Perspectives of the selection scheme of the Sarda dairy sheep breed in the era of genomics

S. Salaris[1], M.G. Usai[1], S. Casu[1], T. Sechi[1], A. Manunta[2], M. Bitti[3], S. Grande[4] and A. Carta[1]

[1]Agris Sardegna, Sassari, Italy
[2]Sardinian Breeders Association of Cagliari , Cagliari , Italy
[3]Sardinian Breeders Association of Nuoro, Nuoro, Italy
[4]ASSONAPA, National Sheep Breeders Association, Rome, Italy

Summary

Over the last decade, a progressive decline of the efficiency of the breeding scheme of the Sarda dairy sheep breed has been observed. In this context, a program for implementing innovative genomic tools has been established. The current state of the selection scheme of the Sarda breed is summarized and the potential impact of innovative genomic tools taking into account their economic sustainability is drawn. A female reference population was created to identify LD o causal mutations and to trigger genomic selection. Results of QTL detection and accuracies of Herd Book rams genomic predictions realized on the basis of the female reference population show that it is a realistic option to increase the effectiveness of the current selection program. The impact of the female nucleus may be increased by an organization of the HB flocks in levels according to the application of selection tools *i.e* the incidence of pedigree known and the engagement in AI program.

Keywords: QTL, MAS, genomic selection, reliability.

Introduction

The efficiency of most dairy sheep selection schemes has been traditionally limited by three main constraints: the scarce diffusion of artificial insemination (AI), the low accuracy of the recording system and the small size of the herd book (HB) relative to the whole population. Thus only few selection programs are currently ongoing in dairy sheep breeds with large differences in terms of genetic gains and cost effectiveness (Carta *et al.*, 2009).

The Sarda is the largest Italian dairy sheep breed with around 90% of the national stock reared in Sardinia (3 million heads). In 1992, an AI program combined with a genetic evaluation based on BLUP AM methodology was implemented. This selection scheme has allowed to achieve satisfying levels of genetic gain for milk yield (Salaris *et al*, 2008b). Over the last decade, the national and local Breeders Associations involved in the official recording of production and pedigree data have been revising the organization of the program. This uncertainty produced a suboptimal application of the selection tools causing a progressive decline of the efficiency of the program

Corresponding Author: slsalaris@agrisricerca.it

which is negatively affecting the introduction of the recording of innovative production quality, health and functional traits. Moreover, the import in Sardinia of improved exotic breeds such as Lacaune and Assaf has been observed.

In this context, the National Breeders Association (ASSONAPA) with the scientific support of the Regional Agency for Agricultural Research (AGRIS) started a program implementing innovative genomic tools in the breeding scheme.

The objective of this study is to summarize the current state of the selection scheme of the Sarda breed and to draw the potential impact of innovative genomic tools taking into account their economic sustainability.

Current state of the selection scheme

The breeding scheme of the Sarda breed is based on the traditional pyramidal management of the purebred population (Carta *et al.*, 2009). Selection objectives are milk yield, scrapie resistance and udder morphology. Milk composition traits are recorded but no breeding value has been provided to breeders. The main feature of the Sarda scheme has been the large application of the single sire mating (SSM) and rates of AI adequate to achieve accurate genetic evaluations not only of AI rams but also of natural mating ones (Salaris *et al.*, 2008a). Milk yield during exclusive milking estimated from test day records with the monthly AC method is currently the selection criterion.

Only lactation records of ewes within 4 years old are retained for genetic evaluation. Udder morphology scoring and milk composition recording started in 1999 and 2000 respectively. Only ewes in first lactation are sampled for milk composition and scored once a year for udder morphology. The official breeding plan for Scrapie resistance started in 2005 (Salaris *et al.*, 2014). The breeding plan established that all the breeding males and the young ewes with high parent average for milk yield must be genotyped. Since 2013 only breeding males have been genotyped.

The highest number of breeding flocks and recorded ewes was registered between 2000 and 2005, when rates of ewes with known sire and from AI sire were 80% and 15% respectively (Table 1). Over the last years a progressive decline of all these parameters has been recorded. In 2016, beside the decreasing of the number of flocks and recorded ewes, the rates of ewes with known sire and from AI sire have reduced to 56% and 3% respectively. Both the number of mating sires and the number of progeny tested rams have decreased from above 1700 and 500 in 2005 to 1100 and 340 in the last years.

