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Reproductive Safety and Protective Efficacy of AVAC ASF LIVE Vaccine in Breeding Female Pigs

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Abstract

Background/Objectives: African swine fever (ASF) live attenuated vaccines have been evaluated in growing pigs, whereas their reproductive safety and protective efficacy in breeding females remain insufficiently characterized. This study evaluated the AVAC ASF LIVE vaccine in sows and replacement gilts. **Methods**: Experiment 1 compared vaccination before mating and during early, mid-, or late gestation, with five vaccinated animals and three controls per group. Clinical and reproductive outcomes, antibody responses, and ASFV DNA detection were assessed. Based on safety findings and practical considerations, pre-mating sows and gilts were selected for Experiment 2, evaluating ten-fold overdose safety and protective efficacy against virulent genotype II ASFV in separate cohorts. **Results**: Vaccination elicited ASFV-specific antibodies across reproductive stages. No abortions occurred following pre-mating or early-gestation vaccination, whereas one of five vaccinated sows aborted in each mid- and late-gestation group. Late-gestation vaccination was associated with fewer live-born and weaned piglets and more frequent ASFV DNA detection in offspring-associated samples, whereas none was detected in available corresponding samples after pre-mating or early-gestation vaccination. A ten-fold overdose was well tolerated, with only transient mild fever in one sow. Following challenge, four of five vaccinated sows (80%) and all five vaccinated gilts (100%) survived, compared with none of the controls. **Conclusions**: Pre-mating and early-gestation vaccination may offer more favorable reproductive safety profiles than later-gestation vaccination. Additionally, pre-mating vaccination was well tolerated at a ten-fold overdose and provided substantial protection against genotype II ASFV challenge. These findings support pre-mating vaccination as a promising strategy for ASF prevention in breeding females.

Keywords: African swine fever; breeding pigs; live attenuated vaccine; reproductive safety; vaccination timing

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1. Introduction

African swine fever (ASF) is a highly consequential viral disease of domestic pigs and wild suids caused by African swine fever virus (ASFV), a large double-stranded DNA virus currently classified as *Asfivirus haemorrhagiae* within the family *Asfarviridae* [1,2]. The disease causes substantial economic losses through animal mortality, disease control measures, production disruption, and international trade restrictions [1,3]. Since the introduction of genotype II African swine fever virus (ASFV) into Asia in 2018, the disease has spread

extensively and become established in several pig production systems [4–6]. Although biosecurity, movement control, surveillance, and stamping-out remain essential for ASF prevention and control, vaccination is increasingly recognized as a potential complementary measure, particularly in endemic regions where continued virus circulation presents persistent challenges [6–9].

Among the vaccine platforms investigated for ASF, live attenuated vaccines (LAVs), particularly those based on genetically modified genotype II ASFV strains, have demonstrated the most promising protective efficacy [9–11]. In Vietnam, licensed ASF vaccines have shown favorable safety, immunogenicity, and protective efficacy in growing pigs under experimental and field conditions [12,13]. However, their approved use is currently restricted to healthy growing pigs raised for meat production, excluding breeding animals [9]. Further evaluation of reproductive safety and protective efficacy in breeding pigs is therefore warranted to support the potential extension of vaccination to these populations.

Breeding females represent a particularly important population for ASF prevention because their health and reproductive performance are essential for maintaining herd productivity, replacement capacity, and the sustainability of pig production [14,15]. However, the use of replication-competent LAVs in these animals raises specific safety concerns that are less relevant to growing pigs. ASFV can affect reproductive tissues and pregnancy outcomes, including abortion and fetal infection [16,17]. Furthermore, pregnancy involves physiological and immunological adaptations that may influence the replication, persistence, and tissue distribution of attenuated vaccine viruses [18,19]. Therefore, vaccine safety established in growing pigs cannot be directly extrapolated to pregnant sows or replacement gilts.

Previous studies investigating ASF LAVs in breeding females have reported variable reproductive outcomes. Certain vaccine candidates have been associated with adverse reproductive effects, including abortion and detection of viral DNA in fetal or neonatal tissues, whereas others have demonstrated favorable clinical and reproductive safety under specific experimental or field conditions [20–23]. These contrasting findings highlight the importance of evaluating reproductive safety for individual vaccine strains rather than assuming comparable outcomes across different LAVs. Moreover, most available studies have focused on vaccination during selected stages of gestation, while direct comparisons between pre-mating vaccination and vaccination at different stages of pregnancy remain limited. Such comparisons are particularly relevant for identifying vaccination schedules that could minimize reproductive risks while maintaining adequate immune responses.

AVAC ASF LIVE is a live attenuated vaccine based on the ASFV-G-ΔMGF-DMAC strain, derived from genotype II ASFV through the deletion of six multigene family genes and subsequent adaptation for vaccine production [12,24]. Although its safety and protective efficacy have been demonstrated in growing pigs [12], its use in breeding females has not yet been reported. In this context, identifying an appropriate vaccination timing for breeding females is particularly important to minimize potential reproductive risks while ensuring adequate protection efficacy against ASF. The present study therefore evaluated AVAC ASF LIVE across five reproductive stages: pre-mating replacement gilts, pre-mating sows, and sows during early, mid-, and late gestation. Clinical and reproductive safety, ASFV DNA detection, antibody responses, and potential exposure of fetuses or offspring to vaccine-derived viral material were assessed to identify the vaccination timing associated with the most favorable safety profile and practical suitability. The selected vaccination stage was then further evaluated for ten-fold overdose safety and protective efficacy against a virulent genotype II ASFV challenge.

2. Materials and Methods

2.1. Vaccine

AVAC ASF LIVE vaccine (batch no. 0923; AVAC Vietnam Joint Stock Company, Hung Yen, Vietnam) was used in the study. The vaccine contains the attenuated ASFV-G-ΔMGF-DMAC strain, a genotype II ASFV derived from the highly virulent Georgia 2007 isolate. The parental attenuated virus was generated by deletion of six multigene family genes, as previously described [24]. For commercial production, the virus was adapted to propagation in the DMAC cell line. Each vaccine dose contained at least $10^{3.5}$ HAD_{50} of ASFV-G-ΔMGF-DMAC [12].

2.2. Challenge Virus

The challenge virus was the highly virulent ASFV isolate Hv-Avac01, recovered from a pig that died during an ASF outbreak in Hung Yen Province, Vietnam, in 2019. The isolate belongs to p72 genotype II and has been characterized previously [12].

2.3. Animals and Housing

Clinically healthy Yorkshire and Landrace breeding females with no history of ASF vaccination were used. The animals represented five reproductive stages: early-gestation sows (T1), vaccinated at 25–30 days of gestation; mid-gestation sows (T2), vaccinated at 55–60 days; late-gestation sows (T3), vaccinated at 90–95 days; pre-mating sows (PM-Sow), approximately two weeks after weaning (second parity, 51–52 weeks of age); and pre-mating replacement gilts (PM-Gilt), approximately two weeks before first mating (29–30 weeks of age).

Before enrollment, animals were screened and confirmed negative for ASFV, porcine circovirus type 2 (PCV2), foot-and-mouth disease virus (FMDV), classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, and *Glaesserella parasuis* by real-time PCR or real-time RT-PCR, as appropriate. Serum samples were also negative for ASFV-specific antibodies by ELISA.

Upon arrival at the experimental facility, animals were acclimatized for five days. Their health status was monitored, and rectal temperature was recorded daily to establish baseline values.

Animals were housed in biosecure facilities at the AVAC research center under appropriate animal-welfare conditions. They were provided with commercial feed and had ad libitum access to water.

2.4. Experimental Designs

The study comprised two sequential experiments. Experiment 1 evaluated the safety and immunogenicity of AVAC ASF LIVE across reproductive stages. Based on the clinical, reproductive, and virological findings from Experiment 1, the reproductive groups considered most suitable for vaccination were selected for Experiment 2, which evaluated overdose safety and protective efficacy.

2.4.1. Experiment 1: Reproductive Stage Safety Evaluation

A total of 40 breeding females were included: T1, T2, T3, PM-Sow, and PM-Gilt groups. Within each reproductive stage group, eight eligible animals were allocated to the vaccinated ($n = 5$) or control ($n = 3$) groups (Figure 1) according to a predefined sequence based on the order in which they were caught.

