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ANALYSIS OF THE SEMINAL CHARACTERISTICS OF A BOAR WITH IMPAIRED FERTILITY

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RESUM

Aquest treball descriu la relació entre la qualitat espermàtica de dos mascles reproductors porcins. Les dosis seminals procedents dels ejaculats dels dos animals han estat examinades al microscopi òptic. L'avaluació de la qualitat espermàtica s'ha dut a terme mitjançant la determinació dels següents paràmetres: pH, ORT, i concentració, motilitat i morfologia espermàtiques. La qualitat espermàtica d'un dels mascles (control) era correcta, si bé es va detectar una concentració espermàtica lleugerament baixa. L'examen de les dosis seminals procedents de l'altre mascle (problema) va revelar les característiques pròpies d'un esperma immadur, amb una elevada incidència d'espermatozoides de cua doblegada per l'anell de Jensen i una motilitat espermàtica molt baixa, fet atribuït a una disfunció epididimària.

RESUMEN

Este trabajo describe la relación entre la calidad espermática de dos machos reproductores porcinos. Las dosis seminales procedentes de los eyaculados de los dos verracos han sido examinadas al microscopio óptico. La evaluación de la calidad espermática se ha realizado mediante la determinación de los siguientes parámetros: pH, ORT, y concentración, motilidad y morfología espermáticas. La calidad espermática de uno de los verracos (control) era correcta, aunque se detectó una concentración espermática ligeramente baja. El examen de las dosis seminales procedentes del otro verraco (problema) reveló las características propias de un esperma inmaduro, con una elevada incidencia de espermatozoides con cola doblada por el anillo de Jensen y una motilidad espermática muy baja, hecho atribuido a una disfunción epididimaria.

ABSTRACT

This report describes the relationship between the sperm quality of two boars. Semen doses coming from the ejaculates of two sexually mature boars were examined by phase-contrast and light microscopy. Sperm quality assessment was carried out by determination of the following parameters: pH, ORT, and sperm concentration, motility and morphology. Sperm quality results were correct for one of the boars (control), although a slightly low sperm concentration was detected. Examination of the sperm doses from the other boar (problem) revealed the inherent characteristics of an immature sperm, with a high incidence of spermatozoa with tail folded at the Jensen's ring and very low sperm motility, which may be attributed to an epididymal dysfunction.

Key words: epididymis, fertility, folded-tail spermatozoa, sperm maturation, sperm quality, *Sus domesticus*.

INTRODUCTION

The male in farm animal reproduction is an extremely important factor for the economy of livestock production (Rasbech, 1984). The world-wide use of artificial insemination (AI) has limited the number of male animals used in the reproductive process and drawn attention to the role of the male in the fertility. Whether the male is used for AI or natural mating, it has become important to identify the male influence on the fertility levels and develop more accurate methods of assessing semen quality and fertility of males. Absolutely sterile males are comparatively seldom found and are usually readily identified, while males with reduced fertility may present serious diagnostic problems and cause economic losses to livestock productions. Nowadays, breeders normally select males for high fertility before they are introduced to the female population, and there are necessary reliable methods for assessing the semen quality with regard to fertility. As for cattle and sheep there are no precise methods to predict the reproductive performance of boars. There does, however, exist varying degrees of infertility in boars arising from low quality of semen as well as from defects in the genital organs which may inflict heavy economic losses to pig breeders.

Mammalian spermatozoa gradually acquire motility and fertilizing capacity while passing through the epididymis and the morphological, chemical and functional changes necessary for motility and fertilizing capacity to be expressed are dependent upon the epididymal environment (Orgebin-Crist and Olson, 1984). Although the mechanisms underlying these changes are poorly understood, it is now well established that there is an extensive modification of the spermatozoon during the process of epididymal maturation (for reviews, see Bedford, 1979 and Orgebin-Crist, 1981).

Indeed male fertility, sperm production, semen quality and male sexual behaviour and its influence on female fertility are traits of direct importance in the economy of animal husbandry. Therefore, it is necessary to deepen our understanding on the fertility problems. This report describes the sperm quality of a problem boar with impaired fertility in comparison with a control boar.

MATERIAL AND METHODS

The semen samples were obtained from the ejaculate of two adult and sexually mature boars (see their characteristics in Table 1). The animals were kept in a controlled environment with an average temperature of 15°C. Their nutrition was based on boar feed plus a vitamin compound supplement ten days per month. One of the boars (nr.1 or problem boar) displayed impaired fertility; it could be observed that this animal was very nervous, with aggressive behaviour and that always climbed several times to the stanchion before the extraction could be carried out. The other boar (nr.2 or control boar) was normal, as much in behaviour as in fertility.