To evaluate the impact of this declining statistics on the effectiveness of the selection scheme, the evolution of the size of the recorded population which is actually exploitable for selective breeding was estimated. Thus, a subset of all data used for the official genetic evaluation including only the portion of the population genetically connected was retained. Practically, only the contemporary groups (CG) i.e the levels of the flock-year-age class-lambing season interaction with at least 5 lactation records of ewes with known sire were retained. Therefore only CG including daughters of sires having offspring in at least one other CG including daughters of other sires were retained. The rate respect to the official dataset of the number of recorded ewes, CG, sires of lactating ewes and the number of sires represented in each CG across production years were calculated (Figure 1).

Table 1. Evolution of the effective size of the Sarda herd book over the last 20 production years

Production year	BF	RF	RF1	MCR	UMR	KSR	AIR	BS	PTS
1997	1,011	93,409	24,984			82.1	13.8	1,397	507
1998	1,133	106,566	29,324			77.6	14.1	1,464	569
1999	1,132	115,658	30,032		42.2	78.9	13.8	1,557	622
2000	1,168	123,737	31,758	47.2	41.5	76.7	14.2	1,594	575
2001	1,176	126,111	33,255	48.0	40.6	77.2	14.9	1,625	482
2002	1,167	129,290	37,343	54.6	33.8	72.4	13.2	1,603	497
2003	1,157	135,858	35,781	51.0	30.8	70.5	11.8	1,617	552
2004	1,141	147,883	41,807	51.7	33.7	69.4	13.0	1,688	519
2005	1,091	142,939	35,326	48.7	22.7	70.8	9.4	1,711	501
2006	1,062	143,581	34,609	50.2	27.4	68.8	8.8	1,613	560
2007	1,075	141,320	31,197	51.7	26.5	66.3	10.0	1,449	461
2008	1,079	135,060	35,216	48.4	25.6	60.8	8.2	1,407	405
2009	1,042	127,231	31,083	49.9	23.9	61.2	7.8	1,371	390
2010	1,035	130,776	31,744	53.6	28.9	60.3	8.0	1,260	416
2011	1,025	126,920	30,491	48.3	30.4	61.2	7.2	1,204	365
2012	972	119,746	28,555	50.6	34.6	62.3	6.9	1,136	350
2013	953	117,404	26,372	48.2	41.7	61.5	4.9	1,093	368
2014	969	112,024	25,172	43.7	26.2	57.5	3.8	1,046	328
2015	943	114,877	27,482	47.3	28.9	57.9	3.7	1,048	307
2016	936	120,604	32,627	40.3	17.2	56.3	3.4	1,142	344

Number of breeding flocks (BF); number of milk recorded females within 4 years old (RF); number of ewes in first lactation (RF1); percentage of RF1 recorded for milk composition (MCR); percentage of RF1 recorded for udder morphology (UMR); percentage of RF1 with known sire (KSR); percentage of RF1 from AI sire (AIS); number of RF1 breeding sires (BS); number of BS progeny tested (PTS).

