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Levels of activity of superoxide dismutase in seminal plasma do not predict fertility of pig AI-semen doses

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ABSTRACT

Superoxide dismutase (SOD) is one of the major antioxidant enzymes present in boar seminal plasma (SP). This study evaluated the role of SP-SOD on sperm performance, including quality and sperm functionality as well as *in vivo* fertility of semen extended and liquid-stored for artificial insemination (AI) of boars included in commercial AI-programs. The SOD activity was assessed in SP-samples from 311 ejaculates (100 boars), recording the fertility (farrowing rate and litter size) of 10,952 AI-sows. Evaluations of sperm motility (by CASA), and viability (by flow cytometry) and intracellular H_2O_2 generation in viable sperm spermatozoa (by flow cytometry) were performed at 0 and 72 h of liquid storage at 17 ºC. The SP-SOD activity differed *(*$P < 0.001$) between boars (n = 50), ranging from 1.16 ± 0.11 to 7.02 ± 0.75 IU/mL. Semen AI-doses (n = 44) hierarchically grouped *(*$P < 0.001$) as with low SP-SOD activity showed lower *(*$P < 0.05$) total and progressive sperm motilities and intracellular H_2O_2 generated by 72 h of liquid storage. Fertility did not differ between AI-boars (n = 39) hierarchically grouped *(*$P < 0.001$) as showing high and low SP-SOD activity in their ejaculates. In conclusion, SP-SOD activity depends on the boar and it is positively related with quality and sperm functionality of liquid-stored semen AI-doses. However, this positive effect is not reflected on *in vivo* fertility post-AI.

Keywords: superoxide dismutase, artificial insemination, fertility, seminal plasma, pig.

1. Introduction

Spermatozoa lack powerful antioxidant defence mechanisms, which lead them to be particularly sensitive to oxidative stress (OS), e.g. the imbalance between the generation of reactive oxygen species (ROS) and antioxidant defences [1,2]. Oxidative stress leads to membrane lipid peroxidation in mature spermatozoa, resulting in sperm dysfunction, including the loss of fertilizing ability [3]. Pig spermatozoa is especially sensitive to OS and subsequent lipid peroxidation due to the high content of polyunsaturated fatty acids in their membranes [2]. Under this scenario, the enzymatic and non-enzymatic antioxidants components of seminal plasma (SP) are the main defences for protecting spermatozoa from OS, by counteracting the excessive generation of ROS [4−6]. Recently, it has been demonstrated that, in pigs, SP antioxidant enzymes paraoxonase type 1 (PON1) and glutathione peroxidase 5 (GPX5) are related with sperm quality and function parameters as well as with fertility outcomes of liquid-stored semen doses of boars included in artificial insemination (AI) programs [7,8]. In addition to these two enzymes, pig SP contains a plethora of enzymes with antioxidant properties, including superoxide dismutase (SOD) [9].

Superoxide dismutase is a biological antioxidant enzyme that plays a key role protecting cells against OS because it is the first of a set of enzymes that catalyze the chemical reactions to scavenge harmful ROS [9]. Particularly, SOD catalyzes the dismutation of O_2^- to H_2O_2 [10] and is one of the major antioxidant enzymes present in the SP of mammalian species [11−14]. However, inconsistent results have been reported about the relationship between SP-SOD and sperm quality and functionality of extended liquid-stored semen in both, human [14−19] and livestock species [20−22]. In this context, the first aim of this study was to evaluate the influence of SP-SOD activity on

the quality and sperm functionality parameters of extended boar liquid-stored AI-semen samples, making special reference to the sperm parameters linked to OS.

Similar to other livestock species, looking for biomarkers for predicting fertility potential of boars used in commercial AI-programs is a challenge for the swine industry. The particular sensitivity of boar spermatozoa to OS prompted us to suggest that OS scavengers, such as SP-SOD, could be a potential fertility biomarker for AI-boars. In fact, while SOD activity has been positively related with fertility in humans [17,23,24], the potential relationship between SP-SOD and *in vivo* fertility has been scarcely studied in livestock species, particularly polytocous, such as the pig. Related with this, since a single study reported that SP-SOD levels in bull semen were unrelated with fertility [25], the current work also aimed to evaluate the putative relationship between SP-SOD and *in vivo* fertility of liquid-stored boar semen AI-doses.

