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INSEMINATION OF SUSCEPTIBLE AND PREIMMUNIZED GILTS WITH BOAR SEMEN CONTAINING PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

C. Prieto,[1] P. Suárez,[1] I. Simarro,[1] C. García,[1] S. Martín-Rillo[2] and J.M. Castro[1]

[1] Departamento de Patología Animal I. Universidad Complutense de Madrid. Facultad de Veterinaria Avda. Puerta de Hierro s/n. 28040 Madrid, Spain

[2] Kubus S.A. Polígono Industrial Europolis. Las Rozas, 28040 Madrid. Spain

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ABSTRACT

Twenty-one gilts without measurable PRRSV serum antibody titres were identified for this experiment. Seven gilts were used as controls (Group C) and 14 as principals. Of these, 7 gilts were preimmunized to PRRSV and constituted Group B, while 7 gilts remained seronegative and constituted Group A. The principal gilts were inseminated with boar semen containing PRRSV and were killed 20 d later. The control gilts were treated similarly but were not exposed to PRRSV. Gilts were observed for clinical signs of infection. The effects on the conception rates were studied and gilts and embryos were tested for PRRSV and homologous antibodies. Group A and B gilts developed signs of PRRS associated with anorexia and slightly elevated body temperatures. Transmission of the infection was demonstrated by the isolation of PRRSV from serum and other tissue samples of principal gilts and also by seroconversion. The results show that early infection may have an insignificant effect or no effect on the conception and fertilization rates. However, exposure to PRRSV at the time of insemination can result in transplacental infection of embryos. In Group A gilts, 5 of 6 litters were infected prenatally with 7.6% of embryos infected. In Group B gilts, 1 of 5 litters and 1.3% of embryos were infected. Moreover, approximately 2 and 4 times more embryos were dead in litters of gilts from Group A and Group B than in gilts from control Group C. The isolation of PRRSV in 3 dead embryos suggests that the embryos may have died as a result of the direct effect of the virus. It can be concluded that the insemination of either seronegative or preimmunized gilts with boar semen containing PRRSV may have an insignificant effect or no effect on conception and fertilization rates, although it can result in transmission of the virus and embryonic infection and death.

Key words: PRRS, boar semen, insemination, virus

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS), which causes abortion and respiratory disease in pigs, was first detected in 1987 in North America (8). The etiological agent was first isolated in 1990 in Europe (28) and designated Lelystad Virus (LV). In 1992, it was isolated in the USA (5). These were antigenically and genotypically distinct but structurally related viruses (13, 15, 21).

The etiological role of PRRS virus (PRRSV) in reproductive failure of swine is firmly established (3, 14, 26), and a substantial amount of evidence indicates that the virus can be a major cause of prenatal death in commercial swine herds. Most reports of field cases of PRRSV-induced

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reproductive failure have described delayed returns to estrus, reduced conception rates, a sudden increase in early farrowing, late term abortions and dead and mummified fetuses (4, 9, 12).

Reproductive sequelae to the disease have generated interest in the possible role of boars in the transmission of PRRSV as well as in the suspected viral effects on male fertility. Experimental infection in boars has led to seminal shedding of virus following infection (17, 23), and epidemiological evidence confirms the transmission of PRRSV from fresh semen of acutely infected boars (20, 29). Meanwhile, there are conflicting results regarding the effect of inseminating gilts with boar semen containing PRRSV. Some authors (24, 29) have expressed the view that low conception rates and returns to service are a feature of the disease while others (27) believe that they are not. Recently, Lager et al. (11) reported no differences in conception rates when PRRSV was infused into the uterus following natural breeding.

The purpose of this study was 1) to determine whether PRRSV can be transmitted to susceptible and preimmunized gilts by insemination with boar semen-containing virus, 2) to establish whether experimentally induced PRRSV infection at the time of breeding has any effect on conception in gilts, and 3) to obtain information on any possible pathological repercussions on gilt exposure to PRRSV at the time of pregnancy.