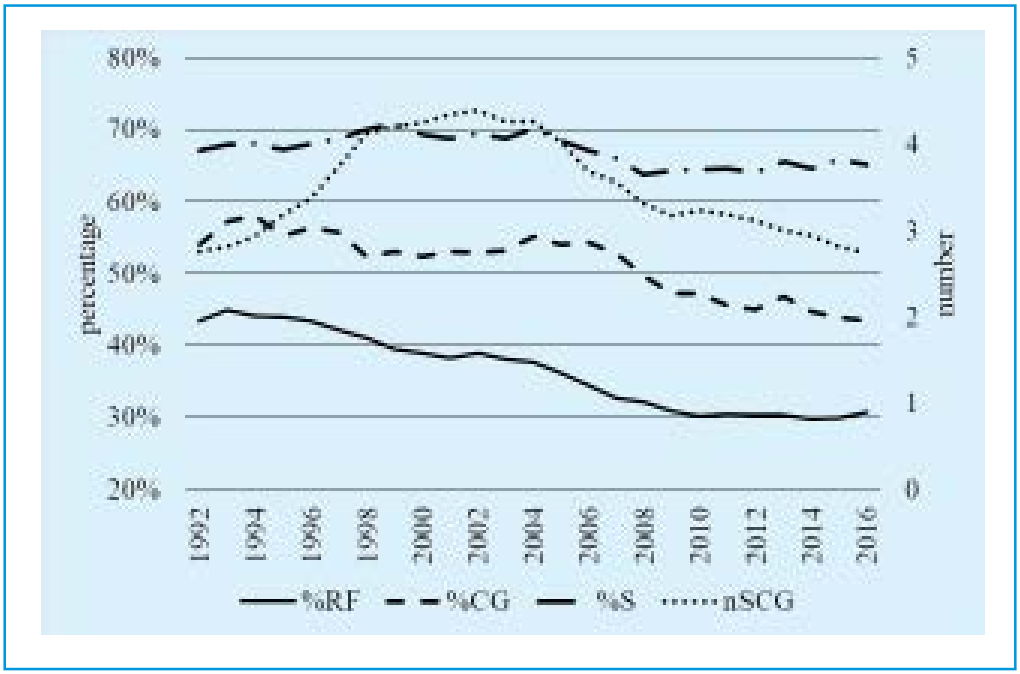

Figure 1. Rates of the number of recorded ewes (%RF), contemporary groups (%CG) and sires of lactating ewes (%S) respect to the official dataset and number of sires represented in the contemporary groups (nSCG) across production years in the genetically connected population.

Moreover, data of the genetically connected population were further split in three subsets according to the degree of application of the main selection tools (from 1983 to 2002, from 1998 to 2011 and from 2006 to 2016) to estimate milk yield heritability and to observe its evolution. A repeatability BLUP animal model was applied on milk yield adjusted for milking length and age-parity-lambing month interaction (Carta *et al.*, 1998) with the interactions "flock-year-class of age-class of lambing season" and "year-class of age-parity-class of lambing month" as fixed effects.

The percentage of the effective size of population remained stable until 2006 whereas successively a progressive decline was observed (Figure 1). Moreover, the number of rams per CG increased before 2000, remained stable until 2006 and then decreased, reflecting the trend of AI rate (Table 1).

The estimates of heritability of milk yield were 0.22, 0.18 and 0.17 in the three periods likely reflecting the reduction of the effective size of the population and of the connectedness.

Between 2000 and 2006, milk composition and udder morphology were recorded on more than 50% and 40% of ewes recorded for milk yield and then the percentages fell to around 40% and less than 20% respectively (Table 1).

Concerning milk composition, the low number of records per lactating yearling (on average 2.3) and the small size of the recorded population (Table 1) led to a lower heritability (unpublished results) compared to literature (Barillet, 2007; El Saied *et al.*, 1999) in particular for fat content. Differently from udder morphology traits, Sarda breeders do not exhibit great interest for milk composition because no payment system based on the chemical quality of milk has been implemented in Sardinia. The amount of rams indexed for udder morphology (Casu *et al.*, 2006) is limited by the low number of scored ewes which can not be extended due to organizational constraints.

As far as scrapie resistance is concerned, the ARR frequency in males moved from 42% to 80% between 2005 and 2015 birth year and since 2010 only homozygous resistant rams are allowed for reproduction.

Potential impact of the implementation of genomic tools

The progressive decline of the selection scheme of the Sarda breed described above combined with the difficulty to measure on large scale innovative traits related to production quality, health and sustainability led breeders to assess the feasibility of two genomic application: the selection assisted by causal or linkage disequilibrium (LD) mutations (MAS) and the genomic selection (GS).

Generally the identification of useful mutations for selective purpose needs long trials based on large populations measured for a lot of traits. GS in dairy sheep should be adapted to the specific preexisting breeding schemes taking carefully costs and benefits into account. The dairy cattle approach (Boichard *et al.*, 2015) has been successfully applied in some breeds such as the French Lacaune where the preexisting selection scheme with a large use of AI and accurate recording schemes for functional and health traits, beside the classical milk yield and composition, have made the GS of breeding males profitable (Baloche *et al.*, 2014).

Considering the above mentioned limitations of the Sarda HB, ASSONAPA and AGRIS created a female reference population (FRP) either for detecting QTLs or predicting genomic effects to be used for GS. In the following sections, we discuss the usefulness of FRP to identify causal or LD mutations and the feasibility of genomic predictions of Sarda rams based on records of FRP only.

