

# Appraisal of the Selection Plan for Scrapie resistance in the Sarda dairy sheep breed.

S. Salaris<sup>1</sup>, F. Ingravalle<sup>2</sup>, A. Pernisa<sup>1</sup>, L. Crasta<sup>1</sup>, A. Fraghi<sup>1</sup>, C. Ligios<sup>3</sup>, S. Murru<sup>4</sup>, G. Ru<sup>2</sup>  
A. Carta<sup>1</sup>

<sup>1</sup>Research Unit: Genetics and Biotechnology, DIRPA-AGRIS Sardegna, 07100, Sassari, Italy.

<sup>2</sup>Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, 10154, Turin, Italy.

<sup>3</sup>Istituto Zooprofilattico Sperimentale della Sardegna, 07100, Sassari, Italy

<sup>4</sup>Associazione Nazionale della Pastorizia – ASSONAPA, 00155 - Rome, Italy

## Abstract

The Sarda is the largest Italian dairy sheep breed. Selection tools are applied in the high genetic merit flocks (HGMF) to generate genetic progress to be disseminated to the commercial population by natural mating rams. The aim of this paper is presenting results and prospects of the breeding plan for Scrapie resistance implemented in Sardinia on the Sarda sheep breed. The breeding plan for Scrapie resistance started in Sardinia in 2005. The plan aimed at reducing the risk of Scrapie by increasing the ARR allele frequency. Two steps were planned: in the first, genotyping and selection of rams were applied mainly in HGMF to increase the availability of ARR carrier rams while preserving the genetic merit for production traits. In the second step, genotyping was extended to the whole population while making compulsory the use of ARR carrier rams. The implementation of this “two-step” plan led to the increase of ARR frequency in HGMF of 28% in 8 years. In the same time no decline of genetic progress for production trait was observed. Furthermore, ARR frequency trend in the commercial population was steady before the start of the second step and increased since its application. The “two-step” plan combined with rules for preserving genetic merit for production traits and facilitate the use of ARR/ARR rams has produced good results in the Sarda breed population. However, further tools to precisely monitor the ram flow from high genetic merit flocks to commercial population should be implemented. Results of selection for Scrapie resistance are more efficient at early stages of the plan whereas subsequently results tend to become more and more negligible. Thus, it is crucial to establish ARR frequency at which the genotyping of all male replacement is still economically suitable.

*Keywords: breeding program, PrP, milk yield*

## Introduction

The Sarda is the largest Italian dairy sheep breed (51% of the national stock) with approximately 2.5 million heads, most bred in Sardinia on 11,000 farms ([www.istat.it](http://www.istat.it)). The selection scheme of Sarda breed is based on a pyramidal management of the population (Barillet, 1997). The high genetic merit population, consisting of around 240,000 ewes in 800 flocks (HGMF), forms the top of the pyramid (Carta et al., 2009). Selection tools are applied in HGMF to generate genetic progress that is successively disseminated to the commercial population flocks (CF), the base of the pyramid, mainly by rams for natural mating. The

genetic gap between HGMF and the commercial population is proportional to the relative size of the recorded population and the flow of breeding animals. In the Sarda breed, the average size of HGMF in the last twenty years was approximately 10% of the whole population. Controlled natural mating, that is managing natural mating by grouping ewes with a single ram (“mating group”) during the reproduction period (Salaris et al., 2008) is the main reproduction technique in HGMF. This breeding strategy makes it easy to determine the correct sire of a lamb based on the lambing date. An artificial insemination (AI) program is realized in HGMF with 2 main purposes: to create genetic links which permit to accurately progeny test young males even for natural mating and to enable planned matings between elite rams and elite ewes. On average, only 8-10% of replacement ewes are born from AI rams. Evolution of the number of flocks and population demographic pattern across years were reported by Salaris et al. (2008).