MATERIALS AND METHODS

Animals and Facilities

Twenty-five crossbred Landrace x Large White gilts (n=25) without measurable PRRSV serum antibody titres, as measured with an ELISA test (INGEZIM.PRRS®[a]), were initially identified for this experiment. For about 3 mo prior to the experiment, the gilts were housed in groups of 8 or 9 in isolated pens with a concrete floor and an automatic watering system. Preimmunized gilts were vaccinated intramuscularly with 2 ml of a killed PRRSV vaccine (Cyblue®[b]) 7 and 4 wk prior to exposure to virus via semen. At the start of the experiment all gilts were approximately 8 mo old. Two Large White boars (n=2) without measurable PRRSV serum antibody titres were kept in the same building. There was direct contact between boars and gilts for 15 min per day for 20 d prior to estrus synchronization. The gilts were fed on a 17% protein diet with 72.5% of total digestible nutrients throughout the trial. Food intake was recorded daily. The gilts were regularly examined for clinical signs of infection with PRRSV. Rectal temperatures were taken daily from the day of mating until 20 d after experimental insemination with boar semen containing PRRSV. At the start of estrus synchronization only 21 gilts were used.

Estrus Synchronization and Artificial Insemination

Synchronizatons were carried out with 5 ml/d of Regumate[c] for 18 d, followed by 800 IU PMSG (Serigan®[d]) 24 h later and 400 IU of hCG (Coriogan®[d]) 72 h later. Gilts showing external signs of estrus 24 to 48 h after the last injection were artificially inseminated. Semen from an artificial insemination center with PRRSV seronegative status (Proinserga, Segovia, Spain) was diluted with MRA® extender[e] to a concentration of approximately 200 million sperm/ml. Each gilt was inseminated with 150 ml of this semen on the 2 consecutive days of estrus. The second day of estrus was designated Day 0.

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[a] INGENASA, Madrid, Spain.

[b] Cyanamid, Madrid, Spain.

[c] Hoechst-Roussel Veterinaria. Roussel Uclaf, France.

[d] Laboratorios Ovejero S.A., León, Spain.

[e] Kubus S.A., Madrid, Spain.

Virus and Cell Culture

Experiments were performed with the seventh passage in swine alveolar macrophages (SAM) of the Spanish PRRSV strain 5710 field isolated in the North of Spain in 1992 (22). This PRRSV strain was propagated on SAM cultures prepared as described by Wensvoort et al. (28). Growth was carried out in 75-cm^2 bottles containing 25 ml of Dulbecco's Minimal Eagle's Medium (DMEM[f]) supplemented with 10% foetal calf serum (FCS[f]). The initial cell concentration was 2.6 x 10^6 cell/ml. After 8 to 16 h of incubation at 37 °C, medium was discarded and 2 ml of a viral suspension with 10^5 $TCID_{50}$/ml were inoculated. After 2 h, 25 ml of fresh medium were added. The virus containing culture medium was harvested 48 to 72 h later, when most of the cells showed cytopathic effect (CPE). After 3 cycles of freezing and thawing, cell debris was removed by centrifugation at 600 x g at 4 °C for 15 min and the supernatant containing virus was frozen and stored at -80 °C. Virus titrations were calculated according to the method of the Reed and Muench (19) and were expressed as log $TCID_{50}$/ml. The PRRSV titre was adjusted to 10^5 $TCID_{50}$/ml with DMEM and constituted the stock virus used to inoculate the gilts.

Experimental Design

At the time of insemination, gilts were assigned to one of the treatment groups. Seven gilts were used as controls and 14 as principals. Of these, 7 were preimmunized to PRRSV and constituted Group B and 7 remained seronegative and constituted Group A. In both groups, 20 ml of PRRSV stock were added to 150 ml of diluted semen immediately prior to insemination. This procedure was repeated on the next day (Day 0), which meant that each gilt was given a total of 4 x 10^6 $TCID_{50}$ PRRSV by the vaginal route. The control gilts constituted the Group C and were inoculated in the same way with a non infected SAM culture.

Blood samples were collected into serum clot vacuum tubes and taken twice before inoculation with PRRSV, once early in the acclimatization period and again 3 d before administering the virus. Four further blood samples were taken on Days +3, +6, +15 and +20 after inoculation with boar semen containing PRRSV. All samples were tested for PRRSV antibodies with an ELISA test (INGEZIM.PRRS®[a]). Moreover, all blood samples obtained on the day of each blood collection were also stored at -80 °C for virus isolation.

All groups (A, B and C) of gilts were killed on Day 20± 1 of gestation.

Post-Mortem Examination

At the time of necropsy, lungs, tonsils, submandibular lymph nodes, iliac lymph nodes, uterine lymph nodes and ovaries were collected. Corpora lutea (CL) were counted, and the uteri were opened and examined. Embryos were counted, identified by their position in the uterus and examined for gross abnormalities. Samples of amniotic fluids and a small portion of the uteri were also obtained.

