Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

Implementation and cost analysis of a regional farm animal cryobank: an Italian case study

Flavia Pizzi, Federica Turri, Teresa M. Gliozzi & Gustavo Gandini

To cite this article: Flavia Pizzi, Federica Turri, Teresa M. Gliozzi & Gustavo Gandini (2016) Implementation and cost analysis of a regional farm animal cryobank: an Italian case study, Italian Journal of Animal Science, 15:2, 207-210, DOI: 10.1080/1828051X.2016.1153406

To link to this article: http://dx.doi.org/10.1080/1828051X.2016.1153406

ITALIAN JOURNAL OF ANIMAL SCIENCE, 2016
VOL. 15, NO. 2, 207–210
http://dx.doi.org/10.1080/1828051X.2016.1153406

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

OPEN ACCESS

Implementation and cost analysis of a regional farm animal cryobank: an Italian case study

Flavia Pizzi[a]*, Federica Turri[a]*, Teresa M. Gliozzi[a] and Gustavo Gandini[b]

[a]Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, Lodi, Italy; [b]Dipartimento di Scienze Veterinarie e Sanità Pubblica, University of Milano, Milano, Italy

ABSTRACT

Scientific and technical reports on farm animal genetic resources (AnGR) cryoconservation activities, and related costs, are needed to optimise conservation programmes. In this paper, we presented the recent Italian AnGR gene banking development, including the creation of the first regional animal cryobank, the 'Lombardia Farm Animal Genetic Resources Cryobank' (LABank). In order to provide indications on cryobanking costs, a detailed analysis of the expenses incurred during the creation of LABank was carried out. Currently, in LABank genetic material (spermatozoa, blood and hair bulbs) of Italian cattle, sheep and goat local breeds is cryopreserved, for a total amount of approximately 2500 semen doses collected from 46 donors of five local breeds. The costs incurred by creating the semen storage showed differences among species and semen collection procedures, providing indications to enhance the setting up of regional cryobanks.

ARTICLE HISTORY
Received 16 October 2015
Accepted 8 February 2016

KEYWORDS
Cattle; costs; goat; gene bank; sheep

Implications

At global-level erosion of farm animal genetic resources (AnGR) is advancing at high speed and livestock genetic diversity is decreasing. Italy is an example of this decreasing trend. Solutions are needed, both at global and local level, to counteract biodiversity losses. In this paper, we reported some recent activities related to farm AnGR conservation in Italy, focusing on the creation of the first Italian regional cryobank. A detailed breakdown of cryoconservation costs was performed, addressed to optimise gene banks management.

Introduction

To date, management of farm AnGR is focused on *in situ* conservation, with *ex situ* conservation representing a primary complementary strategy (Food and Agriculture Organization 2012). The current trend for *ex situ* conservation is to establish national gene banks. These are currently operational in different countries, including Brazil (Mariante et al. 2009), France (Danchin-Burge et al. 2006), United States of America (Blackburn 2009) and the Netherlands (Woelders et al. 2006). In countries where national gene banks are not yet present, within country *ex situ* conservation activities are highly desirable.

Reports on cryobanking costs are scarce, despite costs being a limiting factor in the establishment of gene banks (McClintock et al. 2007; Groeneveld et al. 2008), or only based on simulations (Gandini et al. 2007). Information about cryoconservation costs is needed in order to optimise conservation programmes.

In Italy, agricultural policy and administration is mainly decentralised to 20 administrative regions. Within this regional framework, and in the absence of a national gene bank, the Lombardia Region and the National Research Council (CNR) established in 2008 the first regional cryobank, named the 'Lombardia Farm Animal Genetic Resources Cryobank' (LABank).

Recently, LABank has officially joined the European Genebank Network for Animal Genetic Resources (EUGENA, Hiemstra et al. 2014), as part of the Network of Italian Cryobanks of Farm Animal Genetic Resources – CRIONET-IT (available online at http://www.genrescryonet.unimi.it/).

In this paper, we have reported LABank setting up activities, as case study of AnGR cryobanking activities in Italy, focusing on the expenses incurred by its creation, to provide baseline information about costs of AnGR cryobanking.

