The tubes were incubated at 37°C for 1 hour. The inocula were decanted and 1.5 ml of MEM with 2% fetal calf serum was added. The tubes were maintained at 37°C and were observed daily for cytopathic effect.

When cytopathic effect was observed, the coverslip was harvested and the cells fixed in acetone for FA staining. If cytopathic effect was not observed within 7 days, the medium and cell debris was passed to another freshly prepared Leighton tube culture. Two blind passages

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[1] Bellco Glass Inc., Vineland, NJ.

were performed and the coverslip cultures from the final passage were stained with FA.

A similar procedure was used to test for PrV in the uterine flush fluids except that the original inoculum was 1 ml. Embryos recovered 10 DPB and saved for viral isolation were minced with scissors and placed in the Leighton tubes and tested for PrV as described above.

Fluorescent Antibody Staining

The procedures used for FA staining have been described in detail (Hill et al. 1977). Briefly, tissue samples or embryos were stabilized in carbowax[1] and sectioned at 8 μm thickness in a cryostat. The sections were mounted on glass slides, fixed in acetone, rinsed, flooded with PrV conjugate,[2] incubated at room temperature, rinsed, and a coverslip applied. Coverslip cultures harvested from Leighton tubes were stained in a similar manner, inverted, and mounted on glass slides. In some cases, specificity of the conjugate was confirmed by a FA blocking test (Hill et al. 1977).

Histopathology

Samples for histopathologic examination were fixed in 10% phosphate buffered formalin. The tissues were dehydrated in graded alcohol

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[1] Ames O.C.T. Compound, Ames Co., Elkhart, IN.

[2] Conjugate provided by Dr. E. C. Pirtle, NADC, Ames, IA.

solutions, cleared with xylene, infiltrated with paraffin, and embedded in paraffin blocks for sectioning. Tissues were sectioned at 6 μm thickness and stained with hematoxylin and eosin.

Serology

Serum samples were tested for precipitating antibody to PrV using a microimmunodiffusion test[1] (MIDT) described previously (Gutekunst and Pirtle 1978; Gutekunst et al. 1978). Serum neutralization tests[2] (SN) were performed by the beta method (constant virus and varying serum) using 100 $CCID_{50}$ of PrV as previously described (Stewart et al. 1974; Hill et al. 1977).

Electron Microscopy[3]

The embryos to be examined by electron microscopy were fixed in 2.5% buffered (0.1M cacodylate, pH 7.4) glutaraldehyde. After fixation, the embryos were postfixed with 1% osmium tetroxide, enbloc stained with 2% uranyl acetate in distilled water, dehydrated in graded alcohol solutions, and embedded in epoxy resin. Thin sections were cut with a diamond knife, stained with lead citrate and uranyl acetate, and examined with an electron microscope (Bolin and Bolin 1984).

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[1]Antigen provided by Dr. E. C. Pirtle, NADC, Ames, IA.

[2]Conducted by K. Eernisse, NVSL, Ames, IA.

[3]Conducted by Dr. S. R. Bolin, NADC, Ames, IA.

Sections of each 3- and 6-day embryo, and sections from at least 3 embryos from each gilt (control and exposed to virus) necropsied 10 DPB were examined.

RESULTS

Clinical Signs

All animals maintained longer than 3 DPE became anorectic, listless, and depressed. The anorexia and behavioral changes resolved 10 to 12 DPE. Body temperatures were elevated in gilts euthanized 3, 6, and 10 DPE (Table 1). The temperature of one gilt was elevated at 14 DPE but gilts necropsied 28 DPE had normal body temperatures at necropsy. Clinical signs of pseudorabies were not observed in the control gilts and their body temperatures were normal at necropsy (Table 1).

Gross Lesions

Gross lesions in the reproductive tract of gilts exposed to PrV were infrequent. One gilt (#2) necropsied 3 DPE and two gilts (#7 and #9) necropsied 10 DPE had numerous minute (1 mm x 2 mm) raised, pale foci on the endometrium in the distal 15 cm of each uterine horn. There were multiple subendometrial ecchymoses in the uterus of gilt #16 at 28 DPE (Figure 1). The CL of gilt #11 necropsied 14 DPE had cystic centers bordered by a band of dark brown tissue.

The lymph nodes draining the reproductive tract were enlarged and hemorrhagic in gilts necropsied 3, 6, and 10 DPE. At 14 and 28 DPE the nodes were enlarged but hemorrhage was not a feature. The tonsils and mandibular lymph nodes of each gilt examined at 6, 10, and 14 DPE were enlarged and the tonsils were covered by a scant white exudate.

Table 1. Body temperature, number of corpora lutea, and number of embryos recovered from gilts at necropsy

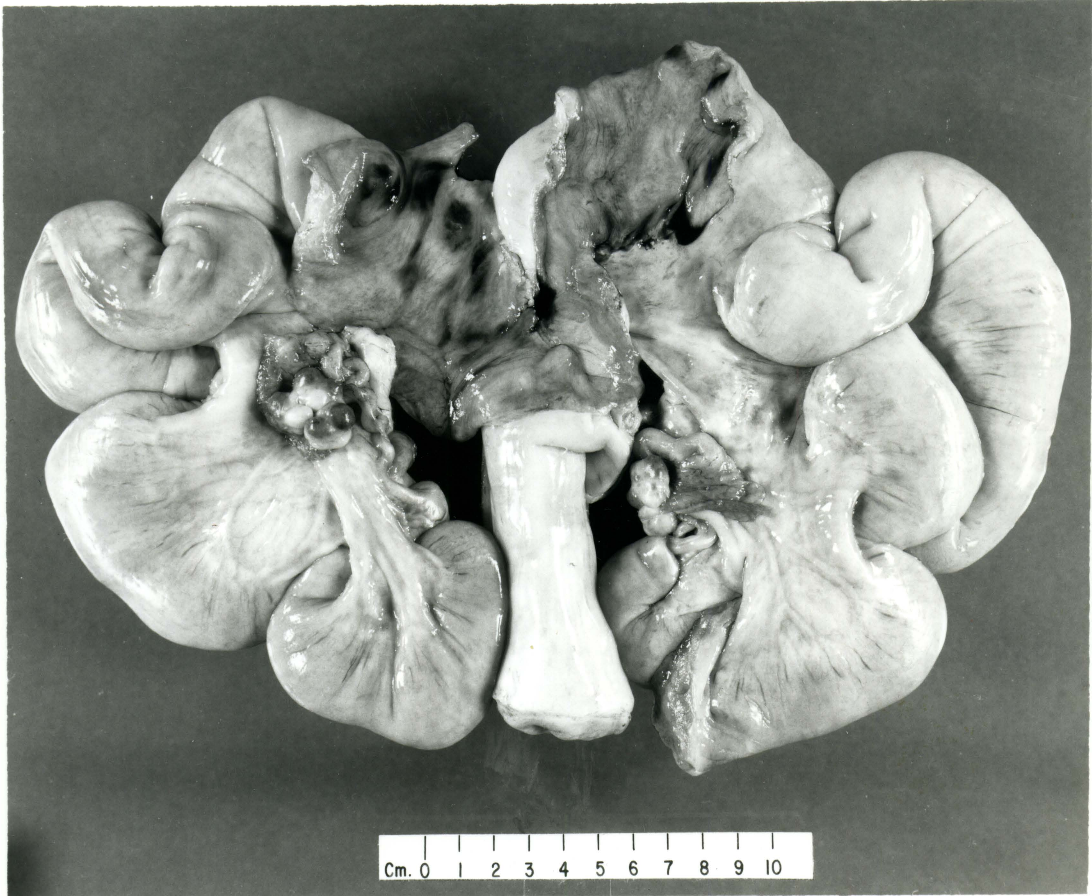
DPB	Gilt #	Body Temperature[a]	Total CL	Embryos Recovered
3	1	40.1	12	11
	2	40.7	15	13
	3	40.5	10	9
6	4	ND[b]	9	1
	5	40.0	12	8
	6	40.4	15	4
	C[c]	38.8	15	6
10	7	39.7	14	6
	8	40.4	10	6
	9	40.4	11	4
	C	38.6	14	14
14	10	ND	17	0
	11	ND	8	0
	12	39.3	15	0
	13	40.1	12	0
	C	38.6	14	0
	C	38.6	11	0
28	14	38.4	14	10
	15	38.8	7	5
	16	39.1	10	0

[a] °C.

[b] Not done.

[c] Control gilt.

Figure 1. Endometrial hemorrhage in the uterus of gilt #16 at 28 DPE


Cm. 0 1 2 3 4 5 6 7 8 9 10

The lung of one gilt (#7) necropsied 10 DPE contained depressed, consolidated areas in the anterioventral portion of each lobe.

Gross lesions were not observed in other organs or in the control gilts.

Embryo Recovery

The numbers of CL and embryos recovered from each gilt are presented in Table 1. At 3 DPE, 4 to 8 cell embryos with an intact zona pellucida were recovered from each gilt. The number of embryos collected corresponds well with the number of CL present on the ovaries.

The number of embryos recovered from gilts necropsied 6 DPB (virus exposed and control) was less than would be predicted from the number of CL. The 6 blastocysts recovered from the control gilt were all flushed from one uterine horn and their number is equal to the number of CL on the ipsilateral ovary. The embryos recovered from gilts #4 and #5 were blastocysts with an intact zona pellucida. Three hatched blastocysts and a degenerating embryo containing 3 blastomeres were recovered from gilt #6.

The number of embryos recovered from the control gilt at 10 DPB was equal to the number of CL on her ovaries. The number of embryos collected from each of the gilts exposed to virus was substantially less than the corresponding number of CL. One embryo recovered from gilt #9 was dark and had an irregular contour.

Embryos were not recovered from gilts (controls or virus exposed) necropsied 14 DPB.

At 28 DPE, two gilts were pregnant and the number of embryos was consistent with the number of CL. Gilt #16 was not pregnant. The ovaries of this gilt contained 13 CL from recent ovulations and 10 degenerating CL from the previous cycle (when breeding and virus exposure occurred).

Tissue Viral Isolation and Fluorescent Antibody Staining

All tissues from control animals were negative by FA staining and viral isolation.

Results of viral isolation and FA staining on tissues from each gilt exposed to PrV are presented in Table 2 and a summary of results in Table 3. Tissues positive by FA staining consistently yielded positive viral isolation results, however, virus was isolated from a number of tissues which were FA negative.

Serology

The sera from gilts necropsied 3 DPE were negative by the MIDT and SN tests. At 6 DPE, serum from 2 of the 3 gilts exposed to PrV were positive for antibodies to PrV by the MIDT but all were negative by the SN test. Serum from each gilt necropsied 10, 14 or 28 DPE gave positive results by both tests with SN titers of 1:16 to 1:32 at 10 DPE, 1:64 to 1:128 at 14 DPE and 1:64 to >1:128 at 28 DPE. Serum from control gilts were negative with both tests.

Table 2. Tissue viral isolation and fluorescent antibody staining from gilts exposed to PrV[a]

		Ton[b]		MLN		RLN		NT		MT[c]		Ov		Ovid		Ut		Vag	
DPE	Gilt #	VI[d]	FA	VI	FA	VI	FA	VI	FA	VI	FA	VI	FA	VI	FA	VI	FA	VI	FA
3	1	+	+	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-
	2	+	+	+	+	+	+	-	-	-	+	-	-	-	-	+	+	+	-
	3	+	+	+	+	+	+	+	-	+	+	-	-	+	-	+	+	+	-
6	4	+	+	-	-	+	+	+	-	+	+	+	-	+	-	+	+	+	+
	5	+	+	+	+	+	+	-	-	+	+	+	-	+	-	+	+	+	-
	6	+	+	+	+	+	+	+	-	+	+	-	-	-	-	+	-	+	-
10	7	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	+	+	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-
14	10	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	11	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	12	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	13	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	14	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	16	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]Only tissues positive in at least one gilt are listed.

[b]Tonsil (Ton); Mandibular lymph node (MLN); reproductive lymph nodes (RLN); nervous tissue (NT); maternal tissue (MT); ovary (Ov); oviduct (Ovid); uterus (Ut); vagina (Vag).

[c]Positive FA staining was found in the spleen (Spl) and not in other tissues included in the pool of maternal tissue.

[d]Viral isolation.

Table 3. Summary of tissue viral isolation and fluorescent antibody staining from gilts exposed to PrV

	Tissue[a]	Date of Necropsy				
		3 DPE	6 DPE	10 DPE	14 DPE	28 DPE
	Ton	3/3[b]	3/3	3/3	4/4	2/3
	MLN	2/3	2/2	0/3	1/2	0/3
	NT	1/3	2/3	0/3	0/4	0/3
	MT	1/3	3/3	0/3	0/4	0/3
VI[c]	Ov	1/3	2/3	1/3	1/4	0/3
	Ovid	2/3	2/3	0/3	0/4	0/3
	Ut	3/3	3/3	1/3	0/4	0/3
	Vag	3/3	3/3	0/3	1/4	0/3
	RLN	2/3	3/3	0/3	0/4	0/3
	Ton	3/3	3/3	3/3	4/4	2/3
	MLN	2/3	2/2	0/3	1/2	0/3
	Spl	2/3	3/3	0/3	0/4	0/3
FA	Ov	0/3	0/3	1/3	1/4	0/3
	Ut	2/3	2/3	0/3	0/4	0/3
	Vag	0/3	1/3	0/3	1/4	0/3
	RLN	2/3	3/3	0/3	0/4	0/3

[a]Tissues not listed were negative in all gilts. Abbreviations are the same as listed in Table 2.

[b]Number positive/number examined.

[c]Viral isolation.

Examination of Embryos and Uterine Flush Fluids

Results of the examination of embryos and uterine flush fluids are presented in Table 4. Virus was not detected in the uterine flush fluids or embryos from the control gilts.