2. Materials and methods

2.1 Reagents and media

All chemicals used in the experiments were of analytical grade. Unless otherwise stated, all media components were purchased from Sigma-Aldrich (St. Louis, MO, USA), with media prepared under sterile conditions using a laminar flow hood (MicroH, Telstar, Terrasa, Spain). Fluorochrome molecules were purchased from Invitrogen™ (Thermo Fisher Scientific, Waltham, MA, USA).

The basic media used to dilute reagents and fluorochromes for flow cytometry was EDTA free phosphate-buffered saline (PBS: NaCl 139 mM, KCl 2.7 mM, KH_2PO_4

1.5 mM, $Na_2HPO_4 \cdot 7H_2O$ 8.1 mM; with 0.058 g/L penicillin G and 0.05g/L streptomycin sulphate; pH 7.1 ± 0.06; 289 ± 3 mOsmol/kg).

2.2 Animals and ejaculates

All procedures involving animals were performed according to European guidelines (Directive 2010-63-EU; European Parliament and the Council of the European Union, 2010) and approved by the Bioethics Committee of Murcia University (research code: 639/2012).

Entire ejaculates were collected from healthy, mature and fertile boars of different breeds (Landrace, Large White, and Pietrain) and crossbreds (Landrace × Large White) housed in a commercial AI-centre (Topigs-Norsvin España) using a semiautomatic collection method (Collectis®, IMV Technologies, L'Aigle, France). Boars underwent regular semen collection for liquid semen AI-doses preparation and all used ejaculates fulfilled the standards of quantity and sperm quality requirements (more than 200 x 10^6 spermatozoa/mL, 70% motile spermatozoa, and 75% of morphologically normal cells). In addition, boars were free of chromosomal translocations and they showed a consistent low sperm nuclear chromatin fragmentation rate.

2.3 Seminal plasma processing and storage

The SP was harvested immediately after ejaculate collection by double centrifugation of the semen at 1,500 × g for 10 min at room temperature (Rotofix 32A; Hettich Zentrifugen, Tuttlingen, Germany). The harvested SP was examined by microscopy (200x; Eclipse E400, Nikon, Tokyo, Japan) to ensure it was sperm-free.

Thereafter, all SP-samples were individually stored in cryotubes (3 mL) and sent in insulated containers with dry ice to the Andrology Laboratory of the Veterinary Teaching Hospital, University of Murcia (Spain). Once in the laboratory, SP-samples were stored at −80°C (Ultra Low Freezer; Haier Inc., Qingdao, China) until SOD activity measurement, and SP-cryotubes were thawed at room temperature immediately before evaluation.

2.4 Measurement of SP-SOD activity

The SP-SOD activity was measured using a commercially available kit (RANSOD kit, RANDOX Laboratories, Crumlin, United Kingdom) following the manufacturer instructions. The method is based on the formation of red formazan dye generated by the reaction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) with the superoxide radical produced by xanthine and xanthine oxidase. The SOD activity is measured as the degree of inhibition of this reaction (one unit of SOD is equivalent to a 50% inhibition of the rate of reduction of INT). The analytical lecture was performed using an automated analyzer (AU600, Olympus, Minneapolis, USA) and the color change was measured at 505 nm. The SOD activity was expressed as IU/mL. The intra- and inter-assay CV were below 10%, depicting high linearity under serial dilutions ($r^2 = 0.982$).

2.5 Assessment of sperm quality and functionality

Sperm assessments included motility (total and progressive), viability and basal production of intracellular H_2O_2 by viable spermatozoa. Sperm viability and H_2O_2

generation were evaluated by flow cytometry using a BD FACS Canto II flow cytometer (Becton Dickinson & Company, Franklin Lakes, NJ, USA).