Milk yield is the main selection goal. Scrapie resistance (Salaris et al., 2007) and udder morphology (Casu et al., 2006) have only been implemented recently. Some breeding for scrapie resistance started in Sardinia since 2000. At the early stage, only AI rams were genotyped at the PrP locus. Successively, in order to estimate allelic frequency, 2,075 rams and 6,424 ewes of HGMF (Salaris et al., 2004) and 5,677 rams of CF (Mura et al., 2004) were genotyped in the framework of projects funded by the national and regional governments. Based on these surveys, the starting frequency of the ARR allele was 42% in HGMF (Salaris et al., 2004) and 40% in CF (Mura et al., 2004). According to these results, Sardinian breed was considered as a medium resistant breed to scrapie deserving a breeding plan for the control of the disease.

However, concern arised over the possibility that selection on the PrP gene might affect other traits of interest (Elsen et al., 2006; Dawson et al., 2008): the PrP gene may be either directly involved or closely linked to a gene affecting the genetic determinism of a trait. In the latter case, the potential effects depend on the strength of the linkage and the phase being positive (favourable alleles inherited with resistant PrP alleles) or negative (unfavourable alleles inherited with resistant PrP alleles). The linkage phase is expected to differ between populations or between families within population, according to the distance between the 2 loci, so that associations that are beneficial in one population (family) may be deleterious in another. In any case, the introduction of scrapie resistance as a breeding objective might have led to a lower selective pressure on the other traits.

The aim of this paper is presenting results and prospects of the official breeding plan for Scrapie resistance implemented on the Sarda sheep breed in Sardinia. In particular, this paper shows how a strategy to control a disease (breeding for resistance to Scrapie) has been implemented without impairing the genetic progress for production traits of the Sarda breed.

## **Material and methods**

The official breeding plan for Scrapie resistance started in 2005 complying with the European Commission requirements issued in 2003 that established the introduction of EU-wide genotyping-based breeding programmes and the guidelines issued by the National Ministry of Health in 2004. The Sardinian Plan aims at reducing the risk of Scrapie in sheep flocks by increasing the frequency of the ARR allele and eliminating the VRQ allele, which is rare in almost all Italian breeds. An advisory group including experts of all the involved institutions was established to manage the plan and provide scientific and technical support. Specifically, the activity in HGMF was assigned to the National Sheep Breeders Association (ASSONAPA) and the Sardinian Agency for Research in Agriculture (AGRI Sardegna). A parallel activity focused on CF was implemented by the regional and national Health

Authorities with the scientific support of both the Istituti Zooprofilattici of Sardinia (IZS) and Piemonte, the latter as National Reference Centre for Animal Transmissible Spongiform Encephalopathies (CEA, Turin) and National Institute of Health.

The plan was conceived in two steps: in the first (up to 2009), genotyping and selection of rams was applied mainly in HGMF in order to exploit the pre-existing breeding structure involved in the selection for production traits. The main purpose of this step was to increase the availability of ARR carrier rams while preserving the genetic merit for production traits.

The breeding plan established that all breeding males and the young ewes with high pedigree genetic merit for milk yield, and therefore candidate to become dams of ram, had to be genotyped. Since in Italy just veterinarians are licensed to take blood samples, in HGMF hair bulbs for DNA extraction were sampled by employees of the official recording organization. Each year, hair bulb samples were collected from all male and female replacement. These samples were classified and stored in the AGRIS lab. Simultaneously, blood samples of rams were collected on a voluntary basis in CF by official veterinarians and analysed in the molecular lab of IZS. These genotypings mainly aimed at identifying homozygous susceptible rams to be replaced by either internal or from HGMF ARR carriers.

Genotypings in HGMF were managed by ASSONAPA and the Regional Ministry of Health; data are centralised within the database of the Italian Breeding Plan based at CEA in Turin.

