Reproductive features of PRRS-convalescent large white pigs after porcine reproductive and respiratory syndrome

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Abstract. Most sows convalescent from porcine reproductive and respiratory syndrome (PRRS) are immune to the re-infection. The PRRS virus's antibodies detected by enzyme-linked immunosorbent assay (ELISA) can persist for a year. The PRRS virus (PRRSV) causes damage to the pigs' reproductive system, manifested by abortions, delivery of non-viable piglets and sows' infertility. We carried out morphometric studies of recovered pregnant pigs' reproductive organs to determine changes in the fetus and placenta system in pregnant PRRS-convalescent sows. Morphological studies of the fetal portion of the placenta indicate that the weight of the placenta in PRRS-convalescent sows was significantly lower as compared to the weight of the placenta in clinically healthy pregnant sows, 0.71 ± 0.05 kg versus 0.92 ± 0.09 kg at the end of the second trimester, and 1.61 ± 0.42 versus 1.75 ± 0.16 kg on day 105-110 of gestation. By day 70-75 of gestation, we observed a significant 31.8% decrease in the fetus's body weight in seropositive pigs compared to the clinically healthy ones and 19.2% decrease on days 105-110. Piglets born from the experimental sows demonstrated physiological abnormalities that allowed for hypotrophy diagnosis. ELISA-tests of sera for anti-Müllerian hormone (AMH) proved the possibility of early assessment of gilts' fertility. This method will enable the premature culling of low-yielding replacement gilts from the breeding stock. Further development of the methods for gilts' fertility determination and ELISA-testing for AMH during the mandatory gynecological screening of the gilts at the pig breeding establishments are economically practical and effective. Key words: porcine reproductive and respiratory syndrome (PRRS), morphometric studies, placenta, umbilical cord, newborn piglets, replacement gilts, anti-Müllerian hormone, fertility.

1 Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first diagnosed in the Kursk region of the Russian Federation in 1993. In later years, there was a rise and fall in PRRSV infection on pig farms. The peak was observed in 1997 when PRRS was widely spread in...
over 50 constituent entities of Russia. Then a tendency to decrease the number of PRRS-infected farms was reported, which could be explained by several reasons. First, the coverage of pig farms by PRRS vaccination increased along with the introduction a set of disease control measures in the infected areas. Second, up-to-date pig breeding holdings were put into operation, which was stocked with imported quarantined pigs [2].

Currently, inactivated vaccines and two MLV vaccines against PRRS are marketed in Russia: AMERVAC-PRRS (Amervac® PRRS) – Laboratorios Hipra, Spain (strain VP046-BIS, Hipra, Spain) and Porcilis PRRS® (strain DV, Intervet International, Netherlands). However, the procedure for MLV vaccine administration in the Russian Federation is limited only by the manufacturer’s instructions, which are inconsistent with the OIE recommendations for MLV vaccines [3].

The high efficacy of MLV vaccines is associated with higher requirements for monitoring their administration, design of a reliable vaccination schedule for a specific production unit and the need for PRRS monitoring in different age and gender groups of animals [4].

The majority of convalescent sows appear to be immune to re-infection. ELISA-detected PRRSV antibodies can persist for a year [5,8].

The porcine reproductive and respiratory syndrome virus causes damage to the pigs’ reproductive system, which can be manifested by abortions, delivery of non-viable piglets, sows’ infertility, etc. [6,7].

In addition, there are difficulties in the determination of the fertility of the replacement gilts obtained from sows demonstrating PRRS clinical signs. Anti-Müllerian hormone (AMH) is recommended to be determined for fertility diagnosis. AMH is one of the main indicators of the normal functioning of the reproductive glands [9].

In females, AMH is produced by granulosa cells of growing follicles starting from the primary stage, reaching its maximum in the small antral follicle and practically disappearing in follicles close to the Graafian vesicle [11, 12].

AMH is a marker of ovarian reserve. AMH biological effects are realized through the effect on two types of serine/threonine receptors: AMGR-1 and AMGR-P. Interaction with the receptors results in the formation of a complicated receptor complex, which takes its effect as soon as it attaches to the cell nucleus [13].