All tissue samples, amniotic fluids and all embryos were tested for PRRSV isolation.

Virus Isolation

For virus isolation in samples, SAM cultures were used. In all tissue samples and embryos, 1 g of each was homogenized in potter with 9 ml of DMEM and subsequently centrifuged at 3000 x g for 15 min at 4 °C. All supernatants were passed through a 0.45-μm filter. The aliquots of sera obtained from blood samples quoted above were thawed to 37 °C and passed through a 0.45-μm filter. Cotton swabs, used for swabbing nasal cavities of all the experimental gilts, were immediately

[f] Gibco/BRL, Uxbrige, UK.

immersed in 2 ml of DMEM and frozen at -80 °C. Each sample was prepared for virus isolation by thoroughly mixing swabs and media and then wringing all the liquid from the swab. Afterwards all samples were passed through a 0.45-μm filter.

All samples in quadruplicate were added (100 μl) to 96-well tissue culture plates (Nunc[g]) seeded with 2 x 10^6 cell/ml of SAM. After 1.5 h of adsorption at 37 °C in humidified 5% CO_2 and air atmosphere, the sample inocula were removed, and 200 μl of fresh DMEM with 10% FCS were added. The culture plates were then incubated at 37 °C in a humidified 5% CO_2 and air atmosphere. Monolayers were examined for CPE on Day 4 to 5 postinoculation. On the fifth day, the monolayers were frozen and thawed twice, the cell debris was removed by centrifugation at 600 x g at 4 °C for 20 min and the supernatants were passed onto fresh monolayers of SAM. These monolayers were observed for a further 5 d before they were discarded. When CPE was observed, the presence of PRRSV was confirmed by the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) following a method previously described in our laboratory (22).

As a positive control, PRRSV strain 5710 was added to DMEM, serum or tissue samples from 1 of the control gilts. Virus was added to give a final concentration of 10^4, 10^3 and 10^2 $TCID_{50}$/ml. Uninfected DMEM, FCS or supernatant from tissue samples of 1 control gilt were used as a negative control. These samples (100 μl) were taken twice through SAM cultures as described above.

Statistical Analysis

Chi-square tests were used to estimate statistical significance of differences between principal and control gilts. When the expected values (E_{ij}) were too small, and the number of individuals is fewer than 20, we used the Fisher's exact probability test (6).

RESULTS

Clinical Signs and Serology

The Group A gilts showed no signs of respiratory distress, and 5 of 7 were anorectic for 2 to 3 d after virus exposure. In Group B, the gilts showed normal health and condition throughout the experiment, with the exception of 1 gilt that was anorectic for 1 d after viral exposure. Rectal temperature increased to 39.5 - 40.5 °C postinoculation in both groups of gilts. The Group C gilts remained in normal health and conditon throughout the experiment and had normal daily rectal temperatures.

The first 4 serum samples from principal gilts were negative to the ELISA test with the exception of 1 Group B gilt that was seropositive (1:200) on Day -3, Day +3 and Day +6 and 2 other Group B gilts that were seropositive (1:200) on Day +6. All samples from Group A and B gilts had antibodies titres › 1:400 on Days +15 and +20. Group C gilts remained serologically negative.

Effects of Maternal Exposure to PRRSV

One gilt in group A and 2 gilts in group B were not pregnant but had old CL at slaughter. Total CL, total embryos and the ratio of embryos to CL were similar for Groups A and B gilts and control Group C gilts (Table 1). However, there were marked differences in that 1 or more embryos in 5 of the 6 Group A litters and only 1 embryo in 1 of 5 Group B litters were infected; none of the embryos of control Group C gilts were infected. The number of embryos infected in Group A gilts (7.6%) was statistically significant when compared with the number of embryos infected in control Group C ($P<0.01$). Moreover, approximately 2 and 4 times more embryos were dead in litters of

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g A/S Nunc Postbox 280-SK-4000 Roskilde, Denmark.

gilts from Groups A and B, respectively, than in gilts from control Group C (Table 1). The difference in the number of dead embryos in Group B gilts and in control Group C gilts was statistically significant ($P<0.001$).

Table 1. Effect of insemination of susceptible and preimmunized gilts with boar semen containing PRRSV on survival of embryos in the first 20 days of gestation.

