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Across borders – the status and future opportunities for long-term conservation of Nordic animal genetic resources

Ellen-Louisa Fagerheim White [a], Maria Kjetså [b], Jaana Peippo [a], Lucy Morgan [b], Juha Kantanen [c], Pierre Comizzoli [d], Lise Lykke Steffensen [a], Morten Kargo [e], Tullis Matson [b], Ian Mayer [f] and Mervi Honkatukia [*,a]

[a] *The Nordic Genetic Resource Center (NordGen), Alnarp, Sweden*
[b] *UK National Livestock Biobank, Whitchurch, Shropshire, United Kingdom*
[c] *Natural Resources Institute Finland (LUKE), Jokioinen, Finland*
[d] *Smithsonian's National Zoo and Conservation Biology Institute, Washington DC, United States of America*
[e] *Center for Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark*
[f] *Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway*

Abstract: The genetic diversity of multiple animal species is now declining rapidly, highlighting the need for action to protect and preserve animal genetic resources for the long term. The Nordic countries house a broad range of farm and companion animal breeds and subspecies that play a critical role in environmental sustainability, food safety and security, and human activities. Unfortunately, close to 80% of these breeds and subspecies are either endangered or critically endangered, with population sizes too small to ensure their long-term survival. In addition, almost half of them have either a declining or unknown demographic trend, and many of them suffer from high inbreeding. Emerging pressures such as climate change, infectious diseases and public unrest further threaten the status of the populations, and urgent action is necessary to ensure their future survival. Consequently, efforts for safeguarding the genetic diversity of animal genetic resources (AnGR) with additional *in vitro* or cryoconservation efforts need further consideration. The Nordic conservation strategies for AnGR have traditionally been based on *in vivo* or live conservation. Although cryoconservation efforts are in place for some species, the number of donors and doses varies considerably between breeds and species. Due to the increasing demand for additional measures for safeguarding AnGR, this document discusses the status of active AnGR conservation measures in the Nordic countries and emphasize the central role of regional cooperation in ensuring AnGR sustainability and long-term viability. Further, the contributions of cryoconservation in mitigating genetic losses are discussed.

Keywords: *In vivo* conservation, live conservation, *in vitro* conservation, genebank, cryoconservation, Nordic food security

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Background

The global decline in biodiversity also includes our farm animal breeds

There is a growing global concern about the continued loss of animal biodiversity. Evidence suggests that we have entered the "sixth mass extinction"; the first in Earth's history to be driven primarily by human

*Corresponding author: Mervi Honkatukia (mervi.honkatukia@nordgen.org)

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activity (Cowie *et al*, 2022; WWF, 2022). Currently, species are disappearing 10 to 1,000 times faster than the normal 'background' rate of extinction, and the number of individuals in multiple species is declining rapidly (WWF, 2022; IUCN, 2024). While most attention has focused on wildlife, the conservation of the world's numerous native breeds of farm animals, and the necessity to maintain genetic diversity within these species also need attention.

The Food and Agriculture Organization of the UN (FAO) published the *Global Plan of Action for Animal Genetic Resources* in 2007 (FAO, 2007b) and the *First State of the World's Animal Genetic Resources for Food and Agriculture* (FAO, 2007a). This was followed up in 2015, when FAO published the *Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture* (FAO, 2015), to further highlight the importance of the need for additional measures under the Global Plan of Action. The world's commitment to the Global Plan of Action was reaffirmed in 2017 by the 16th Regular Session of the Commission on Genetic Resources for Food and Agriculture and the 40th FAO Conference. A third edition of the Global Plan of Action for Animal Genetic Resources is currently in preparation. Despite the various implemented conservation efforts and the continuously increased awareness of the many important roles of native animal genetic resources (e.g. genetically, environmentally, economically and culturally), 6.7% of the worlds native breeds have become extinct, and 26% are classified as being at risk of extinction (FAO, 2019). The loss of heritage breeds in current production systems may have a detrimental impact on efforts to improve future animal production systems (FAO, 2007a; Kantanen *et al*, 2015).

The Nordic commitment to conservation of animal genetic resources

The Nordic countries – Denmark (including Greenland), Finland (including Åland), Iceland, Norway, Sweden, and the Faroe Islands – have a rich and diverse assemblage of local and regional transboundary farm animal breeds and subspecies (Kierkegaard *et al*, 2020; White *et al*, 2024c). These animals hold strong cultural and historical importance, and both livestock species and companion animals like sheep, goats, cattle, horses, dogs and cats have a long history of association with human societies (Ovaska *et al*, 2021; Bläuer, 2024; Kroløkke *et al*, 2024). The ancestors of some of the breeds even go back as far as 3,000–2,000 BCE, and several of them were important during the Viking age (Bläuer, 2015). Thus, these animals represent an invaluable part of the socioeconomic history of the Nordic countries. Notably, the grazing animals also play an essential role in ecosystem biodiversity and sustainability (Bele *et al*, 2018; Hall, 2018; Fraser *et al*, 2022). Unfortunately, at present, many of the local breeds face the risk of extinction due to dimishing population sizes, highlighting the urgent need to implement additional conservation measures before it is too late (FAO, 2007a; White *et al*, 2024c).

Importantly, the Nordic countries are committed to the "conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of benefits arising from genetic resources" as stated in the 1992 UN Convention on Biological Diversity (UN, 1992). This commitment encompasses both wild and domestic animals and is further outlined for domesticated animals in the FAO Global Plan of Action for Animal Genetic Resources (FAO, 2007a), highlighting their responsibility to safeguard the Nordic native breeds.