According to some reports [14], anti-Müllerian hormone or Müllerian-inhibiting hormone is able to reduce the sensitivity of ovarian granulosa cells to follicle-stimulating hormone, thus delaying the follicles at the stage of small antral ones.

2 Materials and Methods

Enzyme-linked immunosorbent assay (ELISA) was used as the basic tool for the detection of PRRSV antibodies in porcine serum and plasma samples. IDEXX PRRS X3 test kit was selected for this purpose. We used recombinant antigen-coated microplates. During incubation of the samples, the PRRSV-specific antibodies bound to the antigen and formed an antigen-antibody complex on the surface of the microplate wells. After washing and unbound material removal, the anti-species HRPO-conjugate was added to the wells, and it bound to the antibodies being in the complex with the PRRSV antigens. The unbound conjugate was removed by washing, and TMB substrate was added to the wells. The subsequent staining was directly proportional to the amount of PRRSV-specific antibodies present in the sample.

The presence or absence of PRRSV antibodies was determined by calculating the S/P value for each sample: samples with an S/P value below 0.40 were considered as having no PRRSV antibodies, and samples with an S/P value over or equal to 0.40 indicated the presence of antibodies.
The diagnosis was confirmed in a real-time reverse transcription-polymerase chain reaction (RT-RT-PCR) at the FGBI “Federal Centre for Animal Health”.

Morphometric studies were carried out in 40 pregnant sows according to generally accepted methods. The experimental pigs received a standard diet and were kept in typical livestock facilities.

The research objects also included clinically healthy and replacement gilts obtained from sows demonstrating PRRS clinical signs. Sera collected from the gilts was used for the determination of AMH concentration. The blood samples were collected from the jugular vein using BD Vacutainer systems. The whole blood was collected in the holdings. The blood samples were centrifuged at 2500 rpm for 10 minutes, and then the resulted sera were aliquoted and frozen for the subsequent delivery to the testing department. Further studies were carried out using ELISA, which was performed using the “Ansh Labs porcine AMH” test kit (USA). 50 μl of the test serum, 50 μl of A-G calibrators and 50 μl of buffer for the AMH test were added to each plate well.

The plate was shaken at 600-800 rpm for 120 minutes at room temperature. Then it was washed five times with solution A, and 100 μl of AMH biotin conjugate (RTU) was added to each well.

The plate was shaken at 600-800 rpm for 60 minutes at room temperature. Hereafter it was washed, and 100 μl AMH streptavidin-enzyme conjugate (RTU) was added. The plate was vigorously shaken (600-800 rpm) for 30 minutes and washed. 100 μl of TMB chromogenic solution were added to the experimental wells avoiding direct sunlight, and the plate was shaken at 600-800 rpm on the orbital microplate shaker for 10-12 minutes at room temperature.

100 μl of stopping solution were added to each well using a repeater pipette. The plate was hereafter transferred into the ELISA reader set to 450 nm wavelength. The calibration curve was used to determine AMH concentration in the gilts’ sera.

Sex hormones in the blood sera of experimental gilts were determined by ELISA using domestically produced test kits.

3 Results

The experimental work was carried out in accordance with the Veterinary rules for the implementation of preventive, diagnostic, therapeutic, restrictive and other measures, establishment and lifting of quarantine and other restrictions aimed at prevention and containment of the spread of porcine reproductive and respiratory syndrome (PRRS) outbreaks [1].

Since the PRRSV causes damage to the reproductive system of pigs, we carried out morphometric studies of the reproductive organs of PRRS-convalescent pregnant sows.

The outer layer of the maternal placenta in the experimental PRRS-convalescent sows was of a lobular cellular structure with the inclusions of the microcrypts, which was physiologically normal. At the same time, larger and thicker microcrypts were located in the center, and the lobules were flattened towards the periphery. The total number of microcrypt nodules ranged from 16 to 22.

