Animal Science Papers and Reports vol. 39 (2021) no. 2, 141-150
Institute of Genetics and Animal Biotechnology
of the Polish Academy of Sciences, Jastrzębiec, Poland

Genetic investigation for the characterization of three indigenous pig breeds of southern Italy: advantages and prospects

Cristina Rossetti[1], Angela Perucatti[1], Filomena Mottola[2], Domenico Incarnato[1], Viviana Genualdo[1]*

[1] National Research Council (CNR), ISPAAM, Laboratory of Animal Cytogenetics and Genomics, P.le Enrico Fermi 1, Italy

[2] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", 81100 Caserta, Italy

(Accepted April 22, 2021)

The characterization and safeguarding of typical and local animal breeds were the focus of this study. These two aspects are crucial for their management, in particular for the Italian pig population. Indeed, Italy is among the top ten nations in terms of the pig population, with the local breeds famous worldwide for their great meat production. In this study we characterized three of these local pig breeds of Italy, i.e. Nero Siciliano (Black Sicily, BS), Casertana (C) and Nero Lucano (Black Lucano, BL), using cytogenetic and genomic techniques. These three local pig breeds of Italy were characterized by Aneuploidies, CAs (Chromosome Aberration) and SCEs (Sister Chromatid Exchange) and RAPD-PCR (Random Amplified Polymorphic DNA – Polymerase Chain Reaction) in order to clarify any genetic differences between the breeds to preserve biodiversity and to guarantee economic development of local pork industries. Comparing the data obtained from the analysis of Aneuploidies, CAs and SCEs, as well as the RAPD-PCR examination, it was concluded that the BS and C breeds are more similar and sufficiently different from the BL breed. The genetic diversity and the characteristic polymorphic profile make BL a breed of high value to be protected, particularly for its much appreciated meat.

The research was carried out as a preliminary phase for the development program for local breeds and will provide useful information for the management, conservation and promotion of these three Italian pig breeds.

*Corresponding authors: viviana.genualdo@cnr.it

KEY WORDS: aneuploidy swine / chromosomal aberration / genetic variability/ RAPD-PCR

Pigs are the most popular farm animals in the world with about a 148.2 million of head only in Europe and their meat is the most widely consumed. Italy is among the top ten nations for number of pig population [Eurostat 2018] However, as resulted from the decline of farm animals, from twenty-one Italian local breeds, at the beginning of the 20th century [Franci and Pugliese 2007] only six autochthonous pig breeds at risk of extinction are currently recognized at the national level in the National Pedigree Register of Italian National Pig Breeders Association (ANAS). These local breeds probably belong to the Mediterranean type, diversifying as late as in the beginning of the last century when in Northern Italy with its extensive farming local breeds started to disappear, while in Southern Italy farmers were more motivated to preserve local breeds [Franci and Pugliese 2007]. This genetic erosion was the effect of globalization, due to increases in the demand for livestock products, converting farming from extensive to intensive production systems, in favor of genotypes coming from northern European countries [Maiorano 2009]. The major genetic erosion risk is the loss of local breeds potentially leading to extinction of an entire species. For this reason, it is essential to characterize typical local breeds in order to conserve biodiversity, which explain the temporal relationship between animals and the environment, such as greater disease resistance [FAO 2007] or the ability to convert natural grazed or low quality food (roughage) into high-quality products (meat), as well as safeguard local economic resources, especially for low-input production systems.

