
Effect of combining controlled natural mating with artificial insemination on the genetic structure of the flock book of Sardinian breed sheep

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The aim of this study was evaluating the effect of combining controlled natural mating with artificial insemination on the genetic structure of the flock book of Sardinian breed sheep. Discussion was focused on the feasibility of a sound genetic evaluation and the strategies to adopt for increasing the efficiency of the selection scheme. Flock and population demographic patterns across years, breeding management and links between contemporary groups were analysed from a dataset including 2 960 169 lactation records from 971 992 ewes in 2 905 flocks from 1986 to 2006. The number of registered flocks, the total female stock and the average flock size increased across years. The average number of mating groups (groups of ewes with a single ram during the reproduction period) per flock was 1.9. The highest incidence of artificial insemination was reached in 2000 with 10% of yearling primiparous born by artificial insemination. Combining the controlled natural mating with artificial insemination led to 68% of connected primiparous contemporary groups and 81% of connected all age classes contemporary groups in the last years.

Key words: *Population demographic patterns, Breeding management, Selected population, Lambing.*

The Sardinian is the largest Italian sheep breed (51% of the national stock) with approximately 3.6 million head, 3.0 of which bred in Sardinia on 12 500 farms, and the rest on the mainland, especially in central Italy (www.istat.it).

In Sardinia the sheep industry is exclusively - the presence of other breeds or crosses with other breeds is negligible - based on the local dairy sheep breed. The typical production pattern is characterised by an out-of-season lambing period. The mating period of mature ewes starts in May and continues until mid-July. Lambings occur in autumn when the grass starts to grow after the summer drought. Lambs are

Summary

Introduction

slaughtered after around 30 days (8-11 kg of live-weight). Yearling primiparous usually lamb between February and March. Milk production is entirely processed into PDO cheeses (Pecorino Romano, Pecorino Sardo and Fiore Sardo). The other important product of the sheep industry is the GPI milk fed lamb named “Agnello di Sardegna”.

The Sardinian scheme is based on a pyramidal management of the purebred population with at the top the registered flocks where artificial insemination (AI), controlled natural mating (NM), official recording and breeding value estimation are carried out to generate genetic progress (Barillet, 1997; Carta and Ugarte, 2003). The genetic progress is then transferred to the base population mainly by natural mating rams. Young rams born from assortative matings between elite rams, used either for NM or AI, and the best dams, are progeny tested every year to select the next generation of elite rams.

The main selection objective is milk yield. Since 1992 breeding values for milk yield have been obtained by Best Linear Unbiased Prediction methodology (Sanna *et al.*, 1994; Carta *et al.*, 1998a). Since 1998 fat and protein content recordings on primiparous ewes have been realized. Till now, dry matter content has not been considered yet as breeding goal due to the lack of a payment system of milk adequate to refund farmers for the potential loss of genetic progress on milk yield. Since 2004, breeding values for teat placement and degree of udder suspension have been estimated (Casu *et al.*, 2006). Scrapie resistance was introduced as breeding goal in 2004 (Salaris *et al.*, 2007).

The main feature of the breeding program is the large application of the controlled natural mating, i.e. grouping ewes with a single ram during the reproduction period (“mating group”). Ewes of a mating group (MG) can be grouped with another ram in the same reproduction season only after a lag of 14 days. This management allows assigning the correct sire of a lamb on the basis of the lambing date. Controlled natural mating is realized either with young rams to progeny test or adult proven rams. The size of MG depends on the sexual aptitude of the ram and the planned lambing period.

AI is usually performed with fresh semen after induced oestrus in June-July. From a genetic point of view, AI is mainly used to create genetic links between flocks either through AI rams or NM rams but born by AI. Moreover, planned matings are realized between elite AI rams and elite dams to generate the new cohort of young rams to introduce in the AI centre.

