

Semen quality of Italian local pig breeds

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RIASSUNTO – Qualità del materiale seminale delle razze suine locali italiane. *Tra il 1996 e il 1999 nell'ambito del progetto europeo "European gene banking project for the pig genetic resources" si è attuato un programma di conservazione delle razze suine locali italiane. Oltre al censimento delle razze e delle organizzazioni coinvolte nella loro gestione il progetto prevedeva la caratterizzazione delle razze per caratteri produttivi e riproduttivi. Nel presente lavoro sono illustrate la qualità, la composizione lipidica e l'attitudine al congelamento del materiale seminale di quattro razze suine locali italiane: Cinta Senese, Casertana, Mora Romagnola e Nero Siciliano. La qualità del seme delle razze locali è risultata simile a quella delle razze commerciali. Considerando complessivamente volume, concentrazione, vitalità e attitudine al congelamento i verri di razza Nero Siciliano hanno prodotto eiaculati di migliore qualità rispetto ai verri di Cinta Senese e Casertana. Per la Mora Romagnola, essendo rappresentata soltanto da due verri, non è stato possibile fare confronti rispetto alle altre razze. La composizione lipidica in PUFA è diversa tra razze. E' interessante sottolineare l'elevata presenza di DHA negli spermatozoi di razza Cinta Senese.*

Key words: pig local breeds, semen quality, lipid composition, freezability.

INTRODUCTION – From 1996 to 1999 a conservation programme was carried out within the framework of EC contract "European gene banking project for the pig genetic resources" (Ollivier *et al.*, 2001) in the Italian local pig breeds. The aims of the program included the primary characterization of the breeds, i.e. information on the organization in charge of the breed, breeding population numbers, breed description and qualifications, and field trials on productive and reproductive performances. In this context the "Semen Bank of Italian local pig breeds" was built. A total of 30,835 straws of four Italian local pig breeds (Cinta Senese, Casertana, Mora Romagnola and Nero Siciliano), collected from 42 sires, have been stored. In this work semen quality traits, lipid composition and freezability of the four Italian local pig breeds are reported.

MATERIAL AND METHODS – Five hundred and fifty seven ejaculates were collected from 42 boars of four Italian local pig breeds from January 1998 to January 2002. Mora Romagnola was less represented than the other breeds because semen has been collected only from two boars. At collection, semen volume, sperm concentration and motility were measured. The volume was measured by weighing the ejaculate. Sperm concentration was evaluated by Coulter counter (Coulter Z1). Motility was assessed subjectively at the microscope (Nikon Diaphot). Semen was frozen according to the procedure of Westendorf *et al.* (1975) modified by Almlid *et al.* (1988). At thawing, normal apical ridge (NAR) was detected using phase contrast microscopy. Motility was assessed after 1, 3 and 5 hours of incubation at 37°C on frozen-thawed samples. On 74 ejaculates, balanced among different breeds, computer assisted semen analysis of motility and related parameters, viability of cells

and lipid analysis were performed. Viability was recorded using a fluorometric ethidium bromide exclusion procedure. Fluorescence measurements were performed at room temperature with a spectrofluorometer (model FP750; JASCO Corporation). Total lipids were extracted in excess chloroform:methanol (2:1 v:v), and then separated by thin layer chromatography (TLC) in the following classes: phospholipids (PL), free cholesterol (FC), free fatty acids (FFA), triacylglycerols (TG) and cholesterol esters (CE). The fatty acid profile of the major classes was determined by gas chromatography on capillary column after trans-methylation. The amount of free cholesterol (FC) was quantified by standard colorimetric assay (Boehringer Mannheim Spa, Germany). Breed effect on semen quality and lipid composition was detected using General Linear Model procedure (SAS, 1998). The model includes the effect of breed and boar within the breed as fixed effect.

RESULTS AND CONCLUSIONS – Volume, concentration and motility evaluated on ejaculates collected in the different breeds are shown in Table 1.

Table 1. Semen quality parameters in four Italian pig local breeds (LSmean ± SE)

	Cinta Senese	Casertana	Mora Romagnola	Nero Siciliano
Volume (ml)	122.26 ± 3.01a	114.01 ± 4.11a	164.76 ± 10.84b	149.48 ± 3.12b
Concentration (sperm 10 ⁶ /ml)	377.67 ± 12.14a	244.81 ± 16.57b	271.25 ± 43.66bc	301.53 ± 12.57c
Motility (%)	59.34 ± 1.09a	53.04 ± 1.48b	29.64 ± 3.91c	62.07 ± 1.12a
Viability (%)	83.83 ± 1.13a	89.89 ± 1.13b	77.74 ± 2.93a	83.39 ± 1.09a

Different letters on the same row correspond to significant differences (a, b: P<0.01)

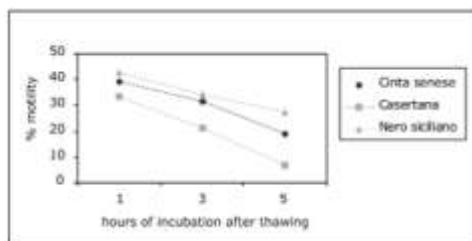
The volume of the ejaculate was slightly lower in Italian local breeds than in commercial breeds. In Mora Romagnola the highest value (164.76±10.84 ml) was recorded, but we remind that for this breed the parameter was estimated on two sires only. Sperm concentration evaluated in the four local breeds was similar than in commercial breeds. The values ranged from a 244.81±16.57 x10⁶ spermatozoa /ml in Casertana and 377.67±12.14 x10⁶ spermatozoa /ml in Cinta Senese.