In this study for the first time we carried out a genetic study by cytogenetic and genomic characterization of the three local pig breeds of southern Italy: Nero Siciliano (Black Sicilian, BS), Casertana (C) and Black Lucano (BL), the latter registered and also known as the Apulo-Calabrese breed (AC) – [ANAS 2020]. Fortunately, the data of these three breeds, as reported in 2019, were increasing: with 7313, 4650 and 871 head, respectively (DAD-IS). The BS, BL and C pigs have very similar morphological, reproductive and productive characteristics [Bozzi *et al.* 2019], but with a genetic diversity, which is essential information for their management [Muñoz *et al*. 2019, Schiavo *et al.* 2020]. These breeds are most appreciated also for the abovementioned characteristics, especially for meat production characterized e.g. by the content of intramuscular fat (IMF) as a result of their high predisposition to the deposit of MUFA (monounsaturated fatty acids), the quality traits that confer excellent seasoned hams [Pugliese and Sirtori 2012].

Our previous cytogenetic study evaluated chromosome stability of the BS and C breeds using the Sister Chromatid Exchange (SCE) test [Genualdo *et al.* 2018], but limited data are available for the Italian local pig population on variation in genome stability [Ciotola *et al.* 2014] and polymorphisms [Schiavo *et al*. 2020]. The research on the variability and stability of the genome is fundamental, as these native breeds have probably been exposed to crossbreeding with other breeds over the centuries, with resulting mutations induced by several extrinsic and intrinsic factors [Wang *et al.* 2016, Rocco *et al.* 2015] having a negative effect on fertility and reproduction [Mottola

et al. 2019, Santonastaso *et al.* 2019]. Nevertheless, evolutionary mechanisms have selected DNA damage repair systems that allow to protect genetic material from induced mutations (epigenetic mechanisms, telomere functionality) (Feng *et al.* 2020); however, not all mutations are repaired, and if they are life-compatible they are passed on to the offspring, creating polymorphic variability within the same breed and unique genetic characteristics.

As regards particularly the BL, it is known that it is an indigenous pig, of the ancient autochthonous genetic type (AAGT), but the information on its genetic characteristics is still scarce, except for those concerning meat products [Perna *et al*. 2015ab, Simonetti *et al*. 2016), some loci [Valluzzi *et al.* 2019] or cytogenetic screening [Genualdo *et al*. 2020]. In the last few years, different researches enriched the knowledge of genetic variation of autochthonous Italian pig breeds, but a simultaneous comparison of their genome stability and the polymorphic differences are still being studied.

For this reason the present work aimed to evaluate the genome of the three breeds used different but complementary aspects: cytogenetics tools to evaluate the genome stability by Aneuploidy, Chromosome Aberration (CA) and Sister Chromatid Exchange (SCE), and the Random Amplification of Polymorphic DNA – Polymerase Chain Reaction (RAPD-PCR) to identify DNA polymorphisms and detect a genetic relationship between the breeds. The research was carried out on the animal resources as a preliminary step for the development of local breeds, and the results obtained will provide useful information for the management, conservation and promotion of these three Italian pig breeds.

Material and methods

All experimental procedures used in this study followed the requirements of the National Community Directive on research in animal science of 7th June 2011, as certified by the Ethical Commission of the National Research Council (CNR), ISPAAM of Naples (protocols no. 643 May 30, 2017 and no. 0001190 December 2018). All pigs selected for this study were registered in the National Pedigree Register of ANAS. In brief, BL were sampled from a family-run piggery in the Basilicata region (Southern Italy), while for the BS and C breeds samples were taken from blood and lymphocyte cell suspensions, stored at -20°C after they had been collected for a previous study [Genualdo *et al.* 2018]. From each BL pig samples were collected to one sodium-heparin tube for cytogenetic screening and tests, and one EDTA (ethylene-di-amine-tetra-acetic acid) tube for DNA extraction. Our research was carried out on a homogeneous group of pigs in terms of age and feeding conditions. Two peripheral blood cultures were set up, according to the standard protocols of our laboratory, as previously published [Genualdo *et al*. 2018]. For each sample the following procedures were performed: normal cultures (without the addition of any analogue base), used for Aneuploidy and CA tests, and cultures treated with the incorporation