The aim of this study was evaluating the effect of combining controlled natural mating with artificial insemination on the genetic structure of the flock book. Results will be discussed focusing on the feasibility of a sound genetic evaluation and the strategies to adopt for increasing the efficiency of the selection scheme of the Sardinian sheep breed.

Materials and method

The database used for the genetic evaluation of 2007 managed by the National Association of Breeders (ASSONAPA) was used to extract a dataset including 2 960 000 lactation records from 971 992 ewes in 2 905 flocks from 1986 to 2006.

To analyse flock and population demographic patterns, the following annual parameters were considered: number of registered flocks (FN) with at least 5 milk recorded ewes; number of new registered flocks (FIN); number of flocks coming out of the flock-book (FOUT); total female stock (FS); total number of lactating ewes (LE).

Flock and population demographic patterns

To analyse the breeding management the following annual parameters were considered: number of replacement ewes (NR); number of breeding males (NS); number of mating groups (NMG).

Breeding management

Generally, a ram performs its first service between 15 and 23 months age (on average 18 months). It could be used in further two mating years, before being officially indexed with at least 15 first lactation daughters. According to this, five classes of rams were identified: sampling rams at their first mating year (S1); sampling rams for which the size of previous mating groups was not sufficient to obtain 15 lactating daughters (S2); sampling rams for which the size of previous mating groups was sufficient to obtain 15 lactating daughters (W); proven rams at first mating year (P1); proven rams at successive mating years (P2).

To evaluate the generation interval on the sire-son pathway, the number of mating years of proven rams was considered. This parameter allows to distinguish the effect of proven rams' use duration from the length of their progeny test.

Genetic links between flocks were evaluated considering the AI rate and the exchange of NM rams. With this aim, contemporary group (CG) was defined as the group of ewes in the same flock-year of production, age and lambing season classes. Finally, three CG were defined within flock-year: yearling primiparous, mature ewes lambing before or after December the 15th.

Links between contemporary groups

Two kinds of NM rams producing genetic links were considered: rams born in one flock and used in another one (external rams) and rams with daughters in more than one flock.

To study the genetic connectedness, a database including only records from CG with daughters of at least one sire who had offspring in at least one other CG including daughters of other sires was built. All CG included in this database were considered connected. For each CG, the number of direct links was calculated as the number of other CG with at least one common sire.

An increase of 48% of FN was observed from 1986 to 2006 (Table 1). The highest values were found from 2000 to 2003 whereas in the last four years there was a decreasing of approximately 100 flocks.

Results

On average, 10-11% of FN has been substituted by new registered flocks. An increasing trend of the percentage of flocks sited in the mainland was observed.

Flock and population demographic patterns

The total female stock quadrupled from 1986 to 2006 (Table 1). The annual increasing rate reached its maximum from 1994 to 1999 (11%) whereas it was around 7% in the previous period and 3% in last years. Difference between FS and LE was due to ewes which had first lambing when they were 2 years old. On average this group represented 9% of LE and 36% of first lactations.

On average, every year 31% of newly recorded ewes (LEIN) appeared: 72% of yearling primiparous in already registered flocks and 28% of ewes in FIN. The average percentage of ewes coming out of the flock-book (LEOUT) was 27%: 81% of culled ewes and 19% of ewes in FOUT. Only in 2001 and 2006, LEIN was lower than LEOUT.

The annual average flock size increased from 78 to 209 lactating ewes (Table 1). On the whole, a great variability of flock size was observed across years with an average coefficient of variation of 0.8. Till 1996 the percentage of flocks with less than 100 lactating ewes was more than 50% whereas in the last four years it has been reduced to approximately 25%. The percentage of flocks with more than 300 lactating ewes ranged from 1% in 1986 to 19% in 2006.