Female reference population

FRP yearly consists of approximately 1,000 milking ewes with a replacement of about 25% generated by mating adult ewes with Sarda HB rams. Ewes are raised in an experimental farm that has a typical Sardinian dairy sheep farming system. The original aim of FRP was to detect QTL segregating in the Sarda breed. Thus, the number of daughters per ram had been 40 ewes on average until 2009. It was reduced to 9 after 2010 with the aim of increasing the number of breeding rams with daughters in FRP and, consequently, the number of represented *bloodlines* from HB. So far, 3,949 ewes have been generated by 161 rams. Ewes are routinely measured for several traits: production traits (milk yield, fat, protein and lactose content, body weight and body condition score), milkability and udder morphology type traits, health traits (somatic cell count, clinical mastitis, faecal egg count, ELISA test for paratuberculosis, ELISA test for visna-maedi, histo-pathological examination for paratuberculosis), fatty acids content and reproduction traits (fertility and prolificacy).

Genotyping and whole genome resequencing

To date, all FRP ewes and their sires have been genotyped with the Illumina Inc. OvineSNP50 Beadchip (50K). Moreover, whole genome resequencing of target animals, chosen on the basis of their genetic impact on FRP or because having high probability to be homozygous at causal mutations is in progress. Presently, the genome re-sequencing with 12X coverage has already been done for 24 animals and further individuals will soon be resequenced with a higher coverage.

The whole genome sequences of target animals jointly to genotypes from DNA arrays allow the imputation of large genome blocks to many individuals of FRP.

Detection of causal or LD mutations

Indeed, QTL detection analysis based on 50K data and a combined Linkage and LD mapping method using a principal component analysis to synthesize identity-by-descent matrix information have already been applied to the first generations of FRP (Usai *et al.*, 2014; Casu *et al.*, 2014b; Carta *et al.*, 2016; Casu *et al.*, 2018). Several significant locations affecting most of the measured traits have been identified (Table 2).

Among those, the most significant ones are chosen for further investigations with the aim of identify causal or LD mutations to be used for selection purposes. Target regions are first of all screened to verify whether they harbour evident candidate genes for the traits of interest, as it was the case for the casein genes cluster interval (Usai *et al.*, 2014) associated to milk protein content; FASN, AACS and SCD genes (Casu *et al.*, 2014b) associated to fat acids content and ratio in milk yield; MUC15 gene (Carta *et al.*, 2016) associated to gastro-intestinal nematode resistance; different regions of the Major Histocompatibility Complex (Carta *et al.*, 2016) associated to gastro-intestinal nematode and paratubercolsis resistance.

In a second step, candidate polymorphisms identified in the region of interest by exploiting re-sequences or imputed genotypes, can be annotated to the reference genome to search for variants potentially affecting gene expression (untranslated regions, splicing sites, CpG island, and promoter regions) or SNPs in coding regions that have nonsynonymous consequences. This approach was applied to explore a region on OAR6 significantly associated with milk protein contents and mapping close to the cluster of caseins genes (Casu *et al.*, 2014a). Genetic variants resulting alternatively homozygous in two sequenced animals alleged to carry the positive or negative alleles were annotated on Oar_v3.1 reference genome and classified using the gene annotation database from Ensembl release 74 with the Variant Effect Predictor

Table 1 - QTL regions significant at 5% genome-wide threshold for measured traits.