of 5-bromodeoxyuridine (BrdU 10 µg/ml) 17 h before harvesting for SCE tests. After cell harvesting the slides were treated as described by Perucatti *et al.* [2016]. For the Aneuploidy and CA tests at least 100 cells per animals were treated as follows: the first 100 cells, captured under a microscope, were counted as incomplete (2n<38) and complete metaphases (2n=38) for Aneuploidy, while for the CA test only 100 complete metaphases were scored. The latter were analyzed according to the Savage classification (2004). In the SCE test for each animal 35 complete metaphases were counted and elaborated from the culture with the BrdU addition. All metaphases were observed under Leica DM2000 and DM5500 LED fluorescence microscopes equipped with 100× oil immersion lenses, FITC-specific filters and a camera. Each image was processed using a dedicated software (CytoVision platform by Leica). The data elaborated were expressed as means and standard deviations (SD). Aneuploidy was evaluated as the percentage of cells with 2n≠38. Total CAs (chromatid and chromosome breaks), aneuploidy and SCEs among the experimental breeds groups were analyzed by the unpaired Student's t-test using GraphPad Prism 6. Only the results with p-value ≤0.05 were considered statistically significant.

Upon the RAPD test, the genomic DNA was isolated from 1 ml of blood stored in EDTA tubes and processed according to the method described by Pauciullo et al. (2012) with some modifications. The DNA pellets obtained were re-suspended in sterile water and quantified using NanoDrop ™ 2000 / 2000c Spectrophotometers (Thermofisher). Six commercial primers (Sigma Aldrich) with variable nucleotide proportions (G-C content above 60-70 %) were used for this study (Tab. 1). All primers were in the 10-mer size range and were selected from the literature as being tested on different species, including pigs, and were found to generate amplification products with a reasonable number of bands [Yeo *et al.* 2000, Huang *et al* 2003, Mottola *et al.* 2019]. From the 6 primers, only P2 and P6 were used for the analysis of the samples having shown a greater share of repeatable bands in the population.

Table 1. List of random gene primers, their nucleotide sequences and GC % contents

Primer name	Sequence (5'-3')	(G+C) contents (%)	References
Primer 1	CCCGTCAGCA	70	Mottola. *et al.* [2019]
Primer 2	GTCCCGACGA	70	Huang *et al.* [2003]
Primer 3	CAATCGCCGT	60	Yeo *et al.* [2000]
Primer 4	AACGGTGACC	60	Huang *et al*. [2003]
Primer 5	TTCCCCCCAG	70	Huang *et al.* [2004]
Primer 6	GTCCACACGG	70	Yeo *et al.* [2000]

The PCR sample volume was 25 ml, containing the DNA template (100 ngr),1X PCR Buffer, MgCl2 (1.5 mM), primer (5 picoM - 5′-d-3′), 1U Taq DNA polymerase (Microgem 01-01-02000) and dNTP mix (0.2 mM). Analyses were performed using a T100 thermal cycler (Biorad). Amplification conditions were as follows: initial denaturation at 94°C for 5 min, denaturation at 95°C for 1 minute, annealing at 36°C for 1 minute, extension at 72°C 2 minutes, all the three steps for 45 cycles, and final

holding at 4°C. The amplification product was separated by electrophoresis on 2% agarose gels in 1X TBE buffer and visualized by staining with ethidium bromide solution. The gel was observed and the image was captured using the GEL DOC EZ System (Biorad) with its dedicated Image Lab Touch Software. The band analysis was carried out by evaluating the presence or absence of characteristic bands using a DNA ladder of 2000 and 10000 bp. Binary coded characters (1, 0) were used for molecular genetic investigation and elaborated by the Genesis software. (Graz University of Technology Istitute for Genomics and Bioinformatics 1.8.1).