Additional approaches are needed

While the conservation of Nordic animal genetic resources (AnGR) has primarily focused on maintaining live populations (*in vivo* conservation), national efforts for cryoconservation of e.g., sperm, embryos and somatic cell tissue (*in vitro* conservation) of AnGR have also been carried out in all the Nordic countries. However, current and future threats such as emerging diseases and climate change, combined with small populations with high inbreeding levels, highlight the importance of additional safeguarding for the breeds. Plant safeguarding initiatives, including backup repositories in the Millennium Seed Bank[1] for wild plants in the UK and the Global Seed Vault in Svalbard[2] for cultivated crops have been successfully operating for some time. However, there have not yet been significant regional efforts for farm animal cryoconservation in the Nordics. The Nordic network project titled 'Nordic animal genebanks - added value through Nordic cooperation' (NordFrost, 2021–2024; White *et al* (2024a)), aimed to strengthen the collaboration and competence for cryopreservation of AnGR in the Nordic region. NordFrost gathered stakeholders in the Nordic countries to discuss the possibilities and challenges of genebanking in the region (White *et al*, 2024a). Concerns on the costs versus benefits of cryoconservation were some of the topics that were raised as obstacles influencing genebanking activities. However, new methods for collection and preservation of material have made cryoconservation and utilization of genetic resources more cost-effective, allowing a wider range of stakeholders to participate in cryoconservation (Blackburn *et al*, 2023; FAO, 2023). Many Nordic countries are now recognizing the need to improve cryoconservation activities in combination with continued efforts for live conservation (for example, the National Strategies of the Nordic Countries, Supplemental Material 1).

[1] https://www.kew.org/wakehurst/whats-at-wakehurst/millennium-seed-bank

[2] https://www.croptrust.org/work/svalbard-global-seed-vault/

Aims and objectives

This paper is a result of the work from the NordFrost network on finding new possibilities for optimized cryoconservation of Nordic AnGR. It aims to advocate for active conservation measures that emphasize the central role of regional cooperation and adaptation of new techniques in ensuring the sustainability and long-term viability of AnGR in the Nordic countries. Thus, the status, challenges, opportunities and future possibilities of cryoconservation in the Nordic countries are presented.

Animal genetic resources in the Nordic countries and conservation strategies

External threats to genetic diversity in animal populations from Nordic countries

The Nordic native breeds face a range of external challenges such as economic and political unrest (e.g. economic fluctuations, warfare influencing prices and availability of feed), modern farming practices (e.g. intensification and industrialization), societal acceptance, climate change, and emerging diseases (FAO, 2007a; Clasen *et al*, 2020, 2021; White *et al*, 2024c,b). The effect of climate change, including global warming and more extreme weather patterns, continues to be of concern as temperatures continues to rise (Simmons, 2022). Overall, the Nordic region is predicted to become both warmer and wetter, and these climatic changes will likely increase the spread and transmission of new disease epidemics threatening both farm animals and wildlife throughout the Nordic region (Gray *et al*, 2009; Wang *et al*, 2011; Yoo *et al*, 2016; Sommer and Cowie, 2020; Ramos *et al*, 2021). Some diseases are already threatening the Nordic farm animal populations. For example, African swine fever (ASF) and highly pathogenic avian influenza (HPAI), infect several populations globally every year (CDC, 2023, 2024). Further, HPAI was reported on 71 fur farms in Finland, causing the euthanasia of nearly half a million animals in 2023 (EFSA *et al*, 2023). The neurodegenerative prion disease classical scrapie has significantly influenced the production of small ruminants in Iceland (Jónmundsson *et al*, 2016; Hauksdóttir, 2021; Thorgeirsdottir, 2022) and recent salmonella outbreaks on poultry farms in Sweden have influenced the national egg supply (SVA, 2024b). More recently, a new strain of vector-borne blue tongue virus has quickly spread through Europe, and has now been detected in Denmark, Sweden and Norway (DEFRA, 2024; Jordbruksverket, 2024; NDCC, 2024; SVA, 2024b,a; Veterinærinstituttet, 2024). Such disease outbreaks can lead to euthanasia of the entire flock of animals on the farm and even on nearby farms to prevent expansion of the epidemic, thereby having critical consequences for the small populations of the local breeds (FAO, 2007a). Some small locally adopted breeds might only exist on one farm or in a small geographical area, which makes them particularly vulnerable.

Internal threats to genetic diversity in animal populations from Nordic countries

Internal threats to animal populations are mainly those of inbreeding and low genetic diversity caused, for example, by a small population size or suboptimal breeding practices. This can lead to the accumulation of deleterious recessive genetic mutations in the population, which, in turn, leads to compromised fertility rates and increased disease rates (Kadri *et al*, 2014). A small population size combined with inadequate breeding strategies can cause loss of genetic variation and lead the population further down in an extinction vortex, as illustrated in Figure 1. Effective management of genetic variation in small populations is therefore critical. Strategies for managing small populations are comprehensively described in *Sustainable Management of Animal Genetic Resources* (Wooliams *et al*, 2005).

