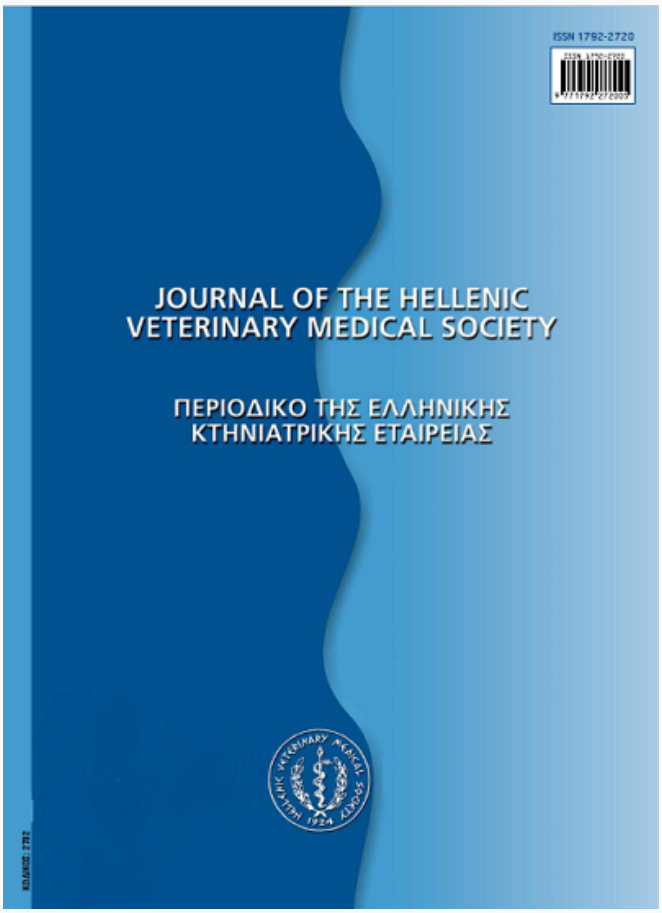
Journal of the Hellenic Veterinary Medical Society

Vol 62, No 3 (2011)



Impact of a porcine reproductive and respiratory syndrome inactivated vaccine on the health and semen traits of boars

V. G. PAPATSIROS (Β. ΠΑΠΑΤΣΙΡΟΣ), C. ALEXOPOULOS (Κ. ΑΛΕΞΟΠΟΥΛΟΣ), C. BOSCOS (Κ. ΜΠΟΣΚΟΣ), S. C. KYRIAKIS (Σ.Κ. ΚΥΡΙΑΚΗΣ)

doi: 10.12681/jhvms.14853

JOURNAL OF THE HELLENIC VETERINARY MEDICAL SOCIETY 2011, 62(3): 221-228
ΠΕΡΙΟΔΙΚΟ ΤΗΣ ΕΛΛΗΝΙΚΗΣ ΚΤΗΝΙΑΤΡΙΚΗΣ ΕΤΑΙΡΕΙΑΣ 2011, 62(3): 221-228

Original article
Ερευνητική

Impact of a porcine reproductive and respiratory syndrome inactivated vaccine on the health and semen traits of boars

Papatsiros V. G.[1*] DVM, PhD, **Alexopoulos C.**[†2] DVM, PhD, DipECAR, **Boscos C.**[2] DVM, PhD, DipECAR, **Kyriakis S. C.**[†3] DVM, PhD, DipECPHM, DipECAR

[1] *Clinical Veterinary Medicine Department, School of Veterinary Medicine, University of Thessaly, 431 00, Karditsa, Greece*

[2] *Clinic of Farm Animal Medicine, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 541 24, Thessaloniki, Greece*

[3] *Foundation of Biomedical Research, Academy of Athens, 115 27, Athens, Greece.*

Η επίδραση ενός νεκρού εμβολίου κατά του αναπαραγωγικού και αναπνευστικού συνδρόμου του χοίρου στην υγεία και στα ποσοτικά και ποιοτικά χαρακτηριστικά του σπέρματος των κάπρων

Β. Γ. Παπατσίρος[1] DVM, PhD, **Κ. Αλεξόπουλος**[†2] DVM, PhD, DipECAR, **Κ. Μπόσκος**[2] DVM, PhD, DipECAR, **Σ. Κ. Κυριάκης**[†3] DVM, PhD, DipECPHM, DipECAR

[1] *Παθολογική Κλινική, Τμήμα Κτηνιατρικής, Πανεπιστήμιο Θεσσαλίας, 431 00 Καρδίτσα*

[2] *Κλινική Παραγωγικών Ζώων, Κτηνιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, 541 24 Θεσσαλονίκη*

