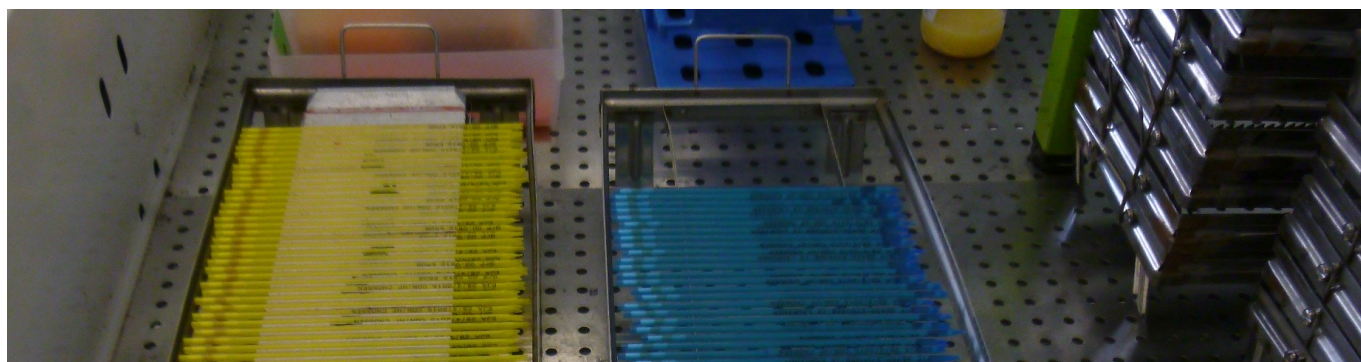


NEWSLETTER

DECEMBER 2018—ISSUE 4



editorial

The last 6 months have seen an intense activity of IMAGE in training and consultation of stakeholders, whereas significant progress was achieved on innovative approaches for animal gene banks.

Two successful training events took place: a post-graduate course on the management of animal genetic resources was organized in Bogota, 1-6 October 2018, by our Colombian partner, AGROSAVIA. It gathered 40 PhD students and researchers from South America and included a practical workshop focusing on reproductive technologies for pig production. Also, a PhD course was organized by the WUR on the use of genomic information for the conservation and management of genetic diversity, which gathered 28 PhD students from a wide geographic origin, beyond Europe. See more in this Newsletter.

“Significant progress was achieved on innovative approaches for animal gene banks”

To answer the concerns raised by the ethical review of IMAGE, we launched a large scale consultation of our stakeholders. Instead of organizing a dedicated meeting for them, we chose to integrate ethical questions in

already planned meetings of various nature by FAO, SAVE and WUR. Opinions have been collected through a survey which has received more than 150 answers until now, covering a wide range

of actors (government, research, associations, students). Preliminary analysis shows a clear consensus on the usefulness of gene banks, but reveals controversial opinions regarding the use of some reproductive technologies.

Cutting-edge technologies are being developed within IMAGE for gene banking, as illustrated in this Newsletter by the microRNA profiling of chicken primordial germ cells (HaGK and NARIC) as well as the validation of an early *in-ovo* genotyping method to increase the efficiency of introgression of a rare trait into a new chicken population (FLI).

A fully novel approach was undertaken by our economist partners in SRUC and UEDIN, who developed a mathematical model to optimize the cost of preservation of genetic diversity in gene banks. Challenging results are described in this Newsletter, where decision makers will discover possible alternatives in order to decrease the cost of gene banking at the European scale.

Michèle Tixier-Boichard

news

IMAGE SAVE-DAGENE Meeting

By Waltraud Kügler
SAVE Foundation

Ethical questions in research must be answered when living material is exchanged or during experimental laboratory work. Gene bank conservation in particular, is subject to far more ethical aspects: What are the criteria for selecting animals or breeds? Which methods of obtaining breeding material are ethically acceptable? Who decides and who pays? Are in situ and ex situ conservation complementary methods or are there conflicting goals? Within the framework of the Horizon 2020 project IMAGE (Innovative Management of Animal Genetic resources, www.imageh2020.eu) the opinions of the stakeholders in the conservation scene should be obtained. Therefore, in addition to an overview of the status of the IMAGE project, these ethical aspects were discussed at the SAVE-DAGENE meeting that took place in Kozard, Hungary, on June 24-27, 2018. Thereafter, participants were asked to complete a questionnaire asking for their opinion. The described ethical aspects of the ex situ conservation will be explained within the framework of the project at as many conferences and events as possible and opinions will be collected by questionnaire. The goal is to create the broadest possible opinion of stakeholders in conservation work, while at the same time awareness of ethics in conservation shall be promoted. The survey was completed by 30 participants.