Advantages and limitations of live animal conservation and cryoconservation

A population's ability to adapt to its environment and achieve genetic gain is an important factor in both conservation and production populations. Live conservation allows continuous adaptation to a changing environment, and breeding can lead to improved breed performance over time. Cryoconservation is described as lacking the ability for continuous adaptation because the genetics are in a frozen state. Nevertheless, cryoconservation can be seen as an important second layer of safeguarding the populations. Further utilizing genetic variation stored in genebanks may help recover lost traits that are potentially valuable to adapt to new environments and improve performance. Therefore, a combination of live conservation and cryoconservation is the optimal way to go. Further, education regarding cultural heritage traditions, such as landscaping, is important for raising awareness surrounding unique breeds and their history as human companions and production animals. Live conservation can directly promote both tradition and raise awareness of the breeds (FAO, 2013), in a way that cannot be done by cryoconservation alone. However, it can be argued that by storing the genetic material, heritage is also conserved.

Key concerns surrounding the management and conservation of AnGR are related to different pressures, especially those relating to climate change, disease outbreaks and decline in genetic variation. Live conservation in a geographically limited area is especially vulnerable to events such as political unrest, natural disasters and emerging diseases, as well as the decline in genetic variation due to suboptimal breeding practices or too small population sizes. While important tools for inbreeding control, such as optimum contribution selection (OCS) (Grundy *et al*, 2000; Woolliams *et al*, 2015) and biosecurity measures, mitigate genetic loss and the risk of disease, they do not remove the threat completely.

Cryoconservation protects against disease outbreaks and provides the possibility to improve the genetic

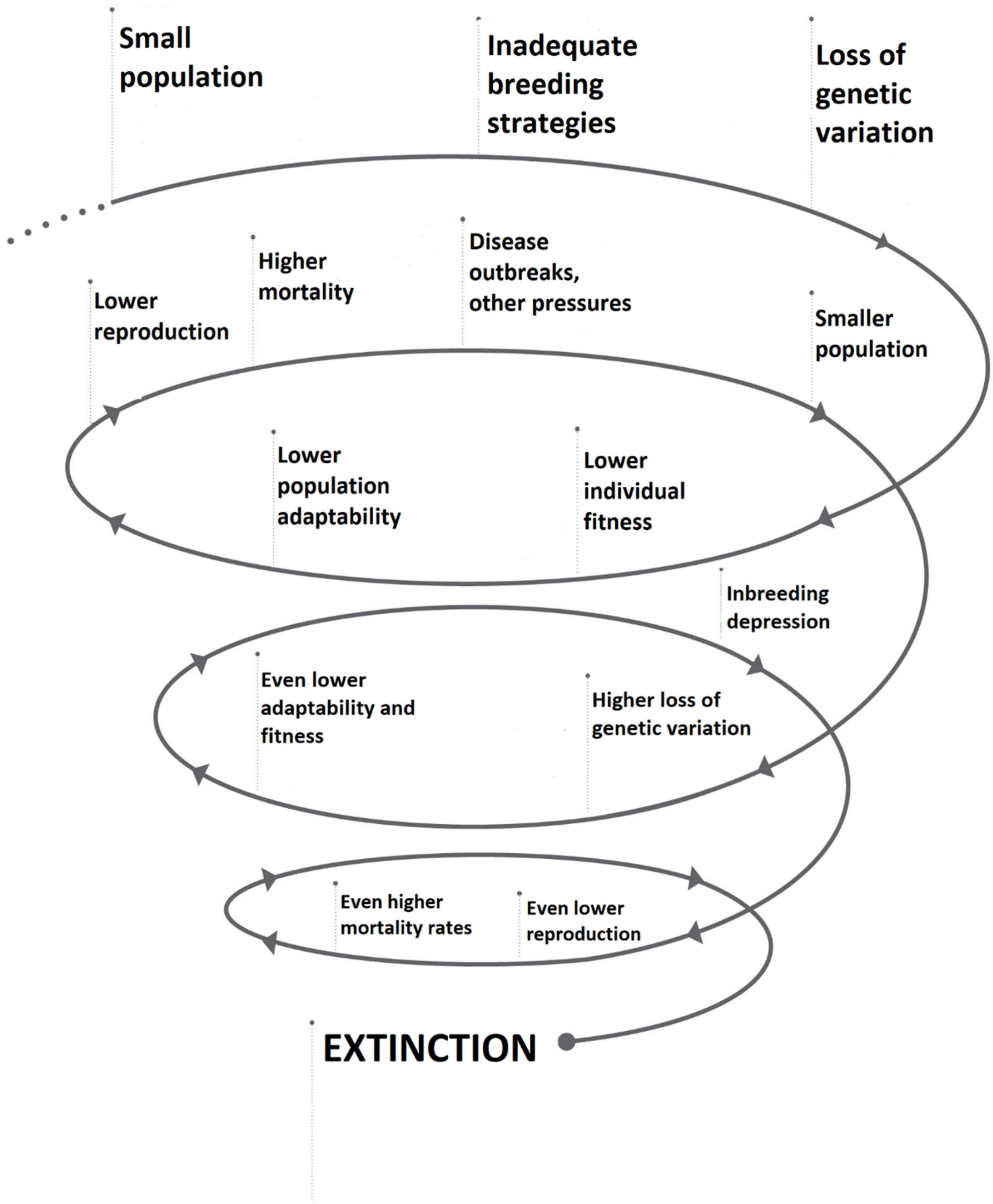


Figure 1. The extinction vortex. The figure above is a simple illustration of the consequences related to the continued loss of genetic variation in an already small population. Loss of variation has a range of driving factors, both related to genetics and inbreeding and human decisions such as breeding strategies, economy and social acceptance.

status of populations that may have suffered large losses. Genetic materials from previous generations can be reintroduced into the populations by using cryopreserved material such as semen (Blackburn *et al*, 2023; FAO, 2023). Implementing cryoconservation combined with live conservation can therefore increase the population carrying capacity, and possibly the effective population size, without influencing the living populations (Eynard *et al*, 2018; Blackburn *et al*, 2023; FAO, 2023). Further, evolving genomic methods and sampling techniques offer tremendous potential for effectively managing the Nordic native breed populations.