Histological and histochemical studies of the maternal and fetal portions of the placentas of control and experimental pigs demonstrated a significant difference (p <0.05).

Analysis of the area of the maternal portion of the placenta demonstrated significant variability in this parameter. Thus, by day 110 of gestation, the average uterine area in clinically healthy gilts was 813.11 ± 36.42 cm², while in PRRS-convalescent sows, this parameter amounted to 732.12 ± 37.23 cm²; the difference is statistically significant (Table 1).
These values are supported by the fetal/placental weight ratio, which fluctuated in PRRS-convalescent pigs from 3.89 ± 0.11 to 6.33 ± 0.19; and in clinically healthy sows from 4.24 ± 0.19 to 7.44 ± 0.41, depending on the gestational age.

Morphological study of the fetal portion of the placenta indicates that the weight of the placenta in PRRS-convalescent sows was significantly lower as compared to the weight of the placenta in clinically healthy pregnant sows; 0.71 ± 0.05 kg versus 0.92 ± 0.09 kg at the end of the second trimester, and 1.61 ± 0.42 versus 1.75 ± 0.16 kg on day 105-110 of gestation.

A similar situation was reported when determining the volume of the fetal portion of the placenta indicates that the weight of the placenta in seronegative pigs increased in comparison with the convalescent ones and by 10.0% at the end of the third trimester.

By the end of the second trimester of gestation, the length of the umbilical cord in PRRS-convalescent pigs was within 17.59 ± 0.72 cm, while in clinically healthy sows, this parameter amounted to 18.94 ± 1.26 cm. Before farrowing, the difference in length of the umbilical cord in seronegative pigs increased in comparison with the convalescent ones and amounted to 22.94 ± 1.16 against 19.33 ± 0.91 cm. Herewith, there was one vein and two arteries in all umbilical cords.

Table 1. Morphological parameters of the placenta and fetuses of clinically healthy and PRRS-convalescent pregnant sows (n = 10). Note: * P< 0.05, ** P< 0.01.

<table>
<thead>
<tr>
<th>Morphological parameters</th>
<th>Trimester of gestation</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End of the second trimester (day 70-75)</td>
<td>PRRS-convalescent</td>
<td>Clinically healthy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>kg</td>
<td>cm²</td>
</tr>
<tr>
<td>Uterus weight.</td>
<td>5.27 ± 0.33</td>
<td>6.26 ± 0.28</td>
<td>8.20 ± 0.41</td>
</tr>
<tr>
<td>Membranes’ weight.</td>
<td>0.71 ± 0.05</td>
<td>0.92 ± 0.09</td>
<td>1.61 ± 0.42</td>
</tr>
<tr>
<td>Uterus size. cm²</td>
<td>549.44 ± 11.81</td>
<td>687.02 ± 19.33*</td>
<td>732.12 ± 37.23</td>
</tr>
<tr>
<td>Thickness of the fetal portion of the placenta, cm</td>
<td>7.82 ± 0.71</td>
<td>7.54 ± 0.83</td>
<td>10.22 ± 2.14</td>
</tr>
<tr>
<td>Placenta volume</td>
<td>203.52 ± 9.7</td>
<td>216.81 ± 8.74*</td>
<td>322.52 ± 10.9</td>
</tr>
<tr>
<td>% of glands in the endometrium</td>
<td>10.6</td>
<td>12.3</td>
<td>14.4</td>
</tr>
<tr>
<td>Height of glandular cells, μm</td>
<td>14.82 ± 0.55</td>
<td>18.72 ± 0.62*</td>
<td>22.31 ± 1.78</td>
</tr>
<tr>
<td>Diameter of glands, μm</td>
<td>34.32 ± 1.25</td>
<td>40.79 ± 1.32*</td>
<td>49.91 ± 2.06</td>
</tr>
<tr>
<td>Umbilical cord length, cm</td>
<td>17.59 ± 0.72</td>
<td>18.94 ± 1.26</td>
<td>19.33 ± 0.91</td>
</tr>
<tr>
<td>Allantoic fluid, ml</td>
<td>321.76 ± 10.71</td>
<td>522.16 ± 2.74**</td>
<td>36.14 ± 2.73</td>
</tr>
<tr>
<td>Amniotic fluid, ml</td>
<td>386.54 ± 27.23</td>
<td>436.38 ± 21.44 *</td>
<td>120.18 ± 12.24</td>
</tr>
</tbody>
</table>
The amount of allantoic fluid normally varies depending on the period of gestation. In the case of the normal course of gestation, its amount ranges from 25 to 350 ml in the first trimester, there is an increase to 500–530 ml in the second trimester, and the amount of fluid gradually decreases to 10–100 ml by farrowing. In our case, on days 70-75 of gestation, the volume of allantoic fluid in the PRRS-convalescent pigs amounted to 321.76 ± 10.71 ml, while in healthy sows, it was 522.16 ± 2.74 ml. By the end of the third trimester, it amounted to 36.14 ± 2.73 ml in the recovered sows; and to 81.24 ± 1.41 ml in clinically healthy ones.

