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in

Almeida J.A. (ed.), Tirapicos Nunes J. (ed.).
Tradition and innovation in Mediterranean pig production

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 41

2000
pages 93-98

Quantitative and qualitative evaluation of Alentejano swine breed boars semen

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SUMMARY - Extensive swine production systems, traditional in Mediterranean Europe, pose some difficulties to the application of reproduction auxiliary techniques, as a result of inadequacies of population size and management practices. Artificial insemination (AI) is seldom used. Several authors point out the lack of AI extend as an obstacle to both genetic improvement in general and reproduction parameters in particular. Alentejano swine semen characteristics are not known and artificial insemination has not been used in Alentejano breed. This trial aimed at studying Alentejano boar semen characteristics and its inherent capability of being stored by using standard extenders (BTS, MR-A and ACROMAX) in order to allow for artificial insemination usage under Alentejo field conditions. 8 Alentejano (AL) boars and 2 contrast boars [1 Duroc x Large White (DL) and 1 Large White (LW)] were used. Initially, all animals were taught to jump a dummy, so that semen could be collected. A total of 177 ejaculates were evaluated. Alentejano boars showed statistically significant differences in the following variables: less volume of semen (rich and poor fractions, P<0.001), greater % of alive spermatozoa and higher % of spermatozoa motility. Although semen quality was influenced by season in both genotypes, Alentejano swine semen was the least affected. Storage of AI doses was negatively affected by the hottest month. Extender had no effect on the studied storage period.

Key words: Local swine breeds, AI, semen, characteristics, conservation.

RESUME - *"Evaluation quantitative et qualitative du sperme de verrats de race alentejana". Les systèmes extensifs de production porcine, traditionnels de l'Europe méditerranéenne, par leurs caractéristiques d'exploitation, posent des difficultés d'utilisation des techniques auxiliaires de reproduction. L'IA a été rarement utilisée. Une telle situation a été indiquée, par plusieurs auteurs, comme étant un obstacle au progrès génétique en général et plus particulièrement aux aspects reproductifs. Peu d'informations sont disponibles sur les caractéristiques de la semence de verrats de race alentejana. Ceci nous a amené à essayer de compléter les données et simultanément tester la possibilité d'application des dilueurs utilisés pour la semence de races améliorées, pour sa conservation en frais, (par périodes équivalentes à celles obtenues chez certaines races). Les dilueurs BTS, MR - A et ACROMAX ont été utilisés dans ces travaux. Au total, 8 verrats de race alentejana (AL), et deux verrats contrastants [1-Duroc x LW (DL) et 1-Large White (LW)] ont été utilisés pour réaliser des tests comparatifs. Tous les animaux ont été soumis à un entraînement initial. Les paramètres quantitatifs et qualitatifs ont été mesurés sur 177 éjaculats. La race alentejana se différencie de façon significative des races standard pour les paramètres suivants : moindre volume total de l'éjaculat, [(fraction riche et pauvre) (*P*<0,001)], % de spermatozoïdes (SPZ) vivants au moment de la récolte et % de SPZ mobiles progressifs au moment de la récolte (*P*<0,001) supérieurs. L'influence de la saison a aussi été testée. Des différences significatives ont été observées pour les paramètres suivants : volume total de l'éjaculat (*P*<0,01), volume de la fraction riche (*P*<0,05) et fraction pauvre (*P*<0,001) pour le verrat DL, volume de la fraction pauvre (*P*<0,001) pour les verrats de race alentejana, % de spermatozoïdes vivants et progressifs au moment de la récolte chez le verrat DL (*P*<0,05) et chez les verrats de race alentejana (*P*<0,001). Concernant l'observation et l'évaluation des doses de IA conservées, le génotype a influencé significativement le % de SPZ mobiles progressifs après 48 heures (L<0,05), 72 heures (*P*<0,001) et 96 heures (*P*<0,01) de conservation. L'époque de récolte a un effet significatif sur la conservation des doses 24 heures après la récolte (*P*<0,001). Le dilueur utilisé n'a eu aucune influence pendant la période de conservation.*

Mots-clés : *Races locales porcines, IA, semence, caractéristiques, conservation.*

Introduction

Several authors refer the scientific and technological advances that have been achieved on the fields of both human and animal reproduction (Terqui *et al*., 1996). New technologies have had an application, almost immediate and universal, on intensive pig production systems. Nevertheless technology transfer to extensive systems, based on local breeds of swine, is seldom applied.