Regarding costs, schematics demonstrating the different components needed for establishing a genebank are listed in Figure 2. There is a lack of research evaluating the cost-effectiveness of cryoconservation compared with live conservation. Some evidence suggests that genebanks can be cost-effective: for example, Silversides *et al* (2012) determined that it could be approximately 90% cheaper to conserve chicken genetic resources by storing cryopreserved germplasm as opposed to living populations. However, it is important to consider that this is an extreme example, only considering genebanking alone. Importantly, it is possible to conserve populations cost-effectively in live populations by developing niche products or creating added value (e.g. animal-assisted therapy, using animals in restoration and management of traditional ecosystems or the production of premium-quality food products).

History, current conservation efforts and risk status

Globally, conservation efforts are still under development or insufficient to ensure the future security of AnGR (FAO, 2015; ERFP, 2021). Meticulous record-keeping and filling the observed gaps in knowledge, such as important genetic parameters and population development, are essential for the success of long-term conservation goals. However, the status of long-term conservation does not only rely on the conservation status of live populations but also on the status of cryoconservation. The Nordic countries have 167 local and regional transboundary farm animal breeds and subspecies. The majority (71%) of the Nordic breeds are endangered or critically endangered, while 5% are considered vulnerable. Only 14% are not at risk, while the remaining 10% have an undetermined risk status (Figure 3; White *et al* (2024c)). Data for the Nordic breeds collected from the Domestic Animal Diversity Information System (DAD-IS) strongly indicate that the numbers of samples stored in national genebanks are not yet sufficient, or representative of the living populations' genetic diversity. FAO has recommended to cryoconserve breeds that are categorized as endangered or critically endangered; however, only 60 of the 119 Nordic breeds with this risk status have cryoconserved materials, and the remaining 59 breeds have no additional safety measures in place (FAO, 2024; White *et al*, 2024c).

The Nordic countries have so far mainly focused on maintaining living populations through live conservation measures. However, all Nordic countries also have some cryoconserved material. The first Nordic cryoconservation efforts included the establishment of semen banks for native cattle breeds in Sweden, Iceland, Denmark and Norway in 1967, 1969, 1971 and 1977, respectively (Maijala, 2011). In Finland, cryopreservation of semen and embryos from the Finncattle breeds began during the 1980s (Pehu *et al*, 2018). The vast majority of the collected material is semen from cattle and sheep breeds (Table 1). The remaining species are highly under-represented in cryoconservation, and no material is stored from any avian species, pigs, cats or bees (Table 1). Cell types that include female genetic material including embryos, oocytes, DNA and somatic cells are also lacking in Nordic cryoconservation according to DAD-IS record of genebanking for conservation.

Number of donors presently used in cryoconservation in the Nordic countries

In addition to having at least some cryoconserved material, the breeds should ideally have sufficient samples preserved to be able to recreate healthy and sustainable future generations (White *et al*, 2024c). Recent species-specific data from the Centre for Genetic Resources, the Netherlands (CGN) of Wageningen University & Research (WUR) evaluates the sufficiency of stored samples according to different criteria. These include the number of donors and samples necessary for future reconstruction of a breed (Van Der Sluis and Schoon, 2024). The general consensus across all species is that a minimum of 50 male donor animals is required to reduce the risk of inbreeding depression. Having 37 donors allow for some selection, but the estimated inbreeding rate per generation will be higher (0.67%), while 25 donor animals yield no possibility for selection because the inbreeding rate per generation will exceed 1%. It is also essential to consider the relationship between the donors for these evaluations (Van Der Sluis and Schoon, 2024).

Data from DAD-IS shows that 19 (24.36%) of the cryoconserved Nordic breeds have sufficient semen doses from enough male donors for population sustainability (> 50 donors) (Figure 4). Furthermore, 7 (9%) of the breeds have cryopreserved embryos. So far, there have been no attempts to preserve somatic cells for genebanking purposes in any country (Table 1, White *et al* (2024c)). Notably, the largest proportion of material cryopreserved is from Norwegian cattle and sheep breeds (Table 1), which also constitute almost half of the breeds with sufficient male donors (Figure 4; FAO (2024); White *et al* (2024c)).

Further, the current collections lack female reproductive material and, in most cases, the genetic characterization of the donor living animals. The scarcity of stored material, combined with the number of breeds that are endangered and critically endangered, highlights the urgent need to improve the efforts to protect and conserve their genetic resources. In addition, the sanitary status of donors collected at artificial insemination (AI) stations is diagnosed according to EU regulations. However, information may be lacking for other genebank collections outside certified collection units (especially for female germplasm). Furthermore, in some cases, current genebanks lack a link to animal pedigree information, genotypes and phenotypes. Utilization of the samples without sufficient information about the sanitary status, pedigree or genotype risks introducing infectious diseases, unintended intensification of breeding, and unwanted genetic defects into the living population later (FAO, 2023). Importantly, utilization of breeding material from genebanks that are not EU-certified also conflicts with EU Animal Health regulations.