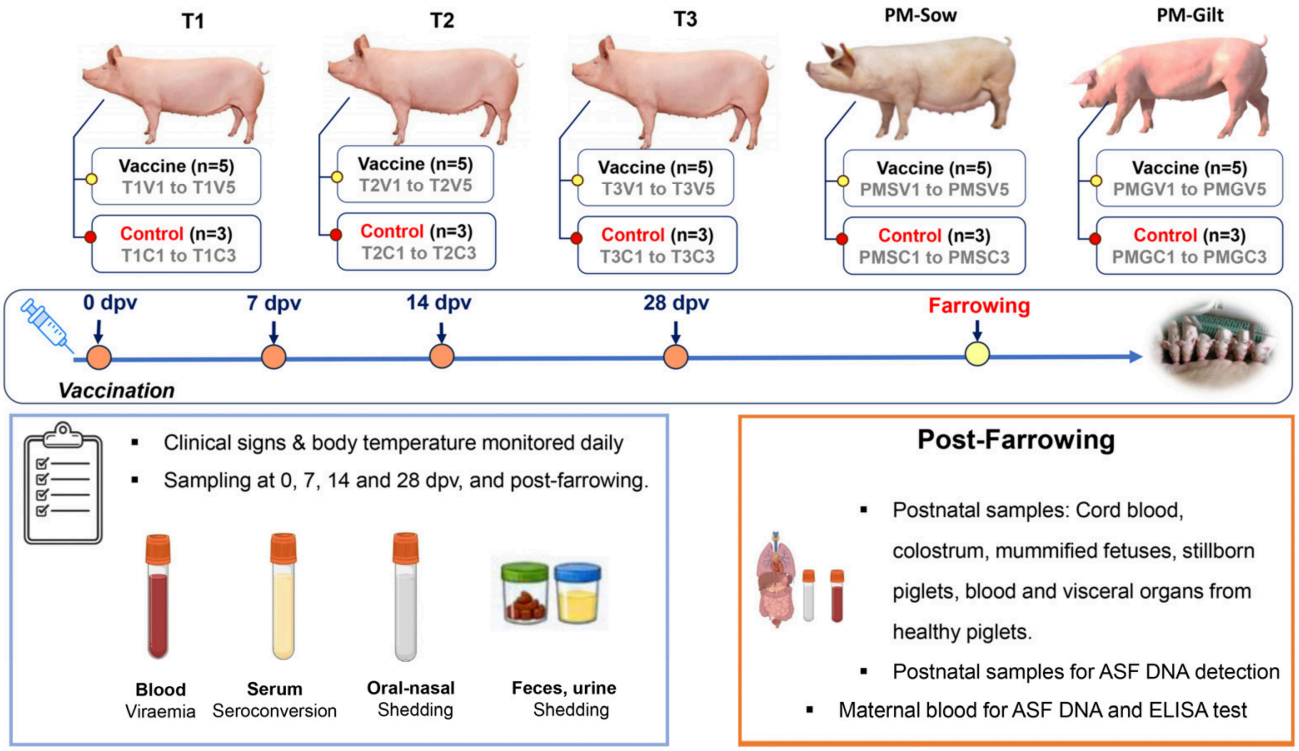

Figure 1. Experimental design of the AVAC ASF LIVE safety study in breeding females vaccinated at different reproductive stages.

Individual animals were identified as follows: T1 vaccinated (T1V1-T1V5) and controls (T1C1-T1C3); T2 vaccinated (T2V1-T2V5) and controls (T2C1-T2C3); T3 vaccinated (T3V1-T3V5) and controls (T3C1-T3C3); PM-Sow vaccinated (PMSV1-PMSV5) and controls (PMSC1-PMSC3); and PM-Gilt vaccinated (PMGV1-PMGV5) and controls (PMGC1-PMGC3).

Vaccinated animals received one 2 mL intramuscular dose of reconstituted AVAC ASF LIVE vaccine according to the manufacturer's instructions. Control animals received 2 mL of sterile physiological saline by the same route.

Animals were clinically examined daily until farrowing, and rectal temperature was recorded daily for 28 days after vaccination. Reproductive outcomes included conception or farrowing status, abortion, total litter size, live-born and stillborn piglets, and piglet survival to weaning.

Blood, oral–nasal swabs, rectal swabs, and urine were collected at 0, 7, 14, and 28 days post-vaccination (dpv) and after farrowing completion, when available, to evaluate viremia and detection of viral DNA in secretions. For animal-welfare reasons, sampling during late gestation was avoided, and samples of the T3 group were collected after farrowing or abortion. Serum collected at the same time points was tested for ASFV-specific antibodies. To assess potential fetal or offspring exposure to vaccine-derived viral material, colostrum, umbilical cord blood, mummified fetuses, stillborn piglets, pre-colostral blood from three piglets per litter, and lung, spleen, kidney, and lymph node tissues from two clinically healthy piglets per litter were collected at farrowing and examined for ASFV DNA.

2.4.2. Experiment 2: Overdose Safety and Protective Efficacy in Pre-Mating Females

Based on the findings of Experiment 1, pre-mating sows and replacement gilts were selected for further evaluation. Experiment 2 comprised two independent studies conducted in separate animal cohorts: a ten-fold overdose safety study and a virulent challenge efficacy study (Figure 2).

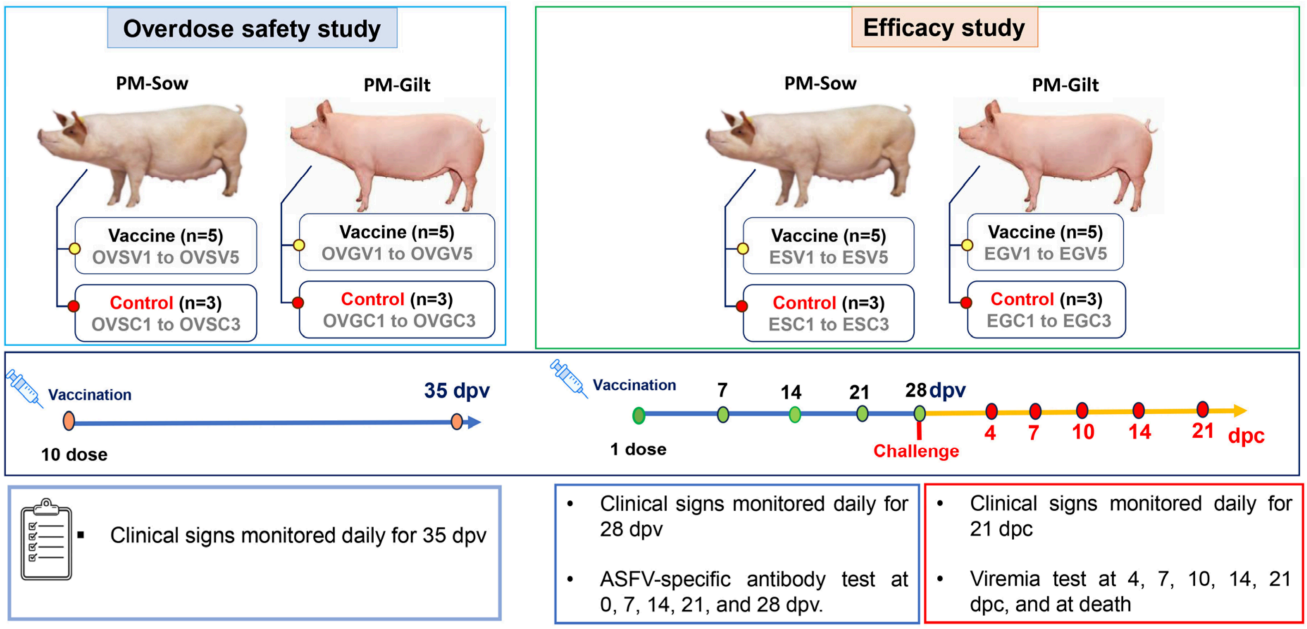

Figure 2. Experimental design of the ten-fold overdose safety and protective efficacy studies in pre-mating breeding females.

For the overdose safety study, 16 animals were enrolled, comprising eight pre-mating sows and eight pre-mating replacement gilts. Within each category, five animals were vaccinated and three served as controls. Animals were designated as vaccinated PM-Sows (OVSV1–OVSV5), control PM-Sows (OVSC1–OVSC3), vaccinated PM-Gilts (OVGV1–OVGV5), and control PM-Gilts (OVGC1–OVGC3). Vaccinated animals received a ten-fold overdose of AVAC ASF LIVE intramuscularly, whereas controls received 2 mL of sterile physiological saline. Clinical signs and rectal temperature were monitored daily for 35 days after vaccination.

For the protective efficacy study, a separate cohort of 16 animals was used, comprising eight pre-mating sows and eight pre-mating replacement gilts. Within each category, five animals were vaccinated and three served as controls. Animals were designated as vaccinated PM-Sows (ESV1–ESV5), control PM-Sows (ESC1–ESC3), vaccinated PM-Gilts (EGV1–EGV5), and control PM-Gilts (EGC1–EGC3). Vaccinated animals received one standard 2 mL dose of AVAC ASF LIVE intramuscularly, whereas controls received 2 mL of sterile physiological saline. The sows and gilts in Experiment 2 were not mated during the study and remained non-pregnant at the time of challenge.