Motility (%) was not statistically different between Nero Siciliano and Cinta Senese (62.07±1.12 and 59.34±1.09 respectively). This parameter was quite low and highly variable in Mora Romagnola (29.64±3.91). In Casertana the percentage of motility was 53.04±1.48.

Viability (%) of sperm cells was similar in Cinta Senese and Nero Siciliano, 83.83±1.13 and 83.39±1.09 respectively, whereas it was statistically different in Casertana and Mora Romagnola. In Casertana breed a high percentage of viable cells was detected (89.89±1.13), in stark contrast to Mora Romagnola (77.74±2.93).

After freezing/thawing motility was recorded after 1, 3 and 5 hours of incubation in Cinta Senese, Casertana and Nero Siciliano. In the latter, motility after 5 hours remained higher than in the other breeds indicating a good attitude of sperm cells to survive in the female tract (Figure 1).

Figure 1. Motility patterns after 1, 3 and 5 hours of incubation in three Italian pig breeds.



The loss of motility after freezing/thawing ranged from approximately 37% in Casertana and 30% in Nero Siciliano. The relative loss of motility in Nero Siciliano indicates a good resistance of sperm cells to cryoconservation injuries. NAR (%) at thawing was low in Casertana (50.93±2.74), showing that only half of the cells had normal apical ridge. In Cinta Senese and Nero Siciliano this value (%) was 65.99±2.10 and 60.63±1.44

respectively; the difference between these two breeds was not significant. Considering the loss of motility after freezing/thawing and NAR we can conclude that freezability was higher in Nero Siciliano and Cinta Senese than in Casertana.

The proportions of the major fatty acids of PL in spermatozoa and seminal plasma are shown in table 2. Saturated fatty acids such as C16:0 and C18:0 were higher in plasma than in spermatozoa in all breeds. As a consequence the proportion of total saturates (SFA) was higher in plasma than in spermatozoa in all the Italian local breeds. The major polyunsaturates in sperm phospholipids were C22:5n6 (DPA) and C22:6n3 (DHA), accounting for around 57% of the total fatty acids in all breeds. The highest value of DPA, 35.87±3.09, was detected in Mora Romagnola, whereas the lowest, 23.06±1.95, in Cinta Senese. The opposite was evidenced in the major omega 3 fatty acid (DHA), with the greater proportion recorded in Cinta Senese (39.56±2.51) and the lowest in Mora Romagnola (24.17±3.96). Polyunsaturates (PUFA) represent around the 70% of PL fatty acids in spermatozoa; a lower proportion, approximately 30%, was detected in seminal plasma. FC content ($\mu\text{g}/10^9$ cells) of spermatozoa was 300.72±86.59, 181.34±24.09 and 255.76±43.31 in Nero Siciliano, Casertana and Mora Romagnola respectively. The fatty acid composition reveals interesting feature of less selected breeds. As a matter of fact, the DHA content of sperm PL of Cinta Senese is rather unique, usual levels of DHA are of about 30% for commercial breeds fed with standard diets. These results may suggest that, to an extent, selection has had an effect on biochemical parameters of boar semen. Our previous studies have indicated correlations between specific fatty acids and sperm quality parameters (Cerolini *et al.*, 2002).

Table 2. PL fatty acid composition of spermatozoa and seminal plasma at collection in different breeds (LSmean \pm SE standard error)

	Cinta Senese		Casertana		Mora Romagnola		Nero Siciliano	
	spermatozoa	plasma	spermatozoa	plasma	spermatozoa	plasma	spermatozoa	plasma
C16:0	14.40±1.56	22.58±1.56	16.20±1.44	24.54±1.44	13.34±2.49	18.69±2.49	16.61±1.17	18.01±1.57
C18:0	7.13±1.23	15.51±1.23	8.41±1.12	15.20±1.12	7.55±1.94	14.99±1.94	6.41±0.91	13.97±1.23
C22:5n6	23.06±1.95	1.80±1.95	26.29±1.78	2.65±1.78	35.87±3.09	10.39±3.09	27.52±1.46	2.64±1.95
C22:6n3	39.56±2.51	2.15±2.51	27.51±2.28	3.35±2.28	24.17±3.96	6.49±3.96	24.52±1.86	1.87±2.51
Total PUFA	71.29±4.54	18.73±4.54	64.8±4.14	31.72±4.14	71.24±7.18	46.26±7.18	59.43±3.38	31.55±4.54
Total SFA	25.64±3.86	54.57±3.86	31.15±3.52	54.03±3.52	25.09±6.10	41.50±6.10	36.78±2.87	40.65±3.86

Semen quality and freezability evaluated in Cinta Senese, Casertana and Nero Siciliano boars was similar to commercial breeds. Mora Romagnola was represented only by two sires therefore only preliminary comparison can be done. Nero Siciliano seems to show a good semen quality and the best attitude to freezing semen, followed by Cinta Senese and Casertana.

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