“The evaluation of the surveys is running yet and the results will be published on our website <http://www.imageh2020.eu>”



A great ambiance at the SAVE-DAGENE meeting

Successful IMAGE session at the 10th ITWG-AnGR

By Tessa Brinker
European Forum of Farm Animal Breeders

Highlights of the IMAGE project were presented at a side event held in conjunction with the Tenth Session of the Intergovernmental Working Group on Animal Genetic Resources for Food and Agriculture (ITWG-AnGR10), held on 27 June in Rome, Italy.

Featuring talks by Michèle Boichard-Tixier from INRA in France and Sipke-Joost Hiemstra from the Centre for Genetic Resources in the Netherlands, focused on the state of play of the IMAGE project at its midterm and the ethical issues raised by cryoconservation. Harvey Blackburn from the National Animal Germplasm Program of the United States of America, who is a scientific advisor to IMAGE, chaired the session and discussion.

The audience comprised of wide variety of professions and nationalities – participants were from governmental institutions, NGOs, extension services, universities, and genebanks; from USA to Vanuatu, and from Norway to Ghana. The enthusiastic participants were involved in a discussion with the speakers and chair about, among others, the choice of breeds, trade-offs between in situ and ex situ conservation; ethics of the funding model; decision-making processes; and consideration about innovative technologies for tissue cryopreservation. Moreover, they reflected their opinions in the survey IMAGE developed on “Innovations and ethical issues for the cryoconservation of animal genetic resources”.



The presenters and chair at the ITWG-AnGR

Ethical aspects of gene banks for livestock— stakeholder perspectives

By Anouk Schurink

Centre for Genetic Resources, the Netherlands

Wednesday evening, 17.10.2018, participants of the IMAGE interactive post-graduate course on genomic diversity of animals, discussed ethical questions related to objectives of gene banks. To inform and to facilitate the discussions, the organisers invited several Dutch stakeholder representatives. Discussions were lively and rendered a general consensus on several topics.

After a warm welcome by Sipke Joost Hiemstra, director of the Centre for Genetic Resources, the Netherlands (CGN), Michèle Tixier-Boichard, co-ordinator of the EU H2020 IMAGE project, introduced the IMAGE project. Prior to the discussion, Sipke Joost provided some background information on the ethical questions to be discussed. Questions were related to the objectives of gene banks, the choice of breeds to conserve, the decision making process, funding of gene banks, the balance between *in situ* and *ex situ* efforts, and the use of innovative cryobiology and reproductive technologies. Henri Woelders explained in more detail about the perspectives and ethical aspects of emerging technologies, such as cryoconservation and transplantation of ovarian tissue and primordial germ cells.

The students and several course teachers formed four groups to represent different stakeholder perspectives. The Dutch stakeholder representatives were: Sijne van der Beek - CRV (cattle breeding company), Rolinka Snijders - Moorkop rare chicken breed society, Geert Boink - chair of the Dutch Rare Breed Survival Trust (SZH) and Karel de Greef - Wageningen Livestock Research, representing a citizen perspective.

Results were discussed in a plenary session, after the individual group discussions. Although groups had different perspectives, there was general consensus on a number of issues. Stakeholders generally agreed about the main objective of gene banks: to provide a backup in case of unexpected threats or events and as an insurance for future needs. Moreover, gene bank collections can play an important role supporting breeding of *in situ* populations. All stakeholders agreed that breeds chosen for cryoconservation should add



Lively discussions about ethical questions

value to the gene bank collection, but different values were considered depending on the type of stakeholder: breeding industry was more interested in (future) production perspectives, whereas other stakeholders considered the cultural value, the endangerment status of a breed, unique characteristics and health aspects of a breed as criteria for conservation. There was general agreement that prioritization and decision making on which breeds and which animals to conserve should be done in a multi-actor setting where scientists, NGOs, breeders and government all contribute. The answer to the question “who should pay?” was that first of all the government is responsible for long term conservation, but breeding industry could also contribute, as well as possibly future users. The position of “the one who pays, is the one who decides” was also discussed but was not so much in line with the preference towards a multi-actor setting of priorities.

When discussing about the balance between *in situ* and *ex situ* conservation efforts, *in situ* efforts were prioritized, but *ex situ* efforts were considered an important back-up strategy. Over time *ex situ* collections might genetically become too distant from the *in situ* breeding population when collections are not regularly updated. *In situ* populations are currently used by farmers and breeders and breeds have different values, including cultural and environmental. Important information that is generally missing is the respective cost of both approaches, which could differ between species as well.

Finally, many questions were raised concerning the use of the latest innovations in cryobiology and reproductive technologies, such as cryopreservation of ovarian tissue, primordial germ cells or somatic cells. Most often,

participants thought that an innovative technique may be used if this is the only way to go, considering that “the target justifies the way to reach it”. Yet, participants often expressed that all possible strategies and techniques should first be reviewed technically and ethically in detail, per species. Before implementing novel techniques in gene banking, the need for any technique should be clear and societal acceptable.