To preserve production traits, in the early stages of the plan even homozygous susceptible rams with high genetic merit for production traits were used for breeding. These rams were preferably mated to ARR homozygous ewes. With the same goal, no distinction was made between homozygous and heterozygous ARR carriers and both were selected according to the genetic merit for production traits. On the other hand, ARR homozygous young rams without progeny test for production traits were licensed as sires of ram when their pedigree index was beyond a specific threshold to increase the availability of ARR carrier rams in HGMF. Use of susceptible rams in HGMF was permitted only within the birth flock up to 4 years from the beginning of the Regional Plan. Furthermore a publicly funded flock was established to produce homozygous ARR rams to supply outbreaks.

In the mating year 2009, the expected genotypic frequencies of the next cohort of males was estimated basing on the genotypic frequencies of breeding males and females. This frequencies were used to calculate the amount of rams both homozygous resistant and with good genetic merit for production traits that HGMF was able to produce. This amount was the base to schedule the second step of the breeding plan. Particularly, it had to be established the exclusion of homozygous susceptible rams from reproduction in CF. These rules had the objective of accelerating the dissemination of rams from HGMF to CF by exploiting the previously large availability of ARR carrier rams generated in HGMF. Blood samples were collected systematically from all CF rams by official veterinarians and analysed in the molecular lab of IZS. Flocks involved in the regional plan are yearly qualified according to the genotype of breeding rams and the length of the period in which just homozygous resistant or at least ARR carriers are used.

Finally association studies between the PrP genotype and production traits were carried out based on the methodology developed by Salaris et al. (2007)

## **Results and discussion**

The strategy of DNA sampling is showed in Table 1. Before the starting of the official plan, hair bulbs samples were collected only from AI rams and from ewes in a AGRIS flock which provided many rams for AI (897 and 1,204 samples respectively).

Table 1 - Number of hair bulbs samples and genotypings in HGMF per sex and year

Year	flocks	hair bulb samples		genotypings	
		ewes	rams	ewes	rams
2005	753	40,633	8,574	5,616	8,418
2006	548	24,907	3,268	4,125	3,040
2007	531	21,071	3,258	5,520	3,449
2008	581	28,562	3,918	2,322	3,567
2009	588	25,939	3,951	2,656	3,893
2010	662	33,577	5,022	5,822	5,242
2011	645	30,106	4,278	8,188	4,364
2012	623	28,356	3,549	1,980	3,539
2013	562	22,891	3,531	2,882	3,616
Total		256,042	39,349	39,111	39,128

Since 2005, when the official Regional Plan started, hair bulbs were collected from males and the whole female replacement at one year of age when young ewes are usually reared in a single group. This last strategy, even if implies the collection of much more samples than those that will be actually genotyped (see Table 1), allowed the punctual genotyping of elite ewes, that are approximately 20% of the whole female population, after they performed at least two lactations and before they were mated with an elite ram to generate a young breeding male, avoiding a further visit in the flocks for sampling. Information obtained from these genotyped ewes allowed farmers to plan assortative matings taking into account Scrapie resistance and genetic merit for production traits. In total, samples from 39,349 rams and 256,042 ewes were collected from 1,455 HGMF (Table 1).

Table 2 – Number of AI doses (percentage ) per year and PrP genotype (R = resistant allele; S = susceptible allele). Starting year of Regional Plan is 2005.

Year	PrP Genotypes		
	RR	RS	SS
2000	3,267 (16%)	10,314 (50%)	6,938 (34%)
2001	2,607 (14%)	8,335 (44%)	7,793 (42%)
2002	2,795 (14%)	9,611 (49%)	7,212 (37%)
2003	4,184 (23%)	8,135 (44%)	6,275 (34%)
2004	3,841 (21%)	9,157 (50%)	5,288 (29%)
2005	3,947 (26%)	6,980 (46%)	4,202 (28%)
2006	3,300 (26%)	5,796 (45%)	3,682 (29%)
2007	5,011 (37%)	7,973 (59%)	552 (4%)
2008	4,192 (40%)	6,372 (60%)	0 (0%)
2009	6,187 (59%)	4,383 (41%)	0 (0%)
2010	7,245 (70%)	3,040 (30%)	0 (0%)
2011	6,635 (88%)	902 (12%)	0 (0%)
2012	4,792 (95%)	246 (5%)	0 (0%)
2013	6,308 (100%)	0 (0%)	0 (0%)

PrP genotyping have been carried out on 39,128 rams and 39,111 ewes of HGMF (Table 1). A peak of genotypings was reached in the first year of activity (2005).