CONTACT Dr. Flavia Pizzi pizzi@ibba.cnr.it Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, c/o Parco Tecnologico Padano, via Einstein, Lodi 26900, Italy
*These authors contributed equally to this work.

Materials and methods

The process of creation of LABank

The primary objectives of LABank are: (1) to secure genetic material to support conservation of local livestock breeds in case of occurrence of genetic problems (e.g. excessive inbreeding, loss of founder genetic variation), (2) to reconstruct breeds in the event of extinction, and (3) for research purposes. The cryobank was initiated for the conservation of local livestock breeds farmed in the Lombardia region and, whenever necessary, it will expand to include breeds farmed in other Regions of Italy.

The primary site of the LABank is located in Lodi at the Parco Tecnologico Padano, and it is under the responsibility of IBBA-CNR. According to FAO Guidelines (Food and Agriculture Organization 2012), in order to reduce risks of loss, a duplicate collection is stored at a commercial artificial insemination (AI) centre 25 km away from the primary site.

The breeds involved in the creation of LABank are five local livestock breeds of conservation interest farmed in the Lombardia region: Varzese cattle breed; Brianzola sheep breed; Frisa Valtellinese, Orobica and Verzaschese goat breeds. A census of the existing semen collections revealed no risk of duplications. As first step, semen doses of Varzese cattle and Verzaschese goat (n. 144 from 2 bulls and n. 670 from 4 bucks, respectively), collected in previous conservation projects, were acquired. It was decided, during the first phase, to collect only semen because of the lower costs and simpler management compared to embryos (Boettcher et al. 2005; Gandini et al. 2007). Pedigree information, and historical information about animal flows among herds, were used to select donors, in order to maximise genetic variation. Information about the stored material is managed by the CryoWEB software (Duchev et al. 2010).

To reduce costs, three different semen collection protocols were used, on the basis of species, reproduction management, and field opportunities. The sheep species is known requiring long training periods for semen collection (Woelders et al. 2012); moreover, AI is not routinely used for Brianzola sheep breed. Thus, for Brianzola sheep, epididymal sperm was extracted from the testicles of slaughtered animals (Turri et al. 2014). Goat semen was collected on farms through repeated semen collections, by using an artificial vagina and females in oestrous as teasers. Cattle semen collection was performed on farm by an AI centre team service.

Analysis of costs incurred during the creation of LABank

Costs related to LABank creation include fixed costs relative to equipment and liquid nitrogen, and variable costs relative to genetic material collection and processing.

Fixed costs include building and maintenance costs. Building costs consider: (1) security equipment of the cryobank storage room, both for employees (detection of oxygen saturation) and for the genetic material stored (detection of liquid nitrogen levels); (2) n. 2 cryogenic tanks, considering the necessity of duplication of the material in two different storage sites, and a maximum amount of 5000 straws stored (i.e. the capacity of one tank); (3) equipment for collection, analysis in the field, and transportation of biological samples (artificial vagina, photometer, portable microscope, portable refrigerator). Building costs are given as total and per year. For annual costs, a depreciation of 5 and 10 years was considered for tanks and for equipment, respectively. Maintenance costs include: (1) liquid nitrogen for the tanks (1170 litres per year); (2) labour costs for the routine activities in the storage room (24 h per year). Rooms for storage at the two sites were provided at no costs.

Variable costs for collection and processing of genetic material were estimated considering three different collection activities as function of species and kind of biological material involved (ejaculates or epididymal sperm) and include: travel, labour, outsourced seem collection and laboratory material. Travel costs consider the expenses to travel between LABank and collection sites, including car rental and travel fees for two employees. Numbers of working days, separately for travelling to the site of collection (W1) and for semen processing (evaluation and freezing procedures for two operators) (W2), and the number of straws produced per working day, are given in Table 2. Labour costs consider a remuneration of 70 € per operator per day. Outsourced semen collection expenses incurred only for the Varzese cattle, to overcome difficulties in handling bulls (for sheep and goats, semen collection and processing were performed by internal staff). Laboratory expenses relate to the material necessary for semen processing and freezing: chemicals, consumables, material necessary for the epididymal sperm extraction as forceps, scalpels, Petri dishes, syringes, needles, and CBS^{TM} straws. Total costs per species were calculated summing labour, outsourced semen collection, laboratory material costs, and considering two scenarios including or excluding travel costs, because the high variation across the three species of

distances between collection sites and LABank. Costs are given in Table 2 by species/collection protocol, per straw and per animal donor.

