ARTICLE *SCIENTIA gerundensis*, 17:77-84 (1991)

THE SPERM QUALITY AND FERTILITY OF BOARS AFTER TWO DIFFERENT EJACULATION FREQUENCIES

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RESUM

Deu ejaculacions de cadascun dels sis mascles Large White, sans i de 22 mesos d'edat, han estat examinades al microscopi de contrast de fases i al microscopi electrònic d'escandallatge per observar-ne la motilitat i concentració, i la morfologia espermàtiques, respectivament. Per estudiar la fertilitat, 75 femelles multípares han estat inseminades per cadascun dels sis mascles als 22 mesos d'edat. A partir de tres mascles (primer grup) l'esperma ha estat recollit a un ritme d'extraccions de dos cops a la setmana, sense períodes de repòs, des dels 10 mesos d'edat. Els altres tres mascles (segon grup) han estat sotmesos a una freqüència de recol·lecció de semen amb intervals de dos dies, sense períodes de repòs, des dels 10 mesos d'edat. Les diferències de fertilitat i de qualitat espermàtica entre els mascles del primer i segon grup han estat significatives ($p<0.01$). En el segon grup, el pH de l'esperma i els percentatges d'espermatozoides amb gota proximal i aberrants han augmentat; mentre que la motilitat i concentració espermàtiques, els percentatges d'espermatozoides madurs i immadurs amb gota distal, i la fertilitat han disminuït. La freqüència de recol·lecció del semen afecta la qualitat espermàtica i la fertilitat dels mascles reproductors.

RESUMEN

Diez eyaculaciones de seis machos Large White, sanos y de 22 meses de edad, han sido examinadas al microscopio de contraste de fases y al microscopio electrónico de barrido para determinar la motilidad y concentración, y morfología espermáticas, respectivamente. Para estudiar la fertilidad, 75 hembras multíparas han sido inseminadas por cada uno de los seis machos a los 22 meses de edad. A partir de tres machos (primer grupo) el esperma ha sido recogido con un ritmo de extracciones de dos veces por semana, sin periodos de descanso, desde los 10 meses de edad. Los otros tres machos (segundo grupo) han sido sometidos a una frecuencia de extracciones de semen con intervalos de dos días, sin periodos de descanso, desde los 10 meses de edad. Las diferencias de fertilidad y de calidad espermática entre los machos del primer y segundo grupo han sido significativas ($p<0.01$). En el segundo grupo, el pH del esperma y los porcentajes de espermatozoides con gota proximal y aberrantes aumentan; mientras que la motilidad y concentración espermáticas, los porcentajes de espermatozoides maduros e inmaduros con gota distal y la fertilidad disminuyen. La frecuencia de recolección del semen afecta la calidad espermática y la fertilidad de machos reproductores.

ABSTRACT

Ten ejaculates were examined from each one of six healthy boars, Large White breed, at the age of 22 months. Quality assesment was carried out by means of phase contrast light microscopy and scanning electron microscopy to determine the motility, concentration and sperm quality. To study their fertility, 75 multiparous sows were inseminated by each one of six boars at the age of 22 months. From three boars (group 1) semen was collected twice weekly, without rest periods, from the age of 10 months. Another three boars (group 2) were subjected to an ejaculation frequency at 2-day intervals, without rest periods, from the age of 10 months. The differences in boar fertility and sperm quality between the first group and the second group are statiscally significant ($p<0.01$). In the second group the semen pH, the spermatozoa with proximal droplet and the aberrant spermatozoa increase, and the sperm motility, the sperm concentration, the mature spermatozoa, the spermatozoa with distal droplet and the fertility decrease. The depletion of the sperm reserves of the tail of the epididymis and a shortened maturation of the spermatozoa in the epididymis can be the reasons of these changes in the quality sperm. The ejaculation frequencies affect the sperm quality and the boar fertility.

Paraules clau: Freqüència d'ejaculació, Fertilitat, Qualitat espermàtica.
Palabras clave: Calidad espermática, Fertilidad, Frecuencia de eyaculación
Key words: Ejaculation frequency, Fertility, Sperm quality.

