Detection of porcine parvovirus in the follicular fluid of abattoir pigs

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Summary

Follicular fluid samples from abattoir-obtained pig ovaries were monitored by polymerase chain reaction for viral pathogens known to cause reproductive failure, including porcine parvovirus, porcine circovirus, porcine reproductive and respiratory syndrome virus, bovine viral diarrhea virus, and border disease virus. Six of 49 animals tested were positive for porcine parvovirus.

Keywords: swine, porcine parvovirus, follicular fluid, polymerase chain reaction

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Resumen – Detección de parvovirus porcino en el fluido folicular de cerdos de matadero

Se monitorearon muestras de fluido folicular de ovarios de cerdas obtenidas de matadero mediante la reacción en cadena de la polimerasa en busca de patógenos virales conocidos como causantes de falla reproductiva, estos incluyeron al parvovirus porcino, circovirus porcino, virus del síndrome reproductivo y respiratorio porcino, virus de la diarrea viral bovina, y el virus de la enfermedad de la frontera. Seis de los 49 animales examinados resultaron positivos a parvovirus porcino.

Résumé – Détection du parvovirus porcin dans le liquide folliculaire chez des porcs à l'abattoir

Des échantillons de liquide folliculaire prélevés à partir d'ovaires chez des porcs à l'abattoir ont été vérifiés par réaction d'amplification en chaîne par la polymérase pour la présence de virus reconnus comme responsable de problèmes reproducteurs, et qui incluaient le parvovirus porcin, le circovirus porcin, le virus du syndrome reproducteur et respiratoire porcin, le virus de la diarrhée virale porcine, et le virus de la maladie de la frontière. Six des 49 animaux testés se sont avéréé positifs pour le parvovirus porcin.

Although it is generally accepted that the use of embryo transfer is safer for the distribution of germ plasm than the movement of live animals, the relative risk of transmitting infectious agents via embryo transfer is not negligible.[1] The epidemiological hazard related to the technology depends on the donor species, the method of embryo production applied, and the specific pathogens involved. In cattle, washing the embryo with or without trypsin after collection is a simple procedure to generate specific-pathogen-free embryos.[2] However, in pigs and small ruminants, the association between embryos and pathogens tends to be stronger.[3] When pig embryos were artificially exposed to seven different pathogens, some embryos were always positive for each pathogen even after extended washing.[4] Although this does not necessarily cause infection in the recipient animal or the offspring,[5,6] the tight embryo-pathogen association in the pig remains a concern.[7,8]

Porcine parvovirus (PPV), a ubiquitous virus that affects swine reproductive performance all over the world, has been identified as a major cause of embryonic and fetal death in pigs.[9-11] Porcine parvovirus infections are generally considered harmless to adult animals because the antibodies produced against the pathogen efficiently neutralize the virus. However, when embryos are infected before their immunological competency is activated (ie, prior to 70 days of gestation), embryonic and fetal death may occur.[12]

According to a recent report, approximately 30,000 swine embryos were transferred in 2005 worldwide.[13] This annual number is likely to increase, and the commercial application of the technology clearly carries a level of biohazard that must be diminished or eliminated with implementation of appropriate biosecurity measures. Material obtained from commercial slaughterhouses is often used to produce embryos in vitro. Since many reproductive problems caused by viral infection exist in livestock species in the field, the epidemiologic potential of current and emerging technologies must be monitored continuously.[1] The objective of the present study was to determine the presence of viral pathogens in follicular fluid samples collected from abattoir-obtained porcine ovaries. We report the presence of PPV in a number of ovarian samples, which identifies a potential hazard affecting the safety of embryo transfer technologies in the pig.

Materials and methods

Ovaries from 49 prepubertal gilts were collected at a slaughterhouse and transported to the laboratory in warm (35°C) physiological saline. The animals were selected from different farms prior to slaughter, over a period of 4 weeks. Chemicals were purchased from Sigma-Aldrich Chemical Company (St Louis, Missouri), unless otherwise indicated. The follicular fluid and oocyte-cumulus complexes were removed

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from the individual ovaries using a disposable syringe and a 20-gauge hypodermic needle, transferred to separate microcentrifuge tubes, and frozen immediately at -80°C. They were subsequently thawed and tested in duplicate by polymerase chain reaction (PCR) for porcine parvovirus (PPV), porcine circovirus types 1 and 2 (PCV1 and PCV2, respectively), porcine reproductive and respiratory syndrome virus (PRRSV), and pestiviruses, namely, bovine viral diarrhea virus (BVDV) and border disease virus (BDV). Viral DNA or RNA was extracted from 0.15 to 0.2 mL of each sample using a commercial kit (Qiagen Inc, Valencia, California) according to the instructions of the manufacturer. Two µL of each nucleic acid extract was used for amplification under previously described PCR conditions.[14-18] The target sequence for each virus was visualized after agarose gel electrophoresis of the PCR products, using 100-bp DNA ladders (Roche; Indianapolis, Indiana) and molecular size determination.