Challenges to conservation initiatives in the Nordics

Challenges in collecting and obtaining samples

One of the main reasons why so few species are represented in cryoconservation is that a lot of the stored

The economics of establishing a new genebank

The costs of establishing a new genebank involves two major costings: (1) Initial infrastructure and equipment costs (long-term investment), and (2) Operational costs. Below estimates for the distribution of operational costs are based on the Dutch Gene Bank for Farm Animals managed by the Centre for Genetic Resources, the Netherlands (CGN) of Wageningen University and Research.

1. Initial infrastructure and equipment costs

These costs represent a major part of the budget, and will cover the initial acquisition of suitable premises, including a cryopreservation laboratory and two storage rooms equipped with liquid nitrogen tanks. Purchase of equipment for quality analysis and the processing and freezing of genetic material (microscope, straw-filling machine, programmable freezer). The facility should have monitoring equipment to detect liquid nitrogen spills, as well as a state-of-the-art database system.

2. Operational costs

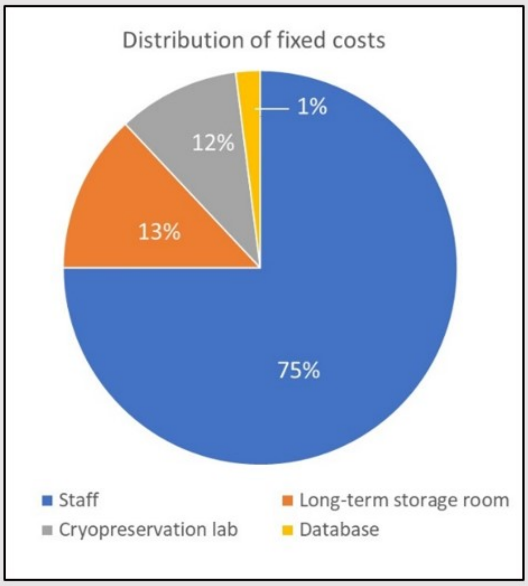
The operational costs consist of a) Fixed costs, and b) Variable costs.

a) Fixed costs

The fixed costs, which represent 80–90% of the operational budget, consist of staff salaries (3-4 positions) as well as rental costs of the cryopreservation lab and the fixed costs associated with storage (liquid nitrogen).

b) Variable costs

The major part of the variable costs is associated with collecting material for the genebank collections. A breakdown of the major costs associated with the fixed and variable costs is shown below.



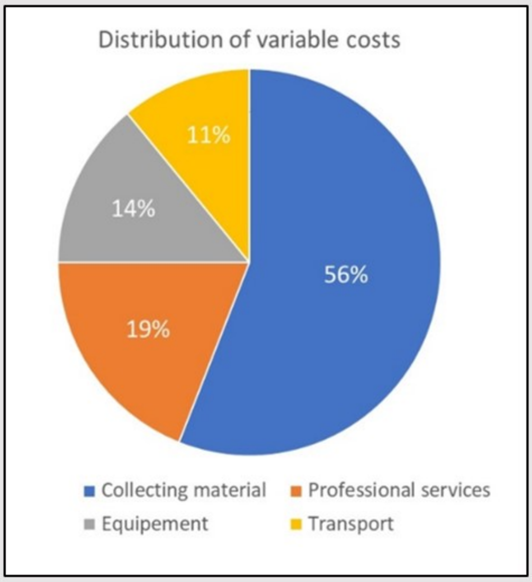


Figure 2. Economic considerations for establishment of a cryoconservation genebank for animal genetic resources. Genebank costs based on the Dutch Gene Bank for Farm Animals.

Table 1. Distribution of cell types of cryopreserved samples between species in the Nordic countries (FAO, 2024). *, Most of the collected DNA and somatic cells until now have been stored to be used in scientific contexts, which is why they have likely not been registered as material that is stored for conservation purposes in DAD-IS.

Species	Number of Breeds	Semen	DNA*	Embryos	Somatic Cells *	Oocytes
Honeybee	0/1	0	0	0	0	0
Cats	0/5	0	0	0	0	0
Cattle	23/28	972,907	190	119	0	0
Chicken	0/21	0	0	0	0	0
Deer	1/1	200	100	1	550	0
Dogs	12/29	1,634	0	0	0	0
Ducks	0/6	0	0	0	0	0
Goats	8/9	21,499	30	4	0	0
Geese	0/6	0	0	0	0	0
Horse	9/14	1,558	34	0	0	0
Pig	2/5	140	0	0	0	0
Pigeon	0/3	0	0	0	0	0
Rabbit	0/5	0	0	0	0	0
Sheep	26/34	75,291	215	108	0	0

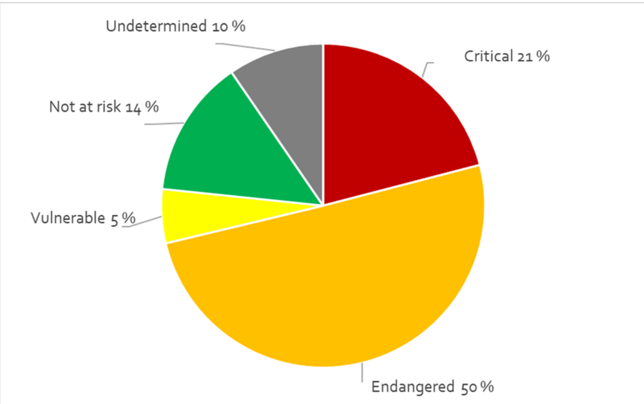


Figure 3. Risk status per breed for the different species of Nordic AnGR. The risk status is based on the classification system from FAO (FAO, 2013). The figure is adapted from White *et al* (2024c).