Breeding management

AI program started at experimental level in 1986 and it has been applied on large scale since 1995. The rate of replacement ewes born by AI ranged from 3% in the period 1985-1994 to 8% in the period 1995-2000 and 7% in the period 2001-2005 (Table 2). In fact, the highest AI incidence was reached in 2000 with 20 773 AI doses

Table 1. Flock and population demographic patterns across years of the Sardinian breed flock-book: number of registered flocks (FN) and percentage of registered flocks sited in Sardinia (SFN), number of newly registered flocks (FIN), number of flocks coming out of the flock-book (FOUT), total female stock (FS), total number of lactating ewes (LE), new registered ewes (LEIN), registered ewes coming out of the flock-book (LEOUT), average (AFS), standard deviation (SDFS) and maximum (MXFS) flock size.

Production year	FN	SFN (%)	FIN	FOUT	FS	LE	LEIN	LEOUT	AFS	SDFS	MXFS
1986	777	89.1	96	70	67 376	60 544	22 923	16 690	78	59	496
1987	803	87.8	107	76	73 429	67 244	23 286	19 080	84	63	525
1988	834	86.2	124	84	79 955	73 852	25 392	21 005	89	71	562
1989	874	86.3	119	94	86 256	79 202	27 723	23 398	91	72	640
1990	899	85.1	103	91	92 124	84 954	29 483	25 430	94	80	736
1991	911	84.2	75	99	98 001	89 643	31 375	27 784	98	82	828
1992	887	85.0	81	91	100 055	93 194	30 372	27 596	105	86	815
1993	877	84.2	87	74	102 760	93 193	31 164	25 319	106	84	630
1994	890	84.3	114	67	110 325	102 121	32 681	26 612	115	93	990
1995	937	84.2	90	71	123 176	114 254	39 275	31 126	122	104	1 206
1996	956	83.5	148	77	130 935	120 988	39 829	32 363	127	109	1 294
1997	1 027	82.9	188	78	147 271	134 994	49 130	33 895	131	110	1 148
1998	1 137	84.4	148	129	173 556	159 434	60 825	41 474	140	117	1 311
1999	1 156	82.9	130	108	195 680	181 439	63 694	51 210	157	129	1 447
2000	1 178	81.8	137	133	208 648	194 764	64 853	61 729	165	137	1 397
2001	1 182	80.0	142	146	206 620	193 678	59 579	60 812	164	142	1 435
2002	1 178	80.0	127	121	210 337	196 095	65 781	53 495	166	144	1 537
2003	1 184	79.3	125	151	224 235	208 898	67 151	56 526	176	150	1 572
2004	1 158	77.5	110	160	241 047	225 458	72 574	66 466	195	162	1 646
2005	1 108	74.7	104	145	241 919	225 770	70 180	67 011	204	168	1 489
2006	1 067	72.5	119	106	238 021	223 227	62 834	68 473	209	178	2 114

and 10% of replacement ewes born by AI. Since then a decreasing trend of AI spread was observed (in 2005, 2006, 2007 AI programs the number of doses were 15 129, 12 778 and 13 536, respectively).

Around 31% of NR was from unknown sire. This value increased from 24% in 1985-1994, to 35% in 1995-2000 and 41% in 2001-2005. Sixty percent of ewes born from unknown sire was in flocks with less than 3 years of milk recording. Around 27% of FN had all ewes from unknown sire.

The annual NS increased according to the increasing NR (Table 2). The average number of AI breeding sires was 41 in 1985-1994, 142 in 1995-2000 and 149 in 2001-2005 (4%, 9% and 8% of the total breeding males in the 3 periods respectively).

The average NMG per flock was 1.9, with 52% of flocks with only 1 sire, 38% with 2 or 3 sires and 10% with more than 3 sires. The frequencies of MG according to sire classes were: 42% of S1 ram, 20% of S2 ram, 15% of W ram, 10% of P1 ram, 13% of P2 ram (Table 3).

As a result of this distribution of breeding males and the average size of MG, the annual distribution of lactating ewes was: 39% from S1 sire, 17% from S2 sire, 18% from W sire, 11% from P1 sire, 14% from P2 sire.