Results

A total of 49 samples was tested, with similar PCR results for each pair of duplicate samples. Six samples (12.2%) tested positive for PPV, with the amplified DNA fragment of the expected size clearly detectable after gel electrophoresis in each case. Nucleic acids of PRRSV, PCV1, PCV2, BVDV, and BDV were not identified in the follicular fluid of the slaughtered animals.

Discussion

In this study, porcine follicular fluid from pig ovaries collected at a slaughterhouse was monitored for a number of viral pathogens, including PPV,[9] that can cause reproductive failure in sows and gilts. The syndrome of stillbirth, mummification, embryonic death, and infertility (SMEDI) was described several decades ago,[19] and subsequent research identified the pathogen associated with the syndrome as a DNA virus belonging to the Parvoviridae family.[20] Later, the virus was isolated from pigs with various manifestations of reproductive failure,[21,22] and results of experiments infecting pregnant animals with the virus established that isolated PPV can infect not only the dam but also the developing fetus.[10,23] Penetration of the placental barrier is believed to occur via maternal macrophages as vehicles.[24] The pathological outcome depends on the time of exposure during gestation. Infection of the embryo before 35 days of gestation causes death and resorption, while fetuses infected in late gestation survive infection due to the immunocompetency acquired approximately day 70 post conception.[9,25] The virus replicates in the proliferating cells of the fetus, and reproductive failure is caused by the direct effect of PPV on a variety of vital tissues and organs, including the placenta.[26,27]

An earlier study reported PPV antibody in the follicular fluid of 94.3% of sows and 78.1% of gilts examined at an abattoir.[28] In the present study, PPV was detected in the antral follicles of 12.2% of animals. The presence of the virus in the oocyte cytoplasm was not monitored. However, even without viral contamination of the oocyte cytoplasm, the presence of the virus on the surface of the zona pellucida poses a risk to the embryo after hatching. Several pathogens have been shown to adhere strongly to the zona of embryos produced in vitro, and mechanical washing or trypsin treatment of in vitro-derived embryos do not provide the same benefit that results from cleansing in vivo embryos.[4] Culture conditions of in vitro embryo production provide an ideal environment for replication of viruses through adequate time, substrate, and permissive cells. This can effectively amplify the number of viruses to high levels by the end of the culture period.[29] Failure to cleanse viruses from in vitro embryos and amplification of viral infections during culturing may be associated with higher embryonic death rates and lower pregnancy rates after embryo transfer. Therefore, testing all abattoir material for contaminating agents is a critical control for production and transfer of in vitro embryos.[1] It remains to be determined whether the quantity of PPV that is associated with the embryos is sufficient to constitute an infectious dose for the recipients. According to one report,[30] PPV was able to cross the intact zona pellucida and infect embryonic cells. If this is verified, it would further emphasize the potential biohazard PPV poses during the application of embryo-associated technologies.

On the basis of our data, we conclude that PPV is a clinically significant pathogen that is present in the reproductive organs of females from a representative swine population. Other viruses known to cause abortion and embryonic losses in pigs (PCV1, PCV2, PRRSV, BVDV, and BDV) were not identified in the follicular fluid analyzed in this study. The results emphasize the importance of using pathogen-free biomaterial for embryo production, especially with the development of new and innovative reproductive technologies that present a level of risk to biosecurity.

Implications

- Porcine parvovirus is present in the follicular fluid of some gilts and sows sent to slaughter.
- When embryos produced by in vitro fertilization of abattoir-obtained oocytes are used for embryo transfer, it is advisable to perform an embryonic wash in an appropriate solution before transfer to eliminate or diminish the transmission of viral pathogens.
- Ideally, oocytes used for in vitro fertilization and subsequent embryo transfer should be collected from females free of known reproductive pathogens.

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