Results and discussion

Fixed costs for building LABank and for maintaining the semen collection are presented in Table 1. Total building costs amount to 18 650.0 €. In terms of annual depreciation, total building expenses amount to 2225.0 €. In addition, each year about 1550.0 € are necessary to maintain LABank collection, 83% of the sum used for liquid nitrogen.

To date, a total of 2497.0 semen doses from 46 donor animals, from 5 breeds, have been collected and stored. Considering the species (Table 2), 1078.0 straws from 3 donors (359.0 ± 59.0 straws per donor), 317.0 straws from 27 donors (12.0 ± 6.8 straws per donor), and 207.0 from 9 donors (23.0 ± 13.7 straws per donor) have been collected for cattle, goat and sheep respectively. The number of straws per donor goat (N 12.0 ± 6.8 SD) and sheep (N 23.0 ± 13.7 SD) donor was highly variable, probably related to the variety of field conditions encountered, e.g. donors trained and not trained, and donors separated from, or kept with, females in oestrus. Blood and hair samples were also collected as DNA sources from the same 46 donors. Total working days (Table 2), including travelling to collection sites and semen processing, ranged from 3.0 d to 14.5 d across species. The highest number of working days was recorded for the goat due to the greater distance to the collection sites that did not allow semen collection and processing within the same working day.

Variable costs are shown in Table 2 by species. Costs varied according to semen collection technique and species. In small ruminants, epididymal semen collection (sheep: 42 semen straws per day) allowed to collect twice the amount of straws compared to the standard semen collection technique (goat: 22 semen straws per day). Consequently, travel costs were much higher in goats because of the greater number of travelling days, and also because the greater distance to the collection sites. The costs of laboratory materials for small ruminants ranged from 233.3 € in sheep to 731.7 € in goat species and were proportional to

Table 1. Fixed costs (€).

	Costs (€)	
Item	Total	Per year
Building costs		
Security equipment for detecting:		
Oxygen saturation	4650.0	465.0[a]
Liquid nitrogen levels	7800.0	780.0[a]
Cryogenic tanks (n. 2)	3600.0	720.0[b]
Equipment for collection and analysis on field	2600.0	260.0[a]
Total	18 650.0	2225.0
Maintaining costs		
Liquid nitrogen (1170 litres per year)		1280.0
Labour costs (24 h per year)		270.0
Total		1550.0

Useful life of assets:
[a]10 years;
[b]5 years.

Table 2. Biological material, labour and variable costs (€).

	Cattle	Goat	Sheep
Biological material	Ejaculate	Ejaculate	Epididymal semen
No. straws	1078.0	317.0	207.0
No. donors	3.0	27.0	9.0
No. straws/donor[a]	359.0 ± 59.0	12.0 ± 6.8	23.0 ± 13.7
Labour – working days			
W1[b]	3.0	9.0	2.5
W2[c]	0.0	5.5	2.5
W1 + W2	3.0	14.5	5.0
N. straws/(W1 + W2)[d]	359.0	22.0	41.4
Costs			
Travel	104.4	1773.8	205.0
Labour	420.0	2030.0	700.0
Outsourced semen collection	1920.0	0.0	0.0
Laboratory material	0.0	731.7	233.3
Total costs, including travel	2444.4	4535.5	1138.3
Per straw	2.3	14.3	5.5
Per donor	814.8	168.0	126.5
Total costs, excluding travel	2340.0	2761.7	933.3
Per straw	2.2	8.7	4.5
Per donor	780.0	102.3	103.7

[a]Results expressed as mean ± standard deviation.
[b]W1 = No. of working days necessary to travel to the site of semen collection;
[c]W2 = No. of working days for semen processing;
[d]No. straws/W1 + W2 = No. of straws/working days.