INTRODUCTION

Subfertility in a reproductive male is of great significance in porcine livestocks for selection and breeding (Bonet & Castellanos, 1989), especially because in farms using artificial insemination, about 20 to 30 sows are inseminated with a single ejaculate.

Cameron (1985) and Colenbrander and Kemp (1989) describe the main factors affecting production and sperm quality of boars, the most important of which are: the size of testis, which depends on the age and breed of each individual, collection frequency, environmental factors (temperature, light and season of the year), nutritional factors, and finally, the housing and management of the boar. Whatever the kind of factor is causing subfertility or sterility, it always results in an alteration of the sperm quality of the ejaculate. According to Bonet (1987), in cases of sterility or subfertility due to a loss of the fertilization capacity of spermatozoa, one can assume three major non-excluding causes: low spermatical concentration, high rate of abnormal and aberrant gametic forms and loss of motility. In porcine livestocks it is extremely important to know exactly which are the factors causing sterility or subfertility, in order to provide the necessary means in case of low reproductive efficiency.

Only a few works have been published dealing with the causes of subfertility in boars and their effect on the ejaculate quality. Kennedy and Wilkins (1984) studied the effect of breed and environmental factors on semen quality in boars. Swierstra (1973), Swierstra and Dyck (1976) and Bonet (1987) examined the ejaculate of boars suffering stress from high collection frequency. Larsson and

Einarsson (1984) and Malmgren and Larsson (1984) carried out a study on the effect of high temperatures on fertility in boars. Trudeau & Sanford (1986) studied the effect of the season of the year and the social environment on the size of the testis and on semen quality of adult Landrace boars.

In this paper, the ejaculate and the fertility of boars have been examined at the age of 22 months after frequent semen collections at 2-day intervals, without rest periods, from the age of 10 months.

MATERIALS AND METHODS

Ten semen samples, from each of six healthy males, Large White breed, have been examined at the age of 22 months. The first group of three boars was kept at a temperature of 18-25°C and semen was collected twice-weekly, without rest periods, from the age of 10 months. The second group of three boars was kept at a temperature of 18-25°C and was subjected to a frequent semen collection for AI at 2-day intervals, without rest periods, from ten months of age.

For each semen sample, pH, sperm motility, sperm concentration and sperm quality were evaluated. The pH was measured directly from the doses at 37°C with a CRISON 506 pH-meter. The sperm motility, expressed as % of spermatozoa capable of progressive movement, was estimated by phase contrast microscopy (Martin, 1982). Sperm concentration was determined using a Thoma counting chamber (Martin, 1982). Sperm quality was evaluated by means of the method of Papanicolau (WHO, 1987) and by scanning electron microscopy, according to the procedure described by Bonet (1990).

To study their fertility seventy five multiparous sows were inseminated (AI) by each one of the six boars at 22 months of age. These 450 multiparous sows were inseminated as follows: a dose of 85ml 12-24 hours after the beginning of oestrus, and another dose of 85ml 24-36 hours after the beginning of oestrus. The seminal doses were prepared with the rich and poor fractions of the ejaculate and the diluent MR-A (dilution titre 1:10), and stored at 15°C during 48 hours (Martin, 1982).

RESULTS

The differences in sperm quality between boars of the first group (subjected to an ejaculation frequency twice-weekly) and boars of the second group (subjected to an ejaculaion frequency at 2-day intervals) affect the semen pH, the sperm motility, the sperm concentration, the mature spermatozoa, the spermatozoa with distal droplet, the spermatozoa with proximal droplet and the aberrant spermatozoa.

Microscopical analysis of the sperm collected at 22 months of age from boars of the first group and the second group gave these results: a/ the pH of the

ejaculate, the spermatozoa with proximal droplet and the aberrant spermatozoa increased, and b/ the motility, the sperm concentration, the mature spermatozoa and the spermatozoa with distal droplet decreased (Table I). The main microscopical changes observed were:

1. The sperm motility decreases from 76.3% in boars of the first group to 35.2% in boars of the second group ($p<0.01$).

2. The spermatozoa with proximal droplet increase from 3.5% in boars of the first group to 67.4% in boars of the second group ($p<0.01$).

3. The spermatozoa with distal droplet decrease from 25.3% in boars of the first group to 1.2% in boars of the second group ($p<0.01$).