Results and discussion

The chromosomal and genetic characterization of animal species facilitates identification of genetic links that exist between both different breeds and between individuals of the same breed. Induced mutations or chromosomal rearrangements can be the cause of genetic variation in a breed. Assessing the degree of genomic stability and probable polymorphisms in different breeds provides important information on species conservation and monitoring, especially if it is a species of interest as a food source. In this study to investigate effective chromosome stability of three pig breeds (BS, C and BL) and provide genomic characterization 36 animals (3600 cells) were examined by CA and Aneuploidy and 50 samples (2030 cell) by SCE of the three breeds with mean values and standard deviations. The data obtained from BS and C in this study confirmed our previous research [Perucatti *et al.* 2013], which proves probably that these two breeds come from similar genetic pools, as also described in genetic research of Schiavo *el al.* [2020]. In fact, there are no statistically significant differences between the two breeds, as the number of chromosome and chromatid breaks and the percentage of aneuploidy were almost identical (Fig. 1). In particular, we observed a very low number of chromosomal aberrations, supporting the hypothesis that their genome is more stable.

Interesting data emerge from the SCEs, CAs and Aneuploidy analysis of BL. We observed a higher frequency of CAs compared to BS and a lower aneuploidy percentage compared to the two other breeds (Fig. 1). The ratio between aneuploidies and chromosomal breaks is higher between BS and C than in BL. More specifically, we found that the percentage of aneuploidies in BS and C is greater than the number of breaks in individual chromosomes, while in BL it is the inverse. It is known that a high frequency of chromosomal aneuploidies can determine the onset of cancer in all living organisms. The cells put in place repair mechanisms to prevent potential damage from aneuploidy accumulation, including apoptosis (alos termed cellular suicide), a standard mechanism to eliminate cells with some kind of defect [Engvild 2018]. This aspect would explain the low percentage of aneuploidies in relation to the high frequency of chromosomal breaks found in BL, indicating that it is a more genetically unstable breed. The hypothesis of BL genetic instability is also supported by the literature, as our results show the highest SCE-mean value in BL (Fig. 1)

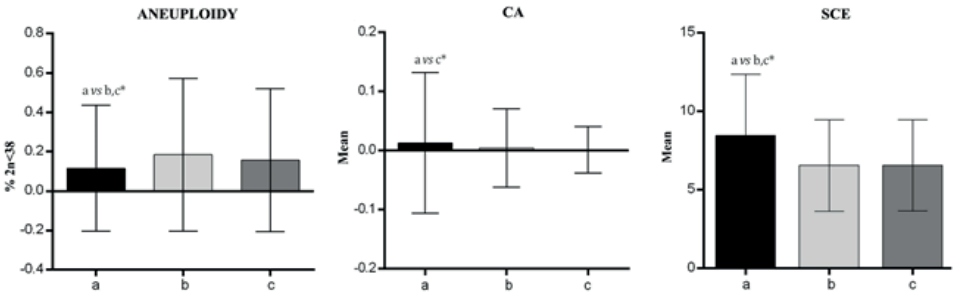

Fig.1. Histograms of % Aneuploidy and mean values ± SD of CA and SCE in three Italian pig breeds: Black Lucano (a); Black Sicilian (b); Casertana (c). *p≤0.05.

compared to other pig breeds studied [Ciotola *et al.* 2014]. Probably, the BL breed is newest or more crossed than the two other breeds examined. This idea was promoted also by a comparison of Schiavo's study [2020], in which the Apulo-Calarese breed developed separately from the other Italian local breeds, differently from C and BS, which instead seem to have the same ancestral genetic pools.