The semen samples were kept at 15°C of temperature until their arrival at the laboratory. The following parameters have been determined for every sample: pH, sperm concentration, sperm motility, osmotic resistance of acrosomes and sperm morphology. The pH was measured using a special indicator paper which has a narrow range between pH 5.5 and 9.0 (Merck-9564). Sperm concentration (number

Table 1. Characteristics relating to the boars: nr.1 or problem and nr.2 or control.

Characteristic	*Boar nr.1*	*Boar nr.2*
Age (days)	260	230
Weight (Kg)	150	160
Semen extraction rhythm/week	1	1
Cell-rich fraction volume (cc)	50	95
Dilution titre (MR-A)	1/10	1/10
Date of penultimate extraction	26.NOV.1991	21.NOV.1991
Date of sample extraction	28.NOV.1991	28.NOV.1991

Table 2. Results of the parameters examined in the semen doses coming from the boars: nr.1 or problem and nr.2 or control.

Parameter				*Boar nr.1*	*Boar nr.2*
pH				6.5-7.0	7.0-7.5
CONCENTRATION (x10^3 spermatozoa/mm^3)				310-360	170-220
MOTILITY	(%)	Motile Spermatozoa		25-50	90-95
		Spermatozoa with good progressive motility		5-10	80-85
		Spermatozoa with slow progressive motility		25-30	10-15
		Immotile spermatozoa		50-75	5-10
ORT	(%)	150mOsm	Normal acrosomes	85-90	94-96
			Damaged acrosomes	10-15	4-6
		300mOsm	Normal acrosomes	90-92	94-98
			Damaged acrosomes	8-10	2-6
			ORT index	135	144
MORPHOLOGY	(%)	Mature spermatozoa		82-86	89-92
		Immature spermatozoa with proximal droplet		0-2	0-1
		Immature spermatozoa with distal droplet		3-4	5-7
		Aberrant spermatozoa		1-4	0-1
		Folded tail spermatozoa		7-12	1-4

of spermatozoa/mm^3) was determined with a Thoma counting chamber under a phase-contrast microscope (Martín, 1982). Sperm motility was estimated by phase-contrast microscopy and classified according to criteria and methods established by the WHO (1987). The osmotic resistance of acrosomes (ORT) was examined by phase-contrast microscopy after having sujected the spermatozoa to the action of an isotonic solution (300mOsm) and a hypotonic solution (150mOsm) of sodium citrate [ORT index = 1/2 (%NAR in 300mOsm) + (%NAR in 150mOsm); being the %NAR the percentage of spermatozoa with normal acrosome] (Schilling and Vengust, 1987). Sperm morphology was studied by light microscopy following Papanicolaou staining (Alvarez, 1989).

RESULTS

The results of the different parameters determined in the semen doses coming from the two boars (nr.1 or problem boar and nr.2 or control boar) are summarized in Table 2.

The pH value obtained in the semen doses from the control boar was correct, although slightly alkaline. The pH slightly acid found in the semen doses from the problem boar was lower than the normal values.

The sperm concentration of the doses from the control boar was low, whereas in the case of the problem boar it was the suitable.

The sperm motility from the problem boar was very low, only between 5-10% of the spermatozoa displayed good progressive motility and, moreover, the percentage of immotile spermatozoa was very high (50-75%)(Table 2).

The osmotic resistance of acrosomes (ORT), either in isotonic (300mOsm) and hypotonic (150mOsm) medium, was lower in the case of the problem boar than in the control boar, whose ORT values were correct (Table 2). Spermatozoa coming from the problem boar showed a particularly high percentage of damaged acrosomes in hypotonic medium (10-15%), this fact revealed that these spermatozoa were very sensitive to osmotic pressure changes in the medium.

Some notable difference between the two boars also occurred in sperm morphology (Table 2). The percentages obtained in the control boar from the different gametic forms were correct, whereas a high percentage (7-12%) of spermatozoa with folded tail at the Jensen's ring was observed in the sperm from the problem boar.