4. The mature spermatozoa decrease from 68.9% in boars of the first group to 25.1% in boars of the second group ($p<0.01$).

5. The aberrant spermatozoa increase from 1.6% in boars of the first group to 6.7% in boars of the second group ($p<0.01$). The spermatozoa with coiled and folded tails are more abundant in the ejaculate of boars of the second group than in the ejaculate of boars of the first group.

The fertility decreased from 73.5% in boars of the first group to 7.7% in boars of the second group ($p<0.01$).

The differences in sperm quality and fertility between boars of the first and the second groups are statiscally significant ($p<0.01$).

DISCUSSION

The diluent is one of the factors that have more important effects on the quality of refrigerated semen. Papiol (1986) compared the diluents Kiew or EDTA, SCK-7 and MR-A used in the preservation of refrigerated boar semen and he concluded that, in the Pietrain race, semen preservation at 15°C in doses containing 3×10^9 spermatozoa, with 80% motility at a dilution titre of 1:10, at least a minimum of 70% fertility is mantained after 4 days and up to 77.5% after 48 hours. In the same conditions, a/ the sperm motility has been 76.3% in boars of the first group and 35.2% in boars of the second group, and b/ the fertility of the studied boars has been 73.5% in boars of the first group and 7.7% in boars of the second group. The kind of diluent used cannot be the cause of these low motility and fertility observed in boars of the second group.

Marcatti et al. (1984) and Horno (1985) studied the optimum time to inseminate multiparous sows, and they verified that maximum fertility (92.6%) in pigs is reached when the insemination takes place between 12 and 18 hours after the beginning of oestrus and a second insemination from 12 to 18 hours later. In this paper, to estimate the fertility of boars, sows were inseminated two times: the first one from 12 to 24 hours after the beginning of oestrus and the second time from 12 to 24 hours later. Thus, the insemination time cannot be the cause of the subfertility observed in the reported boars of the second group.

Table 1. Comparison between values obtained at 22 months of age a/ from boars subjected to an ejaculation frequency twice weekly (first group), from the age of 10 months and without rest periods; and b/ from boars subjected to an ejaculation frequency at 2-day intervals from 10 months of age and without rest periods.

Observed Values	Second Group 2-day intervals		First Group Twice-weekly
Fertility	7,7 ± 2,5%		73,5 ± 2,5%
pH	7,47 ± 0,05		7,15 ± 0,05
Motility	35,2 ± 8,1%		76,3 ± 5,1%
Concentration	210.000 ± 9.500/mm³		300.000 ± 10.500/mm³
Sperm Quality	Light Microscopy	Scanning Microscopy	Light Microscopy
Mature spermatozoa	25,1 ± 5,5%	26,3 ± 5,2%	68,9 ± 16,4%
Spermatozoa with proximal droplet	67,4 ± 5,2%	62,3 ± 3,3%	3,5 ± 2,2%
Spermatozoa with distal droplet	1,2 ± 0,5%	1,7 ± 0,9%	25,3 ± 13,2%
Aberrant spermatozoa	6,7 ± 2,7%	6,6 ± 2,5%	1,6 ± 0,5%

The temperature is another of the factors that has recently received more attention as a cause of low sperm motility and subfertility in pigs. According to Sola et al. (1986), the highest tolerated temperature is 27°C; therefore, boars exposed to temperatures of 30°C or higher show semen malformations that persist up to 2 months after having suppressed the cause. Larsson and Einarsson (1984) observed that after subjecting the boars to a temperature of 35°C for 100 hours, the ejaculate reaches the highest inaptitude after 3 weeks: the motility decreases to 55-60%, the occurrence of proximal droplets increases to 20-25% and the incidence of aberrant forms is of 15-20%. According to Malgrem and Larsson (1984) fertility can reach a minimum of 63% 2 or 3 weeks after the thermal stress at 35°C. The temperature can also be rejected as a factor causing the low sperm quality and subfertility observed in the reported boars of the second group, because the boars were confined in a room at a controlled temperature between 18 and 25°C; on the other hand, neither the subfertility rate (7.7%), nor the ejaculate quality assessment (35.2% motility and 67.4% proximal droplets) can be assumed as disfunctions due to thermal stress.

In artificial insemination the collection frequency is one of the most important factors affecting semen quality.