The frequency of the ARR allele in AI doses reached 49.2% at the start of the Regional Plan in 2005 and 100% in 2013 (Table 2).

*Table 3 - ARR allele frequency and number of genotyped animals according to sex and year of birth*

Birth year	rams		ewes	
	N	ARR frequency	N	ARR frequency
2003	4,725	44.8%	11,932	42.1%
2004	3,064	47.9%	3,646	44.7%
2005	3,697	45.5%	4,880	47.2%
2006	2,910	51.4%	3,649	47.1%
2007	3,397	55.3%	2,342	51.9%
2008	3,581	57.6%	4,687	54.1%
2009	3,635	60.8%	5,444	58.4%
2010	4,650	63.5%	4,826	60.7%
2011	4,253	68.3%	3,108	65.8%
2012	3,473	70.7%	1,245	72.9%
2013	3,337	74.0%		

Taking also into account the mentioned previous surveys (Salaris et al., 2004; Mura et al., 2004), in Table 3 is reported the evolution of ARR frequency per birth year and sex. ARR frequency increased from 46% to 74% for rams born before 2005 and in 2013 respectively. The average yearly gain was approximately 3.5%. A similar pattern was observed in females.

Frequency of ARR/ARR genotype increased from 21.7% to 54.1% and the frequency of ARR carriers increased from 69.3% to 93.9% in rams born in 2005 and 2013 respectively.

Over the entire period, the VRQ allele was found only in 64 animals, 49 females and 15 males, and always at the heterozygous state. The VRQ allele frequency can be estimated 0.21% in female and 0.07% in males. Rams and ewes carrying VRQ allele were not permitted for reproduction.

*Table 4 – Number and proportion of mating group per PrP genotype (R: resistant allele; S: susceptible allele) and mating year*

Mating year	Genotype <sup>1</sup>					
	RR		RS		SS	
2004	260	24.0%	529	48.8%	295	27.2%
2005	264	26.0%	487	48.0%	264	26.0%
2006	304	31.6%	486	50.5%	172	17.9%
2007	355	36.5%	471	48.5%	146	15.0%
2008	397	41.3%	462	48.0%	103	10.7%
2009	470	50.8%	406	43.9%	49	5.3%
2010	514	58.4%	366	41.6%		0.0%
2011	559	69.5%	245	30.5%		0.0%

The proportion of ARR homozygous rams actually used for reproduction increased of 43,5% from the mating year 2005 to the mating year 2011 (Table 4). Use of susceptible rams in HGMF was allowed only within the birth flock up to 4 years from the beginning of the Regional Plan so their frequency was zero since 2010.

Within the commercial population, 40,000 males are mated every year (assuming a sex ratio of 1/50). On average a ram is used over 3 mating years corresponding to a replacement rate 0.33 so the yearly requirement for CF is of 13,200 new rams. In 2009, the expected frequency of ARR/ARR new rams in HGMF resulted 37% (Figure 1) corresponding to 44.400 individuals (240,000 x 0,50 x 0,37). This number was sufficient to select the best 30% for production traits to satisfy CF needs of new rams.

On this basis, the deadline to exclude the use of susceptible rams in CF in the second step of the Regional Plan was fixed at end of 2011.