AT 3 DPE, PrV was isolated from the uterine flush fluids of one gilt. Virus was not detected associated with the zona pellucida or blastomeres of any of the embryos. The embryos collected from gilt #3 had phagocytic cells adhering to and partially within the zona pellucida (Figures 2 and 3). Sections of spermatozoa were present in the cytoplasm of these cells.

At 6 DPE, viral nucleocapsids of a size and morphology consistent with that of PrV were observed in the nucleus of a blastomere from an embryo that had hatched from the zona pellucida (Figures 4 and 5). Pseudorabies virus was isolated from the uterine flush fluids used in collecting that embryo. Virus was not isolated from the uterine flush fluids of the other gilts and virus was not detected in any other embryo by EM.

Viral nucleocapsids and complete virions were observed in cells of one embryo recovered 10 DPE (Figures 6 and 7). A portion of this embryo was positive by FA staining (Figure 8). The FA staining was blocked by incubation of the tissue with pseudorabies antiserum prior to application of the conjugate (Figure 9). Pseudorabies virus was isolated from the uterine flush fluids used to collect this embryo. Virus was not detected in the embryos or uterine flush fluids of either of the other two gilts.

Table 4. Examination of embryos and uterine flush fluids from gilts exposed to PrV

		Embryos			Uterine flush fluid
DPE	Gilt #	EM	FA	VI[a]	VI
3	1	-	ND[b]	ND	-
	2	-			+
	3	-			-
6	4	-	ND	ND	-
	5	-			-
	6	+[c]			+
10	7	-	-	-	-
	8	-	-	-	-
	9	+	+	-	+
28	14	ND	-	-	ND
	15		-	-	
	16		-	-	

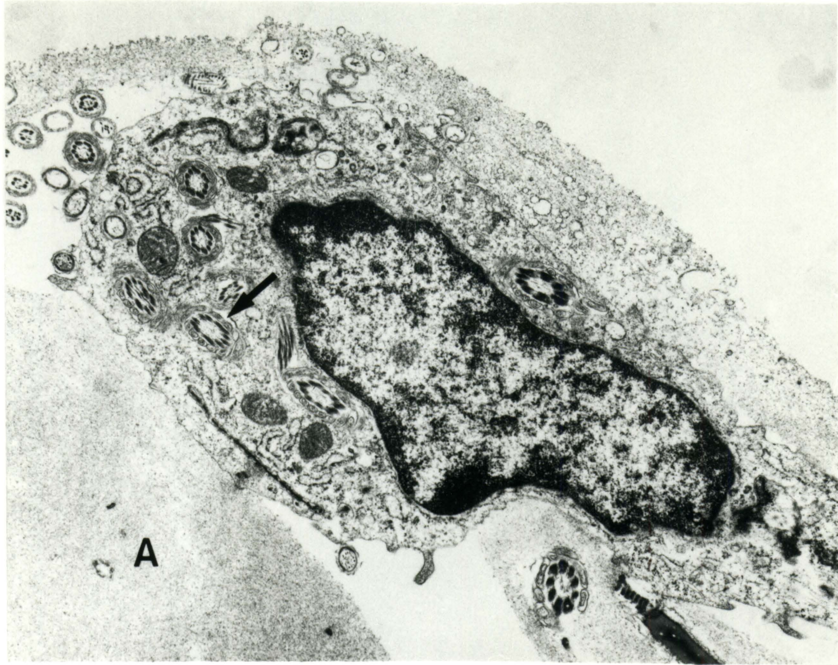
[a]Viral isolation.

[b]Not done. Technique was not applied to embryos at this stage of development.

[c]Virions detected within cells of an embryo.

Figure 2. Cross sections of sperm (arrow) in the cytoplasm of a phagocyte located on the surface of the zona pellucida (A) of an embryo collected 3 DPE, x 7650

Figure 3. Phagocyte within the zona pellucida (A) of an embryo recovered 3 DPE. Note the rim of zona pellucida (arrow) surrounding the cell, x 7200


A

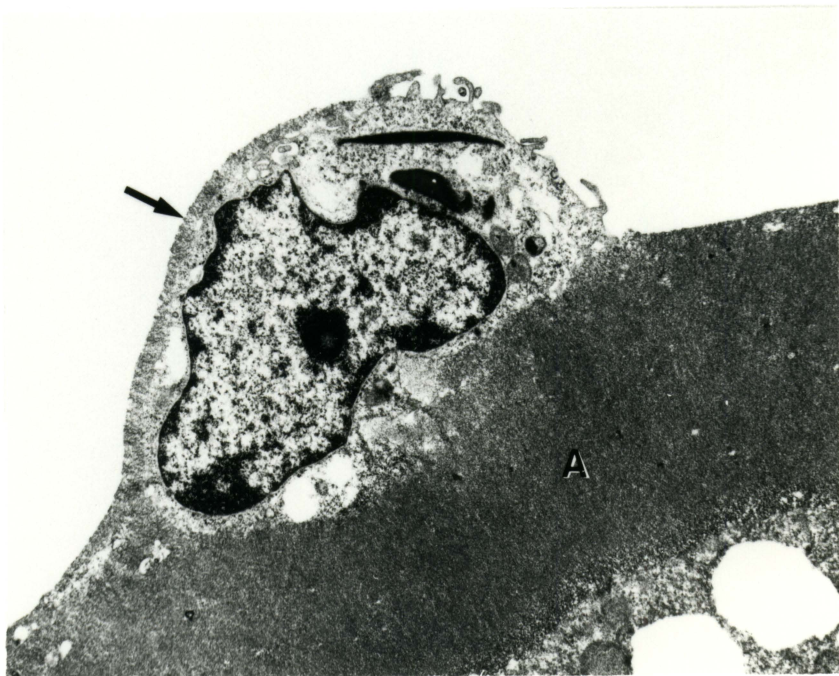
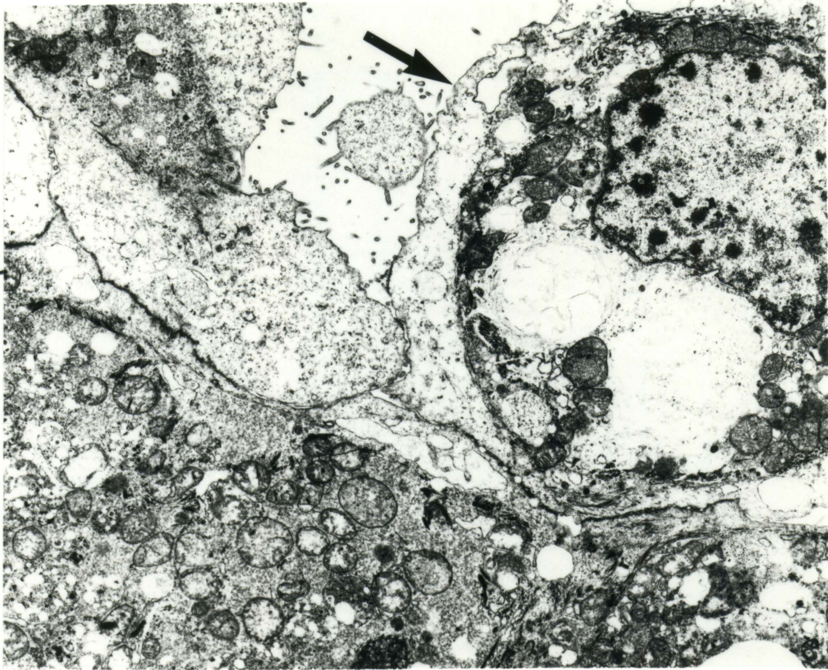

A

Figure 4. Embryonic cell infected with PrV (arrow) located adjacent to the inner cell mass of a 6-day embryo, x 4600

Figure 5. Viral nucleocapsids in the nucleus of the infected cell shown in Figure 4, x 31,000



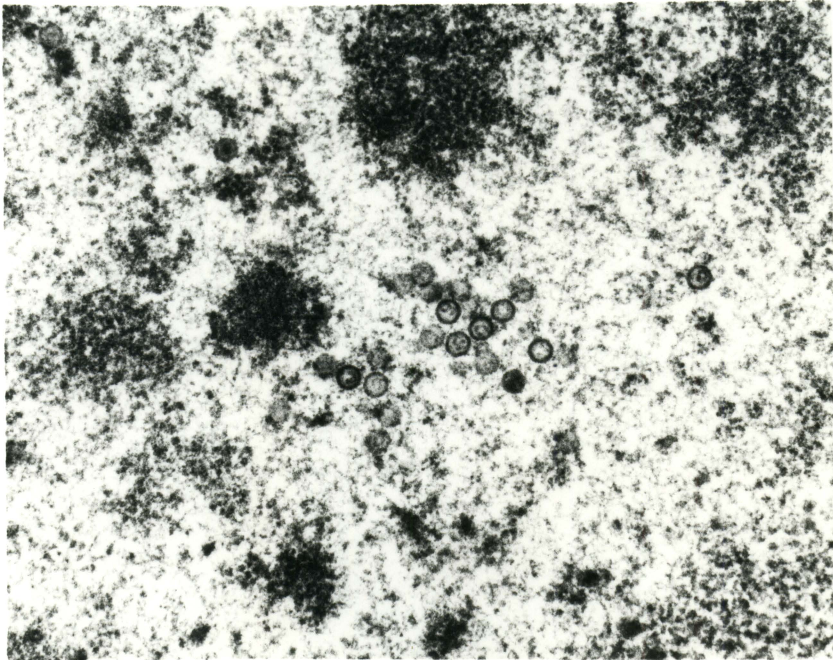
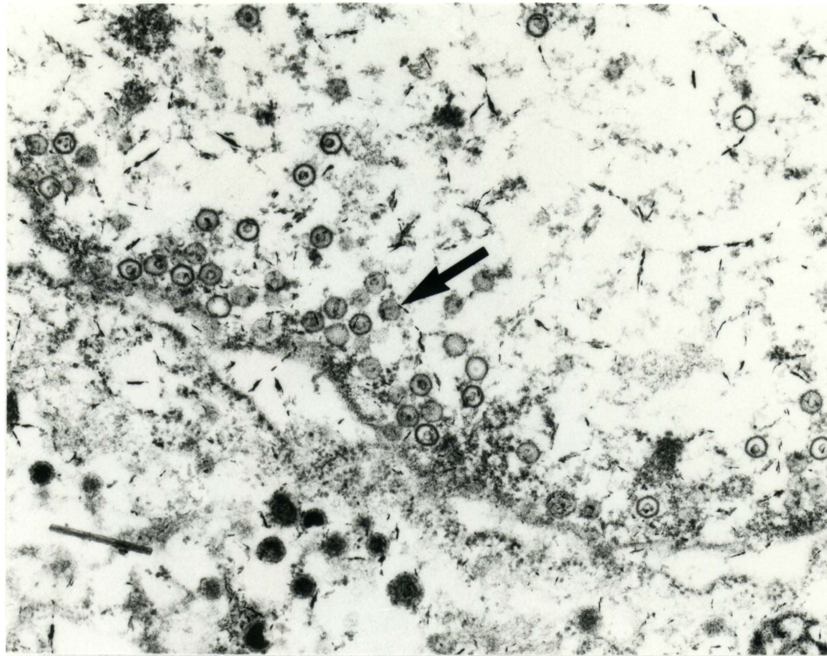


Figure 6. Viral nucleocapsids (arrow) and complete virions in a cell of an embryo recovered 10 DPE, x 25,500