[3] *Ίδρυμα Ιατροβιολογικών Ερευνών, Ακαδημία Αθηνών, 115 27 Αθήνα*

ABSTRACT. The aim of this study was to investigate the effects of a commercial European Porcine Reproductive and Respiratory Syndrome (PRRS) - inactivated vaccine on health status, semen characteristics and semen fertilizing capacity *in vivo* of boars. In a farrow to finish farm that suffered from chronic course of PRRS, 7 donor boars (1.1–2.2 years old) were initially twice vaccinated, with a 4 weeks interval. At the same time, all gilts / sows of the herd were, also, vaccinated. Boars were monitored for abnormal clinical signs 24 h prior to 15 days after each vaccination. Ejaculates were collected 24 h prior, 24 h after and 15 days after each vaccination and the semen characteristics were evaluated. A total of 305 sows were inseminated twice with the collected semen 2 weeks prior up to 6 weeks after the 1st vaccination. No systemic clinical signs and significant differences in semen characteristics, except of sperm viability, were noticed. After the 1st vaccination, sperm viability increased, but this was probably due to the increase of the age of 7 boars during the trial and not due to the vaccination. All semen characteristics were decreased 24h after each vaccination, but they were not lower than the value of accepted criteria semen quality. No change was noticed in sow's fertility parameters, apart from the farrowing rate, that was not, however, of clinical importance. In conclusion, the use of a PRRSV - inactivated vaccine in boars is safe and has not negative effects on health status and their semen characteristics neither on fertilizing capacity *in vivo*.

Keywords: PRRS, vaccine, semen, fertility, boar

Correspondence: V. G. Papatsiros
Department of Medicine, Faculty of Veterinary Medicine, University of Thessaly
224, Trikalon str., 431 00, Karditsa, Greece
Tel.: + 30 24410 66012, Mobile: +30 6977 145703, e-mail: vpapatsiros@vet.uth.gr

Αλληλογραφία: Β. Γ. Παπατσίρος
Παθολογική Κλινική, Τμήμα Κτηνιατρικής, Πανεπιστήμιο Θεσσαλίας
Τρικάλων 224, 431 00 Καρδίτσα
Τηλ.: 24410 66012, Κινητό: 6977 145703, e-mail: vpapatsiros@vet.uth.gr

Submission date: 20.07.2011
Approval date: 26.09.2011

Ημερομηνία υποβολής: 20.07.2011
Ημερομηνία εγκρίσεως: 26.09.2011

ΠΕΡΙΛΗΨΗ. Σκοπός της παρούσας πειραματικής έρευνας ήταν η διερεύνηση, υπό συνθήκες εκτροφής, της *in vivo* επίδρασης του εμβολιασμού των κάπρων με το νεκρό εμβόλιο κατά του Αναπαραγωγικού και Αναπνευστικού Συνδρόμου του Χοίρου (ΑΑΣΧ) στην υγεία, στα ποσοτικά και ποιοτικά χαρακτηριστικά του σπέρματος των κάπρων, καθώς και στη γονιμοποιητική τους ικανότητα. Ο πειραματισμός πραγματοποιήθηκε σε μία «κάθετη» χοιροτροφική εκμετάλλευση, δυναμικότητας 900 συών, στην οποία εκδηλωνόταν η χρόνια και η ενδημική μορφή του ΑΑΣΧ. Η εκτροφή διέθετε εργαστήριο επεξεργασίας και συντήρησης σπέρματος. Αρχικά, οι επτά σπερματοδότες κάπροι της εκτροφής (ηλικίας 1 - 2 ετών) εμβολιάστηκαν με νεκρό εμβόλιο δύο φορές σε διάστημα 4 εβδομάδων. Οι εμβολιασμοί έναντι των υπολοίπων νοσημάτων απείχαν τουλάχιστον 3 εβδομάδες από το συγκεκριμένο εμβολιακό σχήμα. Στη συνέχεια, ακολούθησαν επαναληπτικοί εμβολιασμοί, ανά εξάμηνο. Επίσης, όλες οι σύες της εκτροφής εμβολιάστηκαν με το ίδιο εμβόλιο και για το ίδιο χρονικό διάστημα. Στους εμβολιασμένους κάπρους πραγματοποιούνταν καθημερινά κλινική εξέταση έως και 15 ημέρες μετά από κάθε εμβολιασμό. Τα εκσπερματίσματα που συλλέχθηκαν 24 ώρες πριν, καθώς και 24 ώρες και 15 ημέρες μετά, αντίστοιχα, από κάθε εμβολιασμό, αξιολογήθηκαν με βάση τον προσδιορισμό του όγκου, της ζωτικότητας και της πυκνότητας του σπέρματος. Η εκτίμηση της γονιμοποιητικής ικανότητας του σπέρματος έγινε με βάση την in vivo χρησιμοποίηση των δόσεων του σπέρματος, που προέκυψαν 2 εβδομάδες πριν έως και 6 εβδομάδες μετά από τον πρώτο εμβολιασμό των κάπρων. Με τον τρόπο αυτό, εφαρμόστηκε τεχνητή σπερματέγχυση συνολικά σε 305 σύες. Η σπερματοληψία, η εκτίμηση των χαρακτηριστικών του σπέρματος, ο καθορισμός του αριθμού των δόσεων, η επεξεργασία και η συντήρηση του σπέρματος έγινε σύμφωνα με το πρωτόκολλο που εφάρμοζε η εκτροφή. Κατά τη διάρκεια του πειραματισμού, δεν παρατηρήθηκαν κλινικά συμπτώματα, τοπικές αντιδράσεις στο σημείο της έγχυσης του εμβολίου ή άλλες παρενέργειες στους εμβολιασμένους κάπρους. Από την ανάλυση των αποτελεσμάτων προέκυψε ότι ο εμβολιασμός των κάπρων δεν επηρέασε τον όγκο, την κινητικότητα και την πυκνότητα του σπέρματος. Αντίθετα, επηρεάστηκε η ζωτικότητα του σπέρματος, οι τιμές της οποίας αυξήθηκαν στους επαναληπτικούς εμβολιασμούς, πιθανότατα λόγω της προοδευτικής αύξησης της ηλικίας των κάπρων παρά λόγω του εμβολιασμού. Επίσης, αν και παρατηρήθηκε μείωση στα χαρακτηριστικά του σπέρματος 24 ώρες μετά από κάθε εμβολιασμό, οι τιμές τους κυμάνθηκαν σε φυσιολογικά επίπεδα. Τέλος, όσον αφορά την *in vivo* επίδραση του εμβολιασμού των κάπρων στη γονιμοποιητική ικανότητα του σπέρματός τους, διαπιστώθηκε ότι δεν επηρεάστηκε αρνητικά κατά τη διάρκεια ενός πλήρους σπερματογονικού κύκλου. Το μέγεθος της τοκετοομάδας δεν παρουσίασε σημαντικές διαφορές μεταξύ των διαφόρων πειραματικών ομάδων των συών, ενώ το ποσοστό τοκετών αυξήθηκε σημαντικά στην ομάδα των συών που γονιμοποιήθηκαν με σπέρμα που συλλέχθηκε 1-2 εβδομάδες μετά τον πρώτο εμβολιασμό των κάπρων και μειώθηκε στην ομάδα των συών που γονιμοποιήθηκαν με σπέρμα που συλλέχθηκε 5-6 εβδομάδες μετά τον πρώτο εμβολιασμό. Συμπερασματικά, ο εμβολιασμός των κάπρων παρέχει απόλυτη ασφάλεια, αφού δεν προκαλεί παρενέργειες και δεν επηρεάζει αρνητικά τα χαρακτηριστικά και τη γονιμοποιητική ικανότητα του σπέρματος.