Measured Trait	Chromosome (number of regions in the chromosome)
Production traits[1]	
Milk Yield	2(2), 3(1), 5(1), 10(1), 11(1), 12(1),13(1), 16(1), 20(1), 22(1), 25(1)
Fat Yield	2(1), 10(1), 11(1), 15(1), 20(1), 22(1), 25(1)
Protein Yield	2(1), 3(1), 10(1), 11(1), 12(1), 15(1), 20(1), 25(1)
Fat Content	1(2), 2(2), 3(1), 4(1), 5(1), 6(1), 9(1), 10(1), 12(2), 13(1), 14(1), 16(1), 17(1), 18(1), 19(1), 20(1), 25(1)
Protein Content	1(2), 2(2), 3(2), 4(1), 5(1), 6(1), 8(1), 9(1), 10(1), 11(1), 12(1), 13(1), 15(1), 16(1), 17(1), 18(1), 26(1)
Udder morphology[2]	
Udder depth	9(1), 24(1)
Teat position	3(3), 6(1), 7(1), 8(1), 9(1), 10(1), 16(2), 17(1), 20(1)
Degree of separation of the two halves	2(1), 5(1)
Degree of suspension of the udder	1(1), 2(1), 4(1), 5(2), 7(1), 8(1), 9(3), 10(1), 14(1), 16(1), 20(1), 25(1)
Milk quality traits[3]	
C4:0	17(1)
C10:0	11(1)
C14:0	11(1)
C14:1	7(1), 22(1)
C14:1/C14:0	7(1), 22(1)
C15:0	16(1)
C16:0	26
C16:1	10(1), 22(1)
C16:1/C16:0	22(1)
C18:0	8(1)
C18:1/C18:0	19(1), 22(1)
CLA/vaccenic acid	22(1)
Health Traits[4]	
Somatic Cell Count	2(1), 3(2), 4(1)
Faecal Egg Count	1(1), 3(1), 4(1), 8(1), 12(1), 15(2), 17(1), 19(1), 20(1), 21(1)
ELISA test for paratuberculosis	20(1)
Histo-pathological examination for paratuberculosis	20(1)

[1]Usai *et al.*, 2014; [2]Casu *et al.*, 2018; [3]Casu *et al.*, 2014b; [4]Carta *et al.*, 2016.

Web tool (*http://www.ensembl.org/tools.html*). A manual scanning of the regions corresponding to the 3'UTR of caseins genes was also performed in order to identify potential miRNA target sites, which are known for modulating gene expression. This study allowed to identify, among others, two genetic variants in the CSN1S2 gene strongly candidates to be responsible for the protein content variation in sheep milk: one in positions 85190123 (rs411463377), which defines a splicing region, and another in position 85196954 responsible of the modification of a mi-RNA target site. However, since a new annotation version of Ovis Aries genome (*http://www.ncbi.nlm.nih.gov/genome/annotation_euk/Ovis_aries/102*) has been released a further variant classification analysis is needed in order to take in to account possible updates genes' information also considering that significant discordance remains in variant annotation among the tools, public resources, and literature (Yen *et al.*, 2017). Indeed, as pointed out by McCarthy *et al.* (2014), "Variant annotation is not yet a solved problem. Choice of transcript set can have a large effect on the ultimate variant annotations obtained in a whole-genome sequencing study. Choice of annotation software can also have a

substantial effect. The annotation step in the analysis of a genome sequencing study must therefore be considered carefully, and a conscious choice made as to which transcript set and software are used for annotation".

As an alternative to causative mutation, informative LD SNPs will be identified using the sequences of haplotypes of interest.

Causal or LD mutations associated with complex traits will be used to design low-density SNP panel with high predictive performance for the traits of interest and including SNP selected for parentage assignment.

Genomic selection

A first assessment of the feasibility of genomic predictions for milk yield with the aim of identifying useful criteria to optimize the size and the structure of FRP and modulate the flow of breeding animals from and toward the HB has been performed (Usai *et al.*, 2018).

A validation sample consisting of 537 HB rams was chosen representing all categories of breeding males: 144 AI rams with daughters in FRP, 105 AI rams without daughters in FRP and 288 rams used by SSM in HB with no daughters in FRP. For all these rams official EBV for milk yield were available.

The FRP lactation records were used to predict genomic breeding values (GBV) of the validation sample by exploiting the genomic relationship matrix (**G**). The theoretical accuracies of GBVs were calculated: $rGBV_i = \{1 - [SE_i^2/(\mathbf{G}_{ii}*\sigma_g^2)]\}^{1/2}$. The traditional GBV accuracy obtained through the correlation between GBV and EBV or deregressed proofs of a set of validation individuals with EBV's accuracy so high to be considered a gold standard (i.e. TBV), is not suitable in the Sarda population. In fact, in this population the rate of AI and simplified recording schemes do not allow to select a validation sample. As an example, less than 50% of genotyped HB rams had a number of daughters higher than 50.

