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Multilevel approach to study boar fertility in commercial farm

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INTRODUCTION

Semen quality assessment represents a fundamental step for obtaining successful Artificial Insemination (AI) in pig industries, however the decline in boar fertility, not related to apparent causes, is a common and economically relevant problem. In commercial settings, ejaculates were evaluated at collection, but traditional quality estimates are not able to foretell fertility outcome. New fertility parameters have been therefore studied in vitro and compared with traditional ones.

This study was designed to evaluate if temporary fertility decline could be due to contact with pathogens at level not sufficient to evocate clinical signs of disease but adequate to stimulate the seroconversion.

MATERIALS AND METHODS

Animals: Nine adult boars (2 Large White and 7 Duroc, subjectively named from A to I), routinely utilized as semen donors in an Italian herd (consistency: around 1000 sows), have been monitored during 5 months. All boars were vaccinated against Aujeszky's Disease Virus (ADV) and Swine Influenza Virus (SIV, serotypes H1N1 e H3N2). Every 21 days, sperm and blood samples were collected after clinical assessment.

Semen: For the study of ***in vivo* fertility**, ejaculates were splitted, insemination doses ($3x10^9$spermatozoa) were prepared with a fresh handmade diluent (Swine Fertilisation Medium) and utilized within 4 days (storage at 16°C) for the AI of 230 different sows at estrus after weaning. For the assessment of **fertility** with laboratory ***in vitro*** analyses, we evaluated: Sperm Concentration, Viability (as membrane integrity), Mitocondrial Membrane Potential (SYBR-14/PI and JC-1 staining, Fig.1), and Damaged Acrosome (Coomassie Blue stain, Fig.2).

Microbiological (*E. coli*, *Proteus* spp., *Pseudomonas* spp., *Staphilococcus* spp) as well as **virological** (Porcine Reproductive and Respiratory Syndrome Virus - PRRSV) PCR **analyses** were done.

Blood: Standard **haematology profile** and many **biochemical parameters** were evaluated: Total Protein, Albumin, Urea, Creatinin, Phosporus and Sodium, Total Bilirubin, AST, ALT, GGT. For **serology** profile we researched antibody titers for ADV (ELISA), PRRSV (ELISA), PCV2 (Porcine CircoVirus type 2; ELISA), SIV (H1N1, H2N1, H3N2; HI).

Statistical Analyses: Data were firstly analyzed for confirming the normal distribution. Statistical analysis was performed by **ANOVA**.

RESULTS

Clinical conditions and haemato-biochemical parameters : At semen collections, any disease symptoms have been evidenced in all boars during the experimental period. The blood parameters were always in the normal range.

Bacteriological and virological analyses on semen: All the samples were negative (*E. coli, Proteus* spp., *Pseudomonas* spp., *Staphilococcus* spp, PRRSV).

Serological surveys: All the boars were found positive for PCV2 antibodies for the entire survey period. In two boars (D and I) it has been observed serum conversion for PRRSV whilst in other two subjects (C and E) it has been observed serum conversion for ADV-gE. The boars A, B, E and H were found sero-positive for H1N2.

Fertility in vivo: The outcomes of 230 AI were not statistically influenced by the sero-conversion and sero-positivity found in each donors at time of semen collection.

Fertility in vitro: When the % of spermatozoa with acrosome damages was under the acceptable threshold value of 5% in the ejaculate, the mean of "total piglets born/total inseminated sows" was significantly higher ($F=4,41$; $p=0,045$); also the parameter "pregnancy rate" was near to the statistic significance ($F=3,72$; $p=0,064$). The other in vitro fertility parameters did not express statistically meaningful differences versus in vivo fertility parameters (Tab. 1).

Parameter %	Treshold Value		Pregnancy Rate	Litter Size (n)	Total Piglets born/ AI Sows
Viability	>80	Mean	0,72	10,43	7,58
		SD	0,19	1,65	2,67
	<80	Mean	0,67	9,40	6,97
		SD	0,32	3,12	3,64
	Total	Mean	0,70	9,98	7,32
		SD	0,25	2,42	3,09
Damaged Acrosome	<5	Mean	0,75	10,54	8,00
		SD	0,19	1,46	2,58
	> 5	Mean	0,58	9,03	5,71
		SD	0,30	3,38	3,29
	Total	Mean	0,68	9,92	7,06
		SD	0,25	2,51	3,06
High Mit. Membrane Potential	>70	Mean	0,67	10,55	7,17
		SD	0,14	1,35	2,31
	<70	Mean	0,70	9,93	7,31
		SD	0,29	2,56	3,20
	Total	Mean	0,69	10,00	7,30
		SD	0,27	2,44	3,08

Table 1. Data of in vitro fertility parameters, analysed in ejaculates at the time of collection, and in vivo fertility parameters obtained from the outcomes of 230 sows artificially inseminated.

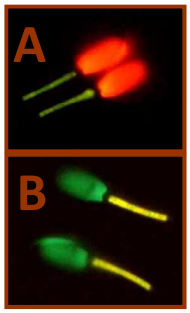

Figure 1. Sample images, at fluorescence, of spermatozoa with damaged membrane and Low MMP (A) and viable spermatozoa with High MMP (B), stained with SYBR-14/PI and JC-1.

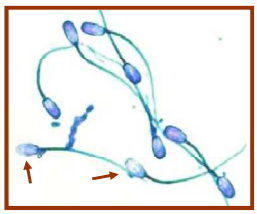

Figure 2. Sample images, at phase contrast microscopy, of spermatozoa stained with Coomassie Blu. Spermatozoa without acrosome is shown (arrows).

DISCUSSION

During the survey period of five months, the nine boars, routinely used as semen donors in a commercial breeding farm, had satisfactory health conditions and did not showed any clinical signs referring to infectious diseases. Although this, we observed sero-conversion in four subjects (2 for RRSV and 2 for ADV) and we found 4 boars with antibodies for SIV-H1N2.

Interestingly only one boar, at same time, resulted positive for H1N2 and presented sero-conversion for ADV during the survey.

From the reproductive point of view, any out of the serological conditions found altered reproductive, in vivo and in vitro, parameters analysed.

These results are really important because it has been ipothesized that temporary decline in male fertility could be due to contact with ethiological agents known to be able to interfere with reproduction, even in the absence of clear signs of disease.

Interestingly the analyses and comparisons of reproductive parameters, obtained in vivo and in vitro, pointed out that some in vitro parameters significantly correlate with in vivo fertility: ejaculates with percentages of spermatozoa with damaged acrosome under the treshhold value (5%) showed significantly higher fertility performances.

Since the analysis of damaged acrosome is an easy staining and requires only the normal microscope, we suggest to perform this test also in farm condition.