		<i>Genotype and frequency of male parent</i>			
		RR	RS	SS	
<i>Genotype and frequency of female parents</i>		51.9	43.3	4.8	
	RR	23.6	12.2	10.2	1.1
	RS	53.4	27.7	23.1	2.6
	SS	23.0	11.9	10.0	1.1

  

<i>Mating</i>		<i>Genotype and frequency of progeny</i>		
<i>Type</i>	<i>Frequency</i>	RR	RS	SS
RR x RR	12.2	12.2	-	-
RR x RS	27.7 + 10.2	19.0	19.0	-
RR x SS	11.9 + 1.1	-	13.1	-
RS x RS	23.1	5.8	11.6	5.8
RS x SS	10.0 + 2.6	-	6.3	6.3
SS x SS	1.1	-	-	1.1
	Sum =	37.0	49.9	13.1

*Figure 1 - Genotype frequencies of all possible matings according to the frequencies of the genotypes of parents (above) and expected genotype frequencies of the progeny of each type of mating (below). R= resistant allele (ARR); S=susceptible allele (not ARR).*

Moving to CF, ARR frequency was steady in rams born by 2008 and increasing until 55% in rams born from 2009 to 2012 (Table 5). This trend was the clear effect of the rules on the rams utilisation included in the last version of the Regional Plan.

Reaching 100% of the ARR allele needs long time selection efforts (Man et al., 2007) and is not an economically efficient target at this stage of the plan as it would require a large amount of genotyping tests to obtain a low marginal increase of resistance. It's more efficient to modulate the breeding plan taking into account the evolution of the prevalence of Scrapie. In any case, strategies to monitor the evolution of the allele frequency should be implemented taking into account genotyping costs. In this sense, the use of DNA of bulk milk to estimate allele frequency in a flock may be an useful and low cost strategy.

*Table 5 – Number of genotyped rams per genotype (R= ARR allele; S= not ARR) and ARR frequency per birth year in the commercial population.*

birth year	SS	RS	RR	Total	%ARR
2004	4,280	6,137	2,458	12,875	42.9%
2005	2,174	3,249	1,206	6,629	42.7%
2006	3,042	4,413	1,747	9,202	43.0%
2007	3,283	5,015	2,038	10,336	44.0%
2008	3,451	5,048	2,098	10,597	43.6%
2009	3,324	5,127	2,255	10,706	45.0%
2010	1,866	3,392	1,599	6,857	48.1%
2011	1,554	2,890	1,655	6,099	50.8%
2012	659	1,580	965	3,204	54.8%

### **Effect of Scrapie selection on the genetic merit for production traits in HGMF**

The introduction of breeding for Scrapie resistance in the breeding scheme of the Sarda breed implied an increasing ARR frequency without slowing down the genetic gain for production and functional traits. Salaris et al. (2007) did not find direct or indirect effects of PrP gene for milk and udder morphology traits in Sarda breed. Other studies on dairy sheep showed no associations between PrP gene and dairy traits (Alvarez et al., 2006; Barillet et al., 2002; De Vries et al., 2005). Moreover, QTL detection studies showed no evidence of genes affecting dairy traits on OAR 13 where the PrP gene is located (Barillet et al., 2005; Elsen et al., 2006). Only 2 QTL affecting somatic cell count in milk (Rupp et al., 2003) and nematode resistance (Moreno et al., 2006) were found on OAR 13. The most probable locations of these QTL were rather far from the PrP locus, suggesting that selecting for scrapie resistance should not change the allele frequency at a major gene for these traits. Many other studies in sheep showed no evidence of associations between PrP and performance traits as reviewed by Sweeney and Hanrahan (2008).

*Table 6 - Contrasts for milk yield (kg) of the genotyped males in a model not including the relationship matrix (from Salaris et al., 2007)*

Genotype class <sup>1</sup>	Contrast	SED	P-value
RR vs. SS	-1.41	0.48	0.009
RR vs. RS	-1.19	0.43	0.015
RS vs. SS	-0.22	0.37	0.82

<sup>1</sup>R = resistant allele; S = susceptible allele.