Λέξεις ευρετηρίασης: Αναπαραγωγικό και Αναπνευστικό Σύνδρομο του χοίρου, εμβόλιο, σπέρμα, γονιμοποιητική ικανότητα, κάπρος

Introduction

Porcine reproductive and respiratory syndrome (PRRS) has caused tremendous economic losses in the global swine industry since the early 1990's. The etiologic agent is an enveloped, RNA virus, named PRRSV (Meulenberg et al. 1997), which is a member of the Arteriviridae family (Cavanagh 1997).

Clinical signs of PRRS include anorexia, lethargy, and respiratory signs, moderate pyrexia, recumbency and in addition may lack libido (Yaeger et al. 1993; Prieto et al. 1996b). A significant decrease of sperm motility and morphological abnormalities (e.g. decrease of number of spermatozoa with intact acrosome) were noticed 2-10 weeks after PRRSV infection sperm motility (Yaeger et al. 1993, Prieto et al. 1996b, Christopher-Henninigs et al. 1997). Although PRRSV can be transmitted through the semen and can be a significant portal entry into susceptible herds (Yaeger et al. 1993, Swenson et al. 1994), it is not clear up today the impact of PRRS viremia on boars at the time of conception (Yaeger et al. 1993, Lager et al. 1996, Prieto et al. 1996a,c).

In the literature, there is little in formations on PRRSV vaccination of boars. Most of the studies are reffered to the use of them, used modified live vaccines (MLV), showing that the use of MLV in boars is under discussion, because it causes clinical signs (anorexia, lethargy, recumbency, fever) and has negative effects on semen characteristics, such as reduction of semen volume and sperm viability (Vilaca et al. 2001). Additionally, it has been shown that MLV virus can persist in boars and can be transmitted through semen (Christopher-Hennings et al. 1997).

At the present, only two studies have been published regarding the use of inactivated vaccines in boars, one with vaccine of American strain (Swenson et al. 1995) and one with the European strain (Nielsen et al. 1997) of PRRS. Swenson et al. (1995) indicated

that the vaccination of boars with inactivated vaccine does not cause clinical signs and may reduce or prevent seminal shedding. On the contrary, Nielsen et al. (1997) using inactivated vaccine, observed a moderate to considerable swelling at the injection-site and no changes in viremia and shedding of virus in semen. However, none of them investigated the effects of inactivated vaccine on semen characteristics and semen fertilizing capacity *in vivo*.