To confirm genomic differences or verify potential genetic relationships between the three livestock breeds considered, we compared the electrophoretic profiles obtained by RAPD-PCR of each sample analyzed. RAPD profiles are a useful tool in DNA fingerprint analysis for gene mapping and breed identification and it has several advantages, including low execution costs and no prior knowledge of the DNA sequence [Mhuka *et al.* 2017]. Considering that we had no information on the DNA sequences for the analyzed breeds, we first tested a series of non-specific sequence primers to obtain an adequate number of bands that guaranteed test quality and provided specific information on the genotypes of the populations. From 6 primers used in RAPD-PCR only P2 (3'-GTCCCGACGA-5') and P6 (3'-GTCCACACGG-5') showed higher shares of bands (Tab. 2), amplifying 45 different loci in total, compared to the other primers, to be particularly suitable for their quality and reproducibility. P1 (3'-CCCGTCAGCA-5'), P3 (3'-CAATCGCCGT-5'), P4 (3'-AACGGTGACC5') and P5 (3'-TTCCCCCCAG-5') proved to be more polymorphic within the same breed and for this reason they were excluded from analysis. P2 amplified 25 bands within a size range of 130-1700 bp, of which only 4 were shared by all the three breeds, ranging from 250 to 500 bp. Among the last twenty-one polymorphic bands evaluated 6 were shared by BL and BS, only 1 by BS and C, while none were shared between BL and C. Moreover, the primers amplified 7 characteristic bands for BL (450-1600 bp), 3 for BS (200-800 bp) and 3 for C (130-420 bp) (Fig. 2). In particular, P6 with 20 bands in total showed more bands (8) shared within the three breeds, with a lower range size of 200-1200 bp and a degree of polymorphism between the breeds smaller than P2. Moreover, only two characteristic bands were amplified with this primer, one each for the BL and BS breeds. Of the 12 remaining polymorphic bands, 6 were shared by BL and BS, and 3 by BS and C (Fig. 2).

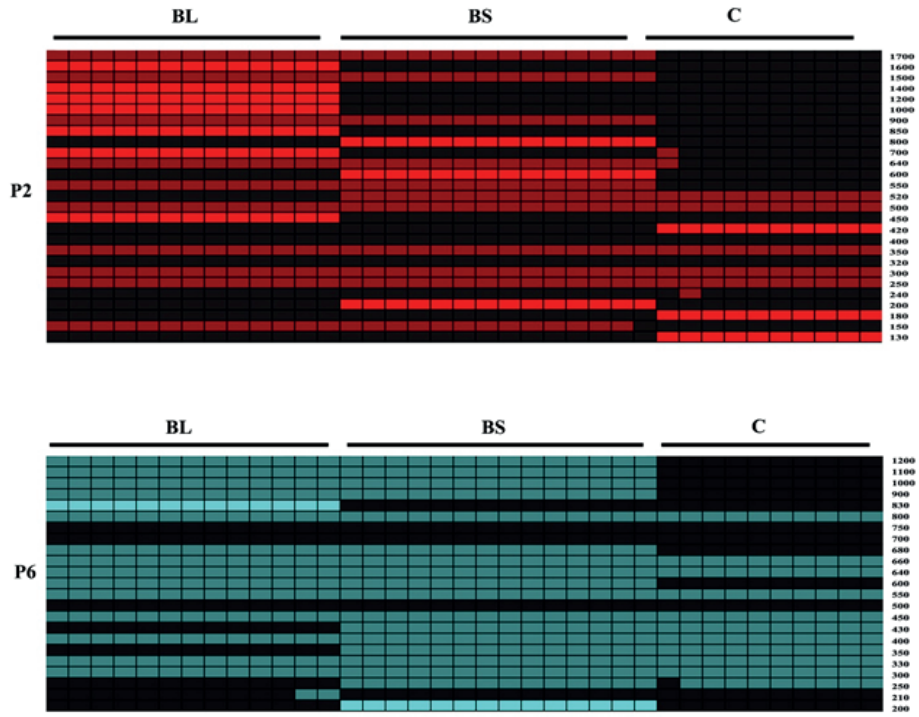

Fig. 2. P2 and P6 primer expression images calculated and displayed by Genesys software of Black Lucano (BL), Black Sicily (BS) and Casertana (C) pig breeds.