Sperm motility was objectively evaluated using a Computer-Assisted Sperm Analysis system (ISASV1® CASA, Proiser R+ D, Paterna, Spain). Briefly, 5 µL of extended semen (20−30 × 10^6 sperm/mL in Biosem+ extender; Magapor, Zaragoza, Spain) was placed in a pre-warmed (38 °C) Makler counting chamber (Sefi Medical Instruments, Haifa, Israel), and six to nine fields, with a minimum of 400 spermatozoa per sample, were analyzed. The sperm motility variables were recorded as the percentage of total motile spermatozoa (average path velocity ≥ 20 µm/sec) and that of motile spermatozoa showing rapid and progressive movement (straight line velocity ≥ 40 µm/sec). For sperm viability assessment, 100 µL of extended semen (30 × 10^6 sperm/mL in Biosem+ extender) were stained with 3 µL Hoechst 33342 (H-42, 0.05 mg/mL in PBS), 2 µL propidium iodide (PI, 0.5 mg/mL in PBS), and 2 µL fluorescein-conjugated peanut agglutinin (PNA-FITC, 100 µg/mL in PBS) and then incubated in the dark for 10 min at 38°C (Sanyo MIR-153 incubator, Gemini BV, Apeldoorn, Netherlands). Before flow cytometry analysis, 400 µL of PBS were added to each sample. Acquisition was stopped after 1 × 10^4 H-42 positive events were acquired for analysis. Data were recorded as the percentage of viable spermatozoa, those exhibiting intact plasma and acrosome membranes (H-42 positive/PI negative/ PNA-FITC negative).

The basal intracellular generation of H_2O_2 was assessed in viable spermatozoa using 5- (and 6-) chloromethyl-20,70-dichlorodihydrofluorescein diacetate acetyl ester (CM-H_2DCFDA). Briefly, 50 µL of extended spermatozoa (30 × 10^6 sperm/mL in Biosem+ extender) were extended in 950 µL of PBS containing 1.25 µL of H-42 (0.05 mg/mL in PBS), 1 µL of PI (0.5 mg/mL in PBS), and 1 µL of CM-H_2DCFDA (1 mM in dimethyl sulfoxide). Samples were incubated at 38°C in the dark for 30 min before flow

cytometry analysis. The mean fluorescence intensity was expressed as fluorescence units (FU) per 10^6 viable spermatozoa.

2.6 Experimental design

2.6.1 Experiment 1: SOD activity in boar SP

The SP-SOD activity was measured in SP-samples from 150 entire ejaculates from 50 boars (3 ejaculates per boar) evaluating the variability in SP-SOD activity between boars and between ejaculates within the same boar.

2.6.2 Experiment 2: Relationship between SP-SOD activity and sperm quality and functionality of liquid-stored semen samples

Semen samples of 44 ejaculates from 11 boars (4 per boar) were used. They were split in two aliquots. One was extended to 30×10^6 sperm/mL in Biosem+ extender, alike a commercial AI-dose, and stored at 17°C (FOC 120E Cooled Incubator; VELP Scientifica, Usmate, Italy) for 72 h. Sperm quality and functionality was assessed at 0 and 72 h of storage. The other aliquot was used for harvesting the SP, as described above, which was used for measuring SOD activity.

2.6.3 Experiment 3: Relationship between SP-SOD activity and *in vivo* fertility of liquid-stored semen AI-doses

Semen AI-doses (2,400 × 10^6 of total spermatozoa in 80 mL) and SP-samples from entire ejaculates collected from 39 boars were used. Weaned Landrace and Large White multiparous (1–7 litters produced) sows housed in different Spanish farms and subjected to the same housing and management conditions were cervically inseminated, 2–3 times per estrus, with AI-doses stored until 72 h at 17ºC. The number of inseminated sows per boar ranged between 103 and 914. Fertility parameters were recorded in terms of farrowing rate (as a proportion of the number of sows inseminated) and litter size (as total number of piglets born per litter) over a 12-month period. The SOD activity levels were measured in three SP-samples per boar obtained from three ejaculates (one sample per boar every 4 months).