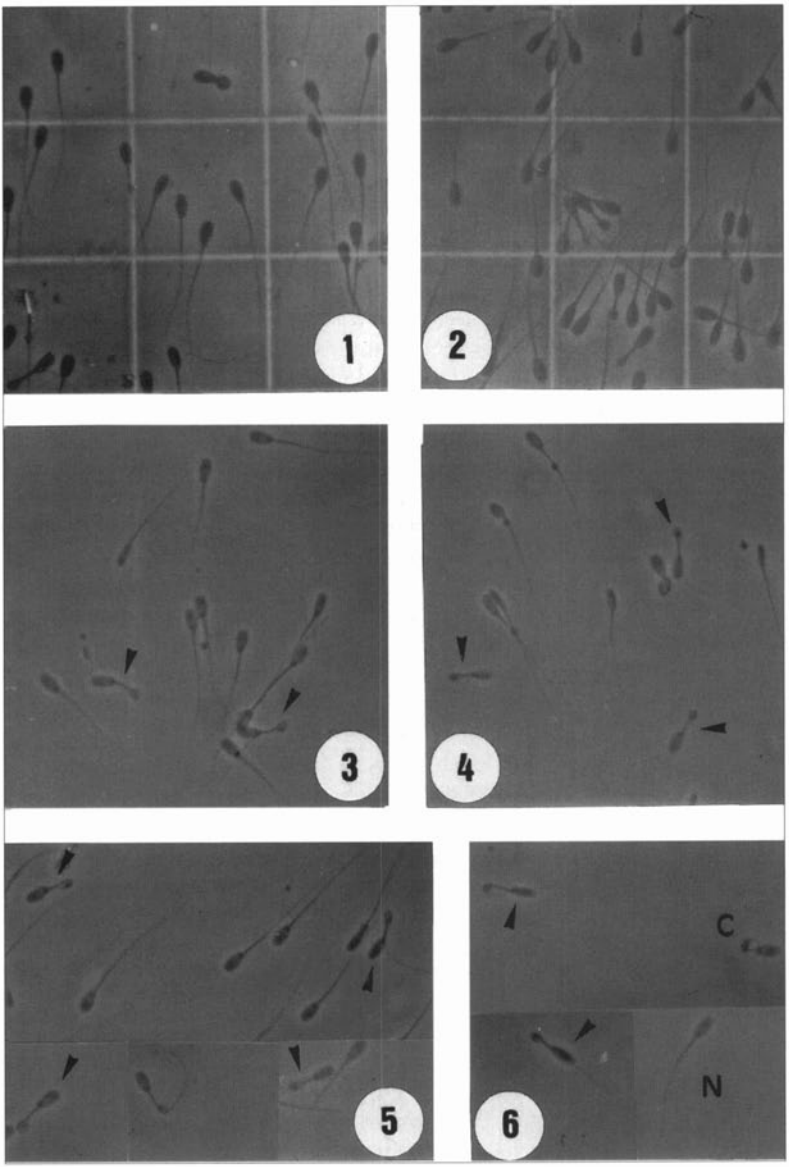
DISCUSSION

The microscopical analysis' results of the sperm from the control boar (nr.2) are within the normal values. The sperm quality of this boar is correct, although in order to gain the maximum profit of its fertility, spermatical doses with a concentration of ca. 300,000 spermatozoa/mm^3 should be established. The low sperm concentration observed might be due to the 7 days period elapsed between the date of the penultimate extraction and the date of the collection of the studied sample, which was too long for this animal, causing a slight decrease of its spermatical production. There are several reports showing that the semen extraction rhythm is one of the most important factors affecting the quantity and quality of the produced

spermatozoa, and therefore the boar fertility; so, either an excessive frequency of semen extractions without rest or too long intervals between the extractions provoke the non total profitability of the animal production ability (Bonet 1987, Bonet et al. 1991, Cameron 1985a and b, Colenbrander and Kemp 1989, Martín 1982, Meding 1975, Swierstra 1973). According to Buxadé (1984), to space out the semen collection from a boar in good conditions more than one week is detrimental, since, in 6 or 7 days the spermatical production fills up the storage capacity of the epididymal tail, and, if there is not ejaculation, the mechanical pressure performed by this accumulation of spermatozoa upon the testis causes the inhibition of the differentation step of spermatogonia into spermatids, this fact has as a consequence an impairment of the spermatical production (furthermore, spermatozoa stored in the epididymal tail may begin to degenerate), and the reproductive potential of the animal is not suitably profited. The boar spermatical production will be appreciably stimulated by emptying regularly the epididymal tail, although respecting the spermatozoal formation and maturation times.