Figure 7. Virions in the extracellular space of a 10-day embryo, x 48,000



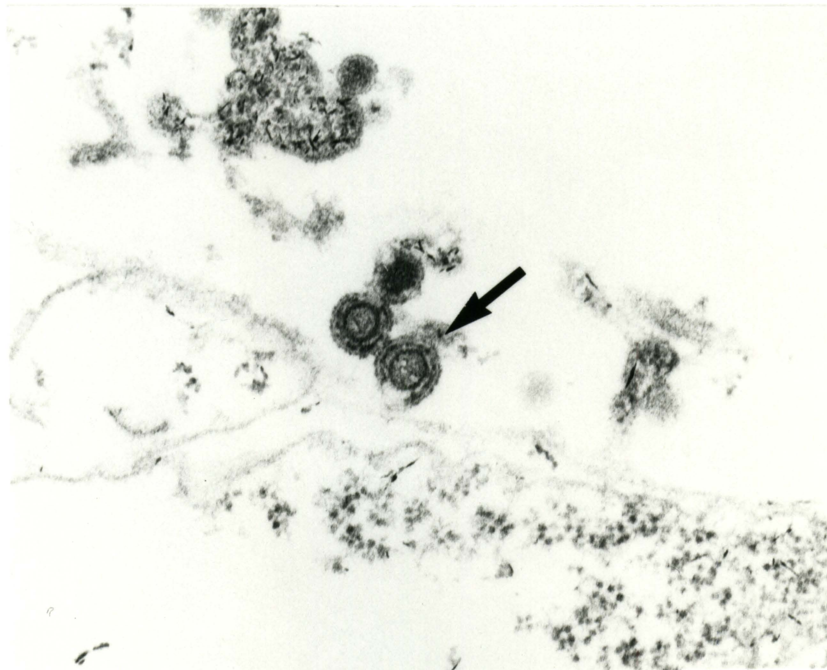
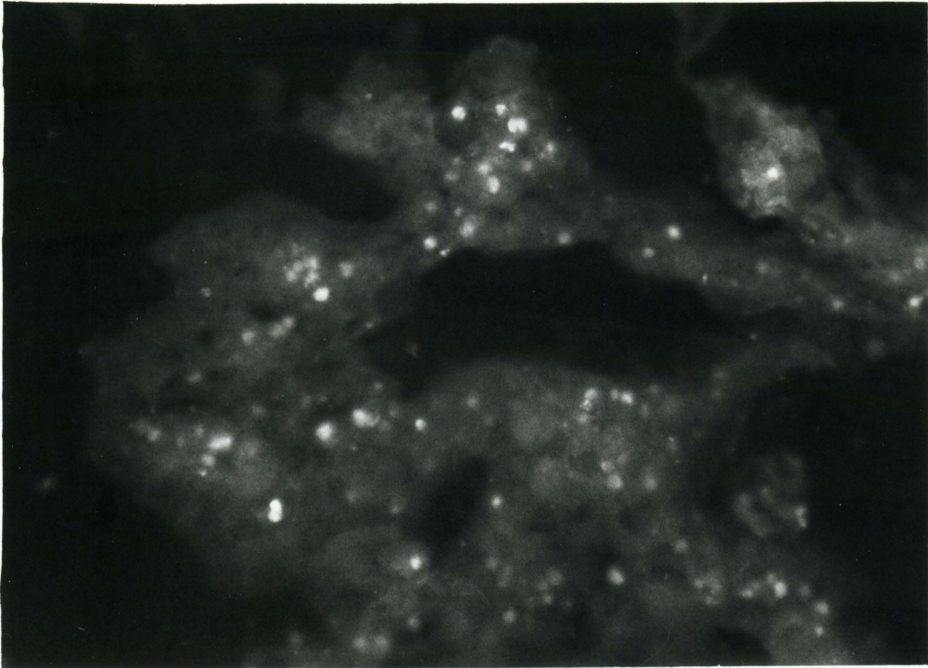
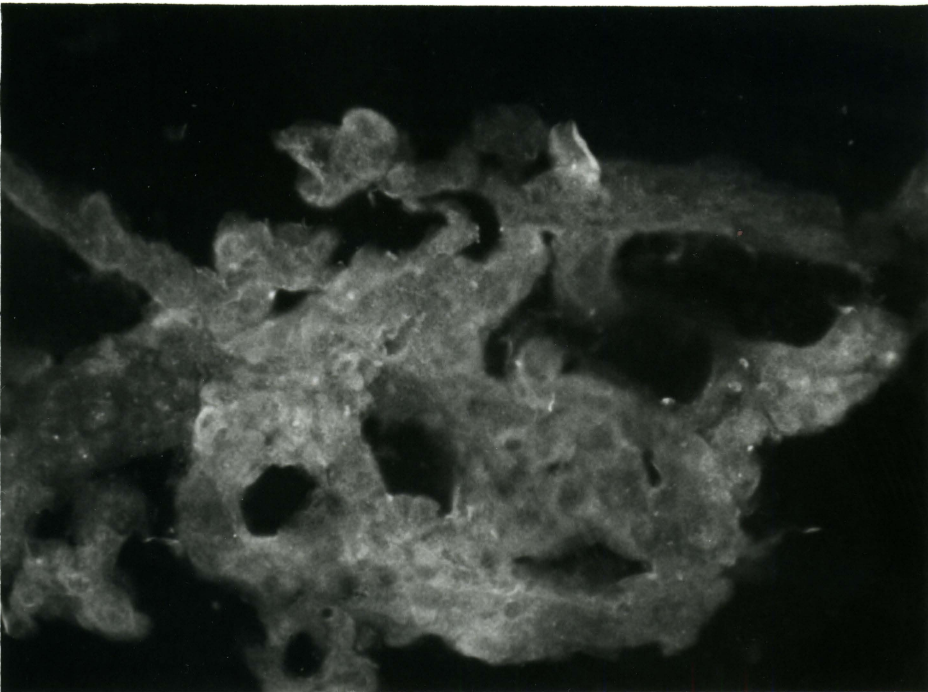


Figure 8. Direct fluorescent antibody stain of a 10-day embryo. Note areas of staining which indicate the presence of viral antigen, x 480

Figure 9. Section of the same embryo shown in Figure 8 which was incubated with unlabeled PrV antiserum prior to the application of PrV conjugate. The fluorescence was blocked by this procedure, x 480





Virus was not detected and microscopic lesions were not present in embryos recovered 28 DPE.

Histopathology

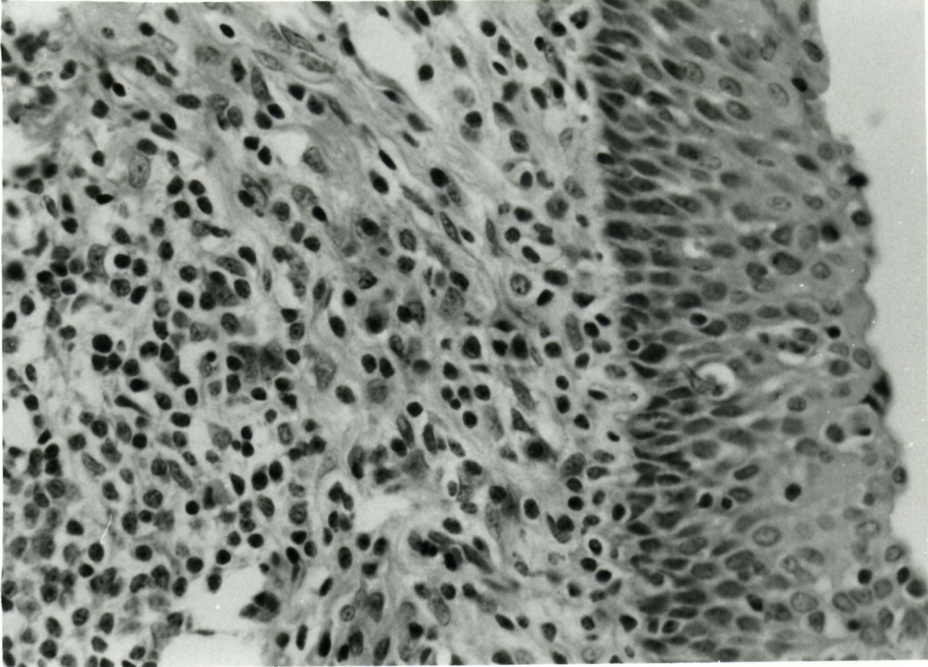
Lesions in the control gilts consisted of tonsilitis and a mild superficial suppurative vaginitis. In addition, there was moderate peribronchial and peribronchiolar lymphoid hyperplasia in the lungs of all gilts used in this study.

All gilts exposed to PrV had vaginal lesions that consisted of focal accumulation of mononuclear inflammatory cells immediately beneath the vaginal epithelium, infiltration of lymphocytes into the epithelium, and lymphocytic perivascular cuffing (Figures 10, 12 and 13). The cellular aggregates were composed of large numbers of lymphocytes with lesser numbers of macrophages and plasma cells. The extent of the cellular reaction was greatest in gilts necropsied 10 and 14 DPE. There were multifocal erosions and ulcerations in the vagina of one gilt (#5) necropsied 6 DPE (Figure 11). Neutrophils, lymphocytes, and macrophages had extensively infiltrated the necrotic areas.

Lesions were present in the uterus of each gilt exposed to PrV, but their extent and severity differed between animals. At 3 DPE, multifocal areas of coagulative necrosis of the endometrium were present in the uterus of gilt #2; these corresponded in location to the raised, pale foci seen grossly. The ulcerations were surrounded by a zone of inflammatory cells composed of neutrophils, lymphocytes and

Figure 10. Lymphocytes and plasma cells beneath and within the vaginal epithelium of a gilt 3 DPE, x 750

Figure 11. Vaginal ulceration with an extensive inflammatory cell infiltrate and perivascular cuffing in a gilt 6 DPE, x 120



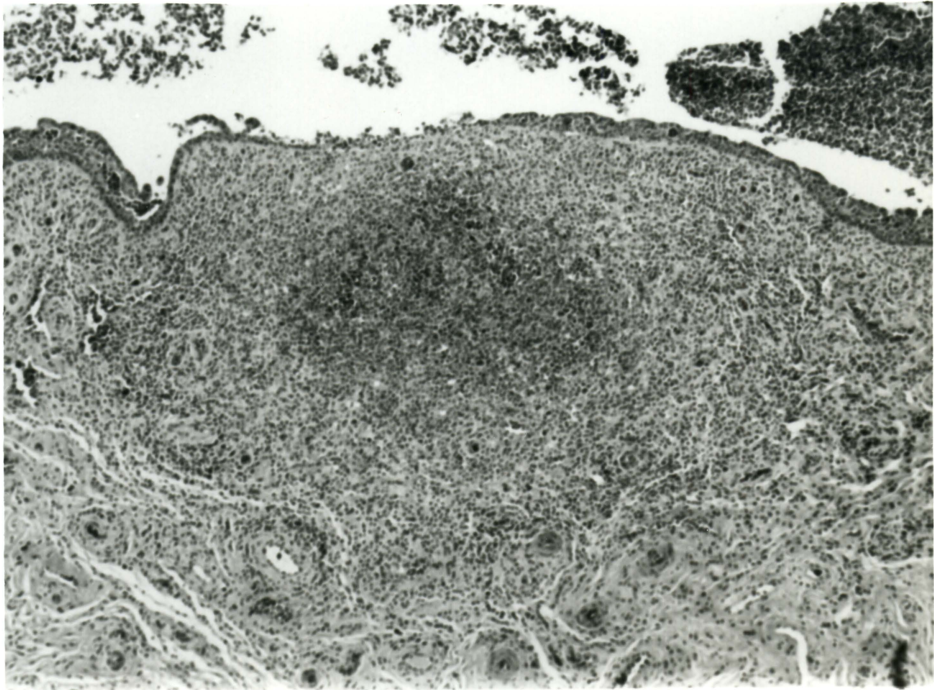
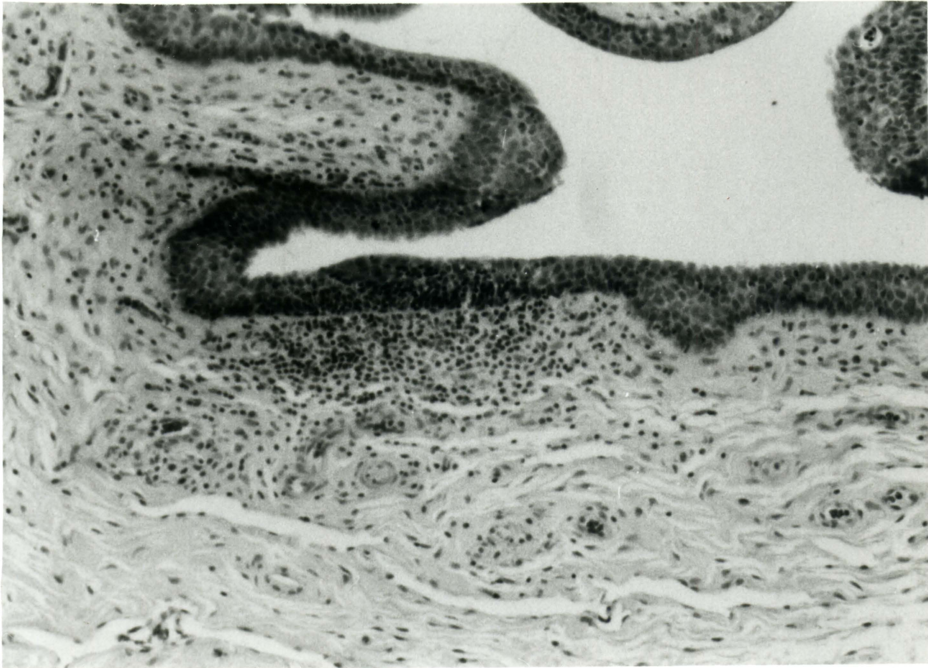
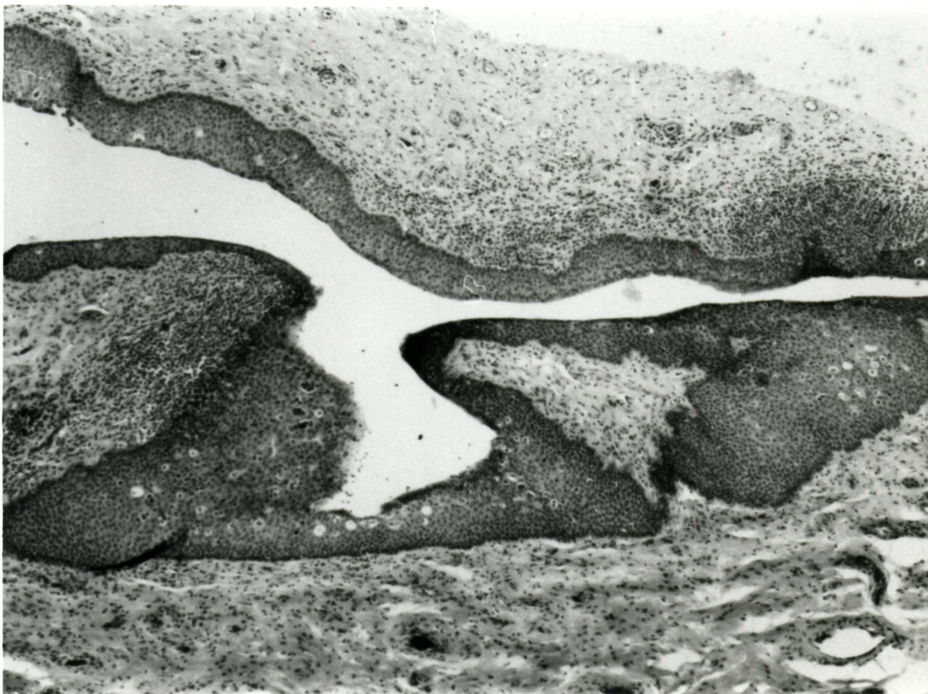


Figure 12. Focal aggregate of lymphocytes in the vagina of a gilt 14 DPE, x 300

Figure 13. Multiple aggregates of mononuclear cells in the vagina of a gilt 14 DPE, x 120





macrophages. In addition, there was a severe, diffuse, lymphohistiocytic endometritis with perivascular and periglandular cuffing in this gilt (Figures 14 and 15). There was a mild increase in mononuclear inflammatory cells in the endometrium of the other two gilts necropsied 3 DPE.