In the present field study, the aim was to investigate the effects of vaccination with a commercial European PRRSV-inactivated vaccine after 18 month-use of donor boars on health status, semen characteristics and semen fertilizing capacity *in vivo*. It should be noted that this study is the first report regarding the testing of the commercial inactivated "PROGRESSIS®" vaccine in boars.

Materials and Methods

Experimental material

The commercial inactivated "PROGRESSIS®" vaccine (Merial, SAS), based on the European P120 strain, was used. The vaccine dose contains $\geq 10^{2,5}$ IF units and is suspended in 2 ml of an oily adjuvant (hydrogenated polyisorbutene is the oily part of the emulsion of mineral oil in water) for intramuscular injection behind the ear.

Trial farm

The trial has been performed in a commercial all-in, all-out farrow-to-finish farm with a capacity of 900 sows located in Katerini, Macedonia, Greece. A grandparent nucleus of 70 sows was kept in the farm for producing own gilts and these animals were separately housed, but in the same premises such as a commercial herd. The farm facilities included 4 farrowing houses, 5 flat-deck units, 6 growing houses, 6 finishing houses, 4 mating-pregnancy (dry period) stables, 1 breeding stock house, a feed mill and an artificial insemination (AI) laboratory. Records in the farm were kept electronically.

Seven healthy crossbred adult boars (1-2 years old) of the same genetic background were included in this study. All boars were housed in individual pens of the mating-pregnancy building under the same environmental, feeding and management conditions. Semen collection was performed one to two times per week according to the routine programme of the trial farm.

An Artificial Insemination (AI) programme with raw semen was applied by the trial farm and sows were inseminated twice with fresh semen from the same boar. Semen collection, dilution and storage were performed in the farm (system "Do-it-yourself AI"). The collected semen was diluted with a commercial BTS (Beltsville Thaw Solution, Androhep® by Minitube International) extender to a concentration of approximately 30 million sperm/ml. Each gilt/sow was inseminated twice 12 and 24 h after the detection of oestrus by a teaser boar.

All gilts / sows of the farm were vaccinated against Aujeszky's disease (AD), swine influenza (SI), parvovirus infection, atrophic rhinitis (AR), erysipelas, *Escherichia coli* and *Clostridium perfringens* infections (*type A* and *C*). All boars were vaccinated every 6 months against erysipelas, AD and SI, fattening pigs against AD and SI and weaners against *Mycoplasma hyopneumoniae*. For the antiparasitic control, all breeding females were treated with a single ivermectin injection 14 days prior to each farrowing, while the boars treated similarly twice a year. The feed provided to the animals was self-prepared, mainly consisted of based corn/barley/wheat–soya meal, depending on the season.

Farm history

The farm had suffered an acute PRRSV infection 5 years prior to the initiation of the trial. Since then, the herd had been infected with PRRSV for some years and had never been vaccinated before against PRRSV. For at least one-year prior the initiation of the trial, the farm was diagnosed PRRS-positive, based on clinical signs (low reproductive performance as was evidenced by increased returns to oestrus, small litters, weak piglets and increased piglet mortality), serology examination of blood samples and detection of viral RNA by PCR from fetuses and newborn piglets. In addition, blood samples of sows were examined for antibodies against a European PRRSV by using indirect immunofluorescence assay in US- or EU-type PRRSV-infected MA104 cells. It was shown that the circulating strain in the farm was a European strain.

The management, vaccination status, nutrient specification and feeding schedule of the farm remained the same during the pre-trial and trial period.

Experimental design

Primary vaccination of all boars was performed by

administering (intramuscularly behind the ear) 2 doses of PROGRESSIS®, 3-4 weeks apart. This 1st vaccination was separated by at least 3 weeks from other boars' vaccinations and all boars were boostered twice per year, for a period of 18 months.

All gilts / sows of the herd were primarily subjected to the first vaccination as previously described, except those being 1 week prior - to 2 weeks post - service. The skipped females were subjected to primary vaccination, starting, however, 3 weeks later. All previously vaccinated animals received a booster vaccination between 55 and 60 days of next gestation and, thereafter, at each gestation for a period of 18 months. The gilts were vaccinated twice prior to breeding (1st vaccination) and boostered in each pregnancy as previously described.

All procedures during this clinical study were carried out according to the Code of Practice for the Conduct of Clinical trials for Veterinary Medical Products and the animals were maintained in accordance with National and European animal Welfare requirements (OECD 1998, European Agency for the Evaluation of Medicinal Products 1999, FVE 2001). In addition, the present study was performed under license for experimenting on animals from the local Veterinary Administration Office (Katerini district Veterinary State Authority, License No 07/1855).

Records

Clinical observations

Boars were monitored for abnormal clinical signs 24 h prior to 15 days after each vaccination. The rectal temperature and bodyweight of the 7 boars were, also, monitored 24 h after each vaccination.

Evaluation of semen characteristics

Ejaculates were collected 24 h prior, 24 h after and 15 days after each vaccination. Semen evaluation was based on microscopic (sperm concentration, viability and motility) and macroscopic (semen volume) characteristics.