Even if no association effect was observed, a loss of genetic gain on production traits might be expected based on the different selection pressure applied on resistant and susceptible genotype classes: resistant rams were selected even if they showed low genetic merit for the production traits, whereas susceptible rams were selected only if they showed high genetic merit for the production traits. Salaris et al. (2007) found that Sarda rams carrying 2 ARR alleles had significantly lower daughter yield deviations for milk yield than those carrying 1 or no ARR alleles when no adjustment for the polygenic value of animals was performed (Table 6). This difference is most likely a result of the breeding strategy that allows the use of homozygous ARR rams even with low genetic merit and of susceptible

homozygous rams with high genetic merit only. In the Sarda breed this effect has been counterbalanced using as sires of ram even homozygous ARR young rams without progeny test but with high pedigree value for milk yield.

After the introduction of breeding for Scrapie resistance, no decline in the genetic trend of milk yield was observed. In the last 7 years genetic gain was on average about 2 litres per year equal to 11% of the additive genetic standard deviation. This result is consistent with Man et al. (2007) that simulated the PrP gene selection effect in a population already submitted to selection for a quantitative trait. They found that in a situation similar to the Sarda one (more than 30% of initial ARR allele frequency and selecting for the ARR carrying rams), the loss of genetic gain for the production trait was negligible and mainly occurring over the first five years of gene selection, when most of the selection pressure was placed on increasing ARR frequency.

## **Conclusion**

The Sarda selection program for production traits is based on a pyramidal management of the population. This breeding structure has been also exploited to select for Scrapie resistance. The basic idea was to create the genetic progress in high genetic merit flocks and then disseminate it to the commercial population. Exploiting the male pathway is the most efficient way to select for Scrapie resistance.

A “two step” plan combined with rules for preserving genetic merit for production traits and facilitate the use of ARR/ARR rams has produced good results in high genetic merit flocks. These results are now being quickly transferred to the commercial population. However, further tools to precisely monitor the ram flow from high genetic merit flocks to commercial population should be implemented.

Results of breeding for Scrapie resistance are more efficient at early stages of the plan whereas subsequently results tend to become more and more negligible. Thus, it is crucial to establish ARR frequency at which the genotyping of all male replacement is still economically suitable.

Changes in genotyping strategies are desirable at high frequency of ARR allele. It would be advantageous to introduce analytical methods at low cost that allow identifying flocks with increased level of risk. As an example, a procedure to estimate flock PrP genotype frequencies by bulk milk analysis has been set up in the Sarda breed.

Acknowledgments: work funded by the Italian Ministry of Health, project “Studio epidemiologico dell’impatto dell’attività di selezione genetica sulla frequenza della Scrapie ovina in Italia”.

## **List of References**

- Álvarez, L., B. Gutiérrez-Gil, F. San Primitivo, L. F. de la Fuente, and J. J. Arranz. 2006. Influence of prion protein genotypes on milk production traits in Spanish Churra sheep. *J. Dairy Sci.* 89:1784-1791.
- Barillet, F. 1997. Genetics of milk production. Pages 523–564 in *Genetics of Sheep*. L. Piper and A. Ruvinsky, ed. CAB International Wallingford, UK.
- Barillet, F., O. Andreoletti, I. Palhière, X. Aguerre, J. M. Arranz, S. Minery, C. Soulas, J. P. Belloc, M. Briois, G. Frégeat, P. Teinturier, Y. Amigues, J. M. Astruc, M. Y. Boscher, and F. Schelcher. 2002. Breeding for scrapie resistance using PrP genotyping in the French dairy sheep breeds. *Proc. 7th World Congr. Genet. Appl. Livest. Prod.* Montpellier, France.



31: 683-686.