Table 2. Number of replacement ewes (NR), number of NM replacement ewes (NMNR), number of AI replacement ewes (AINR), number of breeding males (NS), number of NM breeding males (NMNS), number of mating groups (NMG), AI doses, number of flock with only NM breeding males (NMF), number of flocks with NM and AI breeding males (NMAIF) per birth year of the lactating ewes.

Birth year	AI								
	NR	NMNR	AINR	NS	NMNS	NMG	doses ¹	NMF	NMAIF
1985	18 933	18 933		864	864	918		573	
1986	20 103	20 103		903	903	989		602	
1987	22 454	22 269	185	988	979	1 053	1 753	559	62
1988	23 455	23 159	296	1 083	1 059	1 136	2 062	550	68
1989	24 490	23 994	496	1 197	1 164	1 245	3 610	574	88
1990	26 900	26 089	811	1 264	1 223	1 314	4 943	575	108
1991	26 520	25 252	1 268	1 303	1 253	1 341	8 240	565	145
1992	27 227	26 153	1 074	1 204	1 152	1 215	7 304	538	143
1993	27 069	26 011	1 058	1 240	1 169	1 227	5 218	484	181
1994	32 713	30 784	1 929	1 223	1 151	1 212	8 420	491	196
1995	37 917	35 485	2 432	1 327	1 243	1 328	10 895	460	269
1996	41 172	37 784	3 388	1 490	1 361	1 450	16 273	404	349
1997	46 427	42 658	3 769	1 588	1 447	1 540	17 109	442	361
1998	48 449	44 281	4 168	1 703	1 536	1 617	17 468	450	376
1999	49 483	45 024	4 459	1 726	1 571	1 637	19 043	473	384
2000	49 651	44 833	4 818	1 715	1 524	1 606	20 773	437	371
2001	55 481	51 063	4 418	1 731	1 575	1 638	20 519	456	354
2002	54 804	51 166	3 638	1 772	1 607	1 670	18 735	494	309
2003	61 350	56 815	4 535	1 792	1 629	1 700	19 618	479	351
2004	55 746	52 705	3 041	1 887	1 752	1 836	18 594	499	294
2005	53 609	50 788	2 821	1 776	1 631	1 693	18 286	455	290

¹Represents the number of AI doses realized to obtain the replacement born by AI of that birth year.

Rams' age at first mating ranged from 6 months to 11 years. Out of these, 66% was less than two years old. Usually Sardinian rams are mated the first time at approximately 18 months of age and only a small part are mated at on average 6 months. Seventy eight percent of rams older than two years had their first mating in flocks different from the birth one.

The percentage of S1 natural mating rams officially progeny tested was 73%: 41% by the first mating year and 66% by the first two mating years (Table 4). In AI this value was 94%: 68% by the first use year and 92% by the first two use years. Since 2000 the average annual number of indexed rams has been 531. A proven sire was used on average for 2.0±1.3 years (ranging from 1 to 12 years).

**Genetic links
between
contemporary groups**

The percentage of flocks interested by AI doubled from 14% in 1985-1994 to 28% in 2001-2005 (Table 2). In the same periods the percentage of NM rams born by AI increased from 2% to 32%, connecting 19% of flocks not directly involved in the AI program.

In regard to the genetic links created by NM rams, the average annual percentage of external S1 rams, i.e. rams born in one flock and used in another one, was: 79% in 1985-1994, 73% in 1995-2000 and 62% in 2000-2005. The average annual percentage of NM rams with daughters in more than one flock was: 30% in 1985-1994, 29% in 1995-2000 and 23% in 2000-2005.

Combining controlled natural mating with AI led to 68% of connected yearling primiparous CG and 81% of connected all age classes CG in the last years (Table 5).