Animals were monitored clinically for 28 days after vaccination. Serum samples were collected at 0, 7, 14, 21, and 28 dpv for ASFV-specific antibody testing. At 28 dpv, all animals were challenged intramuscularly with 10^3 HAD_{50} of the virulent genotype II isolate Hv-Avac01 and subsequently monitored for clinical signs and mortality for 21 days. Blood samples were collected at 0, 4, 7, 10, 14 and 21 dpc and at death for ASFV DNA detection. Necropsies were performed on animals that died during the post-challenge observation period. Vaccinated PM-Sows surviving to the end of the observation period were euthanized and necropsied at 22 dpc, and internal tissue samples were collected for ASFV DNA detection. ASFV-positive samples were further analyzed by strain-specific DIVA PCR to distinguish vaccine from challenge virus.

2.5. Pre-Enrollment Pathogen Screening

Before enrollment, animals were screened for ASFV, PCV2, FMDV, CSFV, PRRSV, *Mycoplasma hyopneumoniae*, and *Glaesserella parasuis*. Viral nucleic acids were extracted

from blood using the Virus DNA/RNA Extraction Kit 2.0 (Vazyme International LLC., Nanjing, China) according to the manufacturer's instructions. Commercial assays included VDx ASFV qPCR Ver. 2.1, VDx PCV2 qPCR, VDx FMDV 3Diff/PAN qRT-PCR, VDx CSFV qRT-PCR, and VDx HP-PRRSV qRT-PCR Ver. 1.1 (Median Diagnostics Inc., Seoul, Republic of Korea). Detection of *M. hyopneumoniae* and *G. parasuis* was performed by qPCR using assays previously described [25,26], with Taq Pro Multiple Probe qPCR Mix (QN213-EN02; Vazyme International LLC., Nanjing, China). Only animals negative for all screened pathogens and negative for ASFV-specific antibodies were enrolled.

2.6. Clinical and Reproductive Monitoring

Animals were observed daily for clinical abnormalities potentially associated with vaccination or ASFV infection. An in-house scoring system assessed rectal temperature, appetite, behavior and mobility, skin hemorrhage or cyanosis, joint abnormalities, respiratory signs, ocular discharge, digestive signs, and abortion (Table 1). Rectal temperature was measured daily with a digital thermometer. Mortality and reproductive outcomes, including conception or farrowing status, abortion, litter characteristics, and piglet survival, were recorded as applicable to each experiment.

Table 1. Clinical scoring system used for daily evaluation of experimental animals.

No.	Severity Level (Score) / Clinical Sign/Parameter	Normal (0)	Mild (1)	Moderate (2)	Severe (3)
1	Rectal temperature (°C)	≤40.0	40.1–40.5	40.6–41.0	≥41.1
2	Appetite	Normal	Reduced intake	Picking at feed only	Complete anorexia
3	Behavior/mobility	Normal	Reluctant to move; lethargic	Stands only when stimulated	Recumbent; no response to stimulation
4	Skin hemorrhage/cyanosis	Normal	Mild	Moderate	Severe/black hemorrhagic lesions
5	Joints	Normal	Joint swelling	Lameness or necrotic lesions	Severe lameness; difficulty moving
6	Dyspnea/cough	Normal	Mild	Moderate	Severe
7	Ocular discharge	Normal	Mild	Moderate	Severe
8	Digestive signs	Normal	Diarrhea < 24 h	Diarrhea /vomiting > 24 h	Bloody diarrhea
9	Abortion	No abortion			Abortion

2.7. Collection of Swab and Urine Samples

Oral–nasal and rectal swabs were collected at predetermined time points using Dry Transport Swabs (HP5005; Jiangsu Huida Medical Instruments Co., Ltd., Jiangin, China). Swabs were placed in sterile transport tubes and kept refrigerated until processing. Urine was collected opportunistically during spontaneous urination into sterile tubes. Samples were transported under refrigerated conditions and processed immediately or stored at −80 °C until testing.

2.8. ASFV-Specific Antibody Detection

Serum samples were tested using the VDPro ASFV Ab i-ELISA Version 2.0 Kit (Median Diagnostics Inc., Seoul, Republic of Korea) according to the manufacturer's instructions. Optical-density values were converted to sample-to-positive (S/P) ratios; samples with S/P ratios of at least 0.25 were considered positive.

2.9. ASFV DNA Detection and Strain Differentiation

ASFV DNA was tested in blood, oral–nasal and rectal swabs, urine, colostrum, umbilical cord blood, fetal samples, neonatal tissues, and other study samples. Total DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). ASFV DNA was detected with the VDx ASFV qPCR Kit (Median Diagnostics Inc., Seoul, Republic of Korea) using procedures described previously [12]. Samples with cycle-threshold (Ct) values below 38 were considered positive.

Where differentiation was required, ASFV-positive samples were tested with the strain-specific DIVA PCR described previously [12] to distinguish AVAC ASF LIVE vaccine virus from field or challenge virus. This differentiation is based on two genetic markers: β-GUS, which is present in the vaccine strain but absent from the field or challenge virus, and MGF360-12L, which is deleted from the vaccine strain but retained in the field or challenge virus [12,24]. The MGF360-12L-based DIVA qPCR assay was developed and evaluated by Velazquez-Salinas et al. [27] and is included in the WOAH guidelines for ASF laboratory diagnosis [28]. This assay was applied particularly to samples collected at necropsy after challenge.

2.10. Statistical Analysis

Data are presented as the mean ± SD for the numbers of total-born, live-born and weaned piglets. Differences between two independent groups were analyzed using an unpaired *t*-test with Welch's correction in GraphPad Prism version 10.1. A p value < 0.05 was considered statistically significant. In addition, 95% confidence intervals were calculated for abortion proportions using the exact Clopper–Pearson method and for differences in litter performance using Welch's method (Supplementary Table S2).

3. Results

3.1. Safety and Immunogenicity Across Reproductive Stages

3.1.1. Clinical Signs and Scores

All control animals remained clinically healthy throughout the post-vaccination observation period, with normal feed intake and no clinical abnormalities. Most vaccinated animals also remained clinically normal, and only four animals showed transient clinical reactions (Figure 3A).

In T1, sow T1V3 developed mild fever accompanied by a transient reduction in feed intake at 6–7 dpv. In T2, sow T2V4, vaccinated at gestation day 56, showed reduced feed intake from 11 to 21 dpv and mild fever from 16 to 18 dpv, with abortion of seven fetuses at gestation day 75 (19 dpv). Maternal blood was ASFV PCR-positive at abortion (Ct = 36.14), and 7/7 fetal samples were PCR-positive (Ct = 35.52–37.64). The fetuses exhibited variable development, wrinkled skin, and autolysis, with scattered cutaneous hemorrhages observed on gross examination. In T3, sow T3V3, vaccinated at gestation day 90, showed reduced feed intake from 20 to 26 dpv and mild fever from 19 to 21 dpv, with abortion of 12 fetuses at gestation day 112 (22 dpv). Maternal blood was ASFV PCR-positive at abortion (Ct = 29.59), and 12/12 fetal samples tested were PCR-positive (Ct = 25.17–37.64). The fetuses were near term, with fresh and weak fetuses observed. Gross examination revealed thoracic hemorrhage and localized hemorrhages.

In the PM-Sow group, PMSV1 showed a slight reduction in feed intake at 6–7 dpv without fever or reproductive abnormalities. All clinical reactions were transient and resolved without treatment.