2.7 Statistical Analysis

Data were analyzed using IBM SPSS Statistics 24.0 (IBM, Armonk, NY, YSA). Residual data for each statistical variable were evaluated using the Shapiro-Wilk test to check the assumption of normality, and data not normally distributed were arcsine- (data in percent) or log- (count data) transformed. Homogeneity of variances of raw and transformed data were evaluated with Levene test. In Exp.1 a mixed ANOVA model was performed to evaluate the variability in SP-SOD activity among boars and pig breeds, whereas the reliability among ejaculates within the same boar was assessed by intra-class correlation (ICC; 3, 1) using a 2-way mixed approach. In Exp. 2, a hierarchical cluster analysis was performed to identify naturally occurring groups within the data set of SP-SOD. Two groups of SP-samples with either high- or low- SOD activities were identified. A repeated-measures ANOVA was performed to evaluate putative differences on sperm quality between both SP-SOD groups at the two storage times (0 and 72 h). In Exp. 3,

raw fertility data set were initially corrected for parameters related to farm and sow using a multivariate statistical model [26], which allowed to specifically identify the effect of boar on each fertility parameter. A hierarchical cluster analysis was performed to identify naturally occurring groups within the data set of SP-SOD activity, and boars were clustered into two groups as showing either high- or low- SP-SOD activity. One-way ANOVA was performed to identify putative differences on fertility parameters between the two boar groups. A receiver operating characteristic (ROC) curve was used to determine the value of the SP-SOD activity discriminating boars for high- or low-fertility. Areas under the curve (AUC) and cut-off values were selected by the program. The AUC into the ranges of 1.00-0.90, 0.90–0.80, 0.80–0.70, 0.70–0.60, 0.60–0.50 and <0.50 was considered as excellent, good, fair, poor, fail and no discriminating, respectively. Statistical differences were defined as $P \leq 0.05$. Data are showed as the means ± standard error of the mean (SEM).

3. Results

3.1 Experiment 1: SOD activity in boar SP

The mean activity levels of SP-SOD varied significantly ($P < 0.001$) between boars, ranging from 1.16 ± 0.11 to 7.02 ± 0.75 IU/mL, but not between pig breeds. The measurements of the SP-SOD activity between ejaculates within each boar was highly consistent as the ICC score was 0.87 (95% confidence interval [CI] 0.79–0.92).

3.2 Experiment 2: Relationship between SP-SOD activity and quality and sperm functionality of liquid-stored semen samples

The SP-SOD activity differed widely between the 44 ejaculates, ranging from 1.00 to 5.69 IU/mL. Accordingly, the ejaculates were grouped (hierarchical clustering; $P < 0.001$) into two groups as with low (from 1.00 to 3.26 IU/mL; n = 20) or high (from 3.6 to 5.69 IU/mL; n = 24) SP-SOD activity. Total and progressive sperm motilities of elaborated AI-semen doses differed between the two SP-SOD groups at 72 h of liquid storage (Figs. 1-2). Semen AI-doses from ejaculates with high SP-SOD activity showed higher percentages of both total ($P < 0.05$) and progressive ($P < 0.001$) sperm motilities (75.46 ± 2.24 and 47.46 ± 2.37, respectively) than those from ejaculates with low SP-SOD activity (59.95 ± 6.21 and 28.35 ± 2.92, respectively). Sperm viability did not differ between the semen samples of the two SP-SOD groups, neither at 0 nor at 72 h of liquid storage. The percentages of viable spermatozoa were 91.07 ± 0.95 and 89.17 ± 0.94 in semen from the high SP-SOD group, and 91.37 ± 1.04 and 90.94 ± 0.91 in the low SP-SOD group, at 0 and 72 h of storage at 17°C, respectively. Basal intracellular H_2O_2 generation in viable spermatozoa differed ($P < 0.05$) between SP-SOD groups at 72 h of storage (Fig. 3). In effect, intracellular H_2O_2 levels in viable spermatozoa were significantly higher in semen AI-doses from ejaculates with high SP-SOD activity (mean arbitrary fluorescence intensity was 15.79 ± 1.21 FU $\times 10^6$ viable sperm) than in those from ejaculates with low SP-SOD activity (mean arbitrary fluorescence intensity was 12.22 ± 1.05 FU $\times 10^6$ viable spermatozoa).