Semen collection, evaluation, dilution, estimation of insemination doses and storage of doses were performed in accordance with the protocol of the trial farm. Semen volume was determined by directly reading of the scale marked in ml from the vial of semen collection. Sperm density was determined by using the photometer (Accucell, Product code: 014434, Imv-Technologies) of the laboratory in the trial farm. Sperm viability and motility were estimated immediately after semen collection. Samples of raw semen 1:10 with a commercial BTS (Beltsville Thaw Solution, Androhep® by Minitube International) extender to a concentration of approximately 30 million sperm / ml, microscopic examination (100x) followed after diluting. Microscopic examination of semen slides stained with eosin-nigrosin was performed, in order to confirm the percentage of live spermatozoa. Sperm motility was evaluated by a microscope (Carl Zeiss, KF 2 ICS), equipped with a heated-plate (37°C). A semen sample (10μl) was applied in a pre-warmed slide and covered by a covership. At least ten different slides of each sample were examined by the same person.

Evaluation of semen fertilizing capacity *in vivo*

A total of 305 sows with semen doses from ejaculates that were collected 2 weeks prior up to 6 weeks after the 1st vaccination were inseminated. Farrowing data, including litter size (total born and live born piglets), were recorded for all the above animals.

Data analysis

The results were analyzed with the Statistical Analysis System (SAS) programme, which is installed in the central computer system of the Clinic of Productive Animals Medicine with the code 0084912001 (SAS 2002). The one-way Anova test for quantitative parameters was used; the Tukey's test was, also, used, in order to detect significant differences between the groups. The homogeneity of variance was checked using the test of Levene. In cases when the transformations of real prices did not bring about the expedient homogeneity of fluctuations, the test of Kruskall–Wallis was used. The Fisher's test was used for the qualitative parameters. In all cases, significance was taken at the level of importance $P<0.05$.

Results

Sides effects-Clinical observations

No systemic clinical signs and no local reaction on the area of the injection in all boars after the daily examination, 24 h prior to 15 days after each injection of all vaccinations were observed. Moreover, all boars performed normal appetite, behaviour and normal libido after each vaccination.

The average rectal temperature of each boar 24 h

after 4 vaccinations is shown in Table 1 and Table 2, respectively. The average rectal temperature was always higher than normal mean (38.6˚C) in all boars. No significant statistical difference between the 7 boars regarding the average rectal temperature was observed during the period of vaccination.

Semen characteristics

The mean values for semen characteristics (semen volume, sperm concentration, viability and motility) that resulted from the examination (microscopic and macroscopic) of the ejaculates collected at 24 h prior, 24 h after and 15 days after each vaccination, are shown in Tables 3 and 4.

No significant differences in semen volume and sperm motility prior and after the 1st vaccination were noticed. However, a significant decrease (P≤0,05) in sperm viability 24 h and 15 days after the 1st vaccination compared to the corresponding viability at 24 h prior the primary vaccination was observed. In addition, a significant reduction (P≤0,05) in sperm concentration of ejaculates collected 15 days after the primary vaccination compared to sperm concentration 24 h prior the primary vaccination was, also, observed.

Finally, it should be noted that, during this investigation, all boars were not used with the same frequency for the evaluation of the in vivo fertilizing capacity of sperm. Three out of seven boars were used with a lower frequency of semen collection than the expected frequency of 1-2 collections per week. More specifically, one boar was not used from the 2nd up to the 14th day and two other boars were not used from the 2nd up to the 7th and from the 7th up to the 14th day after the primary vaccination, respectively.

Table 1. Average rectal temperature 24 hours after 4 vaccinations (mean ± SD, n= number of observations)

Boar	Rectal temperature (˚C) (n=4 vaccinations)
1	39.1 ± 0.30 [a]
2	39.2 ± 0.31 [a]
3	39.5 ± 0.55 [a]
4	39.3 ± 0.62 [a]
5	39.1 ± 0.24 [a]
6	39.3 ± 0.17 [a]
7	39.3 ± 0.31 [a]
Total (n=7 boars)	39.3 ± 0.37 [a]

[a] Means in column with same superscripts do not differ significantly (P>0.05).

Table 2. Average rectal temperature 24 hours before the 1st and 24 h after each vaccination (mean ± SD)

