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SUMMARY – Alentejano swine breed (AL) populations raised under extensive production systems have increased during the last years. Selection and preservation of AL to be exploited under this type of production system will require further development of reproductive technologies to the extent of those used nowadays in modern piggeries. Artificial insemination (AI) with fresh or frozen semen will, in the near future, serve both the targets of genetic improvement and germplasm preservation. The project AGRO 204 "Trial to Test the Usefulness of Reproductive Auxiliary Techniques on Alentejano Swine under Extensive Production System" includes the related goal of: evaluating reproductive parameters of boars from different farms. This paper presents results (i) on the possibility of training alentejano boars for semen collection and (ii) their semen characteristics. During 2003 and 2004, a total of 25 AL young boars (aged 8 to 18 months) from different farms, were taught to jump on a dummy, so that semen could be collected. The following semen analyses were performed: semen rich fraction volume (RFV), solid fraction weight (SFW),% of alive spermatozoa (SPZV), motility (MOT), spermatic concentration (CONC), total number of spermatozoa in the ejaculate (SPZT) and % of abnormal spermatozoa regarding: heads, tails and acrosomes. Ejaculation times were also registered. After a very variable training period (1 to 15 attempts per animal), regular semen collection was possible from 17 boars (68% of the total number of trained animals). A total of 127 ejaculates were analysed. The ejaculation time (292 ± 22 sec.), the SFW (31.5±2.4 g) and the RFV (78.6±5.3 ml) were found to be independent of the season of the year. However, seasonal influence was observed regarding the following parameters: SPZV ($p=0.01$); MOT ($p<0.05$); CONC ($p=0.01$); SPZT ($p<0.01$) and acrosome abnormalities ($p<0.001$). Results reported by other authors are in agreement with the ours provided by this work. The exposition to high temperatures reduces semen quality (% of damaged acrosomes), even in a well-adapted breed such as the Alentejano swine.

Keywords: Alentejano swine breed, boars, semen, seasonality.

RESUME – *"Evaluation du sperme collecté sur des verrats Alentejano". La population de porcs de race Alentejana (AL) élevés en conditions extensives a augmenté significativement pendant les dernières années. La sélection et la conservation de la race Alentejana vont demander l'utilisation de technologies reproductives peu utilisées présentement dans la production extensive, contrairement à la production intensive moderne. L'insémination artificielle (IA) à l'aide de semence fraîche ou congelée servira, dans un futur proche, les objectifs de progrès génétique et de conservation de matériel génétique de la race. Le projet AGRO 204 "Test de l'efficacité des techniques auxiliaires de reproduction chez les porcs alentejanos élevés en systèmes extensifs" comprend l'activité "évaluation de paramètres reproductifs de verrats de différentes provenances". Notre travail présente les résultats sur l'aptitude des verrats AL à l'entraînement pour la collecte de semence et les caractéristiques de la semence obtenue. Pendant les années 2003 et 2004 un total de 25 jeunes verrats, âgés de 8 à 18 mois et provenant de différentes exploitations, étaient éduqués à sauter sur un mannequin pour permettre la collecte de la semence. La semence était analysée pour les variables suivantes: volume de la fraction riche (RFV), poids de la fraction solide (SFW),% de spermatozoïdes vivants (SPZV), motilité (MOT), concentration spermatique (CONC), nombre total de spermatozoïdes dans l'éjaculat (SPZT) et % de spermatozoïdes anormaux (anomalies des tête, queue et acrosome). La durée de l'éjaculation à été enregistrée. Après une période d'entraînement très variable entre animaux (de 1 à 15 tentatives) il a été possible de collecter régulièrement la semence de 17 verrats (68% des animaux entraînés). Un total de 127 éjaculats ont été analysés. Le temps d'éjaculation (292±22 secondes), le RFV (78,6±5,3 ml) et le SFW (31,5±2,4 g) n'étaient pas affectés par la saison de l'année. Néanmoins nous avons observé une influence de la saison sur les paramètres suivants : SPZV ($p=0,01$), MOT ($p<0,05$), CONC ($p=0,01$), SPZT ($p<0,01$) et anomalies de l'acrosome ($p< 0,001$). Nos résultats sont en accord avec les observations de plusieurs auteurs. L'exposition à des températures élevées conduit à une diminution de la qualité de la semence (surtout à des dommages aux acrosomes) même chez une race bien adaptée comme la race porcine Alentejana.*

Mots-clés : *Race porcine Alentejana, verrats, semence, saisonnalité.*

Introduction

Alentejano swine breed (AL) populations raised under open field conditions increased during the last years to the point where breeders intent now to establish some genetic improvement programs. Modern breeding requires germplasm exchanges among farms to assess the real improvement potential of boars, independently of farm environment. The AL population is spread across the Alentejo region (Portugal) and the use of artificial insemination (IA) helps to establish genetic connections (besides avoiding sanitary risks). Although preliminary studies on the semen quality of these animals have been made (Charneca *et. al.*, 2000), it is relevant to have more data on AL boar semen collection training and semen characteristics, considering that, in the near future, IA will be increasingly required.

Material and methods

Animals

25 Alentejano swine breed boars (aged 8 to 18 months) from different farms were lodged individually.

Training for semen collection

During the training period, an appropriate swine dummy was daily placed (during 10-15 minutes) in the individual pen of each boar. If the boar jumped onto the dummy, semen collection was attempted. After 3 successful collections in the boar's barn, regular semen collection was then performed in the collection park.