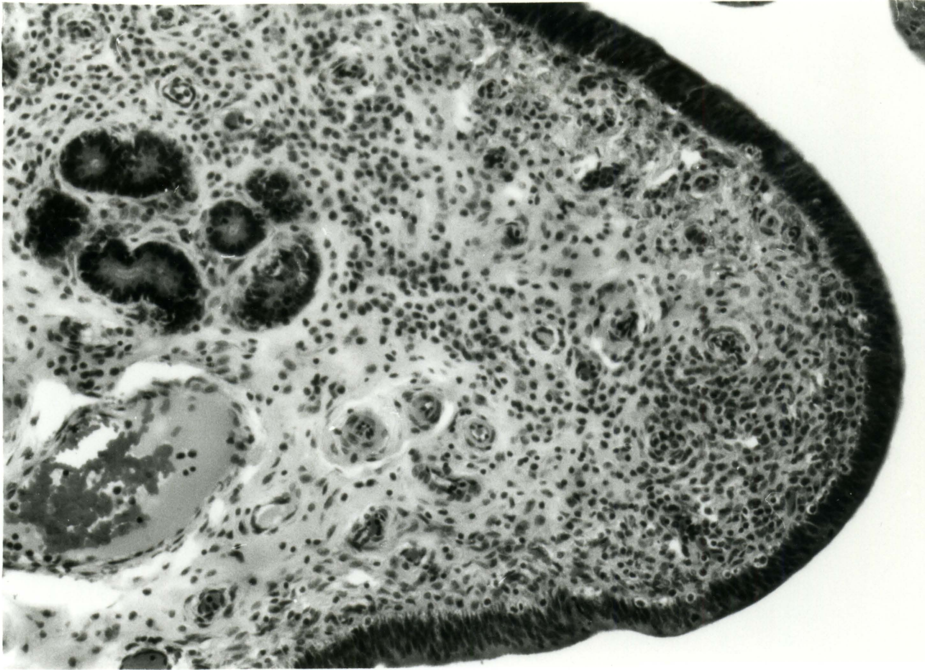
A mild to moderate, diffuse, and focally intense infiltration of lymphocytes and plasma cells with edema and congestion was present in the endometrium of each gilt examined 6 DPE (Figure 16). Gilt #5 also had a large number of eosinophils in the endometrium.

At 10 DPE there were extensive lesions in the uteri of two gilts (#8 and #9). In gilt #9 there were erosions and ulcerations in the endometrium similar to those seen 3 DPE (Figure 17). The uterus of gilt #8 had an acute diffuse endometritis with neutrophils immediately below the epithelium (Figures 18 and 19). In both gilts, there was a moderate multifocal-diffuse lymphoplasmacytic endometritis with marked perivascular cuffing and edema.

The lesions in the uteri of gilts necropsied 14 DPE were similar to those described previously with mononuclear cell infiltration and perivascular cuffing. Lymphoid follicles were present in the endometrium of gilt #2 (Figure 20). By 28 DPE, the inflammatory cell infiltrate in the endometrium was less pronounced and more localized in nature. In the two gilts which were pregnant, there were focal accumulations of lymphocytes and plasma cells in the maternal placenta (Figure 21). In the uterus of gilt #16 there were areas of hemorrhage and mononuclear cell infiltration in the lamina propria and submucosa.

Figure 14. Diffuse lymphohistiocytic endometritis in gilt #2 at 3 DPE, x 300

Figure 15. Marked perivascular cuffing with lymphocytes and plasma cells in the uterus of gilt #2 at 3 DPE, x 300



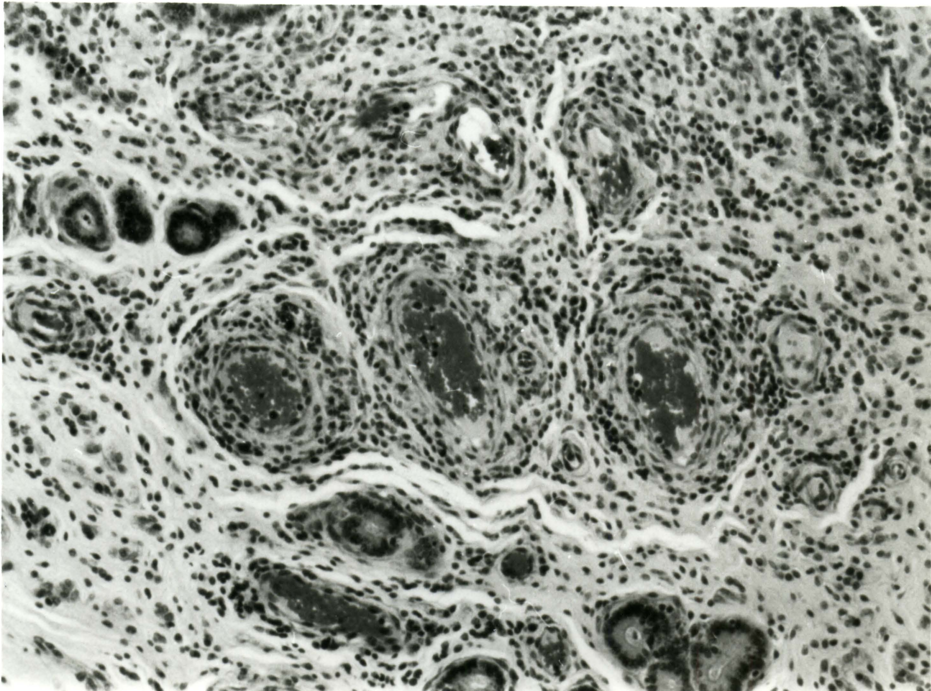
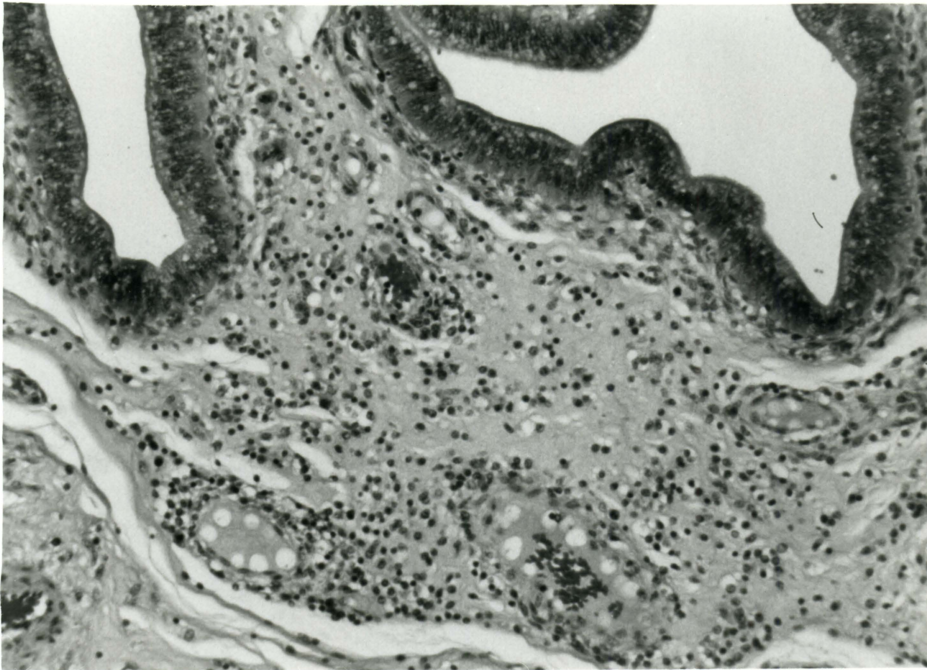


Figure 16. Diffuse lymphoplasmacytic endometritis with edema and congestion at 6 DPE, x 300

Figure 17. Endometrial ulceration at 10 DPE, x 300



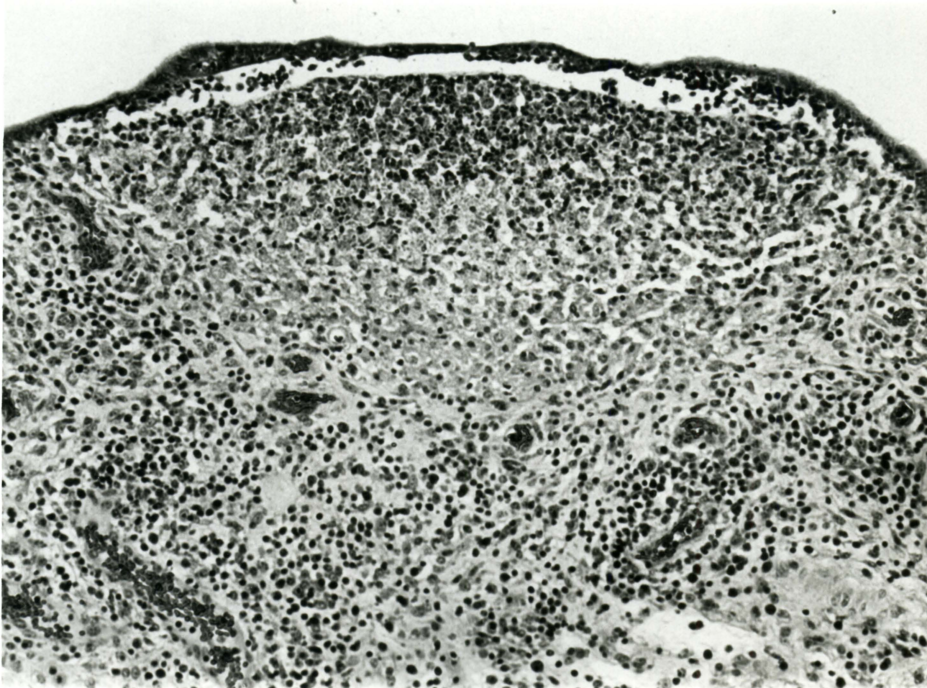
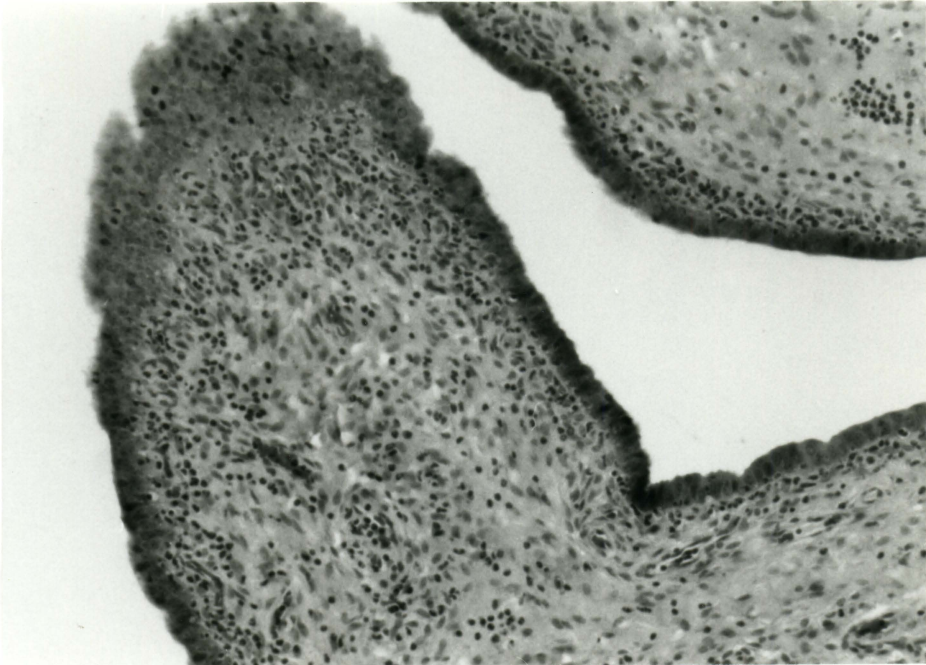


Figure 18. Acute, diffuse endometritis seen in one gilt (#8) at 10 DPE, x 300

Figure 19. Higher magnification of Figure 18 showing neutrophils beneath and within the uterine epithelium, x 750