samples come from species with commercial breeds and are collected in collaboration with commercial breeding partners (e.g. cattle). Sheep, goats, horses and dogs are also species where AI is available, but not as widely used. For some groups of animals, collection of ejaculated semen is also not possible, either because the animals need to be trained beforehand, they are not tame enough, or there are no facilities close by. For avian species, cryopreservation protocols are not yet optimized, which limits the possibility for cryoconservation. Consequently, for example, some poultry are conserved in live genebanks (*ex situ*, *in vivo*) in the Nordics (Brekke, 2017; Sæther *et al*, 2018; Berres *et al*, 2020).

An important challenge is the cryopreservation of female tissue for genebanking. At present, female tissue (oocytes and embryos) is highly underrepresented in the current collections for cryoconservation in the Nordic countries (Table 1). Collection of female reproductive tissues is more expensive than in males (Table 2). However, the cost-effectiveness can be enhanced in species like cattle through hormonal stimulation to induce multiple ovulations. In contrast, for other species, such as horses, even hormonal intervention does not significantly improve efficiency, and collection of even two embryos at a time is rare. Further, the successful use of ovarian tissue as an oocyte reservoir is very limited in domestic animals due to compromised developmental competence of young follicles. Overall, many of the collection techniques require well-established laboratory facilities and staff with sufficient expertise. Thus, establishing sufficient genebanking activities including both male and female tissues within each country can be difficult without enough funding and support. Consequently, there are no collected oocytes at this time, and the cryoconservation of embryos is limited.

Challenges to the establishment and management of long-term genebanks

The most important factors to consider relating to the establishment and management of long-term cryoconservation (genebanking) are long-term storage costs, number of facilities and management tools (e.g. database connecting samples to genotypes and phenotypes), sanitary issues, technical expertise and collection infrastructure, and prerequisites for collaboration (for cross-border cooperation). Cryoconservation is a long-term commitment, and therefore, facility contracts and funding for long-term storage are essential for the management of genebanks (Blackburn *et al*, 2023). In the Nordic countries, nationally cryopreserved genetic material is often stored only in a single location for a given species or breed. Such a form of storage is vulnerable, because it introduces the potential risk of loss of collected material due to unforeseen events such as system malfunction (FAO, 2023). Cryopreserved material is highly valuable and must be safeguarded against poten-

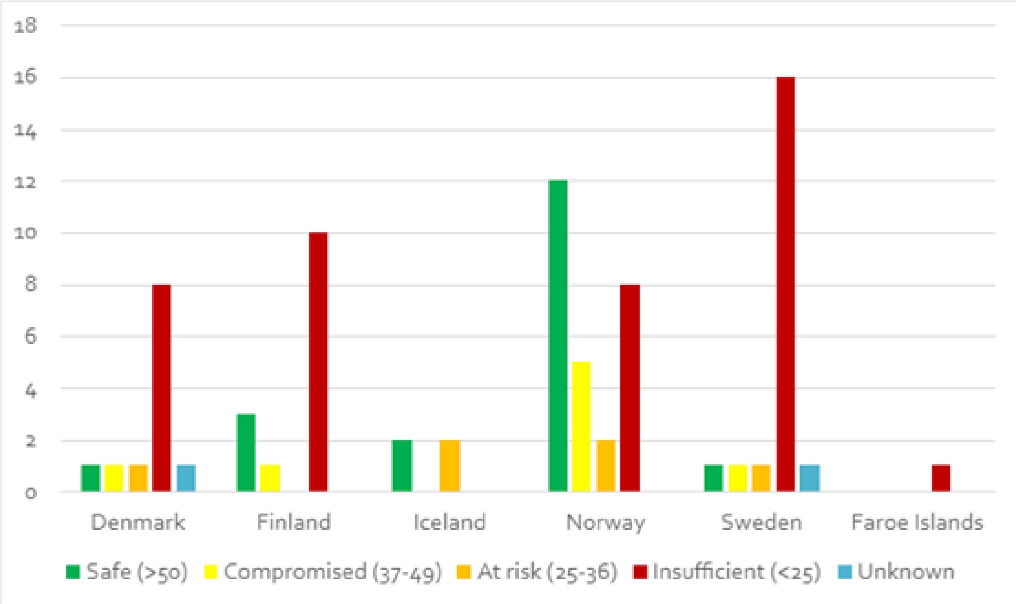


Figure 4. Classification of cryopreserved semen donors from Nordic AnGR breeds based on the criteria developed by the Centre for Genetic Resources, the Netherlands, including the total number of breeds represented in the samples. Total number of breeds with samples: 78 (White *et al*, 2024c).