The pH value of ejaculate in the reported boars of the second group (7.47), although slightly alcaline, does not suggest any alteration in the prostate or the seminal vesicle, since it is very close to acceptable limits (6.9-7.4) (Buxadé,1984),

and the high value observed is probably due to a higher percentage of glandular secretion and to a smaller seminal volume.

The sperm motility in the boars of the second group (35.2%) is decreased to a considerable degree. This fact, together with a high rate of immature gametes with proximal droplet (67.4%), indicates that there is a fast passage through the epididymis and therefore an insufficient sperm maturation (Schilling and Vengust, 1987). The amount of aberrant forms (6.7%), far exceeding normal values (1-2%) (Martín,1982) is also a clear sign that such a complex process of cellular differentiation as spermatogenesis has obviously been forced (Swierstra, 1971) (Barth and Oko, 1989). According to Buxadé (1984), production of spermatozoa is 15 x 10^9 cells/day. The maturation process in the epididymis lasts 7 to 8 days and in 6 to 7 days the tail of the epididymis is completely replenished. In a single ejaculation, 60% of the spermatical population stored in the tail of the epididymis is emptied, and after 3 or 4 ejaculates collected at intervals of 12 hours, the tail of the epididymis is almost completely depleted, and therefore the passage of cells from the head to the tail of the epididymis is forced. This fact gives rise to the high percentage of spermatozoa with proximal droplet, the small number of spermatozoa with distal droplet and the severe loss of motility.

The number of spermatozoa in the ejaculate varies according to the collection frequency (Jonhson et al., 1969; Meding, 1975). Signoret (1974) reported that the total number of spermatozoa in the ejaculate is 100,000 million when collection frequency is one every 7 days, but the total amount decreases to 50,000 million when collection frequency is one every 3 days.

Bonet (1987) studied the ejaculate of a boar subject to series of extractions at intervals shorter than 24 hours, and reported the occurrence of more than 60% immature gametes. This result, due to an intensive stress with rest periods, is similar to the one obtained in this work, in which the boars of the second group have also been subjected to stress, although it was more extensive and without rest periods. According to Pearson (1989) a male older than 10 months can be subjected to a collection frequency of 4 services per week only if these periods alternate with rest intervals; if not, both sperm quality and fertility are affected. Shilling & Vengust (1987) observed that a collection frequency of one extraction every two days during 10 days, results in a decrease of motility (down to 63%) and an important increase in the amount of abnormal acrosomes (up to 70%). The authors explain these results as an incomplete maturation due to a fast passage of spermatozoa through the epididymis. Holt (1982) observed that the origin of spermatozoa with coiled and folded tails is in the epididymis; in our paper, in boars of the second group spermatozoa with coiled and folded tails increased too. The results reported in this paper suggest that collection frequency stress cannot only appear in case of intensive extractions, but also when they are extensive and without rest intervals.

From the results obtained after examining the ejaculates, it can be concluded that the boars of the second group show a low sperm quality and fertility due to an excessive collection frequency for artificial insemination and not to other

etiologies that have a deletereous influence on sperm quality and fertility, such as the diluent of the doses used, the temperature and the moment of insemination. Because the boars were healthy, other causes such as testicular hypoplasia, hermaphroditism, degeneration of germ line cells, etc., or infectious diseases such as brucelosis or tuberculosis, which can affect the sperm quality and fertility, can also be discarded.

Acknowledgements

The authors wish to thank Dr. Josep Egozcue (Universitat Autònoma de Barcelona), Dr. Mercè Durfort (Universitat de Barcelona) and Dr. Marisa Molinas (Estudi General de Girona) for their scientific assessment. This study was made possible by financial support from the CIRIT (Generalitat de Catalunya).

Refereences

BARTH, A.D. & OKO, R.J. (1989). *Abnormal Morphology of Bovine Spermatozoa*. Iowa State University Press, Ames.

BONET, S. (1987). Estudio del eyaculado de un verraco estresado por la frecuencia de recogidas en inseminación artificial. *Scientia gerundensis*, 13: 35-40.

BONET, S. (1990). Immature and aberrant spermatozoa in the ejaculate of *Sus domesticus*. *Anim. Reprod. Sci.*, 22: 67-80.