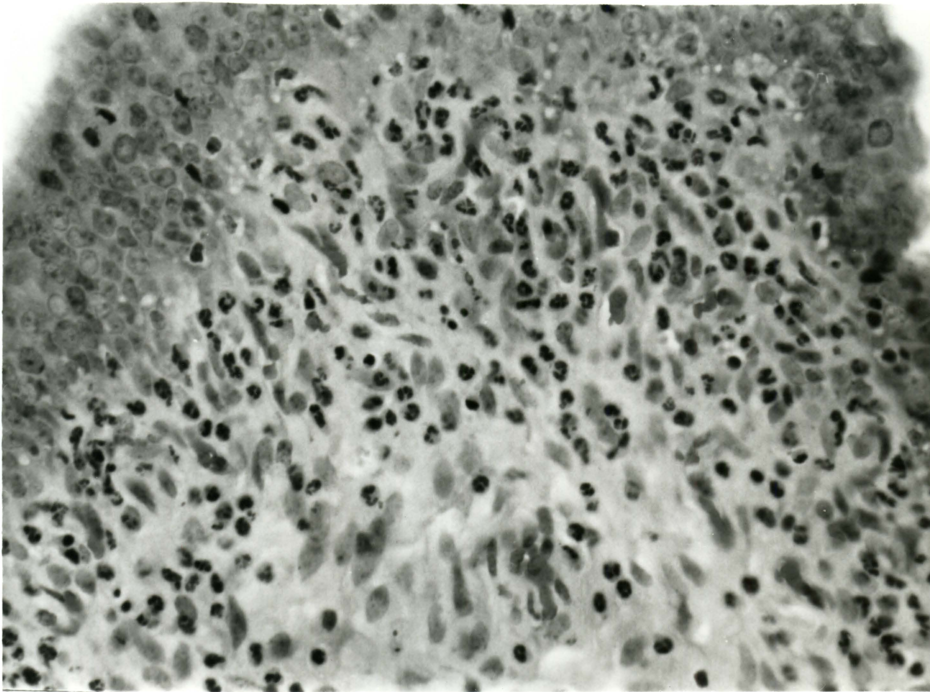
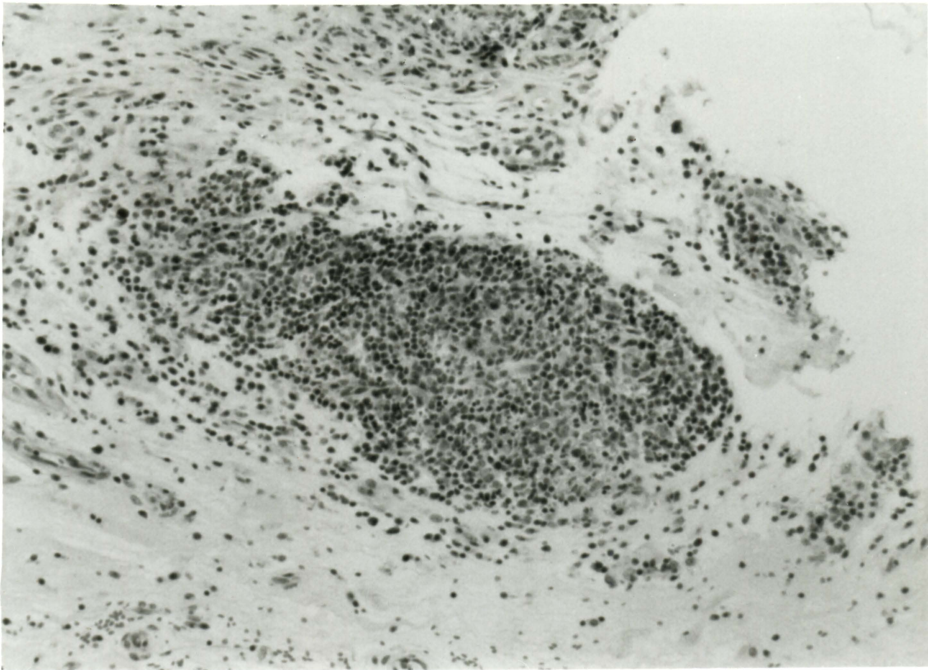
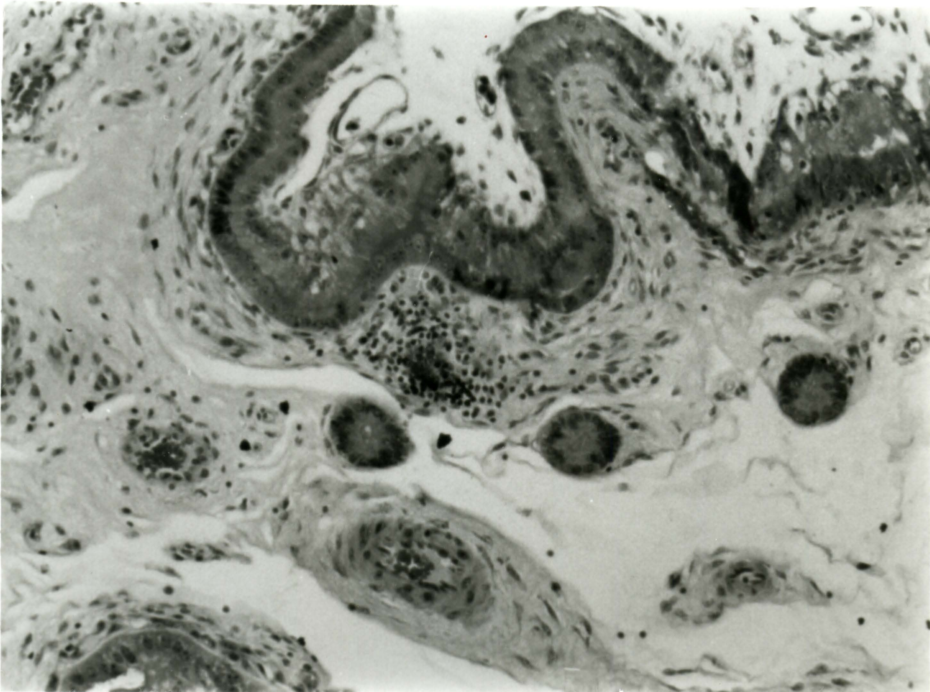


Figure 20. A lymphoid follicle in the endometrium of a gilt necropsied 14 DPE, x 300

Figure 21. Focal aggregate of lymphocytes and plasma cells in the maternal placenta of a gilt 28 DPE, x 750





Lesions in the oviducts of gilts exposed to PrV were mild and limited to lymphoplasmacytic aggregates in the lamina propria (Figures 22 and 23).

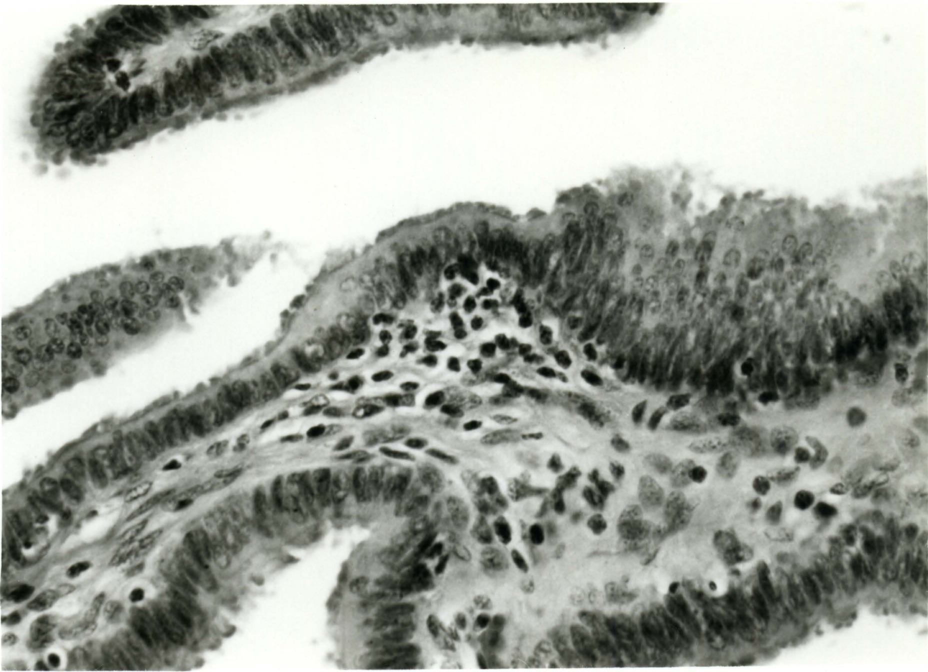
The CL of two gilts (#2 and #3) necropsied 3 DPE were surrounded, but not invaded by, large numbers of neutrophils (Figure 24). The luteal tissue appeared to be forming normally. The ovaries of the remaining gilt necropsied 3 DPE were normal. At 6 DPE the only lesion in the ovaries was the presence of focal collections of 10 to 20 lymphocytes and plasma cells in the CL of two gilts (#4 and #5). An occasional mitotic figure was present.

The ovarian lesions were more extensive at 10 DPE than at 6 DPE. The CL of two gilts (#7 and #9) contained aggregates of large numbers of lymphocytes and plasma cells (Figure 25). The lymphocytes were present at the margin and in the central area of affected CL. The lesion in gilt #9 was more extensive and was characterized by a lymphocytic infiltrate in the surrounding luteal tissue, and the presence of mitotic figures and necrotic lymphocytes (Figure 26).

The ovarian lesions in the four gilts necropsied 14 DPE varied in severity. In three gilts (#10, #12, and #13), there were variously sized lymphoid nodules in the CL. In general, the nodules were larger than those present 10 DPE. In gilt #13, there were lymphoid nodules in the ovarian stroma immediately peripheral to the CL (Figure 27). Within the lymphoid nodules there were many plasma cells, a few mitotic figures, and large lymphoblastic cells (Figure 28). The CL of gilt #11 contained central zones of coagulative necrosis and hemorrhage with

Figure 22. A small accumulation of lymphocytes in the lamina propria of the proximal oviduct at 6 DPE, x 750

Figure 23. Lymphoid aggregate in the proximal oviduct at 14 DPE, x 300



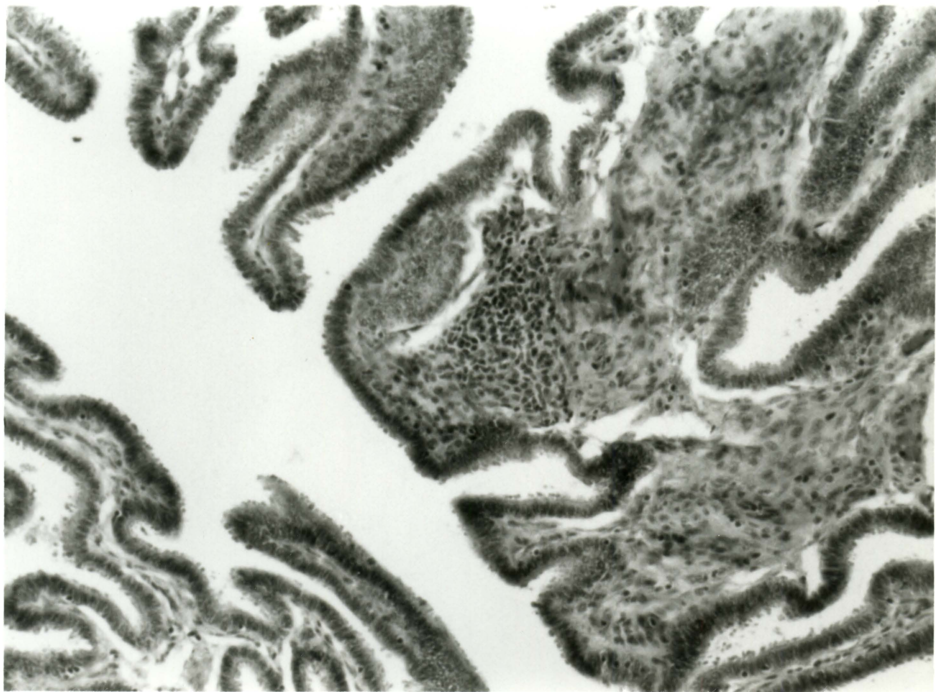
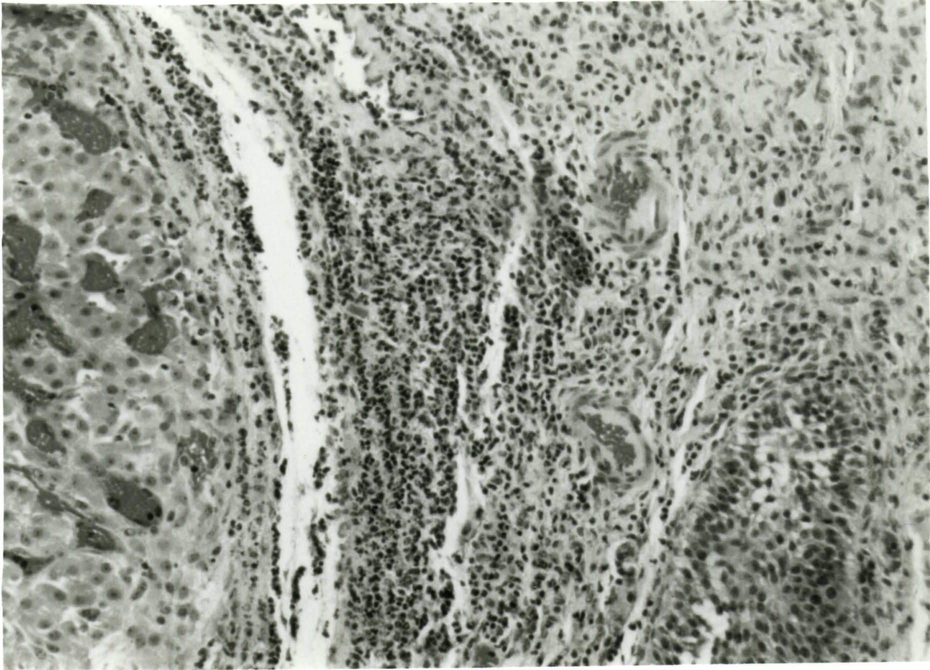


Figure 24. Normal appearing corpus luteum surrounded by large numbers of neutrophils at 3 DPE, x 300

Figure 25. Lymphoplasmacytic infiltrate into a corpus luteum of a gilt at 10 DPE, x 300