- Barillet, F., J.J. Arranz, and A. Carta. 2005. Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheep. *Genet. Sel. Evol.* 37 (Suppl. 1): 109–123.
- Carta, A., Sara Casu, and S. Salaris. 2009. Invited review: Current state of genetic improvement in dairy sheep. *J. Dairy Sci.* 92:5814-5833.
- Casu, Sara, I. Pernazza, and A. Carta. 2006. Feasibility of a linear scoring method of udder morphology for the selection scheme of Sardinian sheep. *J. Dairy Sci.* 89: 2200-2209.
- Dawson, M., R. C. Moore, and S. C. Bishop. 2008. Progress and limits of PrP gene selection policy. *Vet. Res.* 39: 25.
- De Vries, F., H. Hamman, C. Drögemüller, M. Ganter, and O. Distl. 2005. Analysis of associations between the prion protein genotypes and production traits in East Friesian milk sheep. *J. Dairy Sci.* 88: 392-398.
- Elsen, J. M., C. R. Moreno, L. Bodin, D. François, J. Bouix, F. Barillet, D. Allain, F. Lantier, I. Lantier, L. Schibler, A. Roig, J. C. Brunel, and Z. G. Vitezica. 2006. Selection for Scrapie resistance in France: is there evidence of negative effects on production and health traits? *Proc. 8th World Congr. Genet. Appl. Livest. Prod. Belo Horizonte, MG, Brazil. CD Commun. No 15-15.*
- Man W.Y., Lewis R., Boulton K., Villanueva B. 2007. Predicting the consequences of selecting on PrP genotypes on PrP frequencies, performance and inbreeding in commercial meat sheep populations, *Genet. Sel. Evol.* 39. 711–729.
- Moreno, C. R., L. Gruner, A. Scala, L. Mura, L. Schibler, Y. Amigues, T. Sechi, P. Jacquiet, D. François, S. Sechi, A. Roig, Sara Casu, F. Barillet, J. G. Brunel, J. Buix, A. Carta, and R. Rupp. 2006. QTL for resistance to internal parasites in two designs based on natural and experimental conditions of infection. In *Proc. 8th World Congr. Genet. Appl. Livest. Prod. Belo Horizonte, MG, Brazil. CD Commun. No. 15-05.*
- Mura, L., A. Pernisa, A. Fraghì, C. Ligios, and A. Carta. 2004. Frequenze genotipiche al locus PrP nella popolazione ovina di razza Sarda non iscritta al LG. Page 186 in *Proc. XVI Natl. Congr. Italian Soc. Sheep Goat Pathol. Prod. (SIPAOC), Siena, Italy.* Edited by SIPAOC, Sassari, Italy.
- Rupp, R., L. Schibler, E. Cribiu, Y. Amigues, M. Y. Boscher, L. Mura, T. Sechi, A. Fraghì, S. Casu, F. Barillet, and A. Carta. 2003. Evidence of chromosomal regions controlling Somatic Cell counts in Dairy sheep from two QTL detection project. In *Proc. IWMGQSG CD-ROM com n 2-32.*
- Salaris, S., L. Crasta, P. Fozzi, A. Fraghì, S. Casu, S.R. Sanna, and A. Carta. 2004. Selezione per la resistenza alla scrapie nella razza sarda: relazioni con la produzione di latte. In: *Proc. of XVI National Congress S.I.P.A.O.C., 29/09 – 02/10, Siena, Italy.*
- Salaris, S., S. Casu, and A. Carta. 2007. Investigating the relationship between the prion protein locus and udder morphology traits and milk yield in Sardinian sheep. *J. Anim. Sci.* 2007. 85: 2840–2845.
- Salaris, S., S. Casu, P. Fresi, and A. Carta. 2008. 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.
- Sweeney, T., and J.P.Hanrahan. 2008. The evidence of associations between prion protein genotype and production, reproduction, and health traits in sheep (Review). *Vet. Res.* 39:28-45.