	Rectal temperature (ΓC)				
Boar	**24 h before 1st vaccination**	**24 h after 1st vaccination**	**24 h after 2nd vaccination**	**24 h after 3rd vaccination**	**24 hafter 4th vaccination**
1	38.4 ± 0.16 [a]	39.1 ± 0.30 [a]	39.3 ± 0.10 [a]	39.1 ± 0.25 [a]	39.2 ± 0.23 [a]
2	38.4 ± 0.28 [a]	39.2 ± 0.31 [a]	39.1 ± 0.25 [a]	39.4 ± 0.35 [a]	39.2 ± 0.34 [a]
3	38.6 ± 0.10 [a]	39.5 ± 0.55 [a]	39.2 ± 0.10 [a]	39.1 ± 0.15 [a]	39.3 ± 0.45 [a]
4	38.5 ± 0.24 [a]	39.3 ± 0.62 [a]	39.1 ± 0.36 [a]	39.4 ± 0.27 [a]	39.2 ± 0.32 [a]
5	38.4 ± 0.30 [a]	39.1 ± 0.24 [a]	39.2 ± 0.30 [a]	39.3 ± 0.17 [a]	39.2 ± 0.22 [a]
6	38.5 ± 0.15 [a]	39.3 ± 0.17 [a]	39.2 ± 0.40 [a]	39.5 ± 0.47 [a]	39.1 ± 0.36 [a]
7	38.5 ± 0.20 [a]	39.3 ± 0.31 [a]	39.5 ± 0.10 [a]	39.2 ± 0.24 [a]	39.4 ± 0.14 [a]
Total (n=7 boars)	38.6 ± 0.24	39.3 ± 0.37	39.2 ± 0.23	39.1 ± 0.37	39.3 ± 0.37

[a] Means in column and row with same superscripts do not differ significantly (P>0.05).

Table 3. Semen characteristics (mean±SD) after each vaccination

Parameter	Vaccination			
	1st	2nd	3rd	4th
Semen volume (ml)	218.09 ± 46.56 [a]	242.86 ± 48.68 [a]	251.19 ± 48.92 [a]	244.05 ± 52.63 [a]
Sperm concentration (X 10^6/ml)	352.86 ± 57.44 [a]	324.29 ± 68.68 [a]	314.29 ± 74.53 [a]	296.19 ± 43.64 [a]
Sperm viability (%)	75.71 ± 6.22 [b]	80.37 ± 3.47 [a]	81.67 ± 4.30 [a]	81.19 ± 3.81 [a]
Sperm motility (%)	75.48 ± 6.85 [a]	74.76 ± 4.85 [a]	74.29 ± 4.80 [a]	75.95 ± 6.86 [a]

[a,b] Means in a row with different superscripts differ (P<0.05).

Table 4. Semen characteristics (mean±SD) at each time of semen evaluation for the total trial period

Vaccination	Time of vaccination	Semen volume (ml)	Sperm concentration (x10^6/ml)	Sperm viability (%)	Sperm motility (%)
1st	24 h prior	235.71 ± 69.00 [a]	334.29 ± 84.69 [a]	80.00 ± 5.77 [a]	78.57 ± 6.27 [a]
	24 h after	227.86 ± 55.67 [a]	325.71 ± 68.28 [a]	72.86 ± 8.09 [b]	72.86 ± 9.51 [a]
	15 days after	190.71 ± 36.8 [a]	398.57 ± 64.66 [a]	74.29 ± 7.32 [b]	74.29 ± 9.13 [a]
2nd	24 h prior	260.42 ± 59.26 [a]	342.86 ± 64.99 [a]	85.00 ± 4.08 [a]	76.43 ± 5.56 [a]
	24 h after	221.43 ± 46.61 [b]	300.00 ± 58.88 [b]	77.14 ± 4.88 [b]	71.43 ± 5.18 [a]
	15 days after	242.86 ± 53.45 [a,b]	330.00 ± 90.37 [a,b]	82.86 ± 2.67 [a,b]	76.84 ± 4.92 [a]
3rd	24 h prior	253.57 ± 60.26 [a]	324.29 ± 68.03 [a]	82.86 ± 4.88 [a]	77.02 ± 4.32 [a]
	24 h after	225.00 ± 47.87 [b]	295.71 ± 77.86 [a]	77.86 ± 4.32 [b]	70.71 ± 4.50 [b]
	15 days after	275.00 ± 45.64 [a]	322.86 ± 81.18 [a]	84.29 ± 3.45 [a]	75.71 ± 5.34 [a]
4th	24 h prior	254.14 ± 59.36 [a]	320.00 ± 62.98 [a]	84.57 ± 4.45 [a]	77.97 ± 5.01 [a]
	24 h after	214.29 ± 53.73 [b]	262.86 ± 35.46 [b]	76.92 ± 4.12 [b]	71.12 ± 8.52 [b]
	15 days after	264.29 ± 49.70 [a]	305.71 ± 42.76 [a]	82.14 ± 3.93 [a]	77.86 ± 6.99 [a]

[a,b,c] Means in a column with different superscripts differ (P<0.05).

Table 5. Sow fertility parameters indicating semen fertilizing capacity (mean ± SD, n= number of cases)

	Time of semen collection and AI regarding vaccination			
	1-2 weeks	1-2 weeks	3-4 weeks	5-6 weeks
SOWS	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Farrowing rate (%)	60/77 (77,9%[a,b,c])	71/83 (85,5%[a])	45/58 (77,6%[a,b,c])	63/87 (72,4%[b])
Litter size	11,45 ± 3,26 [a] (n=77)	11,85 ± 2,93 [a] (n=83)	11,75 ± 1,64 [a] (n=58)	12,05 ± 2,14 [a] (n=87)

[a,b,c] Means in a row with different superscripts differ (P<0.05).