Moreover, the impact of the information on relatives in FPR on the accuracy of GBV (rGBV) of rams of the validation sample was verified. Usai *et al.* (2018) proposed the diagonal element (diA) corresponding to the ram of the inverted section of the relationship matrix (**A**) that included all FRP ewes and the ram itself as indicator of the relationship of HB rams with FRP.

The authors demonstrated that diA is a good predictor of rGBV. This parameter could be used to select the new sires of FRP in order to maximize the number of HB rams with a sufficient rGBV and, thus, the number of rams that will be accurately evaluated by GBLUP after genotyping.

In fact, the average rGBV of validation sample was 0.58. Average rGBV of rams high related with FRP (diA $\geq$ 2.5) approached the average EBV accuracy of rams progeny tested with 30 daughters in HB (0.82); average rGBV of rams medium related with FRP (diA $\geq$ 1.25 and $\leq$ 2.5) approached the EBV accuracy of rams progeny tested with 2 daughters in HB (0.61); average rGBV of rams low related to FRP (diA < 1.25) was below the average accuracy of parent average of rams entering progeny test in HB (0.47). The average correlation of GBV with official EBV was 0.24. It ranged from 0.18 to 0.47 according to relationship with FRP.

In order to provide practical criteria to modulate the flow of animals from and toward FRP and allow farmers to reach a sufficient estimated rGBV for their rams, an estimate of the expected rGBV on the base of the number of relatives in FRP of a ram was carried out. A linear model (R^2= 0.84) on rGBV was performed using as covariates the

number of relatives in FRP with a relationship coefficient of 0.50 and the number of relatives in FRP with a relationship coefficient of 0.25. Rams having 5 daughters showed a predicted rGBV around 0.58. Sons of rams with 5 daughters (i.e 5 half sisters in FRP) showed 0.54. In the practical management, only young rams entering progeny test will be genotyped and part of those will be used as sires in FRP allowing genomic evaluation of them and their sons. This implies that, considering a replacement rate in the of 250 ewes, at least 50 new rams and their sons will be genotyped and GBV calculated with an accuracy higher than that of young rams in progeny test in the HB (parent average accuracy of 0.52).

Perspectives

Results of QTL detection for routinely and innovative traits and accuracies of HB rams genomic predictions for milk yield realized on the basis of the female reference population showed that it is a realistic option to increase the effectiveness of the current selection program of the Sarda breed. The female reference population was crucial to trigger the application of genomic tools to the breeding scheme. For traits costly to measure on large scale, the female reference population will allow to produce genomic predictions for adult and young rams with sufficient accuracies. On the other hand, for traits routinely measured in HB, the progressive pile up of males genotypes jointly to the application of ssGBLUP methodologies (Mistzal *et al.*, 2009) will allow the increase of the accuracies of genomic enhanced breeding values. Moreover, the use of customer DNA chips including LD or causal SNPs may help to accelerate the genetic progress and to make more feasible the pedigree recording. The impact of the female nucleus and its cost effectiveness may be increased by an organization of the HB in levels of flocks according to the rate of application of the selection tools *i.e* the incidence of pedigree known and the engagement in AI program.

Acknowledgments

This study was funded by the project MIGLIOVIGENSAR, LR n.7/2007, R.A.S.

List of References

Baloche, G., A. Legarra, G. Sallé, H. Larroque, J.M. Astruc, C. Robert-Granié, and F. Barillet, 2014. Assessment of accuracy of genomic prediction for French Lacaune dairy sheep. J. Dairy Sci.,97(2), 1107-1116.

Barillet F., 2007. Genetic improvement for dairy production in sheep and goats. Small Rumin. Res. 70: 60–75.

Boichard, D., V. Ducrocq, and S. Fritz, 2015. Sustainable dairy cattle selection in the genomic era. J. Anim. Breed. Genet., 132(2), 135-143.

Carta, A., P. Carta, S. Casu, S. Miari, G. Mulas, S. Salaris, T. Sechi, M.G. Usai, and S. Sechi, 2016. Regioni cromosomiche associate alla resistenza alle patologie negli ovini: prospettive di applicazione. XXII Congresso Nazionale S.I.P.A.O.C. Cuneo, Italia, 13-16 Settembre.

Carta, A., S. Casu, and S. Salaris, 2009. Invited review: Current state of genetic improvement in dairy sheep. Journal of Dairy Science, 92(12), 5814–5833.