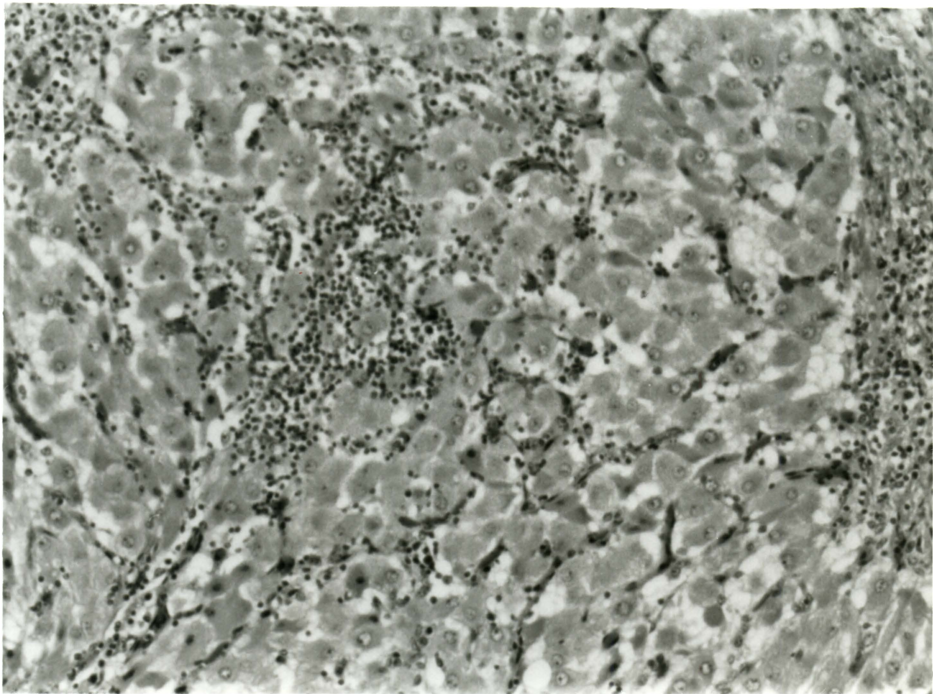
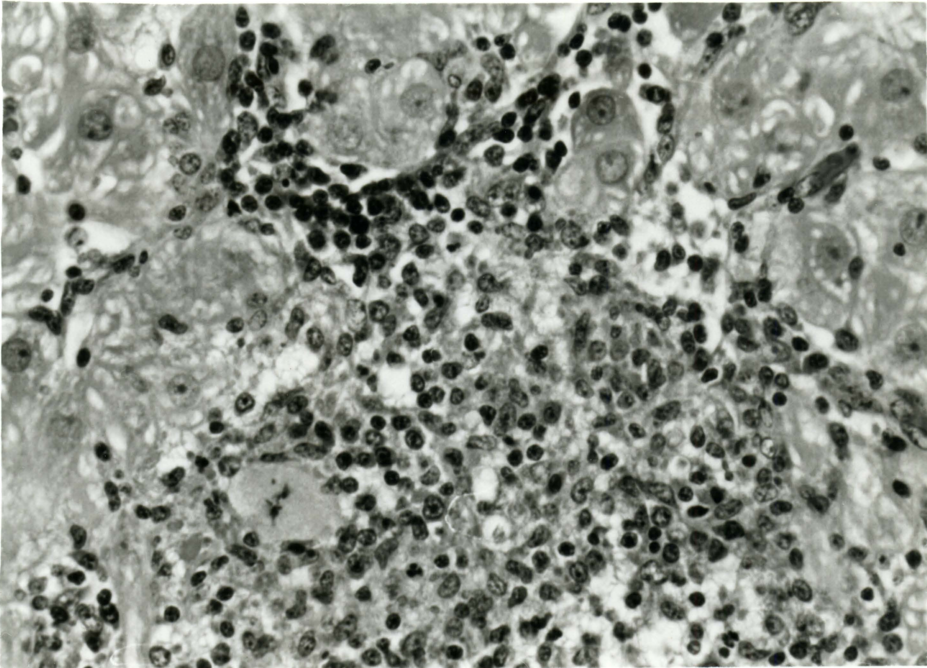
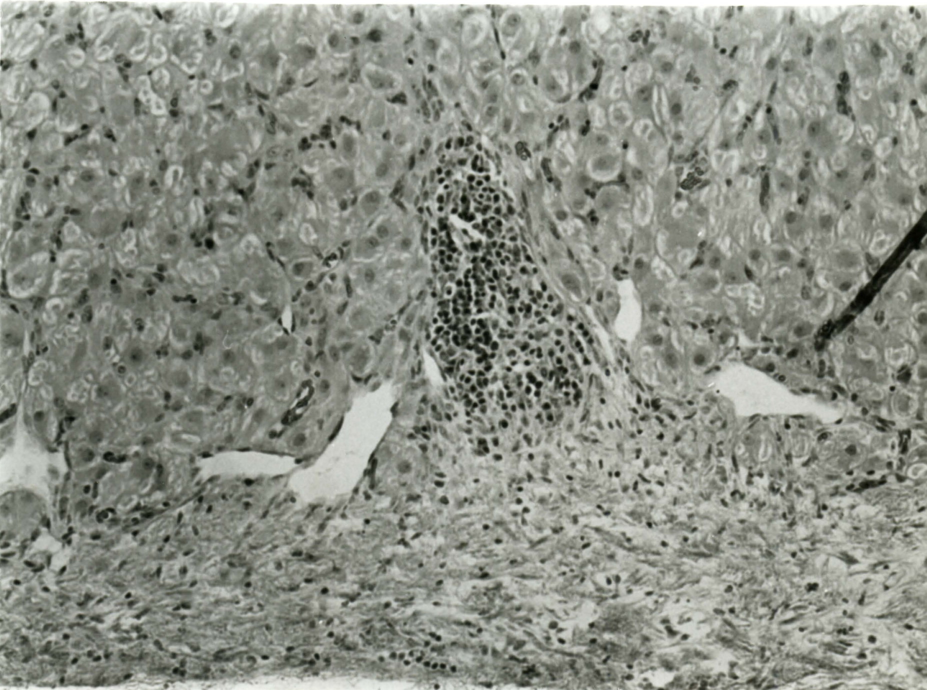


Figure 26. The lymphocytic infiltrate extends into the luteal tissue causing individualization of luteal cells in gilt #9 at 10 DPE, x 300

Figure 27. Focus of lymphocytes and plasma cells in the ovarian stroma adjacent to a corpus luteum, x 300





areas of mineralization (Figure 29). The necrotic areas were bordered by an intense inflammatory infiltrate composed of lymphocytes, plasma cells, macrophages, and lesser numbers of neutrophils and eosinophils.

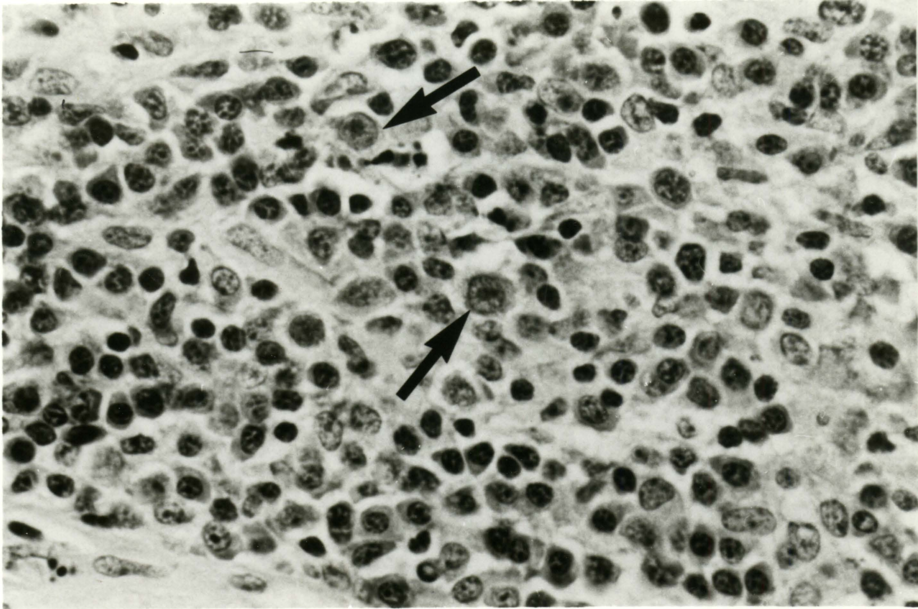
Lesions were not observed in the ovaries of the two pregnant gilts necropsied 28 DPE (#14 and #15). The recently formed CL from the other gilt (#16) were normal but the degenerating CL formed at the time of breeding and exposure to PrV contained lymphoid nodules (Figures 30 and 31).

The reproductive lymph nodes of gilts at 3, 6, and 10 DPE were edematous and contained areas of hemorrhage or congestion. Foci of coagulative necrosis with infiltration of neutrophils were present in the reproductive lymph nodes of gilts necropsied 6 and 10 DPE (Figure 32). Eosinophilic and basophilic intranuclear inclusions were present in cells at the margin of the necrotic areas. The lymph nodes draining the reproductive tract of gilts at 14 and 28 DPE were hyperplastic and had enlarged germinal centers.

Epithelial and lymphoid necrosis with suppuration was present in the tonsilar crypts of gilts necropsied 3, 6, 10, and 14 DPE. The lesion was most severe 6 and 10 DPE and was resolved 14 DPE. Epithelial cell nuclei contained large, eosinophilic inclusions and marginated chromatin (Figure 33). Mandibular lymph nodes contained necrotic foci 6 DPE and were hyperplastic in gilts necropsied 14 and 28 DPE. Acute splenitis with infiltration of neutrophils was present in one gilt (#3) 3 DPE. Necrotic foci were present in the spleen of

Figure 28. High magnification of the cellular infiltrate in the corpus luteum of a gilt at 14 DPE. Note the large lymphoblastic cells, x 1200

Figure 29. A corpus luteum from gilt #11 necropsied 14 DPE. Note the central zone of coagulative necrosis (A), the areas of mineralization (B), and the inflammatory cell infiltrate (C) which was composed of lymphocytes, macrophages, and neutrophils, x 120



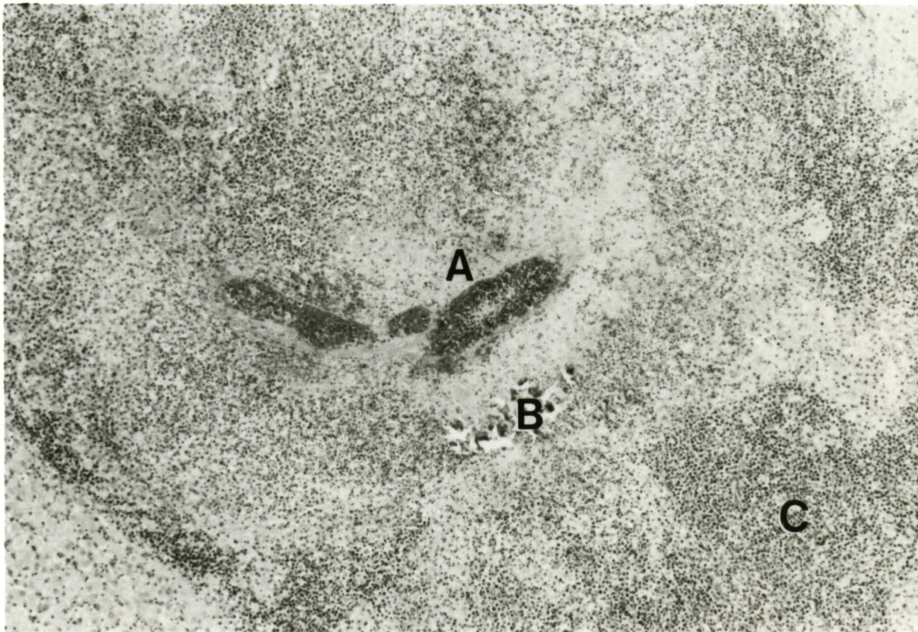
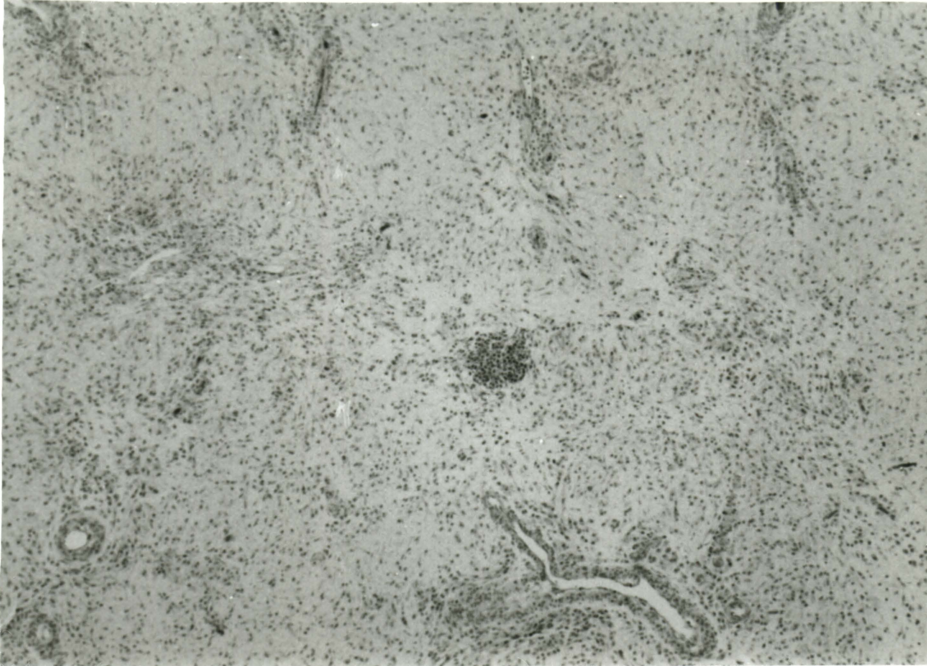

A
B
C

Figure 30. Lymphoid aggregate in a regressing corpus luteum from gilt #16 at 28 DPE, x 120

Figure 31. Higher magnification of the lymphoid aggregate shown in Figure 30, x 750