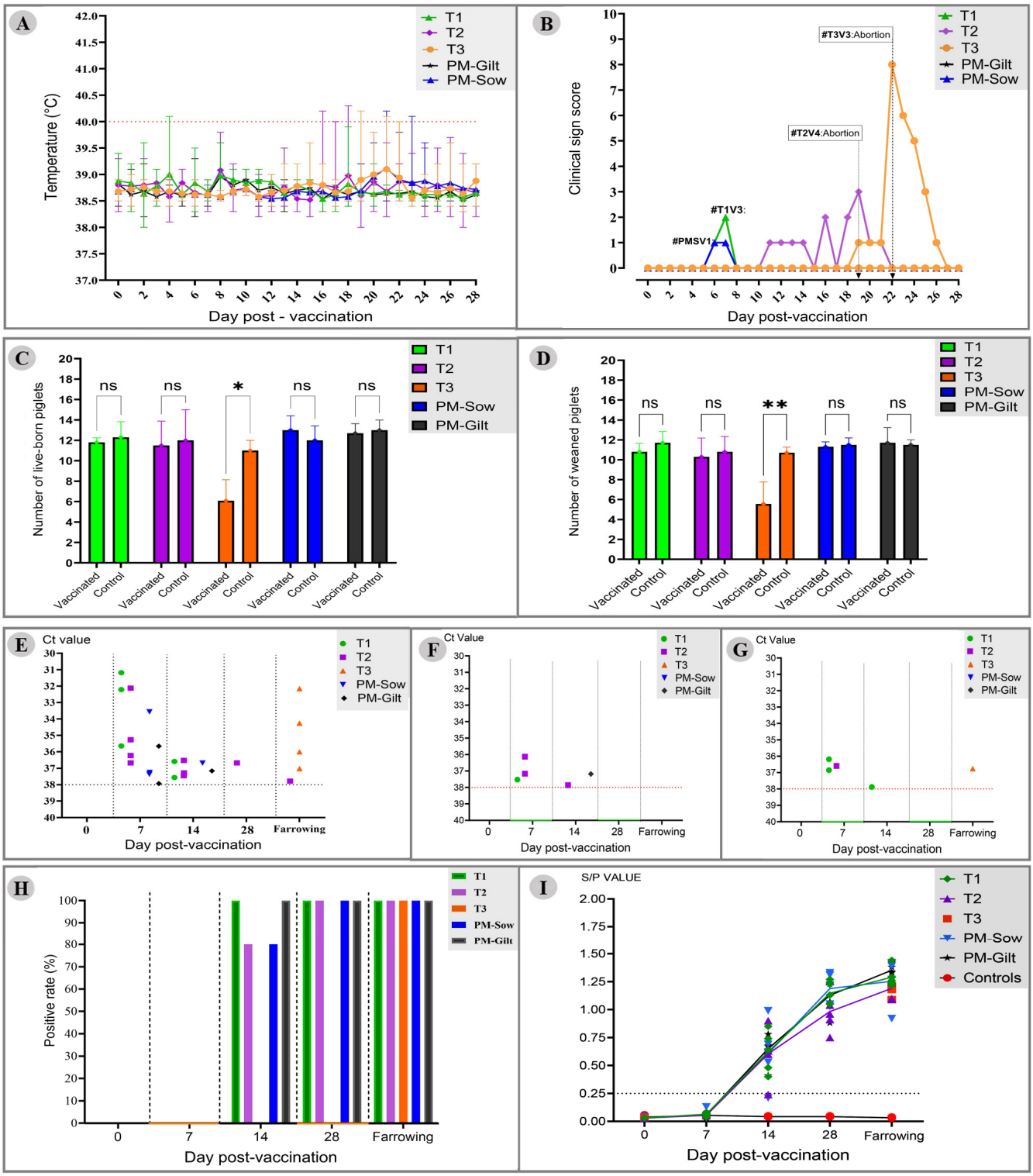

Figure 3. Clinical, reproductive, virological, and serological outcomes after vaccination at different reproductive stages. (**A**) Rectal temperature; (**B**) clinical score; (**C**) live-born piglets per sow; (**D**) piglets alive at weaning per sow; (**E**) ASFV DNA in blood, expressed as Ct values; (**F**) ASFV DNA in oral–nasal swabs; (**G**) ASFV DNA in urine; (**H**) antibody-positive rate; and (**I**) antibody S/P ratio. ns, not statistically significant ($p \geq 0.05$); * $p < 0.05$; ** $p < 0.01$. For reproductive performance analyses (**C**,**D**), the vaccinated/control sample sizes were 5/3 (T1), 4/3 (T2), 4/3 (T3), 4/2 (PM-sSow), and 3/2 (PM-Gilt), representing animals that farrowed.

Clinical scores reflected the observed clinical patterns (Figure 3B). Scores remained at 0 in most vaccinated animals and increased transiently only in T1V3, T2V4, T3V3, and PMSV1. The highest scores were recorded in T2V4 and T3V3, the two animals that experienced abortion.

3.1.2. Reproductive Performance

Reproductive outcomes are summarized in Figure 3C,D and Table S1. Among sows vaccinated during gestation, all five T1 sows completed pregnancy and farrowed, whereas one of five vaccinated sows aborted in each of the T2 and T3 groups; all control sows in T1, T2, and T3 completed pregnancy and farrowed. Among females vaccinated before mating, 4/5 vaccinated PM-Sows and 3/5 vaccinated PM-Gilts conceived, compared with 2/3 controls in each group. All pre-mating females that conceived, whether vaccinated or control, maintained pregnancy to term and farrowed successfully, with no abortions recorded.

Litter performance was then evaluated among animals that farrowed, based on the numbers of total-born, live-born, and weaned piglets per litter. Among females vaccinated before mating, litter outcomes did not differ significantly from those of their respective controls. Mean total-born, live-born, and weaned piglets were 15.0, 12.5, and 10.8 in PM-Sows and 13.3, 12.7, and 11.0 in PM-Gilts, compared with 14.0, 12.0, and 11.5 and 14.5, 13.5, and 12.0 in their respective control groups.

Similarly, among T1 and T2 sows that farrowed, no significant differences in litter outcomes were detected between vaccinated and control animals. Mean total-born, live-born, and weaned piglets were 13.2, 12.0, and 10.8 in T1 and 13.8, 11.5, and 10.3 in T2, compared with 14.0, 12.3, and 11.7 and 13.7, 12.0, and 10.8 in their respective control groups.

In contrast, among the four T3 sows that farrowed, the total number of piglets born per litter did not differ significantly between the vaccinated and control groups (10.0 ± 2.94 vs. 12.0 ± 1.00; $p = 0.277$). However, vaccinated sows had significantly fewer live-born piglets (6.0 vs. 11.0; $p < 0.05$) and fewer piglets surviving to weaning (5.5 vs. 10.7; $p < 0.01$).

3.1.3. ASFV DNA in Blood Samples (Viremia)

All controls remained PCR-negative, whereas ASFV DNA was detected transiently in blood from vaccinated animals. Ct values in positive blood samples ranged from 30.83 to 37.45 (Figure 3E). In T1, 3/5 vaccinated sows were PCR-positive at 7 dpv and 2/5 at 14 dpv. In T2, ASFV DNA was detected in 4/5 animals at 7 dpv, 3/5 at 14 dpv, 1/5 at 28 dpv, and 1/5 at the post-farrowing sampling time point. For animal-welfare reasons, blood samples were not collected from T3 sows during late gestation but were instead collected on the day of abortion or after the completion of farrowing. For T3 sows, the interval between vaccination and farrowing was 22–25 days (T3V1: 24 days; T3V2: 24 days; T3V4: 22 days; and T3V5: 25 days; T3V3: aborted on day 22). At this sampling time point, 4/5 vaccinated T3 sows were PCR-positive.

In PM-sSows, ASFV DNA was detected in 3/5 animals at 7 dpv and 1/5 at 14 dpv. In PM-Gilts, 2/5 were positive at 7 dpv and 1/5 at 14 dpv. No ASFV DNA was detected in either pre-mating group at subsequent sampling points.

3.1.4. ASFV DNA in Secretions

All secretion samples from controls were PCR-negative. ASFV DNA was detected infrequently in oral–nasal swabs and urine from vaccinated animals (Figure 3F,G) and was not detected in rectal swabs. For oral–nasal swabs, 1/5 T1 animals tested positive at 7 dpv. In T2 group, 2/5 were positive at 7 dpv and 1/5 at 14 dpv. Among pre-mating animals, one PM-Gilt was positive at 14 dpv, while all PM-Sow samples were negative. All oral–nasal

swabs collected at farrowing were negative (Figure 3F). For urine samples, 2/5 T1 animals were positive at 7 dpv and 1/5 at 14 dpv, while 1/5 T2 animals was positive at 7 dpv. In T3, 1/5 animals was positive after farrowing. All urine samples from PM-Sow and PM-Gilt were negative (Figure 3G). Positive samples had high Ct values (36.13–37.88), indicating low amounts of detectable viral DNA.

3.1.5. Antibody Responses After Vaccination

All animals were ASFV-antibody negative before vaccination (Figure 3H,I). None of the vaccinated animals reached the seropositivity threshold by 7 dpv. At 14 dpv, all T1 and PM-Gilt animals were seropositive, whereas 4/5 (80%) animals in each of the T2 and PM-Sow groups were positive. By 28 dpv, all animals in these four groups were seropositive and remained positive at farrowing. Blood samples were not collected from T3 sows during late gestation; however, all five vaccinated T3 sows were seropositive when sampled after farrowing or abortion. All control animals remained antibody-negative throughout the study.

3.1.6. ASFV DNA in Reproductive and Offspring-Associated Samples

ASFV DNA results for colostrum, umbilical cord samples, dead fetal tissues (including mummified fetuses and stillborn piglets), and neonatal tissues are summarized in Table 2.

Table 2. Detection of ASFV DNA in reproductive and offspring-associated samples.