New advances on reproduction and genetics (e.g., sex determination at the level of embryo or sperm cells, production of transgenic animals and intracytoplasmatic injection of spermatozoa and spermatides), allow scientists to speed up progress. Meanwhile, researchers have to deal with the local breeds claim for AI, to improve some genetic parameters of autochthonous animals.

AI can be very useful whenever small and spread apart swine populations have to be dealt with because, would allow for the loaning of boars (used in natural mating) to be avoided, simultaneously providing a better control of diseases.

This trial aimed at studying the semen characteristics of Alentejano swine boars, the usefulness of standard extenders applied to native boars and also to assess the relevance of using AI on Alentejano pig breed under Alentejo traditional production systems.

Material and methods

Ten boars: eight Alentejano swine breed, one Duroc x Large White and one Large White were prepared for semen collection. All of them were more than seven months old when the experiment was begun.

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°C. The top of the beaker was covered by a filter to exclude the bulbourethral gel fraction from the collected semen.

Both macroscopical and microscopical semen characteristics of the Alentejano swine breed and Duroc x Large White boars were recorded immediately after collection. The macroscopical characteristics studied in this work were the total volume, the volume of the rich fraction and the bulbourethral gel fraction. The microscopical characteristics that we studied were the % of alive spermatozoa (SPZ), the % of spermatozoa motility (using a phase contrast microscope at x 400) and the sperm cell concentration (via a Neubauer chamber).

Artificial insemination (AI) doses were prepared by diluting rich fractions, from the alentejano swine breed and Large White boars, into three different commercial extenders (BTS, MR-A and ACROMAX). The number of motile spermatozoa in each AI dose was approximately 3×10^9. AI doses were kept for a 2-3 h period under environment temperature (±22°C). Then, they were maintained in a chamber, at 15°C.

The percentage of motile spermatozoa were daily assessed (during a 96 h period of storage) by using diluted semen samples collected from each AI dose.

Statistical analysis

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

The general mathematical model used for semen evaluation was:

$Y_{ijk} = \mu + Gen_i + Est_j + \epsilon_{ijk}$

and for extenders evaluation was: $Y_{ijkl} = \mu + Gen_i + Epc_j + Dil_k + \epsilon_{ijkl}$

Y_{ijk} = value for dependent variable
Y_{ijkl} = value for dependent variable
μ = Mean common effect
Gen_i = fixed effect of genotype (i = 1, 2, 3)
Est_j = fixed effect of season (j = 1, 2, 3, 4)
Epc_l = fixed effect of collect period (l = 1, 2, 3, 4)
Dil_k = fixed effect of extender (k = 1, 2, 3)
ϵ_{ijkl} = standard error of mean

Results

Quantitative and qualitative variables studied on the ejaculates

The total volume, the rich fraction volume and the poor fraction volume of the ejaculate were all significantly smaller (*P*<0.001) in the Alentejano swine breed animals. Nevertheless, these animals had a significantly higher (*P*<0.001) sperm cells concentration, percentage of alive spermatozoa and percentage of spermatozoa motility after collection, compared with the crossed animal (Table 1).

Table 1. Average (± sem) of the various variables studied on the ejaculates

	TV	RFV	PFV	CONC	A. SPZ	MOT
Alentejano	118.9 ± 3.4	91.5 ± 3.0	27.4 ± 0.9	605.5 ± 16.4	86.5 ± 0.4	78.3 ± 0.5
Duroc x LW	212.7 ± 9.0	158.9 ± 7.6	53.8 ± 2.7	426.5 ± 38.4	77.7 ± 3.8	66.9 ± 3.6
SL	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001

TV: Total volume of the ejaculate (ml)
RFV: Rich fraction volume of the ejaculate (ml)
PFV: Poor fraction volume of the ejaculate (ml)
CONC: Spermatozoa concentration (x 10^6/ml)
A. SPZ: Percentage of alive spermatozoa after collection
MOT: Percentage of spermatozoa motility after collection
SL: Significance level

The effect of season on the quantitative and qualitative variables studied on the ejaculates

The season of the year affected significantly (*P*<0.01) the total volume of the ejaculate on both genotypes. Nevertheless, while in the crossed animal both the rich - (*P*<0.05) and poor - (*P*<0.001) fractions differed with the season of the year, in the Alentejano swine breed animals only the poor fraction changed considerably with the season (*P*<0.001). No significant differences (*P*>0.05) were observed on the spermatozoa concentration of the ejaculates. Qualitative variables, such as percentage of alive spermatozoa and spermatozoa motility were significantly different between seasons in the crossed animal (*P*<0.05) and in Alentejano swine breed animals (*P*<0.001). All results (see Tables 2 and 3) pointed to a greater impact of summer on the semen quantity and quality of the crossed animal, a smaller impact being also observed on the alentejano swine breed animals.