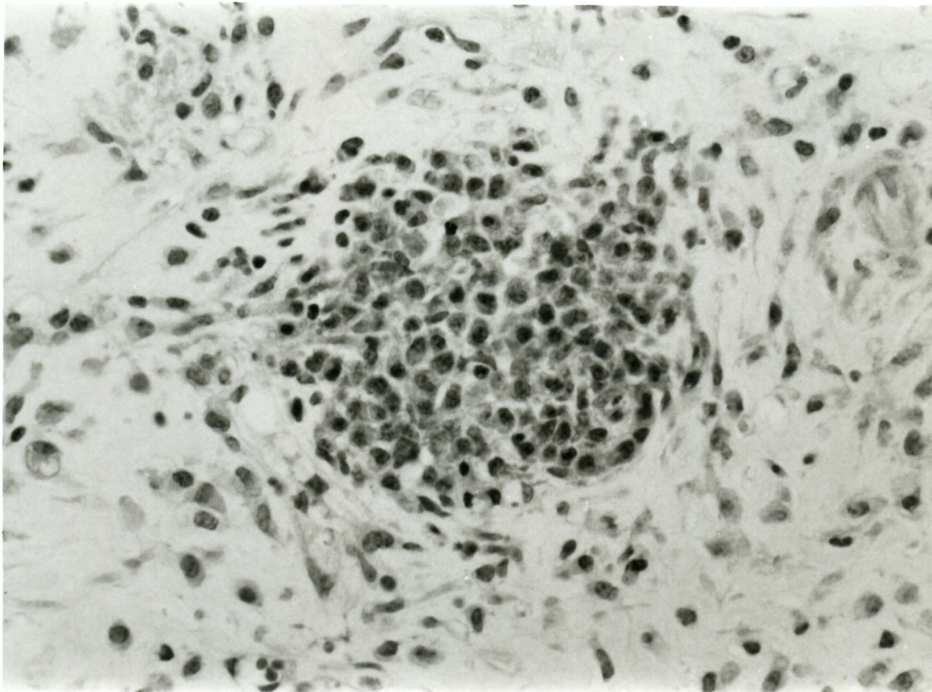
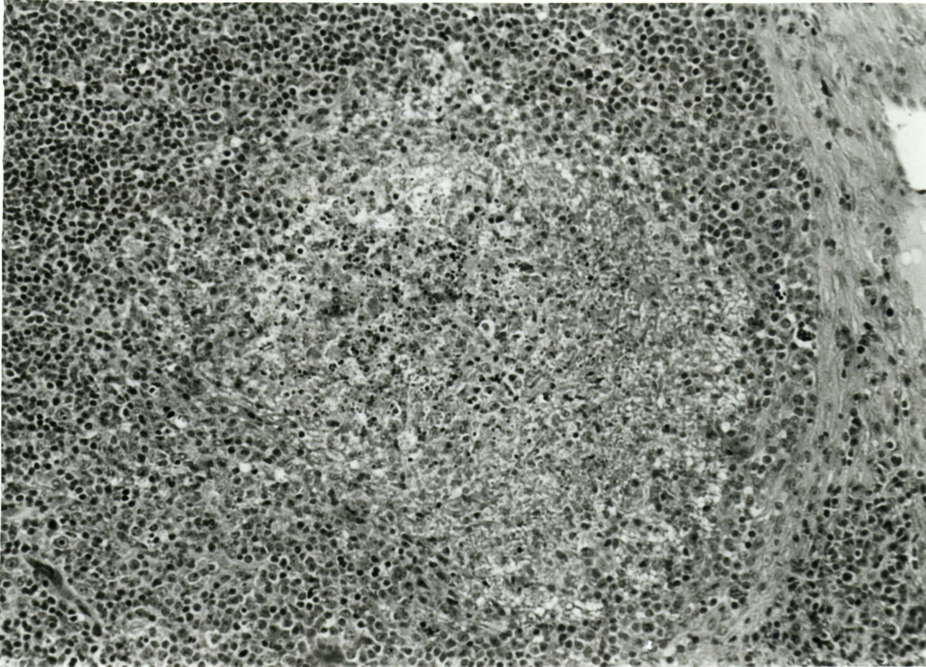
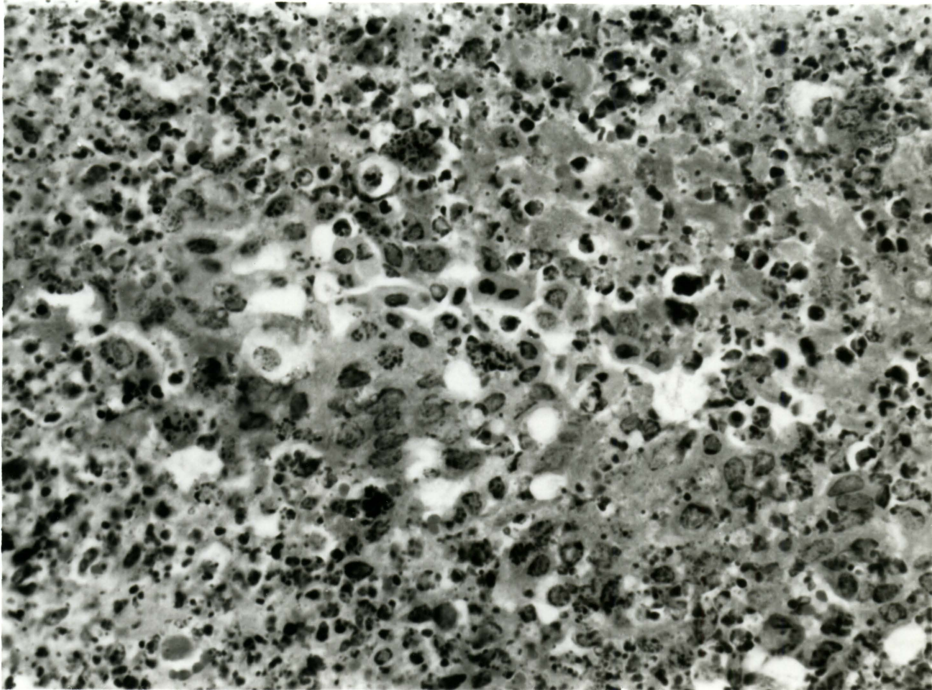


Figure 32. Focus of coagulative necrosis in a lymph node draining the reproductive tract at 10 DPE, x 300

Figure 33. Epithelial and lymphoid necrosis in a tonsilar crypt of a gilt at 3 DPE. Note the large intranuclear inclusions in epithelial cells, x 750





one gilt (#4) 6 DPE. Enlarged lymphoid follicles and germinal centers were present in the spleen of gilts necropsied 10, 14, and 28 DPE.

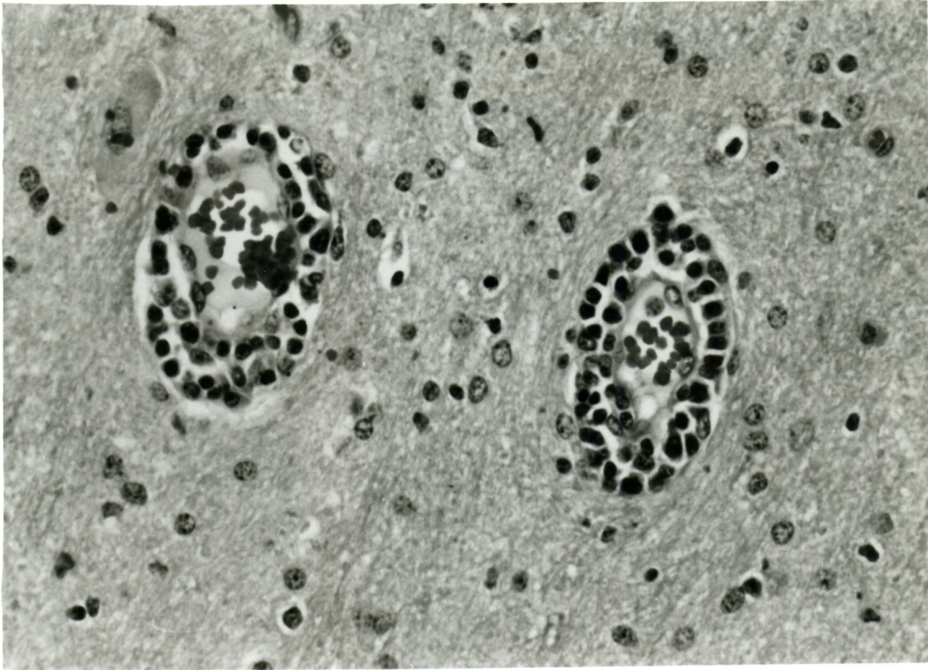
Lesions were first noted in the central nervous system 10 DPE. Mild focal gliosis, neuronophagia, malacia, and lymphocytic perivascular cuffing were present in the cerebrum of one gilt (Figures 34 and 35) and at various levels of the spinal cord in each gilt necropsied 10 DPE. The lesions were located primarily in the grey matter of the brain and spinal cord. At 14 DPE, there were occasional vessels with a cuff of mononuclear cells present in the brain and spinal cord.

In the adrenal cortex of gilt #10, there were multifocal areas of a mixed inflammatory cell infiltrate (Figure 36). The cells were primarily lymphocytes but a few neutrophils were also present. These may represent sites of resolving adrenal necrosis.

The lung of gilt #7 (10 DPE) contained consolidated areas of acute necrotizing pneumonitis with infiltration of neutrophils and intranuclear inclusions in alveolar epithelial cells at the periphery of the necrosis. In addition, the peribronchial and peribronchiolar lymphoid follicles contained areas of necrosis.

Figure 34. Perivascular cuffs in the cerebral grey matter at 10 DPE, x 750

Figure 35. Focal malacia and gliosis in the cerebral cortex at 10 DPE, x 300



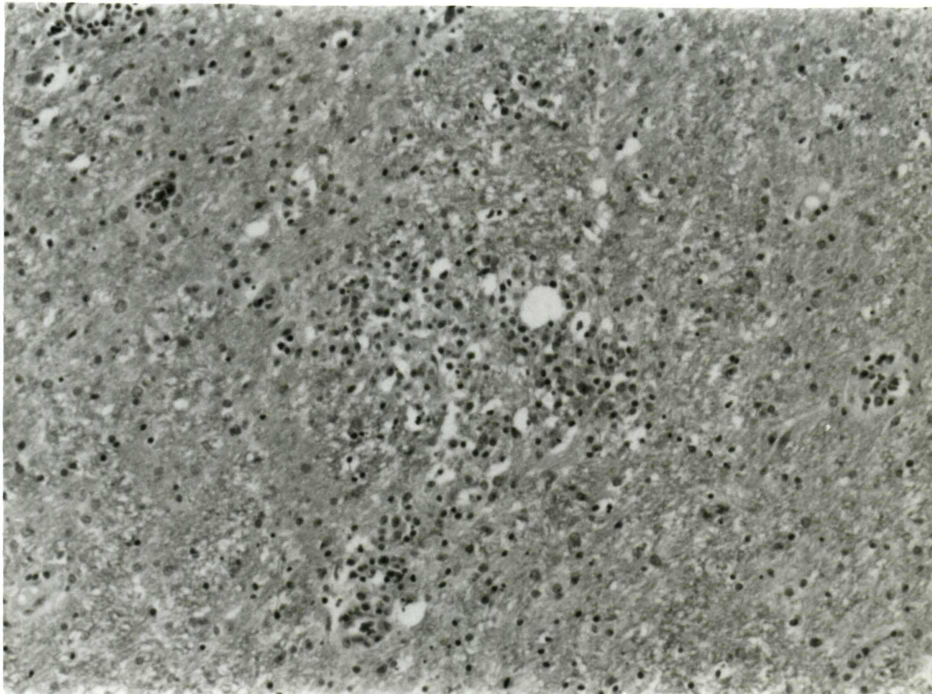
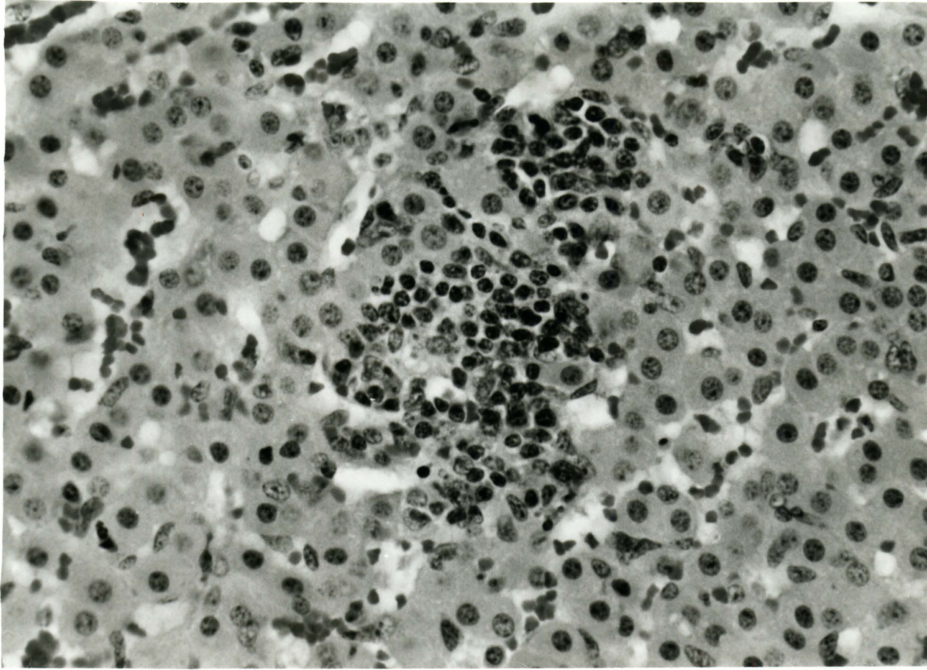


Figure 36. Focus of lymphocytes, plasma cells, and a few neutrophils in the adrenal cortex of gilt #10 at 14 DPE, x 750



DISCUSSION

Gilts exposed to PrV (Funkhouser isolate), in this study, developed only mild clinical signs of infection. Previously, sows given intranasal and intrauterine injections of PrV (Sullivan isolate) at the time of breeding developed similarly mild clinical signs (Bolin 1984). Both the Funkhouser and Sullivan isolates are virulent for suckling pigs (Scherba et al. 1978). Hall (1982), however, reported more severe signs, including central nervous system dysfunction and death, in gilts inseminated with PrV (Iowa isolate) contaminated semen. The variation in clinical signs observed was probably due to strain variation in pathogenicity and dose of virus in the inoculum (Baskerville 1972).