By day 70-75 gestation, in the amniotic cavity of clinically healthy pregnant sows, we noted transparent colourless liquid in a volume of 436.38 ± 21.44 ml, which decreased before farrowing to 120.18 ± 12.24 ml, while the liquid turned brown, mucilaginous and contained large particles of meconium. Herewith, the amount of amniotic fluid in PRRS-convalescent pigs amounted to 386.54 ± 27.23 ml by day 75; and its level decreased to 120.18 ± 12.24 ml by day 110.

Analysis of the resulted data demonstrated a certain pattern being indicative of the condition of the fetuses of the experimental pigs. Thus, in clinically healthy sows, the average fetal weight was 677.83 ± 15.58 g by day 70-75 of gestation and 1124.42 ± 29.51 g by day 105-110. After farrowing, the newborn piglets demonstrated good appetite and well-expressed basic reflexes: movement, digestion, defecation and urination.

In PRRS-convalescent pigs, the average fetal weight was 462.38 ± 12.42 g by the end of the second trimester and 909.54 ± 18.63 g by farrowing. Therefore, there was a significant decrease in fetal body weight as compared to the clinically healthy pigs, which amounted to 31.8% by day 70-75 of gestation and to 19.2% by day 105-110. The piglets born from the experimental sows demonstrated the following abnormalities: shallow, labored breathing, arrhythmic pulse, and poorly pronounced heartbeats. When examining the piglets, we took note of the sparse, hard hair-covering, sunken eyes and soft auricles with the tips hanging down.

Results of the statistical analysis of the internal fetal organs (liver, lungs, kidneys, spleen) demonstrated that by the end of the third trimester, the average weight of the following internal organs of the fetuses of the PRRS-convalescent sows was lower as compared to the fetuses of the seronegative sows: liver – by 10.6%, lung – by 11.8%, kidney – by 11.3% and spleen – by 15.9%.

Therefore, the piglets born from the PRRS-convalescent sows were classified as hypotrophic ones. Their physiological status had been formed in terms of the processes occurring during the prenatal period. In this case, we observed the negative impact on the pregnant sows’ fetus and placenta systems that resulted from the previous PRRSV effect on their reproductive system.

The earliest diagnosis of low-producing gilts is the main task when working with parent females at the holding. Timely determination of fertility in replacement gilts with subsequent culling of defected gilts will help to reduce direct economic losses in raising sows [10].
In the production environment, the replacement gilts were subdivided into two groups: gilts born from PRRS-convalescent sows (experimental group), and gilts born from sows with an average litter (control group). At the study moment, all gilts were clinically healthy, they were 4-4.5 months old, and their live weight amounted to 120-150 kg.

Sex hormones Estradiol $E_2$ and anti-Müllerian hormone (AMH) were determined in the sera in order to establish the hormonal status of the pigs.