Table 2. Comparison of cell types used in cryoconservation, and advantages and limitations of chosen sample types

Sample type	Advantages	Limitations
Semen	Cost-effective (large volumes per individual) Optimized protocols (high survival rate) Can be very useful in managing small population (large quantities can be used for sex-sorted semen Well-known sanitary status Can be used to re-create breeds by back-crossing	Expensive collection infrastructure (fewer males used for collection) Collections done by small numbers of centralized systems of commercial companies (due to legislation) Variation in the semen's freezing ability between individuals.
Epididymal sperm	Can be collected from dead and living donors Supports conservation of genetic diversity (i.e., can be collected from more males) Can be used to re-create breeds by back-crossing Cheap infrastructure	Lower quantity per dose Sanitary status must be defined
Oocytes	Easy to collect Can be collected from both live and dead donors (ovum pick-up, sterilization) Collections can be repeated Cheap infrastructure to collect samples from dead individuals Decision upon mating can be postponed to the future	Developmental competence after cryopreservation is very low Collection infrastructure from living donors is expensive Number of oocyte per donor is variable and unpredictable
Early embryos	Good survival rates (depending on origin – in animal or in lab)	Expensive Unpredictable Mating decisions need to be made prior to cryopreservation
Somatic cells (SC) (e.g. skin tissue)	Can be collected from all individuals 100% of DNA Cheap infrastructure for sampling Continuously developing techniques for using SC	Technologies for using SC can be expensive and not always successful
Ovarian and testicular tissues	Contain thousands of gametes at an early stage that can be developed *in vitro*. Genetic value of prepubertal individuals can also be preserved	Collections can be either after sterilization or post-mortem

tial unexpected risks; therefore, it is recommended to set up and maintain at least two separate storage facilities in different geographical locations (FAO, 2023), similar to recommended safety duplication strategies in plant genebanking.

In cases where multi-country collaboration for cryoconservation is contemplated, there are other important factors to consider, such as legislation for cross-border transportation, sanitary requirements (i.e. biosecurity), and the vulnerability to alterations in national laws or disease outbreaks that could influence the retrieval of samples (Blackburn *et al*, 2023). In Europe, EU regulation 807/2014 (EC, 2014) introduced transitional provisions allowing the transport of samples across borders according to certain sanitary regulations. Legislation and requirements for sanitary status can vary according to national legislation and regulations. In cases of collaboration, it has been recommended to implement the strictest regulations for sanitary issues (Blackburn *et al*, 2023). While multi-country collaboration introduces some concerns, it also provides a range of benefits, such as reduced costs and improved expertise. The initial costs of establishing a facility for cryoconservation are often high, which is why many countries only have one national facility where collections are stored. Collaboration between countries could mitigate the risk of malfunction while reducing the cost.

Obtaining the technical expertise necessary for processes such as collecting, freezing and thawing of samples is a key factor for the success of long-term cryoconservation and the possible use of the stored samples in the future. However, knowing the best practices for the collection and cryopreservation steps for each of the different species requires broad expertise. Cross-border collaboration could promote transparency in knowledge and expertise between the countries involved, positively contributing to conservation.

Successful management of both long- and short-term cryoconservation facilities requires the necessary and relevant knowledge for the collected genetic diversity. Blackburn *et al* (2023) argues that establishing a database with this information is not only important for contemporary management, but also for decision-making processes and the evaluation of the genebanking success. Continuous evaluation of genebanks is essential for the future status of overall conservation, because it will highlight their possible strengths and weaknesses.

Future perspectives on AnGR conservation in the Nordic countries

Collaboration across borders

Collaboration and shared knowledge of strategies and infrastructure offer future collective opportunities for the growth and security of Nordic native AnGR. For plants, the Global Seed Vault in Svalbarddemonstrates that collaboration can be highly successful. Further strengthening the collaboration for conservation of AnGR across country borders could be favourable in several ways. For example, exchanging duplicate material on a regional level could reduce the costs of cryoconservation while mitigating the risk posed by system malfunction.

In Europe, an important stakeholder for regional cooperation in cryoconservation for animals is the European Gene Bank Network for Animal Genetic Resources (EUGENA). This network serves as a coordinated digital platform that facilitates the exchange, management and conservation of genetic data from various farm animal species in Europe. One of the main goals of EUGENA is to improve cryoconservation in Europe by promoting transparency of both information and technology among the network's member countries. In this manner, EUGENA promotes genebanking efforts both at a national level as well as at a regional level by supporting countries in fulfilling their individual roles and objectives, and by facilitating cooperation between their European member countries. All European countries, including the Nordics can join the network if their genebanks meet certain criteria and are certified[3]. This network offers immense possibilities for cross-border collaboration.

Further, in the United Kingdom, a proactive group to recognize the need for comprehensive genebanking of farm animals is the UK National Livestock Biobank (UKNLB, https://www.livestockbiobank.com/), which could act as a valuable networking partner providing peer support for the Nordic countries in their initiative and exploitation of the latest cryo-methodologies. Comparable to the Nordic region, up to 80% of the United Kingdom's native farm animal breeds are at risk of extinction, with the loss of such breeds highlighted as a significant threat to national food security (DEFRA, 2021b,a). The UKNLB is a farm animal biobank based on gamete preservation (sperm, egg and embryo), alongside collection and cryopreservation of skin samples for fibroblast cell line generation. The collection of skin samples alongside gametes is unique within the UK farming cryopreservation sector, and has numerous applications and opportunities, including preservation of the whole genetic profile of farm species and the regenerative genetic capture of female lines (see Supplemental Material 2 for more info). Biobanking permits the indefinite storage of genetics beyond the lifespan of the original animal, ensuring the availability of resources for future applications (ERFP, 2023).