Table 2. Comparison of fingerprint bands generated by P2 and P6 primers among the three pig breeds

Primer	Breed	Total number of bands	Size range (bp)	Total number of bands/ breed	Number of polymorphic bands	Number of characteristic bands	Number of shared bands
P2	Black Lucano	25	1700-150	17	0	7	4
	Black Sicily		1700-150	14	0	3	
	Casertana		700-130	11	3	3	
P6	Black Lucano	20	1200-210	16	1	1	8
	Black Sicily		1200-200	18	0	1	
	Casertana		800-300	11	1	0	

The results showed that P2 and P6 produced a greater number of bands, which were easy to interpret and reproducible (Tab. 2). The analysis of electrophoretic products using the two primers showed no substantial differences in terms of polymorphisms within the breeds, but a specific BL pattern with respect to BS and C was found using the P2 primer. Therefore, the P2 primer was particularly useful for identifying a specific band model, in particular for BL, as it amplified characteristic bands,

which are not found in the BS and C breeds (Tab. 2). The characteristic fingerprints generated in BL from the DNA amplification with P2 (Fig. 2) are of great interest for the purpose of genetic selection and especially for investigations dedicated to breed preservation at risk of extinction, classifying the aforementioned primer as a valid aid for the genomic identification of the BL breed. For the two other breeds P2 generated only 3 bands with high specificity in each sample studied, so further trials with new primers will be necessary to identify a marker test for their genome.

All data support the hypothesis that the BL deviates from the BS and C breeds. It could partly be attributed to the extended breed system, with no systematic selection or a planned mating. Indeed, the breed has probably accidentally crossed with wild boars and experienced limited introgression with other Italians breeds [Guastella *et al.* 2010]. Considering the high consumption of pig meats, the possibility of recovering marginal territories by increasing or creating a market of high quality bio-sustainable meat products, this study would be appropriate to create controlled selection plans to safeguard Italian pig breeds, in particular for the BL breed, which has proved to have great genetic potential.

Acknowledgements. *The authors wish to thank Mr. Giuseppe Grazioli and Giuseppe Auriemma (CNR-ISPAAM) for their technical support and Dr Domenico Mecca as a breeder of BLs. Moreover, heartfelt thanks to Dr Luigi Ascione of Microtech to provide us with an opportunity to test the DNA Taq (Microgem 01-01-02000).*

REFERENCES

1. ABOAGYE G., ZAPPATERRA M., PASINI F., DALL'OLIO S., DAVOLI R., NANNI COSTA L., 2020 – Fatty acid composition of the intramuscular fat in the longissimus thoracis muscle of Apulo-Calabrese and crossbreed pigs. ***Livestock Science*** 232, 103878.
2. ANAS Associazione Nazionale Allevatori Suini, Apulo-Calabrese breed. Retrieved from http://www. anas.it/docum enti/Scheda_apulo calab rese.pdf. (February 23, 2020).
3. BOZZI R., GALLO M., GERACI C., FONTANESI L., BATOREK L.N., 2019 – Nero Casertano Pig. 10.5772/intechopen.83778; 10.5772/intechopen.83760; 10.5772/intechopen.84438. (…)
4. CIOTOLA F., ALBARELLA S., SCOPINO G., CARPINO S., MONACO F., PERETTI V., 2014 – Crossbreeding effect on genome stability in pig (Sus scrofa scrofa). ***Folia Biologica (Krakow)*** 62, 23-28.
5. DAD-IS 2020, Domestic animal diversity information system. Retrieved from http://www.fao.org/ dad-is/brows e-by-country-and-speci es/en/. (June 11, 2020)
6. D'ALESSANDRO A., MARROCCO C., ZOLLA V., D'ANDREA M., ZOLLA L., 2011 – Meat quality of the longissimus lumborum muscle of Casertana and Large White pigs: metabolomics and proteomics intertwined. ***Journal of Proteomics*** 75, 610-627.
7. ENGVILD C.K., 2018 – Cancer follows chromosome missegregation when all endogenous repair mechanisms fail. ***Medical Hypotheses*** 120 121-123.
8. SAVAGE J.R.K. 2004 – On the nature of visible chromosomal gaps and breaks. ***Cytogenetics and Genome*** *Research* 104, 46-55.
9. Eurostat 2020 – Agriculture, forestry and fishery statistics. Chapter 3: Farm production, 50-57. 2018;doi: 10.2785/340432 – https://ec.europa.eu/eurostat/statistics-explained/index.php?oldid=427096 .