Table 3. Annual number of mating groups (MG) and daughters (D). S1: sampling rams at their first mating year; S2: sampling rams for which the size of previous mating groups was not sufficient to obtain 15 lactating daughters; W: sampling rams for which the size of previous mating groups was sufficient to obtain 15 lactating daughters; P1: rams at first mating year as proven; P2: rams at a successive mating year as proven; MGF: annual number of mating groups per flock.

Period	MG	S1	MGS2	MGW	MGP1	MGP2	MG	S1D	S2D	WD	P1D	P2D	MGF
1985-1994	1 165	513	246	161	103	142		16	11	19	19	19	1.8
1995-2000	1 530	617	319	236	156	202		17	15	20	21	21	1.9
2001-2005	1 707	709	316	276	193	214		17	16	22	22	22	2.1

Table 4. Annual average number of sampling rams. PT: at first mating; PTF: progeny tested; PTF1: progeny tested by the first mating year; PTF2: progeny tested by the first two mating years. NM: controlled natural mating; AI: artificial insemination.

Period	NM				AI			
	PT	PTF	PTF1	PTF2	PT	PTF	PTF1	PTF2
1985-1994	482	355	192	319	16	14	10	14
1995-2000	589	448	246	398	48	45	35	44
2001-2005	691	484	282	450	49	47	32	46

No CG resulted completely disconnected in the 2007 genetic evaluation (Table 6). More than 80% of yearling primiparous CG not including AI ewes showed from 1 to 5 direct links. Obviously a better result appeared when yearling primiparous CG including also AI ewes were considered. Higher average number of direct links was observed in all age classes CG. It was due to the effect of grouping lactations performed in the same flock-year by ewes of different age classes (2, 3 and 4 years old) so allowing the comparison in the same CG of sires used in different mating years (Carta *et al.*, 1998b).

The 2007 female stock of the selected population was approximately 8% of the whole population. This percentage is below the optimal threshold indicated by Elsen and Mocquot (1974) for dairy sheep. However, the number of milk recorded ewes of Sardinian breed is one of the largest (www.icar.com). Organizational problems and farms' constraints made difficult applying selection tools and limited the number of registered flocks and flock size. In the last decade, efforts made to educate farmers for managing selection tools as well as improvements in milk recording and selection scheme organization have allowed a constant increase in number and size of registered flocks. The average flock size is important to permit an accurate progeny test either in terms of number of daughters per sire or number of compared rams within flocks. Unfortunately, the increase in number of flocks has been coupled with a high rate of coming out flocks so producing negative effects on the genetic structure of the selected population as it will be discussed later.

Discussion

Table 5. Average annual number of contemporary groups (CG) defined for genetic evaluation. TOT: total; KS: with known sire; CN: connected.

CG	Period	TOTCG	KSCG	CNCG
Only	1986-1995	5 447	4 843	3 183
yearling	1996-2001	11 856	10 433	7 878
primiparous	2002-2006	17 311	14 875	11 754
All age classes	1986-1995	16 432	13 887	13 207
	1996-2001	37 087	31 361	30 560
	2002-2006	54 431	45 323	44 313

Table 6. Contemporary groups percentage (%) of 2007 production year per number of direct links classes (DL) with only NM or with NM and AI sires.

Yearling primiparous				All age classes			
NM		NM+AI		NM		NM+AI	
DL	%	DL	%	DL	%	DL	%
1-3	66.6	1-24	9.3	1-4	13.6	1-99	10.1
4-5	16.9	25-99	29.4	5-9	25.2	100-199	11.2
6-10	14.2	100-200	33.6	10-19	25.0	200-499	26.8
11-20	2.3	200-499	26.2	20-49	28.4	500-999	28.8
> 21	0.0	500	1.4	> 50	7.8	> 1 000	23.0

The high percentage of first lambing ewes at 2 years age could be reduced by management techniques. This is important to fast progeny tests and their accuracy.