Semen fertilizing capacity *in vivo*

Table 5 presents the farrowing rates and mean litter sizes for the four experimental groups. No significant differences between the four experimental groups were observed, except of the farrowing rate in Group 4, which was significantly lower than in Group 2. However, the above decrease of farrowing rate has no clinical importance, probably due to the season of AI (early summer).

Discussion

Safety is one of the major factors that determine vaccine usage. Vaccination continues to be the only safe, reliable and effective way to protect animals against the major infectious diseases. Nevertheless, the use of vaccines is not free of risk. Residual virulence and toxicity, allergic responses, disease in immunodeficient hosts and neurological complications associate with the use of vaccines (Tizard 2004). The absence of general or due to PRRS clinical signs and local reactions on the area of injection in all vaccinated boars used for this study leads to the conclusion that the tested inactivated vaccine is safe. Moreover, the increase of the rectal temperature that was observed in boars at the first 24h after each injection should probably be considered a normal response to the vaccination, as it is well-known that any vaccination induces stress to boars (Flowers 1997). Body temperature fluctuations should not be a measure of the clinical status of the animal, unless accompanied by other clinical findings (Houston and Radostits 2001).

Concerning the safety of the tested inactive vaccine against PRRS, the above findings of the present study are in agreement with the observations reported by Swenson et al. (1995). However, it contradicts with the results of a previous study (Nielsen et al. 1997), where a moderate to considerable swelling at the injection-site was observed. In addition, Vilaca et al. (2001,

2003) noticed that after the use of MLV, boars presented anorexia, lethargy, recumbency and rectal temperature above 39,5˚C for 4-5 days. Thus, the results of the present study, in combination with those referred to in the literature about vaccination of boars, confirm that the vaccination of boars against PRRS with an inactivated vaccine is safer than that with an MLV. Moreover, in our previous study (Papatsiros et al. 2006), it was indicated that the use of the same inactivated vaccine on gilts/sows of the same experimental farm was safe, since no adverse or side effects were observed. Furthermore, the vaccination of sows with "PROGRESSIS®" proved to reduce the negative effects of PRRSV on the breeding herd, especially as it concerns reproductive parameters and litter characteristics.

In addition, in similar studies with MLV, significant changes in the semen quality after the vaccination, such as a reduction in semen volume and sperm motility were observed (Christopher-Hennings et al. 1997, Vilaca et al. 2001, 2003). On the contrary, in this study, the mentioned semen characteristics were not influenced in vivo fertility parameters, which were remained within normal ranges. The semen quality was not influenced, as the values remained significantly higher than 70%, which is considered as the acceptable limit of the raw semen for artificial insemination (Flowers 1997). Moreover, it was, also, noticed that the finding that sperm motility, which is considered as a valuable measure for the evaluation of semen quality (Britt et al. 1999), was not influenced and remained in levels higher than 70%, led to the assumption that semen quality remained unaffected after vaccination. However, it is known that the semen fertilizing capacity decreases (reduction of farrowing rates and litter size) when sperm motility is lower than 60% (Flowers 1997).

The rapid spread and economic impact of PRRS have made it a frequent topic of research regarding its control. As with many other infectious diseases, the most effective means for control often depends on the use of vaccines. Regarding this option, there are currently several commercially available vaccines. These include MLV, as well as inactivated vaccines. However, the pig's immune response to PRRSV makes the development of an unquestionably safe and highly effective vaccine a formidable challenge. However, the results of the present study indicate that the boars' vaccination with the tested inactivated vaccine is safe. Furthermore, all boars can be regarded as normally fertile concerning the following parameters: semen volume, sperm concentration, motility and viability.

Taking into account how significant is the production and use of high quality semen for the global swine industry, the above results have an important financial impact (Leiding 2000). Regarding the biosecurity of swine, it seems that inactivated vaccines have important advantages compared to MLV, herds, since they do not induce shedding of the vaccine virus (Swenson et al. 1995), as it happens with MLV (Nielsen et al. 1997, Vilaca et al. 2001, 2003). Further studies are needed on boars vaccination with the same inactivated vaccine "PROGRESSIS®" and they should focus on the reduction of PRRSV shedding in semen.

Acknowledgements

This work has been supported by Merial SAS (Lyon, France) through the Research Committee of the Aristotle University of Thessaloniki (Code no: 20567).

REFERENCES

Britt JH, Almond GW, Flowers WL (1999). Diseases of the Reproductive System. In: Diseases of Swine. Straw BE, D'Allaire S, Mengeling WL, Taylor DJ, eds. 8th ed., Iowa State University Press, Ames, Iowa: pp. 898-911.

Cavanagh D (1997). Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch Virol 142: 629–633.

Christopher-Hennings J, Nelson EA, Nelson JK, and Benfield DA (1997). Effects of a modified-live virus vaccine against porcine reproductive and respiratory syndrome in boars. Am J Vet Res 58: 40-45.