Implementing state-of-the-art technologies for future possibilities of conservation

The rapid progress in biotechnology paves the way to a future-oriented approach for genebanks. Anticipated future advancements, including precision gene editing, synthetic biology and advanced reproductive

[3] Memorandum of Understanding (MoU) related to the European Genebank Network for Animal Genetic Resources (EUGENA), accessible from https://www.eugena-erfp.net/en/about/how-to-become-a-member

technologies (Blackburn *et al*, 2023), offer promising avenues (Supplemental Material 2). For example, currently explored methods such as dry-preservation of germinal vesicles offer a simpler and less expensive way for long-term conservation because the preserved material can be stored at room temperature (Graves-Herring *et al*, 2013; Lee *et al*, 2019; Lee and Comizzoli, 2024). Further, advances in sequencing and genomics allow improved characterization of traits as well as better representation of the genetic diversity within genebanks and effective evaluation of populations and inbreeding control. Integrating these technologies into genebank models will enhance the ability to revive and restore endangered populations, ensuring the long-term viability of AnGR.

The NordFrost network has introduced state-of-the-art technologies that have previously been underutilized in traditional genebanking approaches to the Nordic stakeholders, such as epididymal sperm collection and the cryopreservation of skin tissue (Table 2; White *et al* (2024a)). Utilizing epididymal sperm in other disciplines is not a new phenomenon, yet it has not been widely used as a cryoconservation method for AnGR. The advantage of using epididymal sperm is that it can be collected from both deceased and living donors as well as during castration, which means that sperm can be collected from more individuals. This supports the conservation of genetic diversity. Further, like traditionally collected semen samples, it can be used to re-create breeds by back-crossing. For this method, it is important to consider that only one collection event is possible, and subsequently, the number of doses is lower than that of ejaculated semen. Nevertheless, this method is highly valuable for species where routine semen collection poses challenges – e.g. for animals that are difficult to train for semen collection, or in situations where the necessary facilities are unavailable, or where animals die due to unforeseen events and the genetic diversity would otherwise be lost. This method has been successfully implemented in countries like Finland, where epididymal sperm has been frozen from a variety of species, including cattle, horses, sheep, reindeer and roosters.

The cryopreservation of skin tissue also holds significant potential, extending the range of future possibilities for long-term conservation and could act as an additional security measure alongside the traditional sampling of reproductive tissues. Utilizing skin tissue offers an effective and less complicated way of capturing the genetic variation of individuals. For example, where gametes only capture half of the genetic information of the donor animal, skin samples and fibroblast cell lines effectively capture the entire DNA (Gorji *et al*, 2021). Furthermore, while effective infrastructures for cryopreservation and thawing of sperm have been established for most breeds, equally effective procedures for female reproductive tissue have yet to be established (Table 2; Li *et al* (2009)). Consequently, storing skin tissue samples in a genebank overcomes challenges that follow cryopreservation and thawing of egg cells (Li *et al*, 2009). Furthermore, there is also significant progress being made in the development of induced pluripotent stem cell technology, which would facilitate the development of sperm and egg cells from reprogrammed fibroblast cell lines (Bhartiya *et al*, 2014; Horer *et al*, 2023). The prospect of this technology is substantial as it would allow for the creation of individuals with a fresh genetic set, from a readily taken and cryopreserved skin sample. Most development so far has been in lab-based mice, with pups already born from reprogrammed skin samples (Mahabadi *et al*, 2018; Moradi *et al*, 2019). More detailed descriptions of the increased recognition and utilization of cryopreserved skin tissue in conservation can be explored in Supplemental Material 2.

Final remarks

To date, the Nordic countries have maintained high sanitary standards for animal health. However, maintaining these standards is expected to become increasingly challenging. Recent outbreaks of blue tongue, HPAI and African swine fever spreading to the Nordics highlight the importance of additional safeguarding measures for the conservation of animal genetic resources. Cryoconservation is a possibility to add a second layer of conservation measures and safeguard animal genetic resources, as it protects against disease outbreaks and provides the possibility to improve the genetic status of populations that may have suffered large losses due, for example, to disease epidemics.

One of the main goals for conservation in the Nordic countries is to maintain healthy and stable populations, which implies maintenance of genetic variation within the populations. Implementing both live conservation and cryoconservation allows for continuous adaptation and promotion of cultural heritage, while maintaining and/or improving genetic variation, and protecting against disease outbreaks that are difficult to contain. Therefore, the combination of both live conservation and cryoconservation enhances the safeguarding of both current and future AnGR (FAO, 2007a; Blackburn *et al*, 2023).

The Nordic countries share the responsibility to secure their AnGR to the best of their knowledge (UN, 1992). Despite the increased efforts for utilizing cryoconservation as an additional safeguard, most of the Nordic breeds are not secured and, in some instances, important information regarding both the samples and donors is missing. The NordFrost project highlighted the benefits of increased Nordic collaboration on cryoconservation of AnGR. Nordic cooperation in the field of live animal conservation and cryoconservation benefits from the growing recognition of the value of genetic diversity, the identification of shared threats and opportunities, and the development of new cost-effective genebanking methods.

Stronger Nordic collaboration on cryoconservation of AnGR could improve both the risk status and genetic diversity of the Nordic AnGR. Collaborative conservation could play an important role in strengthening the future of AnGR by allowing shared responsibility, cross-border cooperation and knowledge surrounding both infrastructure and data collection.

Supplemental data

Supplemental Material 1. National Strategies of the Nordic Countries
Supplemental Material 2. Fibroblast cell lines and advancing technologies

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Author contributions

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