Like in other dairy sheep breeds, AI was introduced in the breeding scheme mainly to increase genetic links between flocks. The decreasing of AI spreading and fertility rates after 2000 was due mainly to the blue-tongue crisis. Furthermore, the negative economic conjuncture determined by the decrease of milk price and the increase of production costs led farmers to limiting the application of selection tools. Big efforts have been made to come back to the same AI rate of the end of nineties.

The annual turnover of flocks led to a high percentage of ewes with unknown parents with two main negative effects: limited depth of the relationship matrix and low effective size of the selected population. Furthermore, the percentage of ewes without pedigree was high even in the already registered flocks due to a low efficiency of the mating group management.

In fact, the mating group management is one of the most important factors affecting the generation interval and the accuracy of the progeny test. The optimal management of controlled natural mating of sampling rams implies a first use of ram by 18 months of age and a size of the mating group sufficient to produce at least 15 yearling primiparous daughters. For this purpose, it is necessary to reduce the number of rams older than 18 months used at first mating, to size mating groups between 40 to 50 ewes and to apply management techniques able to increase the percentage of primiparous ewes of 1 year age.

Another possible intervention could be trying to limit the habit of using rams in the lag between the first mating year and the indexation year. In fact, this practice limits the number of young sampling rams per year so reducing selection intensity on the male pathways.

The other constraint is the high length of the reproductive life of a proven ram. This is not always due to the high genetic value but somewhat to the farmer's difficulty of buying or internally producing elite males.

Although problems related to the application of controlled natural mating, its combining with artificial insemination allowed reaching a good level of direct genetic connectedness in the selection scheme of the Sardinian breed. On the whole, the number of completely disconnected flocks have had a strong decrease in the last 20 years. However, a more precise evaluation of the genetic connectedness in the registered Sardinia breed population is needed to identify the best strategies for combining AI and controlled natural mating.

Conclusion

Artificial insemination is surely the most important breeding tool to permit sound genetic evaluations of dairy sheep breeds. However its large-scale application encountered strong limitations either in some Western Europe countries or in East Europe and North Africa. In these conditions, the strategy of combining small rates of artificial insemination with controlled natural mating could be effective (Carta, 2008). However, an accurate modelling of the selection scheme is needed to optimise the artificial insemination rates and the genetic impact of the controlled natural mating.

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Barillet, F. 1997. Genetics of Milk Production. in Genetics of sheep. CAB International, eds L. Piper and A. Ruvinsky. Wallingford, Oxon, UK, 523-564.

Carta, A., S.R. Sanna, A. Rosati, S. Casu. 1998a. Milk yield adjustments for milking length and age-parity-month of lambing interaction in sarda dairy sheep. *Ann. Zootec.* 47 (1):59-66.

Carta, A., S.R. Sanna, S. Casu. 1998b. The use of equivalent mature ewe for the genetic evaluation of Sarda dairy sheep. 6th World Congr. Genet. Appl. Livest. Prod. Armidale, Australia. 24: 173-176.

Carta, A., E. Ugarte. 2003. Breeding goals and new perspectives in dairy sheep programs. Book of abstract of the 54th Annual Meeting of the European Association for Animal Production No 9. Rome, Italy, 31 August – 3 September. Wageningen Academic Publishers.

Carta, A. 2008. Sheep breeding. FABRE TP society meeting. Brussels, 16 April 2008 <www.fabretp.org/content/view/full/92/77/>.

Casu, S., I. Pernazza, 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

Elsen, J.M., J.C. Mocquot. 1974. Recherches pour une rationalisation technique et économique des schémas de sélection des bovins et ovins. *Bulletin Technique du Département de Génétique Animale, INRA*, n. 17, pp. 76-97.

Salaris, S., Sara Casu, 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

Sanna, S.R., A. Carta, S. Casu, A.M. Pilla, G. Pagnacco. 1994. Valutazione genetica della razza ovina Sarda: III – Indici ed andamenti genetici ed ambientali. *Zoot. Nutr. Anim.*, 20, pp. 313-318

List of references