European Agency for the evaluation of medicinal products (1998). Guideline on Good Clinical Practices. EMEA/CVMP/595/98. London UK.

Federation of Veterinarians of Europe (FVE) (2001). Code of Good Veterinary Practice (GVP Code): pp. 4–7.

Flowers WL (1997). Artificial Insemination in Swine. In: Current Therapy in Large Animal Theriogenology. Youngquist RS ed. WB Saunders Company: pp. 678-683.

Houston DM and Radostits OM (2000). The Clinical Examination In: Veterinary Clinical Examination and Diagnosis. Radostits OM, Mayhew IG, Houston DM, eds. WB Saunders Company: pp. 91-124.

Lager KM, Mengeling WL, Brockmeier SL (1996). Effect of post-coital intrauterine inoculation of porcine reproductive and respiratory syndrome virus on conception in gilt. Vet Rec 138(10): 227-228.

Leiding C (2000). Prevention of Disease Transmission by the Use of Semen in the Porcine AI Industry. Liv Product Sci 62: 221-236.

Meulenberg JJ, Petersen den Besten A, de Kluyver E, van Nieuwstadt A, Wensvoort G, Moormann RJ (1997). Molecular characterization of Lelystad virus Vet Microbiol 55:197-202.

Muelenberg JM, Hulst MM, de Meijer EJ, Moonen PL, den Besten A, de Kluyver EP, Wensvoort G and Moormann RJ (1993). Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS) is related to LDV and EAV. Virology 19: 62-74.

Nielsen TL, Nielsen J, Have P, Bækbo P, Hoff-Jorgensen R and Botner A (1997). Examination of virus shedding in semen from vaccinated and from previously infected boars after experimental challenge with porcine reproductive and respiratory syndrome virus. Vet Microbiol 54: 101-112.

Organization for Economic Co-operation and Development (OECD) (1998). Series on Principles of Good Laboratory Practice (GLP) and Compliance Monitoring, Organization for Economic Co-operation and Development, Paris, France: pp. 2–41.

Papatsiros VG, Alexopoulos C, Kritas SK, Koptopoulos G, Nauwynck HJ, Pensaert MB and Kyriakis SC (2006). Long-term administration of a commercial porcine reproductive and respiratory syndrome virus (PRRSV)-inactivated vaccine in PRRSV-endemically infected sows. J Vet Med B, Infect Dis Vet Public Health 53: 266-272.

Prieto C, Sanchez R, Martin-Rillo S, Suarez P, Simarro I, Solana A, and Castro JM (1996a). Exposure of gilts in early gestation to porcine reproductive and respiratory syndrome virus. Vet Rec 138 (22): 536-539.

Prieto C, Suarez P, Bautista JM, Sanchez R, Rillo SM, Simarro I, Solana A, and Castro JM (1996b). Semen changes in boars after experimental infection with porcine reproductive and respiratory syndrome (PRRS) virus. Theriogenology 45: 383-395.

Prieto C, Suarez P, Simarro I, Garcia C, Martin-Rillo S, Castro JM (1996c). Effect of porcine reproductive and respiratory syndrome virus (PRRSV) on development of porcine fertilized ova in vitro. Theriogenology 46 (4): 687-693.

Swenson SL, Hill HT, Zimmerman JJ, Evans LE, Landgraf JG, Wills RW. Sanderson TP, McGinley MJ, Brevik AK, Ciszewski DK, and Frey ML (1994). Excretion of porcine reproductive and respiratory syndrome virus in semen after experimentally induced infection in boars. J Am Vet Med Assoc 204: 1943-1948.

Swenson SL, Hill HT, Zimmerman JJ, Evans LE, Wills RW, Yoon KJ, Schwartz KJ, Althouse GC, McGinley MJ and Brevik AK (1995). Preliminary assessment of an inactivated PRRS virus vaccine on the excretion of virus in semen. Swine Health Product 3: 244-247.

Tizard IR (2004). The Use of Vaccines. In: Veterinary Immunology, An Introduction. Tizard IR ed. 7th ed. WB Saunders Company, Philadelphia: p. 265.

Vilaca KJ, Dewey C, Pettitt M and Friendship R (2001). The effects of a PRRS vaccine on the semen quality of boars In: Proceedings of 32th American Association of Swine Veterinarians Meeting, Nashville, Tennessee, USA, 32: pp. 59-61.

Vilaca KJ, Dewey C, Friendship R, Plante C, Buhr MM (2003). Semen quality parameters in naïve boars vaccinated with a MLV porcine reproductive and respiratory syndrome virus vaccine. In: Proceedings of 34th American Association of Swine Veterinarians, Orlando, Florida 32: pp. 25-26.

Yaeger MJ, Prieve T, Collins J, Christopher-Hennings J, Nelson E, and Benfield D (1993). Evidence for the transmission of porcine reproductive and respiratory syndrome (PRRS) virus in boar semen. Swine Health Product 1(5): 7-9.