Table 2. The effect of season on the different semen variables studied on the Alentejano swine breed animals (mean ± sem)

Season	TV	RFV	PFV	CONC	A. SPZ	MPT
Summer	107.0 ± 4.5^{a}	88.9 ± 4.0	18.0 ± 1.3^{a}	590.2 ± 21.4	88.7 ± 1.0^{a}	81.3 ± 1.0^{a}
Fall	113.3 ± 7.5^{a}	86.6 ± 6.6	26.6 ± 2.1^{b}	637.3 ± 35.4	87.3 ± 1.6^{ac}	79.2 ± 1.7^{ac}
Winter	138.9 ± 7.2^{b}	102.7 ± 6.4	36.3 ± 2.0^{c}	640.9 ± 34.1	84.6 ± 1.6^{b}	75.0 ± 1.7^{b}
Spring	116.4 ± 8.1^{a}	87.7 ± 7.2	28.5 ± 2.3^{b}	553.9 ± 38.5	85.5 ± 1.8^{bc}	77.7 ± 1.9^{bc}
NS	*P*<0.01	NS	*P*<0.001	NS	*P*<0.001	*P*<0.001

TV: Total volume of the ejaculate (ml)
RFV: Rich fraction volume of the ejaculate (ml)
PFV: Poor fraction volume of the ejaculate (ml)
CONC: Concentration in spermatozoa (x 10^6/ml)
A. SPZ: Percentage of alive spermatozoa after collection
MOT: Percentage of motility after collection
SL: Significance level

Table 3. The effect of season on the different semen variables studied on the Duroc x LW animal (mean ± sem)

Season	TV	RFV	PFV	CONC	A. SPZ	MPT
Summer	166.9 ± 12.0^{a}	133.0 ± 10.7^{a}	33.8 ± 3.4^{a}	563.5 ± 57.0	68.0 ± 2.6^{a}	59.0 ± 2.8^{a}
Fall	251.0 ± 12.0^{b}	187.9 ± 10.7^{b}	63.1 ± 3.4^{b}	414.5 ± 57.0	89.0 ± 2.6^{b}	80.0 ± 2.8^{b}
Winter	238.7 ± 22.0^{bc}	167.0 ± 19.6^{ab}	71.7 ± 6.2^{b}	261.7 ± 104.2	66.7 ± 4.8^{ab}	50.0 ± 5.1^{c}
Spring	194.3 ± 14.4^{ac}	147.7 ± 12.8^{a}	46.7 ± 4.0^{a}	466.4 ± 68.2	87.1 ± 3.2^{b}	78.6 ± 3.3^{b}
NS	P<0.01	P<0.05	P<0.001	NS	P<0.05	P<0.05

TV: Total volume of the ejaculate (ml)
RFV: Rich fraction volume of the ejaculate (ml)
PFV: Poor fraction volume of the ejaculate (ml)
CONC: Concentration in spermatozoa (x 10^6/ml)
A. SPZ: Percentage of alive spermatozoa after collection
MOT: Percentage of motility after collection
SL: Significance level

Conservation of AI doses

The animals used for this experiment were Alentejano swine breed (AL) and Large White (LW).

Between genotypes significant differences were observed according to the month of collection and fresh-extended storage. A minor storage capacity was observed during August. In this month, the % spermatozoa motility observed at 24 hours of storage was always significantly lower (P<0.001) than in the other studied months.

Statistically significant differences between months were not observed on the initial spermatozoa motility (MOT 0). For the remaining studied motilities means (%) were as follows: MOT 24 h = 63.3 ± 1.5; MOT 48 h = 49.4 ± 1.3; MOT 72 h = 39.9 ± 1.3; MOT 96 h = 29.4 ± 1.3. This pattern of decreasing motility was observed on both genotypes. Table 4 contrasts average spermatozoa motilities between months.