BONET, S. & CASTELLANOS, M. (1989). Estudi de la qualitat espermàtica de Sus domesticus i la seva repercussió en les explotacions porcines. *Biologia de la Reproducció*, 1: 204-210.

BUXADÉ, C.C. (1984). *Ganado Porcino: sistemas de explotación y técnicas de producción*. Mundi Prensa, Madrid.

CAMERON, R.D.A. (1985). Factors influencing semen characteristics in boars. *Aust. Vet. J.*, 62: 293-297.

COLENBRANDER, B. & KEMP, B. (1989). Factors affecting semen quality in the boar. *Proceedings 10th Symposium Anaporc*: 58-66.

HOLT, W.V. (1982). Epididymal origin of a coiled-tail sperm defect in a boar. *J. Reprod. Fert.*, 64 : 485-489.

HORNO, L.A. (1985). Fertilidad y concepción. Momento óptimo de la inseminación en cerdas multíparas y nulíparas. *Anaporc*, 37: 3-10.

JONHSON, L.A.; GERRITS, R.J & YOUNG, E.P (1969). Quantitative analysis of porcine spermatozoa and seminal plasma phospholipids as affected by frequency of ejaculation. *J. Reprod. Fert.*,19 : 95-102.

KENNEDY, B.W & WILKINS, J.N. (1984). Boar, breed and environmental factors influencing semen characteristics of boars used in artificial insemination. *Can. J. Anim. Sci.*, 64: 833-843.

LARSSON, K. & EINARSSON, S. (1984). Seminal changes in boars after heat stress. *Acta Veterinaria Scandinavica*, 25: 57-66.

MALMGREN, L. & LARSSON, K. (1984). Semen quality and fertility after heat stress in boars. *Acta Veterinaria Scandinavica*, 25: 425-435.

MARCATTI, N.A.; NOGUEIRA, L.A.G.; SILVA, F.J.M.; BERGMANN, J.A.G. & SILVA, M.I.F. (1984). El efecto del momento de la cubrición sobre la fertilidad y el tamaño de la camada en cerdas multíparas y nulíparas. *Proceedings VIII Congress IPVS*: 355.

MARTIN, R.S. (1982). *Reproducción e inseminación artificial porcina*. Aedos, Barcelona.
MEDING, H.J. (1975). Effect of frequency of semen collection on semen production and breeding efficiency of A.I. boars. *Nord. Veterinaermed*, 27: 153-160.
PAPIOL, G.C. (1986). Comprobación de diferentes diluyentes para la conservación de semen de verraco refrigerado a 15ºC. *Anaporc*, 48: 95-103.
PEARSON, M. (1989). Managing the modern boar. *Pigs* (march-april): 22.
SCHILLING, E. & VENGUST, M. (1987). Frequency of semen collection in boars and quality of ejaculates as evaluated by the osmotic resistance of acrosomal membranes. *Anim. Reprod. Sci.*, 12: 283-190.
SIGNORET, J.P. (1974). Ultimas aportaciones sobre la reproducción del cerdo y su aplicación a las prácticas de producción. *Actas I Simposio Internacional Porcino*: 113-131.
SOLÀ, P.J. & CARMENES, P. (1986). Trastornos de la reproducción en el ganado porcino. *Proceedings IX Congress IPVS* : 3-8.
SWIERSTRA, E.E. (1971). Sperm production of boars as mentioned from epididymal reserves and quantitative testicular histology. *J. Reprod. Fertil.*, 27: 91-99.
SWIERSTRA, E.E. (1973). Influence of breed, age and ejaculation frequency on boar semen composition. *Can. J. Anim. Sci.*, 53: 43.
SWIERSTRA, E.E. & DYCK, G.W. (1976). Influence of the boar and ejaculation frequency on pregnancy rate and embryonic survival in swine. *J. Anim. Sci.*, 42(2): 455-460.
TRUDEAU, V. & SANFORD, M. (1986). Effect of season and social environment on testis size and semen quality of the adult Landrace boar. *J. Anim. Sci.* 63: 1211-1219.
WHO (1987). *Laboratory Manual for the Examination of Human Semen and Semen Cervical Mucus Interaction*. Cambridge University Press, Cambridge.