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Review article

Susceptibility of porcine preimplantation embryos to viruses associated with reproductive failure

Haijing Zhao, Guangyuan Zhao, Wenjun Wang*

Center for Reproductive Medicine, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou, P. R. China

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ABSTRACT

In the modern biological area, the applications of pig as a laboratory model have extensive prospects, such as gene transfer, IVF, SCNT, and xenotransplantation. However, the risk of pathogen transmission by porcine embryos is always a topic to be investigated, especially the viruses related to reproductive failure, for instance, pseudorabies virus, porcine reproductive and respiratory syndrome virus, porcine parvovirus, and porcine circovirus type 2. It should be mentioned that the zona pellucida (ZP) of porcine embryos can be a barrier against the viruses, but certain pathogens may stick to or even pass through the ZP. With intact, free, and damaged ZP, porcine preimplantation embryos are susceptible to these viruses in varying degrees, which may be associated with the virus-specific receptor on embryonic cell membrane. These topics are discussed in the present review.

1. Introduction

Along with the rapid development of embryo transfer (ET) technology in pigs, there are still some sanitary risks inherent to ETs that warrant more research on embryo–pathogen interaction [1], especially the *in vitro* interaction of porcine preimplantation embryos with viruses, such as pseudorabies virus (PRV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), and porcine circovirus type 2 (PCV2), which are considered to be the main pathogens to affect the reproductive system of swine [2].

Taking sanitary risks into consideration (i.e., to prevent disease transmission), the transfer of *in vivo*–derived porcine embryos is relatively safe when the operational guidelines recommended by the International Embryo Transfer Society are strictly followed [3,4]. However, it should be mentioned that since the *in vitro* embryo production technology (i.e., parthenogenetic activation and somatic cell nuclear transfer) began to develop, the accompanying biohazards have attracted much attention because *in vitro*–produced porcine embryos are vulnerable to be contaminated by numerous sources under the external environment [5]. Most porcine viruses have specific attachment to or can penetrate through the zona pellucida (ZP) of the embryos leading to their contamination.

In the present review, we summarize some researches on the susceptibility of porcine preimplantation embryos to the viruses, namely PRV, PRRSV, PPV, and PCV2, which have detrimental effect on reproductive tract and even result in abortion. The report mainly states from the following aspects: first, how do these viruses contact with porcine embryos; second, the role of ZP on the embryo–pathogen interaction; third, the susceptibility of porcine preimplantation embryos to these viruses *in vitro*; finally, the specific receptors of the viruses on embryonic cell membrane.

2. Some basic factors for the embryo–virus interaction

Porcine viruses, such as PRV, PRRSV, PPV, and PCV2, may spread from the primary site of infection or replication to swine genital tract where the embryos float freely. First, if the enough high dose of the viruses to be present in the reproductive tract, an embryo has the first chance to be infected, and then the viruses could reach the cellular

* Corresponding author. Tel.: 020-81332333; fax: 020-81332011.
E-mail address: zhaohj0502@163.com (W. Wang).

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membrane in their unique ways. Next, the embryonic cell surface must have specific receptors for the viruses [6]; the absence of specific receptors on cell surface is one of the most important resistance factors to prevent the entry of viruses into the cell. Finally, an active intracellular mechanism is needed for the viruses to replicate their genomes and produce viral proteins [3].

According to the foregoing conditions, it is obvious that the embryonic cell surface receptor is the crucial factor to determine whether porcine preimplantation embryos are susceptible to the virus. However, it is notable that embryos are surrounded by the ZP, which is considered to be not only a barrier against viral infection [7] but also a carrier of certain pathogens [3].

3. Zona pellucida plays a dual role on the embryo–virus interaction

In pigs, the ZP, with a thickness of approximately 16 μm, is a kind of glycoprotein secreted by oocyte and cumulus cells. It is necessary to know something about the structure and functional properties of ZP considering its dual role. Studies in the late 1970s have showed that the ZP presents a complex and fenestrated appearance with many pores [8]. According to several researches, we can conclude that the ZP is dynamically changed based on different embryonic stages [9,10]. Moreover, the ZP of *in vitro*–developed embryos was thinner than that of *in vivo*–developed embryos in pigs [11].