Semen collection and processing

Semen was collected by the gloved-hand method into a beaker held in a thermos bottle containing water at 35-37 ºC. The beaker was covered by a filter to exclude the bulbourethral gel fraction from the collected semen.

Macroscopical and microscopical semen characteristics of AL boars were immediately assessed after collection. The macroscopical semen characteristics studied in this work were the total volume, the volume of the rich fraction and the bulbourethral gel fraction weight. The microscopical semen characteristics considered were the % of alive spermatozoa (SPZ), the % of spermatozoa motility (using a phase contrast microscope at x 400), the sperm cell concentration (via a Neubauer chamber), the total number of spermatozoa in the ejaculate (SPZT) and the % of abnormal spermatozoa (regarding: heads, tails and cytoplasmatic droplets).

Statistical analysis

Data were subjected to analysis of variance, using a least square mix model (maximum likelihood; Harvey, 1990).

The general mathematical model used for semen evaluation was:

$$Y_{ij} = \mu + Est_i + I_j$$

where:

Y_{ij} = value for dependent variable;

μ = Mean common effect;

Est_i = fixed effect of season (i = 1, 2, 3, 4)

I_{ij} = standard error of mean

Results

Training of Alentejano boars for semen collection

After the training period for semen collection it was possible to regularly collect semen from 17 boars (68% of the total number of trained animals).

Semen characteristics

The season of the year did not affect significantly the ejaculation time, the sperm solid fraction weight or the rich fraction volume. On average, we found values (mean ± sem) of 291.5 ± 21.9 seconds, 31.5 ± 2.4 g and 78.6 ± 5.3 ml, respectively.

However, a seasonal effect was found on the other studied variables. Tables 1 and 2 reveal the impact of the season on spermatozoa numbers, movement and morphology.

Table 1. The effect of season on the percentages of alive spermatozoa and spermatozoa motility and on the spermatozoa concentration and total number of spermatozoa on the ejaculates (mean ± sem)

	SPZV	MOT	CONC	SPZT
Winter (n=62)	82 ± 1^{a}	67 ± 1^{a}	389.2 ± 26.8^{a}	33699.5 ± 2563.6^{a}
Spring (n=33)	87 ± 2^{b}	73 ± 2^{b}	562.6 ± 39.6^{b}	41138.2 ± 3786.7^{a}
Summer (n=17)	88 ± 2^{b}	69 ± 3^{ab}	564.3 ± 55.1^{b}	54772.3 ± 5275.8^{b}
Autumn (n=15)	84 ± 4^{ab}	72 ± 5^{ab}	462.5 ± 101.6^{ab}	22204.9 ± 9728.1^{a}
SL	p = 0.01	p < 0.05	p = 0.01	p < 0.01

SPZV: percentage of alive spermatozoa
MOT: Percentage of spermatozoa motility
CONC: Spermatozoa concentration (x 10^6)
SPZT: Total number of spermatozoa in the ejaculate (x 10^6)
SL: Significance level

Table 2. The effect of season on the occurrence of spermatozoa abnormalities (as percentage of total spermatozoa)

	Head	Tail	DCD	PCD
Winter	8.1 ± 1.2^{a}	3.5 ± 0.6	13.5 ± 2.2^{a}	1.1 ± 0.1^{ab}
Spring	7.1 ± 1.7^{a}	2.8 ± 0.9	14.3 ± 3.2^{a}	0.9 ± 0.2^{bc}
Summer	22.7 ± 2.7^{b}	2.7 ± 1.3	0.8 ± 0.5^{b}	0.4 ± 0.3^{c}
Autumn	2.3 ± 4.0^{a}	2.0 ± 1.3	3.0 ± 0.8^{b}	2.2 ± 0.5^{a}
SL	p<0.001	ns	p<0.05	p<0.05

Head: Abnormalities at spermatozoa head level (%)
DCD: Distal cytoplasmatic droplets (%)
Tail: Abnormalities at spermatozoa tail level (%)
PCD: Proximal cytoplasmatic droplets (%)
SL: Significance level

The majority of abnormalities at head level were damaged acrosomes.

Discussion and conclusions

Boar's ability for semen collection seems to be dependent on the breed and age of the animals. In this study, 68% of the boars were successfully trained. Some difficulties to train Iberian boars have been reported by Martín Rillo *et al*., 1999 (with a result of 30% of Iberic breed trained successfully).

Better results obtained in this trail can be related with the boar's age at the beginning of the training, once our animals had 8-18 months of age (while in the study cited above, the age of animals were between 1.5 and 5 years). Therefore, our observations are in accordance with those reported by Martín Rillo *et al*. (2001), where younger animals showed better response to the training for semen collection, allowing us to recommend to breeders that boars shall be trained as young as possible, so that the best success rate can be attained.

As far as results on the ejaculates are concerned, the rich fraction volume, the sperm motility and the sperm concentration agree with values reported by Martín Rillo *et al*. (1999) and Charneca *et al*. (2000). The sperm output increased during summer (higher concentration and total number of spermatozoa in the ejaculate), although associated with an increase on the spermatozoa abnormalities (specially at acrossome level). Several authors reported a negative effect of boars exposure to high temperatures on their sperm morphology. During late Spring and Summer, local temperatures are quite high (30º C to 36ºC). These environmental conditions can explain the increasing percentage of morphological abnormalities. Although local breeds are very well adapted to Mediterranean climate, we recommend that breeders implement shadow areas and water baths, which can reduce the negative environmental impact of high summer temperatures on boar's semen quality. Based on our results, artificial insemination can be performed with Alentejano boars, since training procedures and animals' lodgement are in accordance with physiological development and animal welfare.

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