Carta, A., S.R. Sanna, A. Rosati and S. Casu, 1998. Milk yield adjustments for milking length and age-parity-lambing month interaction in Sarda dairy sheep. Annales de Zootechnie, 47(1), 59–66.

Casu, S., I. Pernazza, and A. Carta, 2006. Feasibility of a Linear Scoring Method of Udder Morphology for the Selection Scheme of Sardinian Sheep. Journal of Dairy Science, 89(6), 2200–2209.

Casu, S., T. Sechi, M.G. Usai, S. Miari, M. Casula, G. Mulas, R. Giannico, B. Lazzari, A. Stella, and A. Carta, 2014a. Investigating a highly significant QTL for milk protein content segregating in Sarda sheep breed close to the caseins cluster region by whole genome re-sequencing of target animals. Proceedings, 10th WCGALP. Vancouver, BC, Canada, 17-22 August. Paper n. 188

Casu, S. , M.G. Usai, S. Miari, T. Sechi, S. Sechi, A. Carta, 2014b. Identificazione di regioni del genoma ovino che modulano la variabilità della composizione acidica del grasso del latte nella razza Sarda. Large Animal Review, suppl. 1 al n.4 – August 2014, year 20. Proc. XXI Congresso Nazionale S.I.P.A.O.C. Foggia 9-12 Settembre. Pag. 124.

Casu, S., M.G. Usai, T Sechi, S.L. Salaris, S. Miari and A. Carta, 2018. A genome scan to detect QTL affecting udder morphology traits in dairy sheep. Proceedings, 11th WCGALP. Auckland, New Zealand, 11–16 February. In press

El Saied, U.M., J. A. Carriero, L. F. de la Fuente, and F. San Primitivo. 1999. Genetic parameters of lactation cell counts and milk and protein yield in dairy ewes. J. Dairy Sci. 81: 2956–2961

McCarthy, D. J., P. Humburg, A. Kanapin, M.A. Rivas, K. Gaulton, J.-B. Cazier, and P. Donnelly, 2014. Choice of transcripts and software has a large effect on variant annotation. Genome Medicine, 6(3), 26.

Misztal, I., I. Aguilar, D.L. Johnson, A. Legarra, S. Tsuruta, and T.J. Lawlor, 2009. A unified approach to utilize phenotypic, full pedigree, and genomic information for a genetic evaluation of Holstein final score. Interbull Bulletin 40, 240-243.

Salaris, S., S. Casu, P. Fresi, and A. Carta, 2008a. Effect of combining controlled natural mating with artificial insemination on the genetic structure of the flock book of Sardinian breed sheep. In Proc. 36th ICAR biennial session, Niagara Falls, USA. ICAR Technical series No 13. Identification, breeding, production, health and recording on farm animals. Editor: J. D. Slatter. Pages 113-122.

Salaris, S., S. Casu, P. Fresi, and A. Carta, 2008b. Analisi della variabilità del progresso genetico entro allevamento nello schema di selezione della razza Sarda. Large Animal Review, 14, 221.

Salaris, S., F. Ingravalle, A. Pernisa, L. Crasta, C. Ligios, S. Murru S., G. Ru and A. Carta, 2014. Appraisal of the Selection Plan for Scrapie resistance in the Sarda dairy sheep breed. 39th ICAR Session, Berlin (Germany). 19-23 May.

Usai, M.G., S. Casu, T. Sechi, S. Miari, G. Mulas, G.B. Congiu, S. Sechi, S. Salaris, and A. Carta, 2014. Genome wide association study on milk production traits in a nucleus flock of Sarda breed sheep using a novel method combining linkage and linkage disequilibrium mapping. Proceedings, 10th WCGALP. Vancouver, BC, Canada, 17-22 August. Paper n. 187

Usai M.G., S. Salaris, S. Casu, T. Sechi, S. Miari, P. Carta, and A. Carta, 2018. Feasibility of genomic predictions of Sarda breed rams using a female reference population. Proceedings, 11th WCGALP. Auckland, New Zealand, 11–16 February. In press

Yen, J. L., S. Garcia, A. Montana, J. Harris, S. Chervitz, M. Morra, J. West. R. Chen, and D.M. Church, 2017. A variant by any name: quantifying annotation discordance across tools and clinical databases. Genome Medicine, 9(1), 7.