We can liken the ZP to a coat that surrounds porcine oocytes and embryos [12]. The main functions of the ZP are to avoid polyspermy during fertilization; to maintain the integrity of porcine embryos and a relatively stable microenvironment; and to protect porcine embryos against bacteria, fungi, virus, and mechanical injury as the embryos travel through the oviduct [13,14].

However, in terms of functional properties, certain glycosylation site in porcine ZP may enable corresponding virus to bind firmly onto its surface [3]. Moreover, on the basis of the sizes of the pores in ZP, it cannot be excluded that some small pathogens may pass through the ZP to reach the embryonic cells [15].

The ZP is considered to be a firm physical and chemical barrier against viruses [7]. Whether its barrier function remains guaranteed under all circumstances is not clear. Embryo handling itself, or routinely used techniques, such as intracytoplasmic sperm injection, blastomere biopsy, and embryo cryopreservation, may induce ZP damage [16] and form ports for viral entry. Thus, it is necessary to have better insights into the suscepitibility of porcine preimplantation embryos with intact, free, or damaged ZP to viruses with a tropism for the reproductive tract.

4. The *in vitro* interaction of porcine preimplantation embryos with some viruses

4.1. Pseudorabies virus

Pseudorabies virus is a member of the alphaherpesviruses family. It can cause return to estrus, abortion, and birth of weak or dead piglets in sows.

When ZP-intact porcine embryos were inoculated with PRV *in vitro*, Bolin et al. [17] observed that the virus could adsorb to the ZP and remain on the outer surface of the ZP under electron microscopy. After *in vitro* inoculation of ZP-free 2- to 16-cell stage embryos with PRV, the researchers did not detect antigen-positive cells at 48 hours after incubation, so they concluded that 2- to 16-cell stage porcine embryos were resistant to PRV infection [18]. From here, we see that intact ZP plays a major role in protecting the preimplantation embryos against PRV infection. However, why ZP-free 2- to 16-cell stage embryos are still refractory to PRV?

A more detailed research on the interaction of porcine preimplantation embryos with PRV was carried out [1]. Zona pellucida–intact embryos were incubated with the virus; no antigen-positive embryonic cells were detected after a 48 hours culture period. After incubation of ZP-free embryos with PRV, embryonic cells were still negative for viral antigens. However, PRV-positive cells were present after the incubation of hatched blastocysts with the virus and affected the further embryonic development. In addition, they found that antigen-positive blastomeres were detected in ZP-intact 5- to 8-cell stage embryos, morulae, and blastocysts at 48 hours after subzonal microinjection with PRV; the developmental potential of the embryos was significantly lower than that of control embryos.

It is worth noticing the differences in the results of the microinjection experiments compared with the results obtained by incubation of ZP-free embryos with PRV after protease treatment. Krummenacher et al. [19] demonstrated that protease treatment caused a disruption of the dimensional structure of PRV receptors. This likely explains why Bolin et al., who also used protease to remove the ZP from pig embryos, found that porcine embryonic cells up to the 16-cell stage were resistant to PRV infection. In addition, hatched blastocysts were susceptible to PRV because of the lack of ZP protection and the presence of the specific receptors on the embryonic cell membrane without being disrupted. Mateusen et al. [1] concluded that the appearance of PRV receptor expression coincided with the development of precompact 5- to 8-cell stage embryos; this is why the virus can be detected in that time.

4.2. Porcine reproductive and respiratory syndrome virus

As a member of the *Arteriviridae* family, PRRSV results in late-term abortions, reduced conception rates, and an increase in dead or mummified fetuses.

The incubation or microinjection of ZP-intact 4- to 16-cell stage porcine embryos with PRRSV did not lead to virus-positive blastomeres by reverse transcription polymerase chain reaction and indirect immunofluorescence, so the researchers made a conclusion that 4- to 16-cell stage embryos were not susceptible to PRRSV infection [20].

For a better insight into the resistance of porcine preimplantation embryos to PRRSV, ZP-intact and ZP-free 2- to 4-cell stage, 5- to 8-cell stage, morulae, blastocysts, even hatched blastocysts were incubated or microinjected with the virus; no viral antigen-positive cells were detected at 48 hours after PRRSV exposure [1].