Importance of the artificial insemination centers. The role of semen

Enric Mateu
Senior lecturer at the Department of Animal Health and Anatomy at the Faculty of Veterinary Medicine/Science (Autonomous University of Barcelona) Researcher at CReSA-IRTA

Cinta Prieto
Senior lecturer at the Department of Animal Health at the Faculty of Veterinay Medicines/ Science (Complutense University of Madrid)

Highlights

The infection with the virus can lead to the shedding of the virus in the ejaculate. This virus is transmittable and it can cause the infection of the inseminated sows with the semen doses prepared with the ejaculate.

It is difficult to establish the clinical suspicion of the contamination of the semen since the infection goes clinically unnoticed in most of the animals, and also, the excretion in the semen can go on for various periods of time fluctuating between a couple of days and up to three months after the infection.

It is currently accepted that the AICs must have a negative status against the virus and that they have to implement a monitoring system that allows detecting quickly a possible infection.

A way of detecting the infection of the boars is establishing the presence of the virus in semen samples. Since the shedding in the ejaculate is normally intermittent, it makes it harder to find a positive sample in routine tests than with other kinds of samples. Nevertheless, it can be used to guarantee that some of the ejaculate is virus-free, whether it comes from a positive animal or as a way of confirming the negative status of the boar.

The most efficient method for monitoring an AIC is the systematic determination of the existence of viraemia in the boars. This is because all the animals will be viraemic in the acute stage of the infection, and the amount of viruses present in the serum is relatively high.

The study of the seroconversion can provide information on the status of an AIC, but we have to bear in mind that the amount of time required for the seroconversion to appear is higher than the time needed for the establishment of the viraemia or the beginning of t he virus excretion in the semen; resulting on a higher amount of time needed for diagnosing an infection.

The establishment of the presence of the virus and of its antibodies can be carried out using oral fluids samples, although its use has not become generalised due to the difficulties that sometimes are implied in the taking of the samples. It is more common to take blood samples during the semen extraction.

The time needed to detect an infection in an AIC will depend not only on the kind of sample chosen, but on the design of the sampling, including the number of samples tested and the frequency in which the tests are performed.

Keeping the AICs infectiously negative is very important because it produces a maximization of the biosecurity procedures, including a correct use of the quarantines, a total and absolute restriction on visitation and, sometimes, the use of air filters.

After the boars' infection, the viraemia allows the arrival of the virus to their reproductive system and its shedding in their ejaculate. This virus is transmittable and can cause the infection through the venereal route in the inseminated sows with the semen doses. Although the amount of viruses present in the semen is low, especially as of the second or the third week after the infection, and since the amount of virus needed to cause the infection via the venereal route is relatively high, the fact that the infection can be transmitted through the venereal route is very relevant; epidemiologically speaking. Also, the characteristics of the infection with PRRSV in the boar help to increase its relevance.

In this sense, we must underline that the infection in these animals frequently goes clinically unnoticed, and this complicates a quick diagnosis and the prompt removal of the contaminated doses from the market. In the second place, the excretion of the virus in the ejaculate has a highly variable duration, being frequently longer than the viraemia. The virus can be detected in the semen, although generally for relatively long periods of time in an intermittent way, and naturally in the absence of symptoms. The longest excretion in the semen described lasted 3 months, although in most of the studies it has been shorter and does not exceed six weeks after the infection. The time during which the virus can be detected in the semen of a boar has a very important individual component, although a breed effect on the duration of the excretion has also been described. Anyhow, regardless of the individual effect, the duration, and especially the intermittence of the excretion complicates considerably the prediction of which ejaculates from infected boars will be infected and which will not. Some years ago, this fact was not given too much importance. It was believed that the presence of virus in the semen doses did not entail a problem when those doses were allocated to positive farms, which were the majority of the farms. Nevertheless, currently, this perception has changed, because the use of contaminated doses may lead to the infection of some sows and to the appearance of an outbreak on the destination farm, whether previously positive or negative.

All this has currently generated the essential thought that the artificial insemination centres (AICs) are negative to the virus and that they have developed monitoring systems to detect an infection as soon as possible. There are several ways to detect the infection with PRRSV in an AIC, among which we highlight the following:

1. Detection of the presence of the virus in the boars' ejaculate. This system was the first one developed for the control of the AICs, because years ago many of them were positive and the only way to exert an effective control on the semen doses was to check that the boars' ejaculates were virus-free. It has the problem that the amount of viruses in the semen is low in most of the cases. This, together with the existence of toxic factors and PCR inhibitors in this kind of sample complicate the determination of the presence of the virus. Nevertheless, with time, ARN extraction techniques have been optimised to improve the sensitivity and, therefore, the reliability of the results, so nowadays, the results obtained with a RT-PCR carried out in semen samples must be considered reliable. Nevertheless, we must bear in mind that the intermittent virus excretion only allows to confirm that a certain ejaculate is negative to the virus, and it does not give any guarantee about samples obtained before or later. These limitations (i.e. the low amount of viruses in the semen and the presence of PCR inhibitors in these

samples), together with the development of other control systems easier to implement have made the testing of the semen samples to monitor the AICs increasingly disused. Nevertheless, we must underline that the testing of semen samples is still justified when we wish to use the semen of positive boars for their genetic value; for example, or when a high health farm wants to be completely sure that the introduction of semen doses entails no risks and wants to make additional verifications in doses that come from AICs classified as negative to the virus.