From previous studies in boars we know that, along the passage through the epididymal duct, the sperm changes from a slightly acid pH in the head region to a slightly alkaline pH in the tail region of the epididymis. The spermatozoa coming from the body and tail regions of the boar epididymis are highly resistant to the differences of osmotic pressure in the medium, while the spermatozoa from the head region are most sensitive to osmotic changes in the medium; therefore, the osmotic resistance of the acrosomes is a property acquired by the spermatozoon on reaching the epididymal body. The sperm motility is nil in the first two regions of the boar epididymis and, the sperm from the tail region possess varying degrees of a slight motility (~5%) of the progressive but slow type or non progressive type (Briz et al., 1991). The epididymal duct provides the sperm with the potential for motility, even when in the epididymal environment the spermatozoa are motionless. According to Jouannet (1981), Dacheux et al. (1983) and Orgebin-Crist and Olson (1984), sperm motility appears when the spermatozoa leave the epididymis or when they are transferred to a buffered solution; in this way, when they are removed from the head region of the epididymis they show virtually no motility, while when they are taken form the epididymal tail they describe a restricted circular movement. Schoysman (1981) reported that any epididymal pathology gives rise to an oligospermic and asthenospermic ejaculate. A high incidence of abnormal spermatozoa (teratospermia) in mammalian semen is often indicative of impaired fertility. The presence of a high number of aberrant gametic forms points out that the long and extremely complex process of cellular differentiation, named spermatogenesis, has been forced (Swierstra 1971, Barth and Oko 1989). According to Martín (1982), in healthy and sexually mature boars, the normal percentage of aberrant spermatozoa is between 1-2% per ejaculate. Spermatozoa with tails folded at the Jensen's ring level are the most common aberrant form in the ejaculate of *Sus domesticus* (Bonet, 1987). The spermatozoon with folded tail at the Jensen's ring is an immotile gamete which originates in the cauda epididymidis from immature spermatozoa that have not released the distal cytoplasmic droplet (Bonet et al., 1992).

Regarding to the sperm analysis of the problem boar (nr.1), the slightly acid pH of the semen doses, the incidence of a high percentage of folded tail spermatozoa, the very low motility and the insufficient osmotic resistance of acrosomes, allows us



FIGURES. 1. Phase contrast photomicrograph of the sperm concentration, as determined by Thoma Chamber, from the semen dose of the control boar (nr.2). X400. **2.** Phase contrast photomicrograph of the sperm concentration, as determined by Thoma Chamber, from the semen dose of the problem boar (nr.1). X400. **3.** Spermatozoa submitted to a hypotonic medium (150mOsm), from the semen dose of the control boar (nr.2).[arrows, damaged spermatozoa]. Phase contrast. X400. **4.** Spermatozoa submitted to a hypotonic medium (150mOsm), from the semen dose of the problem boar (nr.1).[arrows, damaged spermatozoa]. Phase contrast. X400. **5.** Spermatozoa submitted to an isotonic medium (300mOsm), from the semen dose of the problem boar (nr.1).[arrows, damaged spermatozoa]. Phase contrast. X400. **6.** Folded tail spermatozoa [arrows] coming from the semen dose of the problem boar (nr.1). C, coiled tail spermatozoon; N, normal spermatozoon. Papanicolaou. X400.

to affirm that the yielded sperm is immature due to an incomplete process of sperm maturation, and being therefore the result of an epididymal dysfunction. The epididymal maturation of sperm is a slow and complex process, and the ejaculate's sperm quality depends on a complete maturation process. The presence of epididymal gamete forms in the ejaculate is a sign of an incomplete sperm maturation (Bonet and Briz, 1991). This boar shows symptoms of nervous stress, so then, the passage of the sperm through the epididymis is likely to be too fast, not allowing its correct maturation; and as a consecuence, it is yielded an immature sperm with characteristics inherent to the proximal regions of the epididymis (caput-corpus) and unable to fertilize. It takes some time for spermatozoa to pass through the epididymis and become mature. Singh (1962) and Swierstra (1968) measured this time by means of the transport of labelled spermatozoa in boars, and established that the duration of sperm transit through the epididymal duct varies from 9 to 12 days [mean transit time= 10.2 days]. During this transit through the epididymis, sperm undergoes a complex maturation process which fundamentally takes place in the first two regions of the epididymis, to be stored once mature in the epididymal tail until the moment of ejaculation (Holtz and Smidt, 1976). The maturational changes that occur in the sperm during its journey through the epididymis have been attributed to the secretory action of the epididymal epithelium (Cooper, 1986).

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