Group	Treatment	Colostrum	Umbilical Cord	Dead Fetal Tissues	Neonatal Blood	Neonatal Tissues
T1	Vaccinated	0/5	0/5	0/1	0/5	0/5
	Control	0/3	0/3	No death	0/3	0/3
T2	Vaccinated	1/4 (Ct 36.05)	0/4	0/2	0/4	1/4 (Ct 30.34)
	Control	0/3	0/3	No death	0/3	0/3
T3	Vaccinated	3/4 (Ct 34.34–37.07)	4/4 (Ct 31.07–37.64)	2/3 (Ct 29.18–29.82)	3/4 (Ct: 32.09–37.11)	4/4 (Ct 31.07–37.91)
	Control	0/3	0/3	No death	0/3	0/3
PM-Sow	Vaccinated	0/4	0/4	No death	0/4	0/4
	Control	0/2	0/2	No death	0/2	0/2
PM-Gilt	Vaccinated	0/3	0/3	No death	0/3	0/3
	Control	0/2	0/2	No death	0/2	0/2

Values indicate the number of PCR-positive sows or litters relative to the number tested. Where applicable, samples were pooled by sow or litter before real-time PCR analysis. Aborted fetal samples from T2V4 and T3V3 were analyzed separately and are not included in the dead fetal tissue results presented in this table.

All samples from control animals were PCR-negative. No ASFV DNA was detected in any available reproductive or offspring-associated sample from vaccinated T1, PM-Sow, or PM-Gilt animals. In T2, ASFV DNA was detected in colostrum from 1/4 sows (Ct 36.05) and in pooled neonatal tissue from 1/4 litters (Ct 30.34). Umbilical cord samples and available dead fetal tissues from T2 were PCR-negative. In T3, colostrum was positive in 3/4 sows (Ct 34.34–37.07), pooled umbilical cord samples were positive for all four sows (Ct 31.07–37.64), dead fetal tissues were positive in 2/3 sows from which such samples were available (Ct 29.18–29.82), and pooled neonatal tissues were positive in all four litters (Ct 31.07–37.91).

Taken together, the clinical, reproductive, and ASFV DNA findings from Experiment 1 indicated that both pre-mating and early-gestation vaccination were well tolerated and provided a favorable safety profile. Of these two stages, pre-mating administration was selected for subsequent evaluation because it is more practical for routine use in breeding herds.

3.2. Ten-Fold Overdose Safety in Pre-Mating Females

Pre-mating sows and replacement gilts given a ten-fold overdose of AVAC ASF LIVE were monitored for 35 days. Rectal temperatures remained within the normal physiological range except in one vaccinated sow (OVSV3), which developed mild, transient fever for two consecutive days and recovered without treatment (Figure 4A). No reduced feed intake, lethargy, respiratory or gastrointestinal signs, hemorrhagic signs, or mortality were observed. Clinical scores therefore remained 0 except for a low transient score in OVSV3 during the period with fever (Figure 4B).

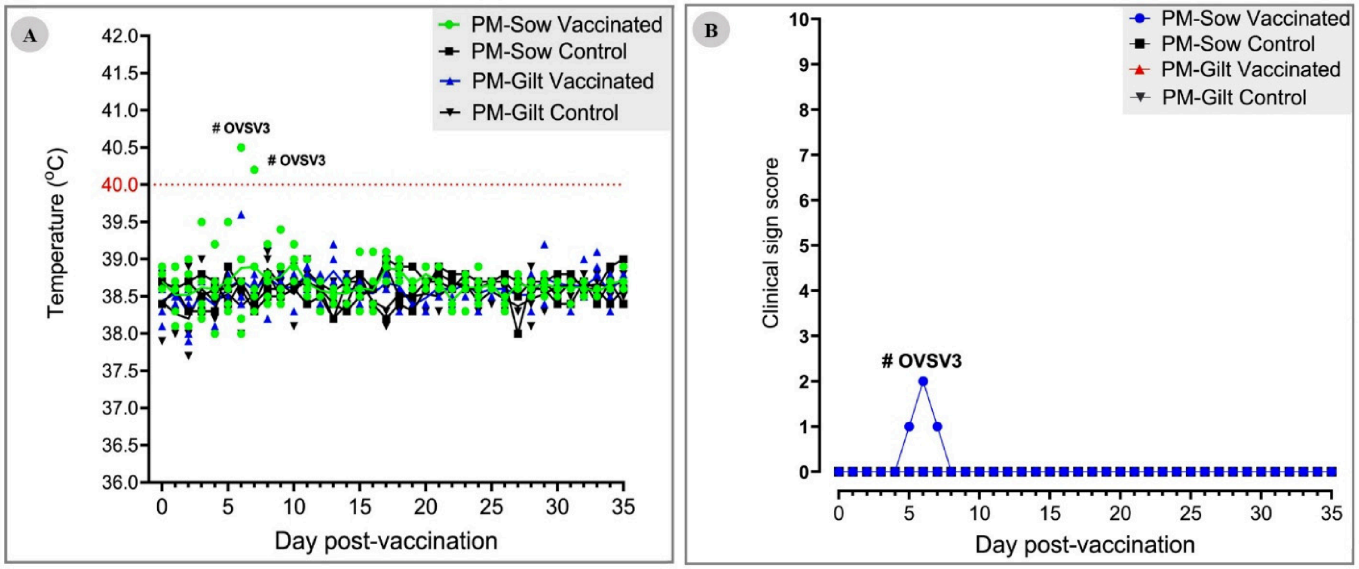

Figure 4. Rectal temperature and clinical scores of pre-mating sows and replacement gilts after ten-fold overdose vaccination with AVAC ASF LIVE. (**A**) Rectal temperature; (**B**) clinical score.

3.3. Protective Efficacy in Pre-Mating Sows and Replacement Gilts

3.3.1. Clinical Outcome and Survival After Challenge

After administration of one standard vaccine dose, vaccinated PM-Sows and PM-Gilts remained clinically healthy throughout the 28-day pre-challenge period. Rectal temperatures remained within the normal range and clinical scores remained 0. Control animals were also clinically normal before challenge.

After intramuscular challenge with virulent genotype II Hv-Avac01, control animals developed fever, increasing clinical scores, and signs including reduced feed intake and diarrhea from approximately 3–4 dpc, followed by progressive deterioration. All control animals in both groups died between 4 and 9 dpc (Figure 5A,B). Most vaccinated animals remained clinically normal or showed only mild, transient changes. Four of five vaccinated PM-Sows survived the challenge at 21 dpc. One vaccinated PM-Sow (ESV4) developed progressive fever and severe clinical disease and died at 10 dpc. All five vaccinated PM-Gilts survived the challenge. Survival was therefore 80% in vaccinated PM-Sows and 100% in vaccinated PM-Gilts, compared with 0% in both control groups (Figure 5C). Necropsy of control animals and ESV4 showed lesions consistent with acute ASF, including splenomegaly, hemorrhagic lymph nodes, multifocal hemorrhages in internal organs, and pericardial effusion.