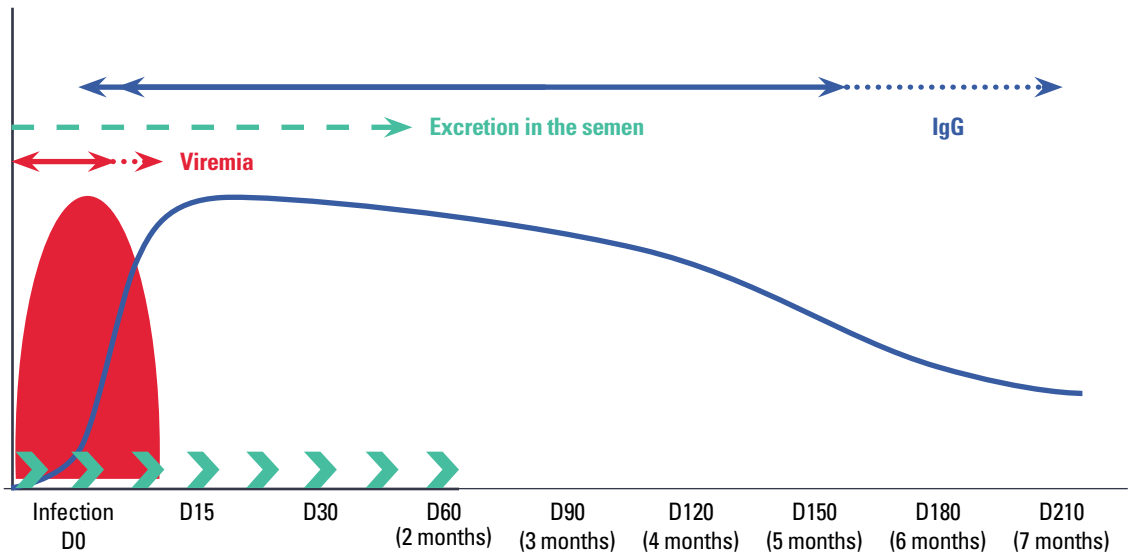
2. Detection of the viraemia. The detection of the viraemia has proven to be the quickest and most effective way of detecting the infection in AICs. This is so because all the infected boars establish a viraemia period in a very short time (typically in the first 24 hours) after the infection, before the shedding in the semen starts. Also, the amount of viruses present in the serum is higher than in the ejaculate, and the serum samples do not exhibit the inhibition problems of the RT-PCR techniques so, in practice, the sensitivity is higher. The main problem is that the viraemia ends before the period of excretion of the virus in the semen and therefore, if the sampling periodicity is low, the animals may be excreting viruses in the semen after a viraemia period that has gone unnoticed. Nevertheless, this problem is solved with a good sampling design in the AICs (i.e. sampling frequency and number of samples taken according to the size of the population and the expected prevalence).

3. Determination of the seroconversion. Another way of establishing if an AIC has become infected is the detection of specific antibodies against the PRRSV. As we have mentioned, the AICs are currently mainly negative, so the boars are seronegative. The finding of specific antibodies against the virus confirms the infection in the AIC. This system has the disadvantage that more time is needed to confirm the infection than with the direct determination of the virus, although it has the advantage that the antibodies persist for longer periods and once the seroconversion has happened, the animals will be positive for months. We can also combine the detection of the virus with the detection of antibodies in the same sample.

Currently, both, the establishment of the presence of the virus and the presence of antibodies can be carried out using oral fluids samples. Theoretically, this system facilitates the taking of samples, which is especially difficult in the case of the boars, mostly if we bear in mind that the monitoring of the AICs forces the taking of samples systematically and repeatedly. The main disadvantage is that some animals do not show interest in the ropes used for collecting the samples, or they lose interest over time, so although the existing studies show that there is a good correlation between the results obtained with serum samples and with oral fluids samples, the use of this latter kind of sample has not become widespread, and the taking of blood samples during the semen extraction is preferred, whereas it is by means of a conventional tube system or using FTA cards.

A critical aspect for a quick confirmation of the infection in an AIC is the frequency of the sampling and the number of samples tested, as well as the detection system chosen. Studies performed in the US in AICs with a high number or boars show that the quickest way to detect the infection is by taking serum samples from up to 60 boars 3 times per week. This system allows detecting, with RT-PCR, 95% of the infections in a period of 13 days. These figures give an idea of the sampling intensity needed to detect the infection in an AIC quite fast. Nevertheless, the quick detection of the infection is essential to

Figure 1. Dynamics of the infection in boars



Enric Mateu and Cinta Prieto (2018)

stop as soon as possible the distribution of infected semen doses and to avoid the infection of an important number of farms, as has happened in several cases of infection in AICs in the past.

Finally, we must underline that, due to the importance of keeping the AICs negative to the PRRSV, the implementation of strict biosecurity guidelines is compulsory. Specifically, the new AICs are built in isolated places, without other farms near them. Also, visits are strictly forbidden, and the premises are only visited by those people whose entrance is strictly necessary. Together with the measures previously mentioned, quarantines are designed and generally used and managed more effectively than those on the sow farms, since they normally involve a real all-in-all-out organization and the systematic monitoring of animals as well as the handling by independent staff and the use of their own equipment. It is also increasingly easy to find AICs with air filtering systems to minimise the possibilities of an airborne transmission.

References

- Benfield *et al.*, 2000. Proc. Am. Assoc Swine Vet.
- Christopher-Hennings *et al.*, 1995. J Vet Diagn Invest, 7: 456-464
- Christopher-Hennings *et al.*, 2001. J Virol, 71: 9170-9179.
- Han *et al.*, 2011. Clin Vaccine Immunol, 18: 1600-1607
- Kittawornrat *et al.*, 2010. Virus Res, 154: 170–176
- Prieto and Castro, 2005, Theriogenology, 63: 1-16.
- Rovira *et al.*, 2007. J Vet Diagn Invest, 19: 502-509
- Wasilk *et al.*, 2004. J Clin Microbiol, 42:4453-4461