Table 4. Average spermatozoa motility (%) contrasts between months (mean deviation)

Month	MOT 24 h	MOT 48 h	MOT 72 h	MOT 96 h
June	4.4 ± 2.1^{A}	6.0 ± 1.9^{a}	10.7 ± 1.9^{a}	12.9 ± 1.9^{a}
July	2.2 ± 1.7^{A}	6.1 ± 1.5^{a}	2.2 ± 1.6^{b}	5.0 ± 1.6^{b}
August	-10.0 ± 1.6^{B}	-14.5 ± 1.5^{b}	-13.6 ± 1.5^{c}	-18.2 ± 1.5^{c}
September	3.4 ± 2.4^{A}	2.4 ± 2.2^{a}	0.7 ± 2.2^{b}	0.2 ± 2.2^{b}
SL	P<0.001	P<0.001	P<0.001	P<0.001

MOT 24 h: Motility of stored spermatozoa 24 h after dilution
MOT 48 h: Motility of stored spermatozoa 48 h after dilution
MOT 72 h: Motility of stored spermatozoa 72 h after dilution
MOT 96 h: Motility of stored spermatozoa 96 h after dilution
SL: Significance level

Spermatozoa motility decreased on both genotypes. Nevertheless, statistically significant between genotype differences were observed after 48 hours of storage, with a higher decrease on spermatozoa motility observed on the LW genotype (Table 5).

Table 5. Average spermatozoa motility (%) contrasts between Alentejano swine breed boars and LW boar (mean deviation)

	MOT 0	MOT 24 h	MOT 48 h	MOT 72 h	MOT 96 h
AL	-0.16 ± 0.8	3.6 ± 2.7	4.7 ± 2.3	8.4 ± 2.4	6.4 ± 2.4
SL	NS	NS	P<0.05	P<0.001	P<0.01

AL: Alentejano swine breed boars
SL: Significance level
MOT 0 h: Motility after collection
MOT 24 h: Motility of stored spermatozoa 24 h after dilution
MOT 48 h: Motility of stored spermatozoa 48 h after dilution
MOT 72 h: Motility of stored spermatozoa 72 h after dilution
MOT 96 h: Motility of stored spermatozoa 96 h after dilution

Discussion

A wide variation in sperm production was observed, both in qualitative and quantitative terms. Many factors are associated with sperm production. The influence of factors such as season, social environment, nutrition, breed, age and testis size have been discussed by Colenbrander and Kemp (1990) (cit. by Colenbrander *et al.*, 1993).

Our results show that all the ejaculate variables we studied were differed significantly between breeds (AL *vs*. Duroc x LW). Individual analysis of some of these variables showed that the volume of the Duroc x LW genotype ejaculates are normal (Hafez, 1993), on the contrary, the volume of the Alentejano breed boar ejaculates are lower than normal (Hafez, 1993). This may be a specific characteristic of the later breed. The sperm cells concentration observed in this study was superior to what has been considered normal (Hafez, 1993), particularly within the alentejano breed. Again, the specificity of the breed is one of possible explanations for such a higher sperm cells concentration in the alentejano breed animals [twice the value refereed by Hafez (1993)]. The spermatozoa motility % observed on semen of both genotypes may be considered normal (Colenbrander, *et al.*, 1993 and Hafez, 1993).

Regarding the variable season of the year, many authors have pointed out seasonally reduced fertility traits in the sow and boar (also known as "summer infertility") that contribute to low prolificacy and economic losses under practical conditions (Foxcroft *et al.*, 1994; Thornton, 1995). Martinat-Botté *et al.*, 1986 and Hafez, 1993, reported the sensibility of the boar to extreme temperature conditions. The total sperm cells per ejaculate, the % of alive spermatozoa, the motility and fertility rate are all known to decrease during high temperature periods. The effect of high temperature is increased by increasing day lengths.

Our results show a decrease in the total volume of the ejaculate during summer, in both genotypes. However, the sperm concentration doesn't decrease during the summer period and the spermatozoa motility only decreased on the Duroc x LW animal. In general, Alentejano boars seem to be less affected by temperature seasonal changes, suggesting that this breed is well adapted to the climatic conditions of Alentejo.

As far as semen conservation is concerned, AL boar doses allowed better results to be achieved compared with LW boar ones. On average, the spermatozoa motility observed on both genotypes was lower than that reported by Pérez Marcos *et al*. (1995). Nevertheless, they used a different extender and semen samples were evaluated mixed with caffeine. The lowest dose conservation capabilities were observed in August, possibly related with high temperatures observed on July and August, which may have lead to a lower conservation capability of the AI doses.

Acknowledgements

Authors would like to thank to programme PAMAF for the financial support of Project No. 3032 which allowed them to run up this experiment and express their gratitude to the Professor A. Murilhas for his help on English version revision.

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