number of donors, rather than number of straws. The lowest cost per straw was recorded in cattle due to the higher number of semen doses obtained from a single donor; however, cattle had the highest cost per donor. Sheep and goat are species similar from a reproductive point of view, with costs differing between epididymal and 'standard' semen collection techniques. In particular considering epididymal extraction technique, in our case applied in sheep species, costs per straw excluding travel expenses were almost half (4.5 €) of that for standard semen collection (8.7 €), mainly due to the greater number of straws obtainable by using this technique (+48%). Costs for epididymal sperm of our study were similar to those documented in the Netherlands (McClintock et al. 2007): labour cost 700.0 € versus 600.0 €; total cost 1138.3 € versus 1084.0 €; cost per donor 126.5 € versus 135.0 € (current study versus Dutch study, respectively). Total variable costs, including and excluding travel expenses, were 2444.4 and 2340.0, 4535.5 and 2761.7, 1138.3 and 933.3, in cattle, goat and sheep respectively.

Conclusions

The report on the 'LABank' presented here shows the need of sharing field data, in particular to increase knowledge about gene banking costs addressed to improve the management of existing and planned banks. Reports should be published on both successful and not successful cases.

To reduce costs, our experience demonstrates the advantage of using different semen collection protocols (epididymal sperm extraction or standard semen collection technique) according to specific species and field conditions, and of using both in-house and outsourced resources. In addition, the participation of the private sector (e.g. AI centres) can reduce costs in a win–win situation.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

Blackburn HD. 2009. Genebank development for the conservation of livestock genetic resources in the United States of America. Livest Sci. 120:196–203.

Boettcher PJ, Stella A, Pizzi F, Gandini G. 2005. The combined use of embryos and semen for cryogenic conservation of mammalian livestock genetic resources. Genet Sel Evol. 37:657–675.

Danchin-Burge C, Verrier E, Moureaux S, Txier-Boicgard M, Bibe B. 2006. Sampling strategies and overview of the French National Cryobank. Proceedings of 8th World Congress on Genetics Applied to Livestock Production; Belo Horizonte, Brazil; 33:1–4.

Duchev Z, Cong TVC, Groeneveld E. 2010. CryoWEB: web software for the documentation of the cryopreserved material in animal gene banks. Bioinformation. 5:219–220.

Food and Agriculture Organization. 2012. Cryoconservation of animal genetic resources. FAO Animal Production and Health Guidelines No. 12. Roma (Italy): Food and Agriculture Organization.

Hiemstra SJ, Martyniuk E, Duchev Z, Begemann F. 2014. European Gene Bank Network for Animal Genetic Resources (EUGENA). Proceedings of 10th World Congress of Genetics Applied to Livestock Production, Poster n. 437; Vancouver, Canada.

Gandini G, Pizzi F, Stella A, Boettcher PJ. 2007. The costs of breed reconstruction from cryopreserved material in mammalian livestock species. Genet Sel Evol. 39:465–479.

Groeneveld E, Tinh NH, Kues W, Vien NT. 2008. A protocol for the cryoconservation of breeds by low cost emergency cell banks – a pilot study. Animal. 2:1–8.

Mariante AS, Albuquerque MSM, Egito AA, McManus C, Lopes A, Paiva SR. 2009. Present status of the conservation of livestock genetic resources in Brazil. Livest Sci. 120:204–212.

McClintock S, Groeneveld E, Hiemstra S, Blackburn H. 2007. Cryobanking animal genetic resources. Available from: http://www.fao.org/ag/againfo/programmes/en/genetics/ITC_sidevent.html.

Network of Italian Cryobanks of Farm Animal Genetic Resources – CRYONET-IT. Available from: http://www.genrescryonet.unimi.it/.

Turri F, Madeddu M, Gliozzi TM, Gandini G, Pizzi F. 2014. Effect of testicle postmortem storage on goat frozen-thawed epididymal sperm quality as a tool to improve genebanking in local breeds. Animal. 8:440–447.

Woelders H, Windig J, Hiemstra SJ. 2012. How developments in cryobiology, reproductive technologies and conservation genomics could shape gene banking strategies for (farm) animals. Reprod Domestic Anim. 47:264–273.

Woelders H, Zuidberg CA, Hiemstra SJ. 2006. Animal genetic resources conservation in the Netherlands and Europe: poultry perspective. Poult Sci. 85:216–222.