All gilts exposed to PrV and maintained 10 or more days after breeding developed precipitating and neutralizing antibodies to the virus. Precipitating but not neutralizing antibody to PrV was detected in the serum from two of three gilts necropsied 6 DPE. Previous reports indicate that precipitating antibody to PrV can be detected as early as 7 days after virus exposure with the MIDT test (Kelling et al. 1978; Pirtle and Gutekunst 1978).

A temporal correlation has been reported between disappearance of detectable PrV from the animal following primary infection and the development of viral neutralizing antibody (McFerran and Dow 1965; Sabó 1969). Other work does not support this finding (Wittman et al. 1980). In the current study, virus was isolated from the tonsils, lymph nodes, and reproductive tracts of gilts with SN titers of 1:64 or greater.

Virus was isolated from the reproductive tract of gilts exposed to PrV for as long as 14 DPE, indicating that infection was established by the route of inoculation used. At 3 and 6 days DPE, PrV was isolated from the lymph nodes which drain the reproductive tract. The development and regression of lymphatics which drain the reproductive tract has been shown to coincide with events of the estrous cycle. Maximum vessel development and efferent lymph flow occur during ovulation and the luteal phase of the cycle (Morris and Sass 1966). This provides a mechanism whereby the virus may rapidly gain access to the lymphatics and be disseminated throughout the body following intrauterine infection. This was the likely route of infection for organs outside the reproductive tract, although an intranasal exposure to virus in the runback of the inoculum from the cervix, cannot be excluded. However, virus was not isolated from the nasal swabs of gilts exposed to PrV, which suggests that a significant intranasal exposure did not occur.

The cellular inflammatory response to the presence of PrV was similar in the different portions of the reproductive tract which were sampled in this study. The response consisted primarily of the accumulation, proliferation, and differentiation of lymphocytes. The proliferation and differentiation of the lymphocytes is likely an inflammatory response to replication of virus in surrounding tissues. In addition, focal necrosis of the vagina, uterus, or ovaries was present occasionally.

Lymphohistiocytic vaginitis, as reported in this study, has been described previously in gilts exposed to PrV (Hall 1982). The presence of ulcerations in the vagina has not been previously described in swine with PrV, but similar lesions occur in other herpes virus diseases of mammals (Kendrick and McEntee 1967; Bittle and Peckham 1971b; Hill and Maré 1974).

The uterine lesions in gilts exposed to virus varied in severity, but were generally mild to moderate in intensity. The lesions were more severe in the caudal portions of the uterus, near the site of virus deposition. Similarly, cattle exposed to BHV-1 by intrauterine inoculation through an insemination tube had more severe lesions in the caudal portion of the uterus (Miller and Van Der Maaten 1984). Focal endometrial necrosis and diffuse endometritis observed in this study were not observed in gilts, similarly exposed, in a previous study (Hall 1982).

Acute oophoritis was observed in 2 of 3 gilts necropsied 3 DPE. Neutrophils were located exclusively around the developing corpora lutea. Following ovulation, eosinophils and mast cells are normally present in the ovarian stroma surrounding the ruptured follicle (Priedkalns 1976), but suppurative inflammation in this organ is rare (Jubb and Kennedy 1970). It is not clear whether this is a physiologic or pathologic change in these gilts as a control gilt was not examined 3 days after breeding.

The lesions in the ovary of other gilts exposed to PrV consisted of a lymphoplasmacytic infiltrate in the corpora lutea, which increased

in extent, with time, until 14 days after exposure. Virus was not consistently isolated from the ovaries containing these lesions. Hall (1982) observed similar lesions in the CL of pregnant gilts which aborted following intranasal exposure to PrV.

Extensive necrosis was present in the CL of one gilt necropsied 14 DPE. Virus was isolated from the ovaries of this gilt and viral antigen was detected at the periphery of the necrotic tissue by fluorescent antibody staining. Luteal necrosis has not been reported in swine with PrV but similar lesions have been demonstrated in cattle exposed to BHV-1 (Miller and Van Der Maaten 1984; Van Der Maaten and Miller 1984), in mice exposed to MCMV (Mims and Gould 1979), and in nonhuman primates exposed to herpesvirus T or herpes simplex (King 1978).

The path by which virus reaches the ovary following intrauterine deposition of virus is not clear. Intralumenal spread of virus from the site of deposition to the ovary is a possible route. The distance to be traveled and the mild nature of the lesions in the cranial portions of the uterus and oviducts make this an unlikely possibility. It is more likely that ovarian infection occurred as a result of systemic spread of the virus. The extensive vasculature of the corpus luteum would predispose it to infection during a viremia. Similarly, infection of the ovary in cattle following intravenous, intramuscular, or intrauterine exposure to BHV-1 is thought to occur as a result of hematogenous spread of virus (Van Der Maaten and Miller 1984).

The clinical significance of the ovarian lesions in gilts exposed to PrV is difficult to assess. It is unlikely that the lymphoid aggregates present in the CL would interfere sufficiently with normal CL function to terminate pregnancy. However, the extensive necrosis of the CL observed in one gilt might result in loss of luteal function with subsequent reproductive failure. Further investigation of the consequences of PrV-induced ovarian lesions is needed.

Isolation of virus from and the observation of lesions in the central nervous system were infrequent in this study. This was likely because of inoculation route and age resistance since the Funkhouser strain of PrV readily infects the central nervous system of young pigs exposed intranasally.

The microscopic lesions observed in other organs were similar to those described previously (Olander et al. 1966; Baskerville et al. 1973; Alva-Valdes 1981; Hall 1982).

An attempt was made to demonstrate virions in cells associated with the necrotic lesions in the uterus and ovary. Selected hematoxylin and eosin stained sections were examined by electron microscopy utilizing the technique of Halvorsen (1978). Technical difficulties and the small size of the lesions precluded a thorough search. Virions were not observed in the few specimens which were available.

The detection of viral nucleocapsids and virions in a recently hatched 6-day embryo and a 10-day embryo indicated that preimplantation porcine embryos were susceptible to PrV _in vivo_, after the zona pellucida is shed. The recovery of substantially fewer embryos than

would have been anticipated from the number of CL at 6 and 10 DPE suggested that the presence of virus had a deleterious effect on the embryo. Viral infection leading to degeneration of the embryo is one explanation for the discrepancy between the number of CL and the number of embryos collected.

Another possible explanation is that virus induced inflammation of the uterus may have caused embryonal degeneration. Prior to implantation, porcine embryos are maintained in the fluid present in the oviducts and uterus (Hunter 1977). Virus induced uterine inflammation was most extensive during this period which may have led to an alteration of the microenvironment of the embryo and subsequent embryonal degeneration. A similar mechanism has been proposed to result in a reduced pregnancy rate and embryonal growth retardation in mice infected with MCMV (Neighbour 1976).

It is also possible that heat stress, as a result of fever, may have caused some embryonal degeneration in the gilts necropsied 6 and 10 DPE. Environmental thermal stress of gilts following breeding has been shown to decrease the number of viable embryos at 35 days of gestation (Tompkins et al. 1967; Omtvedt et al. 1971). However, embryos appear to be the most sensitive to heat stress during the period of implantation (Omtvedt et al. 1971) which occurs after 10 days of gestation (Hunter 1977).

There are other factors, not related to viral infection, that could account for the poor rate of embryo recovery at 6 and 10 days postbreeding. Reports indicate that 30 to 35% of the fertilized

porcine oocytes (estimated from the number of CL) fail to become established as viable embryos with the major loss occurring before or during implantation (Rasbech 1969; Holness 1982). In addition, porcine embryos are difficult to identify near the time of hatching from the zona pellucida and thus we may have failed to identify and recover a few 6-day embryos.

Embryo recovery from the control gilt was also poor at 6 days after breeding. In addition to the nonspecific factors previously mentioned, an anatomic defect may have been present in the oviduct of this gilt which prevented fertilization and/or passage of the embryos to the uterus on the affected side. Anatomical defects in the female reproductive tract of swine are common (Rasbech 1969; Singleton 1980).

The excellent recovery rate of embryos from gilts 3 days after breeding and exposure to PrV, supports a previous observation that PrV infection of the uterus at the time of breeding does not impair ovulation or fertilization (Bolin 1982).

Although PrV was isolated from the oviducts, uterus, and uterine flush fluids of gilts exposed to PrV, virus was not observed in association with any of the embryos which were surrounded by a zona pellucida. In similar studies with bovine embryos that were collected before the zona pellucida was shed, bluetongue virus and BHV-1 were isolated from the uterine flush fluids but neither virus was found associated with the embryos (Bowen et al. 1983: Singh et al. 1983; Thomas et al. 1983). However, studies conducted *in vitro* have shown that PrV and BHV-1 will interact with the zona pellucida of porcine and

bovine embryos, respectively (Singh et al. 1982; Bolin et al. 1983). Transmission of PrV to embryo recipients occurred following the transfer of extensively washed embryos collected from gilts exposed to PrV at breeding (Bolin et al. 1982). This suggests that an interaction between PrV and the zona pellucida does occur in vivo but the results of the current study failed to prove this.

The embryos collected from one gilt (#3) at 3 DPE had phagocytic cells adhering to and within the zona pellucida. The pathogenesis of this lesion is not clear as the embryos appeared to be normal. It seems likely that the embryos were being attacked by these cells as a result of the uterine inflammation induced by the virus infection. It is not clear, however, whether the presence of the phagocytes would have been detrimental to the further development of these embryos.

Embryos were not recovered from gilts necropsied 14 DPB due to faulty collection technique. At 14 DPB, we anticipated that implantation would have occurred and that the embryos would be readily identifiable with the unaided eye. Subsequent to the completion of this study it was determined that a uterine flushing procedure, similar to that used 10 DPB, would have been appropriate to recover 14-day embryos.

Two of three virus exposed gilts were pregnant 28 DPE which indicated that exposure to PrV at breeding did not necessarily terminate pregnancy. One gilt necropsied 28 DPE was not pregnant and the morphology of the CL on her ovaries suggested that ovulation had occurred 1 to 2 days prior to necropsy. The slight delay in the second

cycle suggests that this gilt conceived but that the pregnancy was not maintained. The degenerating CL (those from the previous cycle) present in this gilt contained lymphoplasmacytic infiltrates which suggested that infection of the ovaries occurred.

Pseudorabies virus has been isolated from semen of naturally and experimentally infected boars (Gueguen et al. 1980; Medveczky and Szabó 1981) but it is not likely to be present in the amounts used to inoculate gilts in this study. However, the results presented here and those of others (Hall 1982) suggest that venereal transmission of PrV (by natural breeding or by artificial insemination) may result in infection of the female reproductive tract and subsequently a systemic infection. In addition, infection of the uterus at the time of breeding poses a threat to the early porcine embryo.

SUMMARY AND CONCLUSIONS

Sixteen gilts were exposed to PrV by intrauterine inoculation within 6 hours after natural breeding and subsequently necropsied to determine the effect of PrV on reproductive performance. Four gilts from the same source were treated similarly except for exposure to PrV. Gilts exposed to virus were necropsied 3, 6, 10, 14 or 28 days postexposure (DPE). Controls were necropsied 6, 10, and 14 days postbreeding (DPB). Maternal and embryonic tissues were collected for viral isolation, fluorescent antibody staining, histopathology, and electron microscopy. Maternal blood samples were taken prior to breeding and at the time of necropsy and tested for antibody to PrV.

Pseudorabies virus infection was established in each gilt exposed to PrV and virus was recovered from the reproductive tract up to 14 DPE. Antibody for PrV was detected only in blood collected 6 or more DPE. Lesions in the reproductive tract varied in severity and were composed of multifocal to diffuse lymphohistiocytic vaginitis and endometritis, and lymphoplasmacytic oophoritis. Multiple focal ulcerations were observed in the vagina or endometrium of several gilts at 3, 6, and 10 DPE. The corpora lutea of one gilt at 14 DPE were necrotic and contained large numbers of inflammatory cells. Focal aggregates of lymphocytes were present in the vagina, endometrium, and ovary at 28 DPE.

Viral nucleocapsids or virions were detected in cells from a 6-day and a 10-day embryo. Virus was isolated from the uterine flush fluids

used to collect those embryos. Virus was not detected in association with any other embryos.

Neither lesions nor PrV were detected in the reproductive tract or embryos of controls and all blood samples were free of antibody for PrV.

The results of this study suggest that PrV may cause early embryonic death in swine when it is present in the reproductive tract during early gestation. Several possible causes of early embryonic death were demonstrated to occur following PrV infection of the reproductive tract. First, porcine preimplantation embryos are susceptible to PrV infection *in vivo* and death of the embryo is a likely sequela of infection. Secondly, uterine inflammation produced by PrV infection would likely have a detrimental effect on the preimplantation embryo. Finally, luteal necrosis may sufficiently interfere with the endocrine function of the corpora lutea to cause termination of pregnancy.

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