10. FAO 2007 – The State of the World's Animal Genetic Resources for Food and Agriculture. Rischkowsky B & Pilling D. Rome.
11. FENG J.X., RIDDLE N.C., 2020 – Epigenetics and genome stability. ***Mammalian Genome*** 10.1007/s00335-020-09836-2.
12. FRANCI O., PUGLIESE C. 2007 – Italian autochthonous pigs: progress report and research perspectives. ***Italian Journal of Animal Science*** 6, 663-671.
13. HUANG M., HORNG Y., HUANG H., SIN Y., CHEN M., 2003 – RAPD Fingerprinting for the Species Identification of Animals. ***Asian-Australasian Journal of Animal Science*** 16, 1406-1410.
14. GENUALDO V., PERUCATTI A., MARLETTA D., CASTIGLIONI B., BORDONARO S., IANNACCONE M., CIOTOLA F., PERETTI V., IANNUZZI A., 2018 – Cytogenetic investigation in two endangered pig breeds raised in Southern-Italy: Clinical and environmental aspects. ***Livestock Science*** 216, 36-43.
15. GENUALDO V., ROSSETTI C., PAUCIULLO A., MUSILOVA P., INCARNATO D., PERUCATTI A., 2020 – A de novo reciprocal chromosomal translocation t(3;6)(p14;q26) in the black Lucano pig. ***Reproduction of Domestic Animals*** 10.1111/rda.13664.
16. GUASTELLA A.M., CRISCIONE A., MARLETTA D., ZUCCARO A., CHIES L., BORDONARO S., 2010 – Molecular characterization and genetic structure of the Nero Siciliano pig breed. ***Genetics and Molecular Biology*** 33, 650-656.
17. HILSDORF A.W.S., HALLERMAN E.M., 2017 – Characterization of Genetic Resources. In: Genetic Resources of Neotropical Fishes. Springer, Cham.
18. MAIORANO G., 2009 – Swine production in Italy and research perspectives for the local breeds. ***Slovak Journal of Animal Science*** 42, 159-166.
19. MOTTOLA F., IOVINE C., SANTONASTASO M., ROMEO M.L., PACIFICO S., COBELLIS L., ROCCO L., 2019 – NPs-TiO2 and Lincomycin Coexposure Induces DNA Damage in Cultured Human Amniotic Cells. ***Nanomaterials*** 9(11), 1511.
20. MHUKA C., FUNGAY C., CHIDZWONDO F., SITHOLE-NIANG I., MAKUZA S., MLAMBO S., 2017 – Use of RAPD-PCR for breed/genotype identification in Zimbabwean cattle. ***Journal of Cellular Biotechnology*** 2, 131-137. 10.3233/JCB-15033.
21. MUÑOZ M., BOZZI R., GARCÍA-CASCO J., et al., 2019 – Genomic diversity, linkage disequilibrium and selection signatures in European local pig breeds assessed with a high density SNP chip. ***Scientific Reports*** 9, 13546.
22. PAUCIULLO A., COSENZA G., STERI R., COLETTA A., JEMMA L., FELIGINI M., DI BERARDINO D., MACCIOTTA N.P., RAMUNNO L., 2012 – An association analysis between OXT genotype and milk yield and flow in Italian Mediterranean river buffalo. ***Journal of Dairy Research*** 79, 150-156.
23. PERNA A., SIMONETTI A., INTAGLIETTA I., GAMBACORTA E., 2015 – Fatty acids composition, cholesterol and vitamin E contents of Longissimus dorsi and Semitendinosus muscles of Suino Nero Lucano pigs slaughtered at two different weights. ***Animal Production Science*** 55, 1037-1043.
24. PERNA A., SIMONETTI A., INTAGLIETTA I., GAMBACORTA E., 2015b – Quality and sensory characteristics of culatello dry-cured products obtained from the Italian autochthonous pig Suino Nero Lucano and from a modern crossbred pig. ***Animal Production Science*** 55, 1192-1199.
25. PERUCATTI A., GENUALDO V., IANNUZZI A., PAUCIULLO A., PUCCIARELLI L., INCARNATO D., MARLETTA D., SCOPINO G., CASTIGLIONI B., PERETTI V., IANNUZZI L., 2013 – Sister Chromatid Exchange (Sce) In Two Endangered Pig Breeds Raised in Southern-Italy. 19th International Chromosome Conference – Bologna, Italy 106.