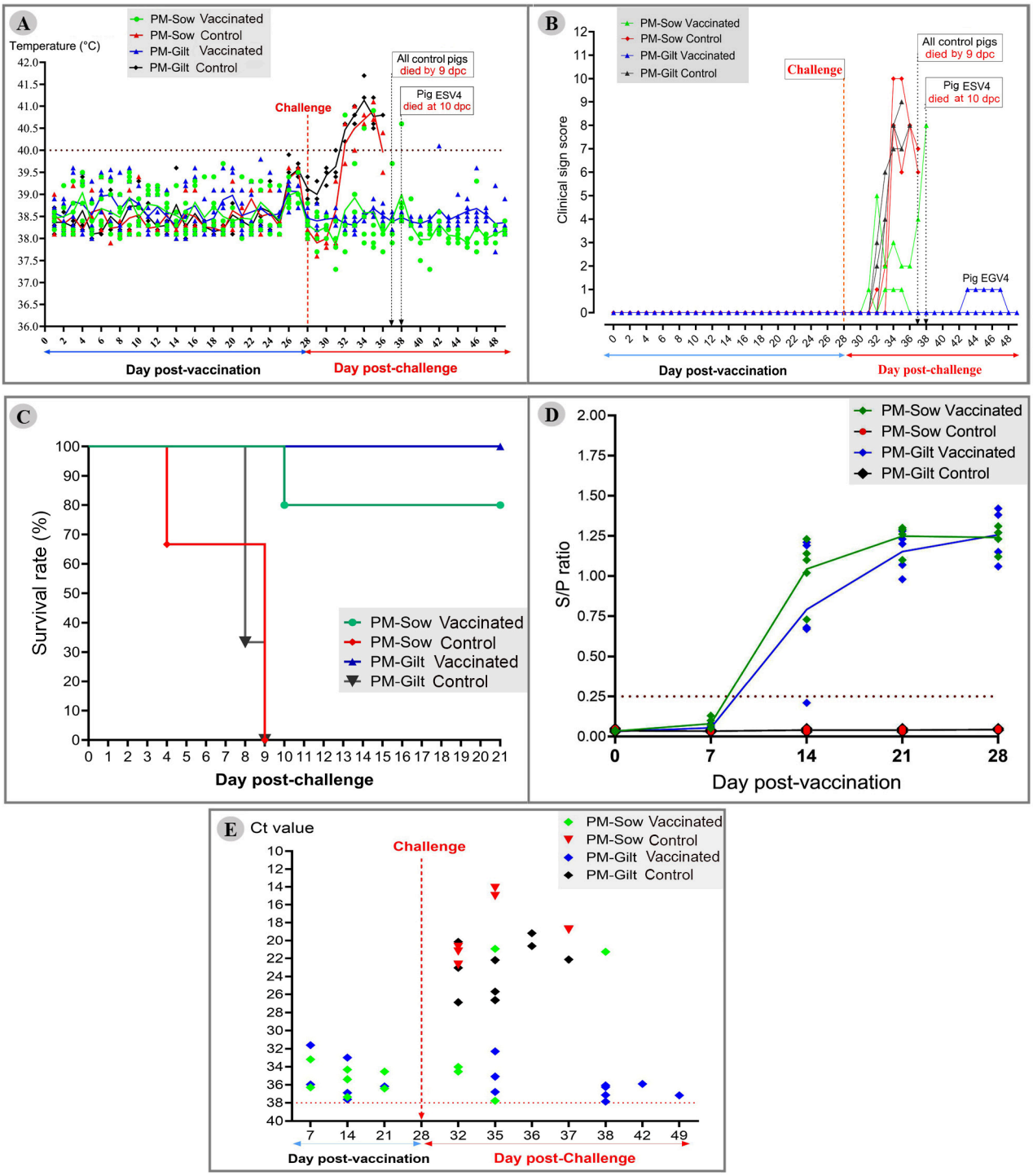

Figure 5. Clinical, survival, virological, and serological responses of pre-mating sows and replacement gilts after vaccination and virulent ASFV challenge. (**A**) Rectal temperature; (**B**) clinical score; (**C**) survival; (**D**) ASFV-specific antibody responses (S/P ratio at least 0.25 considered positive); and (**E**) ASFV DNA in blood after vaccination and challenge.

3.3.2. ASFV-Specific Antibody Responses

All vaccinated and control animals were seronegative before vaccination (Figure 5D). Antibody responses became detectable from 7 dpv, 80–100% of vaccinated animals were seropositive at 14 dpv, and all vaccinated PM-Sows and PM-Gilts were seropositive by 21 dpv. Seropositivity was maintained through 28 dpv immediately before challenge. Controls remained seronegative throughout the pre-challenge period.

3.3.3. Viremia After Vaccination and Challenge

Transient ASFV DNA in blood was detected in a proportion of vaccinated animals during the early post-vaccination period, whereas controls remained PCR-negative (Figure 5E). Vaccine-associated ASFV DNA was not detected in the blood of any vaccinated animals immediately before challenge.

After challenge, all control animals became PCR-positive, with Ct values of 14.11–26.85. In vaccinated PM-Sows, ASFV DNA was detected in 2/5 animals at 4 dpc and 2/5 at 7 dpc. In PM-Sow ESV4 that later died, Ct values decreased from 34.53 at 4 dpc to 20.94 at 7 dpc and remained low (21.27) at 10 dpc. The surviving vaccinated PM-Sow showed only intermittent positivity with Ct values of 34.02–37.96. In vaccinated PM-Gilts, ASFV DNA was detected in 3/5 animals at 7 dpc, 4/5 at 10 dpc, 1/5 at 14 dpc, and 1/5 at 21 dpc. Ct values in positive samples ranged from 32.28 to 37.85.

3.3.4. ASFV DNA in Tissues at Death or Study Completion

Tissue samples were collected at necropsy from animals that died during the post-challenge observation period and from the four vaccinated PM-Sows that survived to 21 dpc and were euthanized at 22 dpc. ASFV DNA was detected in tissues from all three control PM-Sows (ESC1–ESC3) and the vaccinated PM-Sow ESV4 that died following challenge. In contrast, no ASFV DNA was detected in any of the examined tissues from the four surviving vaccinated PM-Sows (ESV1, ESV2, ESV3, and ESV5) at 22 dpc (Table 3).

Table 3. ASFV DNA detection in tissues collected at necropsy from control and vaccinated pre-mating sows following virulent ASFV challenge.

Samples	Controls (n = 3)			Vaccinated (n = 5)				
	ESC1	**ESC2**	**ESC3**	**ESV4**	**ESV1**	**ESV2**	**ESV3**	**ESV5**
	4 dpc	**9 dpc**	**9 dpc**	**10 dpc**	**22 dpc**	**22 dpc**	**22 dpc**	**22 dpc**
Blood	19.08	17.46	17.86	21.27	–	–	–	–
Lung	19.23	19.54	20.30	25.83	–	–	–	–
Spleen	19.08	17.46	17.86	24.24	–	–	–	–
Liver	21.21	17.76	18.37	N/A	–	–	–	–
Kidney	21.27	18.99	20.83	26.29	–	–	–	–
Inguinal lymph node	19.09	17.36	19.36	26.16	–	–	–	–
Tonsillar lymph node	19.48	17.39	18.69	N/A	–	–	–	–
Submandibular lymph node	N/A	N/A	N/A	21.29	–	–	–	–
Uterine mucosa	24.97	23.10	18.67	24.75	–	–	–	–
Uterine horn mucosa	22.21	N/A	N/A	27.40	–	–	–	–
Ovary	21.32	21.00	18.80	28.21	–	–	–	–

Note: Samples were collected at death from three control sows (ESC1–ESC3) and one vaccinated sow (ESV4) and at 22 dpc from four surviving vaccinated sows (ESV1, ESV2, ESV3, and ESV5). Ct values are shown for ASFV PCR-positive samples; "–" indicates a negative PCR result, and "N/A" indicates that the sample was not available for testing.

3.3.5. DIVA Differentiation in Vaccinated Sow ESV4

DIVA PCR of ESV4 tissue samples detected the challenge-virus marker MGF360-12L, whereas the vaccine-virus marker β-GUS was not detected. Together with the increasing ASFV DNA levels after challenge and the characteristic clinical and pathological findings, these results support breakthrough infection with the virulent challenge virus rather than vaccine-associated disease.

4. Discussion

This study evaluated AVAC ASF LIVE vaccination at different reproductive stages and demonstrated stage-related differences in reproductive outcomes and ASFV DNA detection in offspring. Although the vaccine was well tolerated clinically and remained immunogenic across all stages examined, early-gestation and pre-mating vaccination showed more favorable profiles than vaccination during mid- and late gestation. Pre-mating vaccination was subsequently shown to be well tolerated at a ten-fold overdose and to provide substantial protection against virulent genotype II challenge.

The reproductive findings and ASFV DNA detection patterns showed a broadly consistent stage-related trend. Abortion occurred only in vaccinated animals in the mid- and late-gestation groups, and late-gestation vaccination was additionally associated with reduced numbers of live-born and weaned piglets. In contrast, litter outcomes after pre-mating and early-gestation vaccination were comparable with those of controls. A similar pattern was observed in ASFV DNA detection. Following pre-mating and early-gestation vaccination, no ASFV DNA was detected in the available reproductive or offspring-associated samples. In T2, ASFV DNA detection was limited to colostrum and pooled neonatal tissues, whereas umbilical cord samples and available dead fetal tissues were negative. In contrast, T3 showed more frequent and widespread ASFV DNA detection, including in colostrum, umbilical cords, dead fetal tissues, neonatal blood, and neonatal tissues. These findings indicate that primary vaccination during mid- and late gestation was associated with less favorable reproductive outcomes and increased detection of ASFV DNA in offspring-associated samples, particularly following late-gestation vaccination.

Previous studies have demonstrated that ASFV can infect reproductive tissues and reach fetal compartments, although these findings do not necessarily indicate a specific reproductive tissue tropism [16,17]. Schlafer and Mebus (1987) recovered infectious ASFV from placental and fetal tissues but suggested that reproductive failure was more closely associated with the systemic effects of maternal infection than with direct fetal damage [16]. Lohse et al. (2022) detected ASFV DNA in fetal tissues following experimental infection of late-gestation sows with virulent genotype II ASFV, although transplacental transmission appeared limited [29]. ASFV DNA has also been detected in offspring-associated samples following vaccination of pregnant sows with some live-attenuated ASFV strains [20,22]. Consistent with these reports, the detection of ASFV DNA in fetal and neonatal samples in the present study provides molecular evidence supporting possible vertical transmission of the vaccine virus, particularly in T3, whereas the limited detection in T2 provides weaker evidence. In contrast, no molecular evidence of vertical transmission was detected in the available samples following early-gestation or pre-mating vaccination. The higher frequency of ASFV DNA detection in T3 may reflect gestation-stage-dependent differences in maternal immune responses affecting vaccine virus replication and clearance, although the shorter interval between vaccination and sample collection at farrowing may also have contributed to this finding.