3.3 Experiment 3: Relationship between SP-SOD activity and in vivo fertility of liquid-stored semen AI-doses

The 39 AI-boars were classified (hierarchical clustering; $P < 0.001$) into 2 groups as showing low (from 1.18 to 3.97 IU/mL; 29 boars) and high (from 4.79 to 7.00 IU/mL; 10 boars) SP-SOD activity (Table 1). The SP-SOD activity did not influence fertility of sows inseminated. Farrowing rates and the total number of piglets born per litter did not differ between boars of both SP-SOD groups. The ROC curve revealed that SP-SOD activity did not have a discriminating ability to predict whether a boar will exhibit high fertility outcomes, neither for farrowing rate (AUC = 0.42) nor litter size (AUC = 0.41) (Fig. 4).

4. Discussion

To the best of our knowledge, this is the first report evaluating the influence of SP-SOD on sperm performance, including *in vivo* fertility, of extended AI-doses of boars used in commercial AI-programs. The results demonstrated that SP-SOD activity was positively related with sperm motility parameters and with the basal H_2O_2 generated by viable sperm of extended liquid semen stored at 17ºC during 72 h, a storage time usual for semen AI-doses used in commercial swine AI-programs. However, these differences in sperm quality did not influence the fertility outcomes of the sows inseminated.

The first experiment clearly demonstrated that SP-SOD activity varied between boars but not between pig breeds, in agreement with previous observations of Kowalowka et al. [9] and Zura-Zaja et al. [22]. Differences for other SP-antioxidant enzymes, such as PON1 and GPX5, are also known to exist between boars [7,8]. Such individual differences in SP-SOD activity levels could have a genetic origin, more linked to the individual than to the breed [27], and would be a hereditary trait [22]. These assumptions would be supported by the findings of Yan et al. [14], who demonstrated that -in men-

polymorphisms in the SOD gene determine the levels of SP-SOD activity and that Ala16Val polymorphism in the SOD2 gene was related with lower SOD activity in SP.

The second experiment demonstrated that SP-SOD activity was positively related with total and progressive sperm motilities of extended liquid semen stored 72 h at 17ºC. This positive relationship is reasonable considering that SOD metabolizes O_2^-, one of the most dangerous ROS for spermatozoa that particularly impairs sperm motility [28,29]. However, our results differ from those reported by Zakosek Pipan et al. [21], showing a negative relationship between SP-SOD activity and boar sperm motility in liquid-stored semen samples. Differences in the time of SP harvesting after ejaculation could explain such differences in results, as while SP-harvesting was performed immediately after ejaculation in our study, it was obtained after an unspecific time of liquid semen storage in that of Zakosek Pipan et al. [21]. The SOD is present in both SP and boar spermatozoa, the leakage of SOD from spermatozoa to SP being possible due to the destabilization of sperm membrane during liquid semen storage [22]. In contrast, our results were consistent with those reported in human semen showing a positive relationship between SP-SOD and sperm motility [14,19,24,30]. These studies indicated that SP-SOD, through reducing OS, would also indirectly decrease sperm membrane lipid peroxidation and, thereby, preserving sperm functionality, particularly the motility. In this regard, Tavilani et al. [18] reported a negative relationship between SP-SOD and malondialdehyde content in human semen, a sensitive marker of lipid peroxidation.

The SP-SOD activity was also positively related with intracellular H_2O_2 generation in viable spermatozoa stored 72 h at 17ºC. These results were partially expected, since SOD catalyzes the dismutation of O_2^- into H_2O_2, allowing an equilibrated balance between both ROS [10]. Thereby, SP-SOD would protect spermatozoa against harmful effects of an excess of O_2^- [10]. It is well known that controlled amounts of ROS

are essential for regulating many physiological sperm processes [31]. Specifically, H_2O_2 plays a crucial role stimulating the protein tyrosine phosphorylation, which is related with motility, capacitation and sperm fertilizing ability [31,32]. This would explain that semen AI-doses from ejaculates with higher SP-SOD activity also showed higher percentages of motile spermatozoa.