Table 2. Concentration of sex hormones in the sera of replacement gilts (n = 10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Hormone levels</th>
<th></th>
<th>Estradiol $E_2$, pM / l</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>Anti-Müllerian hormone, (ng/ ml)</td>
<td>2.09±1.14</td>
<td>81.23±1.78</td>
</tr>
<tr>
<td>experimental</td>
<td>Anti-Müllerian hormone, (ng/ ml)</td>
<td>6.59±2.11</td>
<td>101.28±1.36</td>
</tr>
</tbody>
</table>

The data in Table 2 demonstrate that the level of AMH level was higher in replacement gilts born from sows demonstrating PRRS clinical signs as compared to clinically healthy gilts (6.59 ± 2.11 versus 2.09 ± 1.14 ng / ml).

During the experiment, the reproductive capacity of gilts was assessed according to generally accepted indicators: the day of oestrus onset, fertility, superfetation and viability of piglets by the two-month age.

Table 3. Diagnostics of the first oestrus in various groups of replacement gilts (n=10).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>First oestrus identified, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>140-150</td>
</tr>
<tr>
<td>Experimental (AMH level 6.59 ± 2.11 ng / ml)</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Control (AMH level 2.09 ± 1.14 ng / ml)</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

Studies of AMH level demonstrated that its amount in gilts is inversely correlated with their reproductive indicators. Thus, sufficiently high AMH concentrations in the serum (6.59 ± 2.11 ng/ml) were indicative of rather low fertility.

At the end of the studies, only eight out of ten gilts born from PRRS-diseased sows were fertilized with further gestation (Table 3).

The data in Table 4 demonstrates a significant difference in fertility and multifetation percentage difference in pigs with low and high AMH levels. Thus, the gilts born from PRRS-diseased sows delivered 7.7 ± 0.5 piglets in the first farrowing, while clinically healthy Large White gilts delivered 14.4 ± 0.3. There was no significant correlation between the AMH level and the viability of piglets by the age of two months.

Table 4. Reproductive capacity of gilts in different groups according to the results of the first farrowing.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fertility, %</th>
<th>Multifetation</th>
<th>Viability up to 2 months of age, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental (AMH level 6.59 ± 2.11 ng / ml)</td>
<td>59.5</td>
<td>7.7±0.5</td>
<td>79.6</td>
</tr>
<tr>
<td>Control (AMH level 2.09 ± 1.14 ng / ml)</td>
<td>72.4</td>
<td>14.4±0.3</td>
<td>75.4</td>
</tr>
</tbody>
</table>
4 Conclusion

The most effective measure for PRRS control currently involves total destruction of PRRSV infected herd. Such drastic measure is economically unacceptable for the Russian pig farms; therefore, in the current situation, measures should be developed in the infected settlements that will allow to avoid complete replacement of the pig population and take account of morphological changes in PRRS-convalescent pigs.

Results of the studies designed to establish possible changes in the fetus and placenta systems of the PRRS-convalescent pregnant sows suggested that the weight and volume of the fetal portion of the placenta are significantly reduced in PRRS-convalescent sows as compared to similar indicators in clinically healthy pregnant sows.

During the experiments, we observed a significant decrease in fetal body weight in seropositive pigs as compared to clinically healthy ones: 31.8% by day 70-75 of gestation and 19.2% by day 105-110. The piglets born from the experimental sows demonstrated physiological abnormalities, thus allowing hypotrophy diagnosis.

During the selection of the replacement gilts, determination of AMH level in sera of gilts can be used along with other diagnostic tests to select replacement gilts, thus increasing the reproductive characteristics of the herd.

The research demonstrated a correlation between AMH levels in sera of the experimental animals and their fertility. The proposed method allows detecting low-fertile gilts even in the first months of their life, which can significantly reduce the cost of keeping animals with health problems.

The studies also demonstrated that at E2 level above 100 pM/L the reproductive capacities of gilts are significantly reduced, which is confirmed by other published reports [15]. This may be a direct indication for further examination of the gilts for ovarian pathologies.

The introduction of AMH ELISA into the mandatory gynecological clinical examination of gilts at pig holdings is therefore expedient and effective.

References

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