26. PERUCATTI A., GENUALDO V., COLONNA M., FRANCESCO G., INCARNATO D., LUBRANO LAVADERA G., IORIO C., VONGHIA L., CAPUTI-JAMBRENGHI A., IANNUZZI L., IANNUZZI A., 2016 – Chromosome instability in lymphocytes of Friesian cows naturally exposed to dioxins being raised close to a metallurgic factory area in Italy. ***Caryologia*** 69, 1-8.
27. PUGLIESE C., SIRTORI F., 2012 – Quality of meat and meat products produced from southern European pig breeds. ***Meat Science*** 90, 511-518.
28. ROCCO L., SANTONASTASO M., NIGRO M., MOTTOLA F., COSTAGLIOLA D., BERNARDESCHI M., GUIDI P., LUCCHESI P., SCARCELLI V., CORSI I., STINGO V., FRENZILLI G., 2015 – Genomic and chromosomal damage in the marine mussel Mytilus galloprovincialis: effects of the combined exposure to titanium dioxide nanoparticles and cadmium chloride. ***Marine Environmental Research*** 111-144.
29. SANTONASTASO M., MOTTOLA F., COLACURCI N., IOVINE C., PACIFICO S., CAMMAROTA M., CESARONI F., ROCCO L., 2019 – In vitro genotoxic effects of titanium dioxide nanoparticles (n-TiO2) in human sperm cells. ***Molecular Reproduction and Development*** 86, 1369-1377.
30. SCHIAVO G., BOVO S., BERTOLINI F., TINARELLI S., DALL'OLIO S.N., COSTA L., GALLO M., FONTANESI L., 2020 – Comparative evaluation of genomic inbreeding parameters in seven commercial and autochthonous pig breeds. ***Animal*** 14, 910-920.
31. SIMONETTI A., GAMBACORTA E., PERNA A., 2016 – Antioxidative and antihypertensive activities of pig meat before and after cooking and in vitro gastrointestinal digestion: Comparison between Italian autochthonous pig Suino Nero Lucano and a modern crossbred pig. ***Food Chemistry*** 212, 590-595.
32. VALLUZZI C., RANDO A., DI GREGORIO P., 2019 – Genetic variability of Nero Lucano pig breed at IGF2, LEP, MC4R, PIK3C3, RYR1 and VRTN loci. ***Italian Journal of Animal Science*** 18, 1321–1326.
33. YEO J., LEE J., LEE C., JUNG Y., DOO N., 2012 – Identification of genetic markers for Korean native cattle (Hanwoo) by RAPD analysis. Biotechnology and Bioprocess Engineering 5,23-26 10.1007/BF02932348.
34. WANG J., LINDAHL T., 2016 – Maintenance of Genome Stability. ***Genomics Proteomics Bioinformatics*** 14, 119-121.