Reproductive outcomes following ASF LAV vaccination have varied among vaccine candidates. ASFV-G-ΔI177L was associated with marked adverse reproductive outcomes in pregnant sows [20], whereas ASFV-G-ΔI177L/ΔLVR was clinically well tolerated in advanced pregnancy despite detection of ASFV DNA in offspring [21]. Favorable reproductive outcomes were reported for HLJ/18-7GD in a large field study including sows vaccinated during early, mid-, and late gestation [22], while ASFV-MEC-01 showed favorable maternal and fetal safety findings during pregnancy under the conditions evaluated [23]. Thus, molecular evidence suggesting fetal or offspring exposure to vaccine virus has been reported for several replication-competent ASF LAVs, although the associated reproductive and clinical outcomes have varied among vaccine strains [20–23]. Notably, AVAC ASF

LIVE and HLJ/18-7GD share deletions in six MGF360/505 genes, although HLJ/18-7GD additionally lacks EP402R and is derived from a different parental strain [12,22,24]. Both studies reported ASFV DNA detection in offspring-associated samples following vaccination during mid- or late gestation. However, unlike the present study, HLJ/18-7GD was not associated with significant impairment of reproductive performance [22]. These findings suggest that reproductive safety may vary according to vaccine strain, genetic background, additional gene deletions, and study conditions, highlighting the need to evaluate each vaccine candidate and vaccination schedule individually.

Although both pre-mating and early-gestation vaccination showed favorable profiles, vaccination before conception may be preferable for routine use because it avoids vaccine administration during pregnancy and may provide an additional margin of safety. This interpretation is consistent with the proposed use of vaccination before mating/insemination to mitigate potential safety concerns associated with vaccination during pregnancy [30]. Recently, a preprint using ASFV-G-ΔI177L reported more favorable outcomes in animals immunized before pregnancy and subsequently vaccinated during gestation than in naïve animals first vaccinated during pregnancy [31].

The ten-fold overdose study further supported the safety of pre-mating vaccination, with no clinically important adverse reactions observed during 35 days of follow-up apart from mild transient fever in one sow. Importantly, vaccination before mating elicited consistent antibody responses and provided a high level of protection against virulent genotype II ASFV challenge. Seroconversion occurred in most vaccinated animals by 14 dpv and in all animals thereafter, consistent with previous observations for AVAC ASF LIVE in growing pigs [12]. Following challenge, survival reached 80% in vaccinated sows and 100% in vaccinated replacement gilts, whereas all controls developed acute ASF and died. As the animals in the challenge study were not mated, these findings demonstrate protection at 28 days post-vaccination but do not establish the duration of protective immunity throughout subsequent pregnancy, farrowing, or lactation. The generally mild clinical responses and low-level, intermittent ASFV DNA detection among survivors further supported effective control of challenge-virus replication. Protection was not complete, however, as one vaccinated sow developed acute ASF and died at 10 dpc. Detection of challenge virus, but not vaccine virus, by DIVA PCR in this animal suggests breakthrough infection rather than vaccine-associated disease.

The breadth of protection observed here should be interpreted in the context of the challenge virus used. AVAC ASF LIVE is derived from genotype II ASFV, and efficacy in this study was assessed against a virulent genotype II isolate. Experimental evidence indicates that a gene-deleted genotype II LAV can provide partial cross-protection against genotype I but no protection against genotype IX [32], suggesting that cross-protection may be limited against genetically divergent ASFVs. This limitation is particularly relevant in Vietnam, where recombinant genotype I/II ASFVs were first identified in 2023 [33]. Genotype II vaccine strains, including ASFV-G-ΔMGF-DMAC, failed to provide complete protection against a Vietnamese recombinant genotype I/II isolate [34], while subsequent epidemiological investigations documented an increasing proportion of recombinant genotype I/II viruses [35]. Thus, the protective efficacy demonstrated in the present study is specific to the genotype II challenge strain and should not be extrapolated to recombinant genotype I/II viruses. These findings underscore the importance of considering the locally circulating ASFV strains when interpreting vaccine efficacy and planning vaccination strategies, as previously recommended [9].

A major strength of this study is the evaluation of AVAC ASF LIVE across multiple reproductive stages, enabling direct comparison of stage-specific reproductive and virological outcomes and supporting pre-mating vaccination as a promising approach for further

evaluation in breeding herds. Several limitations should, however, be acknowledged. The small group sizes limited the statistical power to detect modest but clinically meaningful differences in reproductive outcomes, and isolated events such as abortion should therefore be interpreted cautiously. The study was conducted under controlled experimental conditions and was limited to a single reproductive cycle, limiting assessment of longer-term reproductive safety and repeat-use effects. Larger studies under commercial conditions and across multiple parities are needed to confirm reproductive safety and protective efficacy. Further investigations should characterize maternally derived ASFV-specific immunity in offspring and its implications for the timing of piglet vaccination.

5. Conclusions

Among the reproductive stages evaluated, early-gestation and pre-mating vaccination showed favorable reproductive outcomes and no detectable ASFV DNA in offspring-associated samples, whereas vaccination during later gestation was associated with less favorable reproductive outcomes and more frequent ASFV DNA detection in offspring. Pre-mating vaccination was also well tolerated at a ten-fold overdose and provided substantial protection against virulent genotype II challenge. These findings suggest that pre-mating vaccination may represent a promising strategy for ASF prevention in breeding female pigs, warranting further field evaluation to confirm its reproductive safety and protective efficacy across multiple parities under commercial production conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/vaccines14121030/s1, Table S1: Reproductive performance of breeding female pigs following vaccination at different reproductive stages; Table S2: 95% confidence intervals for reproductive outcomes following vaccination at different reproductive stages.

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References

1. Dixon, L.K.; Chapman, D.A.G.; Netherton, C.L.; Upton, C. African Swine Fever Virus Replication and Genomics. *Virus Res.* **2013**, *173*, 3–14. [CrossRef] [PubMed]
2. International Committee on Taxonomy of Viruses (ICTV). *Master Species List 41: 2025–2026 ICTV Taxonomy Release*; ICTV: Alice Springs, Australia, 2026.