Many efforts are currently done to identify biomarkers of functionality and boar sperm fertility among the SP components and some recent reports highlighted the potential biomarker role of some SP proteins linked to redox signaling and sperm capacitation [33−36]. In this context, some SP antioxidant enzymes, specifically PON1 and GPX5, would be included among the SP biomarkers of boar fertility [7,8]. ~~New insights into pig SP-composition have reported that some SP- antioxidant enzymes could be considered as potential fertility biomarkers [7,8].~~ Our results demonstrated that SP-SOD activity was positively related with total and progressive motile spermatozoa, which is in turn related with *in vivo* fertility [37]. Then, it would be reasonable to assume that AI-boars showing the highest SOD activity in the SP could also exhibit higher fertility outcomes. Moreover, mounting evidence indicates that SP-SOD plays a positive key role in men fertility, the men with low SP-SOD activity being often infertile [17,23,24,38]. While no previous study addressing this topic has been conducted in pigs, a single empirical study showed a positive effect of exogenous SOD added to semen extender upon the *in vitro* ability of frozen-thawed spermatozoa to produce embryos [39]. With this background, it was surprising that SOD activity of SP was not related with fertility outcomes of semen AI-dose. Similarly, Kumar et al. [25] reported that sub- and high-fertile AI-bulls exhibited similar SP-SOD activity in their ejaculates. So, it seems that SP-SOD would be positively related to fertility in men but not in livestock sires, at least in those species, as bull, that has a single ejaculate against the fractionated semen ejaculation

of humans (or boars, for that matter). In terms of fertility, men are either fertile or infertile, while livestock sires are either fertile or sub-fertile but rarely infertile, since those infertile sires are quickly culled from production. Thereby, the subtle differences in SD-SOD between livestock sires would be not important enough to explain by themselves the subtle differences in fertility that they show.

In summary, the present study evidences that the activity of SOD in SP differs between AI-boars. Such a difference positively influences the motility and H_2O_2 generated by spermatozoa of liquid semen AI-doses stored 72 h at 17ºC. However, these differences in SP-SOD would not explain subtle differences in fertility, either as farrowing rate or as litter size, among AI-boars. Thereby, the measurement of SP-SOD activity is, a priori, not a useful tool to predict fertility potential of AI-boars.

Conflict of interest

The authors have declared no conflict of interest.

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Author contributions

J.R., E.A.M. and H.R.M. obtained the funding to carry out the study. I.B. and J.R. conceived and designed the study. J.R., H.R.M., E.A.M. and M.Y. directed the experiments. I.B., L.P., A.T. and I.P. performed the experiments. I.P., H.R.M. and J.R. analyzed and interpreted the data. I.B., L.P and A.T. wrote the draft manuscript. J.R., H.R.M., E.A.M. and M.Y. revised and discussed the manuscript. All authors read and approved the manuscript for publication.

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Figure captions

Fig. 1. Relationship between activity of superoxide dismutase in boar seminal plasma (SP-SOD) and total sperm motility. Box-whisker plot showing the percentage of total motile spermatozoa in AI-semen samples, assessed at 0 and 72 h of storage 15–17°C. Up to 44 boar ejaculates (four per boar) were hierarchically grouped as having low- (from 1.00 to 3.26 IU/mL; n = 20) or high- (from 3.6 to 5.69 IU/mL; n = 24) SP-SOD activity. Boxes enclose the 25th and 75th percentiles, the whiskers extend to the 5th and 95th percentiles and the line is the median. (*) indicate significant differences *(P* < 0.05) among different SP-SOD groups.

Fig. 2. Relationship between activity of superoxide dismutase in boar seminal plasma (SP-SOD) and progressive sperm motility. Box-whisker plot showing percentage of progressive motile spermatozoa of AI-semen samples, assessed at 0 and 72 h of storage 15–17°C. Doses were prepared from 44 boar ejaculates (four per boar) hierarchically grouped as with low- (from 1.00 to 3.26 IU/mL; n = 20) or high- (from 3.6 to 5.69 IU/mL; n = 24) SP-SOD activity. Boxes enclose the 25th and 75th percentiles, the

whiskers extend to the 5th and 95th percentiles and the line is the median. (*) indicate significant differences *(P* < 0.001) among different SP-SOD groups.