	Group A gilts	Group B gilts	Group C gilts
No. pregnant gilts examined	6	5	7
No. gilts repeating oestrus	1	2	0
No. litters infected	5	1	0
Percentage of litters infected	83.3	20	0
Total corpora lutea (range)	120 (15-26)	93 (16-24)	144 (15-29)
Total embryos (range)	92 (7-22)	76 (10-20)	112 (6-24)
Total embryos/ Total corpora lutea	0.77	0.82	0.78
Total live embryos (%)	77 (83.7)	44 (57.9)	101 (90.2)
No. live embryos infected	4	1	0
Total dead embryos (%)	15 (16.3)	32 (42.1)	11 (9.8)
No. dead embryos infected	3	0	0
Percentage of embryos infected	7.6	1.3	0

Detection of PRRSV in Samples Collected from Gilts

All sera collected from gilts before they were exposed to PRRSV were free of virus by virus isolation. After exposure, PRRSV was isolated in Group A gilts in serum (7/7), lung (5/7), tonsil (7/7), submandibular lymph node (5/7), iliac lymph node (4/7), uterus lymph node (3/6), ovary (2/7), uterus (1/7) and amniotic fluid (1/54). In Group B, virus was isolated in serum (7/7), lung (5/7), tonsil (4/7), submandibular lymph node (6/7), iliac lymph node (5/7) and uterus lymph node (3/4). The PRRSV was not isolated from any of 7 uteri and 37 amniotic fluid samples collected from Group B gilts, nor from any samples collected from control Group C gilts.

DISCUSSION

The transmission of infection in gilts following intrauterine inoculation of PRRSV was demonstrated by the reisolation of virus from the serum and other tissue samples of gilts of both inoculated groups and was also demonstrated by seroconversion. The infection produced clinical signs of the disease in either infected groups and may have insignificant effect or no effect on the conception rates. This suggests either that PRRSV has a reduced effect on fertility or that the virus-associated infertility is dependent on strain pathogenicity, because other experimental and field reports have shown variable reductions in conception rates (9-11, 24, 25, 27). On the other hand, marked differences in pathogenicity of PRRSV isolates have also been reported (7).

Since fertilization rates were unaffected by insemination with semen containing virus, embryonic losses were apparently caused by factors common to all groups. Moreover, previous studies have demonstrated that the 4- to 16-cell stage embryos are not susceptible to productive infection with PRRSV (18).

However, the results do indicate that the exposure of seronegative and preimmunized gilts to PRRSV at the time of conception can result in the transplacental infection of the embryos. The extent of prenatal infection of embryos is very similar to that previously found when the gilts were infected intranasally in early and mid-gestation (2, 14, 16), and is markedly lower than that found when the

gilts were infected in later gestation (14). Initially, the authors believed that the difference might be due, as suggested by Christianson et al. (2), to the fact that as gestation advances the vascular beds of the placenta are brought closer together by assuming subepithelial positions and by the invasion of the trophoblast by embryonic capillaries. As a consequence, the materno-fetal capillary interrelationship of the placenta becomes a more efficient organ for exchange as gestation progresses, making greater the likelihood of transplacental infection with PRRSV. These findings may help to explain why many natural epizootics of PRRS-induced reproductive failure are manifested principally as late-term abortions, early farrowing, still-births and the like, although early embryonic deaths and possibly even early losses of whole-litters may be seen occassionaly.

Despite preimmunization in Group B gilts, similar results in both experimental groups could be due to the absence of specific antibodies in most of preimmunized gilts after vaccination. However, the only gilt that developed antibodies before inoculation with the PRRSV had a response similar to that of Group A gilts and additionally, virus was successfully isolated in some of the tissues samples and in 1 of the embryos. An adequate efficacy of the vaccination is also not evident from the observed rate of dead embryos. Although it was never possible to isolate the virus in Group B dead embryos, the difference between Group A and B gilts is that in the latter, most of the dead embryos were in an advanced stage of reabsorption, which permits inactivation of the virus and makes it impossible to isolate it in SAM cultures.

On the other hand, the PRRSV has been observed in ovarian tissues, a finding which has been previously described in other studies (16, 25). The effect of the virus on the ovary and the estrus cycle is not kown, especially taking into account the fact that it has been isolated in the ovaries of non-pregnant gilts as well as in the ovaries of pregnant gilts.

Finally, the results of this study show that insemination of either seronegative or preimmunized gilts with boar semen containing PRRSV may have little or no effect on conception and fertilization rates, although it can result in transmission of the virus and in embryonic infection and death.

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