3. You, S.; Liu, T.; Zhang, M.; Zhao, X.; Dong, Y.; Wu, B.; Wang, Y.; Li, J.; Wei, X.; Shi, B.; et al. African Swine Fever Outbreaks in China Led to Gross Domestic Product and Economic Losses. *Nat. Food* **2021**, *2*, 802–808. [CrossRef] [PubMed]
4. Mighell, E.; Ward, M.P. African Swine Fever Spread across Asia, 2018–2019. *Transbound. Emerg. Dis.* **2021**, *68*, 2722–2732. [CrossRef] [PubMed]
5. Ito, S.; Kawaguchi, N.; Bosch, J.; Aguilar-Vega, C.; Sanchez-Vizcaino, J.M. What Can We Learn from the Five-Year African Swine Fever Epidemic in Asia? *Front. Vet. Sci.* **2023**, *10*, 1273417. [CrossRef] [PubMed]
6. Dixon, L.K.; Stahl, K.; Jori, F.; Vial, L.; Pfeiffer, D.U. African Swine Fever Epidemiology and Control. *Annu. Rev. Anim. Biosci.* **2020**, *8*, 221–246. [CrossRef] [PubMed]
7. World Organisation for Animal Health (WOAH). ASF: WOAH Stresses the Importance of Using High-Quality Vaccines That Comply with Newly Adopted Standard. 2025. Available online: https://www.woah.org/en/asf-woah-stresses-the-importance-of-using-high-quality-vaccines-that-comply-with-newly-adopted-standard/ (accessed on 10 September 2026).
8. Food and Agriculture Organization of the United Nations (FAO). *Considerations for Competent Authorities and Livestock Industries on Use of African Swine Fever Vaccines (Genotype II) to Enhance Disease Control*; FAO Animal Production and Health Position Paper; FAO: Rome, Italy, 2026.
9. Diep, N.V.; Doanh, P.N. Live Attenuated Vaccines for African Swine Fever: Perspectives on Their Use and Strategic Directions. *Transbound. Emerg. Dis.* **2026**, *2026*, e2020820. [CrossRef] [PubMed]
10. Zhang, H.; Zhao, S.; Zhang, H.; Qin, Z.; Shan, H.; Cai, X. Vaccines for African Swine Fever: An Update. *Front. Microbiol.* **2023**, *14*, 1139494. [CrossRef] [PubMed]
11. Wang, L.; Shi, J. African Swine Fever: Vaccine Advancement and Major Gaps. *Microorganisms* **2026**, *14*, 706. [CrossRef] [PubMed]
12. Diep, N.V.; Ngoc, N.T.; Duc, N.V.; Dang, V.X.; Tiep, T.N.; Quy, C.T.; Tham, B.T.; Doanh, P.N. Safety and Efficacy Profiles of the Live Attenuated Vaccine AVAC ASF LIVE for Preventing African Swine Fever in Pigs. *Transbound. Emerg. Dis.* **2025**, *2025*, 8623876. [CrossRef] [PubMed]
13. Tran, X.H.; Phuong, L.T.T.; Huy, N.Q.; Thuy, D.T.; Nguyen, V.D.; Quang, P.H.; Ngon, Q.V.; Rai, A.; Gay, C.G.; Gladue, D.P.; et al. Evaluation of the Safety Profile of the ASFV Vaccine Candidate ASFV-G-ΔI177L. *Viruses* **2022**, *14*, 896. [CrossRef] [PubMed]
14. Soltesz, A.; Hunyadi, A.B.; Kusza, S.; Balogh, P. Survival Analysis of Sow Longevity and Lifetime Reproductive Performance—Review. *Acta Agrar. Debr.* **2016**, *70*, 75–80. [CrossRef] [PubMed]
15. Patterson, J.; Foxcroft, G. Gilt Management for Fertility and Longevity. *Animals* **2019**, *9*, 434. [CrossRef]
16. Schlafer, D.H.; Mebus, C.A. Abortion in Sows Experimentally Infected with African Swine Fever Virus: Pathogenesis Studies. *Am. J. Vet. Res.* **1987**, *48*, 246–254. [CrossRef]
17. Sehl-Ewert, J.; Friedrichs, V.; Blome, S. Infection of the Conceptus with African Swine Fever Virus Following Artificial Insemination in Sows: A Pathological Study. *Transbound. Emerg. Dis.* **2026**, *2026*, 7164197. [CrossRef] [PubMed]
18. Yang, F.; Zheng, Q.; Jin, L. Dynamic Function and Composition Changes of Immune Cells during Normal and Pathological Pregnancy at the Maternal-Fetal Interface. *Front. Immunol.* **2019**, *10*, 2317. [CrossRef] [PubMed]
19. Tizard, I.R. *Vaccines for Veterinarians*, 1st ed.; Elsevier: Amsterdam, The Netherlands, 2021.
20. van den Born, E.; Olasz, F.; Mészáros, I.; Gölti, E.; Oláh, B.; Joshi, J.; van Kilsdonk, E.; Segers, R.; Zádori, Z. African Swine Fever Virus Vaccine Strain ASFV-G-ΔI177L Reverts to Virulence and Negatively Affects Reproductive Performance. *npj Vaccines* **2025**, *10*, 46. [CrossRef] [PubMed]
21. Lee, S.J.; Kim, Y.; Choi, S.A.; Ahn, K.S.; Lee, S.Y.; Zheng, X.; Kim, D.S.; Kim, W.; Shin, Y.; Kim, S.J.; et al. Safety of Live Attenuated ASFV-G-ΔI177L/ΔLVR Vaccination in Sows with Advanced Pregnancies. *Transbound. Emerg. Dis.* **2025**, *2025*, 8007143. [CrossRef] [PubMed]
22. Liu, J.; Ding, L.; Chen, W.; Zhao, D.; Liu, W.; Liu, Y.; Li, F.; Liu, R.; Huo, H.; Zhu, Y.; et al. Live-Attenuated African Swine Fever Vaccine HLJ/18-7GD Is Safe in Pregnant Sows with No Impact on Reproductive Performance or Offspring Health at a Field Farm. *Emerg. Microbes Infect.* **2026**, *15*, 2614713. [CrossRef] [PubMed]
23. Tran, L.H.; Bui, A.N.; Kwon, H.I.; Dao, T.D.; Weerawardhana, A.; Tran, T.M.; Vu, H.T.; Nguyen, Q.D.; Gamage, N.; Kim, M.H.; et al. Evaluation of the Vaccine Candidate ASFV-MEC-01: Safety, Efficacy, Transmission Dynamics, and Assessment of Reversion to Virulence. *Emerg. Microbes Infect.* **2026**, *15*, 2608395. [CrossRef] [PubMed]
24. O'Donnell, V.; Holinka, L.G.; Gladue, D.P.; Sanford, B.; Krug, P.W.; Lu, X.; Arzt, J.; Reese, B.; Carrillo, C.; Risatti, G.R.; et al. African Swine Fever Virus Georgia Isolate Harboring Deletions of MGF360 and MGF505 Genes Is Attenuated in Swine and Confers Protection against Challenge with Virulent Parental Virus. *J. Virol.* **2015**, *89*, 6048–6056. [CrossRef] [PubMed]
25. Marois, C.; Dory, D.; Fablet, C.; Madec, F.; Kobisch, M. Development of a Quantitative Real-Time TaqMan PCR Assay for Determination of the Minimal Dose of *Mycoplasma hyopneumoniae* Strain 116 Required to Induce Pneumonia in SPF Pigs. *J. Appl. Microbiol.* **2010**, *108*, 1523–1533. [CrossRef] [PubMed]
26. Turni, C.; Pyke, M.; Blackall, P.J. Validation of a real-time PCR for *Haemophilus parasuis*. *J. Appl. Microbiol.* **2010**, *108*, 1323–1331. [CrossRef] [PubMed]

27. Velazquez-Salinas, L.; Ramirez-Medina, E.; Rai, A.; Pruitt, S.; Vuono, E.A.; Espinoza, N.; Gladue, D.P.; Borca, M.V. Development Real-Time PCR Assays to Genetically Differentiate Vaccinated Pigs From Infected Pigs with the Eurasian Strain of African Swine Fever Virus. *Front. Vet. Sci.* **2021**, *8*, 768869. [CrossRef] [PubMed]
28. World Organisation for Animal Health (WOAH). *Addressing African Swine Fever: Protocols and Guidelines for Laboratory Diagnosis*; WOAH: Paris, France, 2024.
29. Lohse, L.; Nielsen, J.; Uttenthal, Å.; Olesen, A.S.; Strandbygaard, B.; Rasmussen, T.B.; Belsham, G.J.; Bøtner, A. Experimental Infections of Pigs with African Swine Fever Virus (Genotype II); Studies in Young Animals and Pregnant Sows. *Viruses* **2022**, *14*, 1387. [CrossRef] [PubMed]
30. Blome, S.; Friedrichs, V.; Schäfer, A.; Beer, M. Benefit–Risk Considerations for African Swine Fever Virus Live Attenuated Vaccines. *npj Vaccines* **2025**, *10*, 147. [CrossRef] [PubMed]
31. Friedrichs, V.; Schäfer, A.; Mehinagic, K.; Holzum, T.; Hrabal, I.; Lazar, A.; Blohm, U.; Viaplana, E.; Balasch, M.; Schröder, C.; et al. Timing and Immune Status Shape the Safety of ASFV-G-ΔI177L Vaccination in Pregnant Sows. *preprint* **2026**. [CrossRef] [PubMed]
32. Rathakrishnan, A.; Hemmink, J.D.; Petrovan, V.; Reis, A.L.; Dixon, L.K. Gene-Modified Genotype II Live Attenuated African Swine Fever Virus Induces Cross-Protection against Genotype I but Not against Genotype IX. *Emerg. Microbes Infect.* **2025**, *14*, 2505645. [CrossRef] [PubMed]
33. Le, V.P.; Nguyen, V.T.; Le, T.B.; Mai, N.T.A.; Nguyen, V.D.; Than, T.T.; Lai, T.N.H.; Cho, K.H.; Hong, S.K.; Kim, Y.H.; et al. Detection of Recombinant African Swine Fever Virus Strains of p72 Genotypes I and II in Domestic Pigs, Vietnam, **2023**. *Emerg. Infect. Dis.* **2024**, *30*, 991–994. [CrossRef] [PubMed]
34. Diep, N.V.; Duc, N.V.; Ngoc, N.T.; Dang, V.X.; Tiep, T.N.; Nguyen, V.D.; Than, T.T.; Maydaniuk, D.; Goonawardene, K.; Ambagala, A.; et al. Genotype II Live-Attenuated ASFV Vaccine Strains Unable to Completely Protect Pigs against the Emerging Recombinant ASFV Genotype I/II Strain in Vietnam. *Vaccines* **2024**, *12*, 1114. [CrossRef] [PubMed]
35. Nguyen, T.V.H.; Kim, Y.H.; Nguyen, V.D.; Tran, T.C.G.; Vu, N.D.; Vu, T.T.H.; Nguyen, V.T.; Kim, D.Y.; Cho, K.H.; Hong, S.K.; et al. Rapid Spread of Recombinant African Swine Fever Virus Genotypes I and II, Vietnam, 2023–2024. *Emerg. Infect. Dis.* **2026**, *32*, 649–652. [CrossRef] [PubMed]

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