Fig. 3. Relationship between activity of superoxide dismutase in boar seminal plasma (SP-SOD) and basal intracellular H_2O_2 generation by viable spermatozoa. Box-whisker plot showing basal intracellular generation of H_2O_2 in viable spermatozoa (mean arbitrary fluorescence units [FU] per 10^6 spermatozoa) of AI-semen doses, assessed at 0 and 72 h of storage 15–17°C, from 44 boar ejaculates (four per boar) hierarchically grouped as with low- (from 1.00 to 3.26 IU/mL; n = 20) or high- (from 3.6 to 5.69 IU/mL; n = 24) SP-SOD activity. Boxes enclose the 25th and 75th percentiles, the whiskers extend to the 5th and 95th percentiles and the line is the median. (*) indicate significant differences *(P* < 0.05) among different SP-SOD groups.

Fig. 4. Boar seminal plasma superoxide dismutase (SP-SOD) as potential fertility biomarker. Nonparametric Receivers Operating Characteristic (ROC) curves showing the ability of boar SP-SOD activity to predict farrowing rates and litter size of AI-semen doses stored at liquid state. AUC: area under the ROC curve.

Table 1. Seminal plasma superoxide dismutase activity (SP-SOD, IU/mL) and deviations in fertility (farrowing rate [FR] and litter size [LS]) of 39 AI-boars measured following a direct boar effect. Boars were hierarchically *(P* < 0.001) grouped as with low- (1-29, in grey) or high- (30-39, in white) SP-SOD activity levels (assessed in 3 ejaculates per boar). The FR and LS deviations were calculated regarding to average values of its genetic line averaged.

Boars	SP-SOD (UI/mL) mean ± SEM	Sows inseminated (n°)	FR deviation	LS deviation
1	1.18 ± 0.10	211	0.41	0.20
2	1.40 ± 0.18	334	3.70	1.02
3	1.43 ± 0.16	679	3.60	-0.44
4	1.61 ± 0.47	107	-0.74	-0.23
5	1.76 ± 0.37	133	2.82	0.24
6	1.77 ± 0.33	397	0.87	1.09
7	1.81 ± 0.14	171	0.45	0.18
8	2.09 ± 0.81	132	2.22	0.03
9	2.20 ± 0.39	158	-0.85	-0.19
10	2.20 ± 0.25	108	3.32	0.11
11	2.28 ± 0.27	203	0.08	-0.23
12	2.33 ± 0.13	164	-0.16	0.87
13	2.35 ± 0.07	103	1.30	0.15
14	2.42 ± 0.39	290	-0.12	-0.18
15	2.48 ± 0.53	586	3.59	0.37
16	2.73 ± 0.14	108	1.44	-0.15
17	2.75 ± 0.37	556	0.70	0.86
18	2.84 ± 0.58	914	2.01	0.94
19	3.21 ± 0.59	201	1.01	0.27
20	3.27 ± 0.36	169	-0.13	-0.32
21	3.36 ± 0.41	336	-1.50	-0.36
22	3.40 ± 0.61	148	-2.02	-0.15
23	3.50 ± 0.31	174	-0.26	1.13
24	3.59 ± 1.04	518	0.94	-0.02
25	3.70 ± 0.54	605	4.89	0.97
26	3.87 ± 0.76	182	-0.80	0.30
27	3.91 ± 0.47	540	1.43	0.34
28	3.92 ± 1.41	275	2.10	-0.26
29	3.97 ± 0.33	115	-1.53	-0.06
30	4.79 ± 0.25	104	-0.49	-0.31
31	4.89 ± 0.51	366	2.16	0.31
32	4.91 ± 0.55	335	1.88	0.04
33	4.95 ± 0.42	125	7.54	0.02
34	5.30 ± 0.03	124	-0.90	0.16
35	5.30 ± 0.16	118	1.27	-0.14
36	5.45 ± 1.16	567	-2.25	-0.43
37	5.68 ± 0.14	123	-2.55	0.52
38	6.39 ± 1.27	258	-4.90	0.41
39	7.00 ± 0.74	215	3.22	-0.56