Results from the discussion will be included in the overall ethical review of the IMAGE project. At the end of the discussion a written questionnaire was distributed among the participants to have their personal views as well. More information on the course can be found in the news item below [“Everything you need to know about genetic diversity in one course”](#).

Everything you need to know about genetic diversity in one course

By Aniek Bouwman
Wageningen University & Research

An enthusiastic group of 28 participants from Europe, Africa, and America followed the Interactive post-graduate course on characterization, management and exploitation of genomic diversity in animals, held from 15-19 October 2018 at Wageningen University & Research.

The course was organised by IMAGE. A team of international lecturers, mostly involved in the IMAGE project, put together a challenging program. All relevant topics, needed to understand and make best use of genetic diversity, were covered in the course.

Genetic diversity topics. Gabor Meszaros (BOKU,

Austria) kicked off teaching about measures of genetic diversity. The second day Steffen Weigend (FLI, Germany) took over explaining aspects of the analysis of across population genetic diversity and introgression of specific genetic regions into another breed. Wednesday Mirte Bosse and Martien Groenen (WUR, the Netherlands) jumped into linking phenotype, genotype and selection history, using GWAS, selective sweeps, and functional genomics. Thursday Michèle Tixier-Boichard (INRA, France) taught the participants more on building gene bank collections. Jack Windig (WUR, the Netherlands) finished the course with optimal contribution selection and management of small populations with nice exercises on dog populations.

Excellent group work

During the week the participants worked in groups applying the material learned to actual datasets. Although time was limited, all groups worked very hard and with great enthusiasm to present some nice results on population structure, measures of hetero- and homozygosity, effective population size and more on Friday afternoon.

Ethical questions related to gene banks

Wednesday evening we had a discussion session with invited stakeholders about several ethical questions related to the objectives of animal gene banks led by Sipke Joost Hiemstra (CGN, the Netherlands) and Michèle Tixier-Boichard (INRA, France). The participants divided in different stakeholder-groups representing citizens, the breeding industry, and rare breed societies to discuss topics such as criteria to choose breeds for conservation, who should pay for gene banks, and which technologies are acceptable. More information about the discussion evening can be found in the previous article [“Ethical aspects of gene banks for livestock—stakeholder perspectives”](#).



Enthusiastic group of course participants

Comparison of the MicroRNA Expression Profiles of Male and Female Avian Primordial Germ Cell Lines

By Bence Lázár

Research Centre for Farm Animal Gene Conservation

Associated with the gene preservation effort of the scientist of the HÁGK (Gödöllő, Hungary), in collaboration with scientists of the Animal Biotechnology Department of Agricultural Biotechnology Institute, NARIC (Gödöllő, Hungary), within the framework of the IMAGE and GÉNNET-21 projects, a new [research article](#) was published recently.

In this article we presented a detailed study on the proliferation and developmental properties of male and female chicken primordial germ cells (PGC) lines, furthermore analyzed the miRNA expression in four cell lines with a microarray assay. The main aim of this complex microarray study was to get an overview about the miRNAs expressed in the male and female chicken PGCs.

PGCs are the precursors of adult germ cells, and are easily accessible from the embryonic blood and are suitable for genome preservation (gene banks). PGCs can transmit the genetic information to the next generation. In this paper we compared 12 newly established chicken PGC lines derived from two different chicken breeds (Image 1, 2). The Partridge Coloured Hungarian chicken breed was kept in the HÁGK.

We discovered specific miRNAs expressing differently in PGC lines. MicroRNAs are endogenously expressed small non-coding RNAs. They regulate multiple biological processes such as cell growth, differentiation, apoptosis and development. The main function of the mature miRNAs is post-transcriptional regulation of mRNAs. Microarray assay kit was performed by the LC Science Company.

We found that gga-miR-2127 expresses differently in female and male cell lines. The microarray analysis also revealed high expression level of the gga-miR-302b-3p strand (member of the miR-302/367 cluster) in slowly

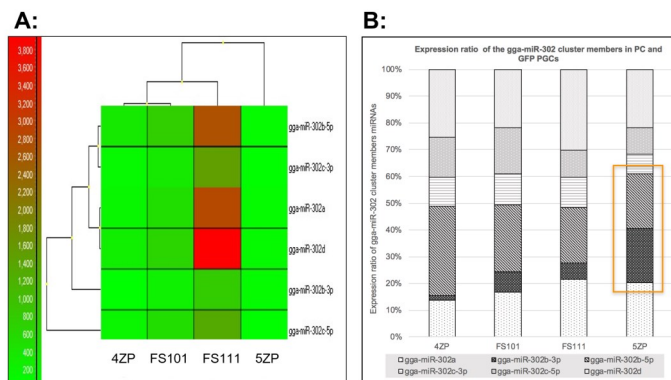


Image 3. Expression of the gga-miR-302 cluster members in PC and GFP PGCs

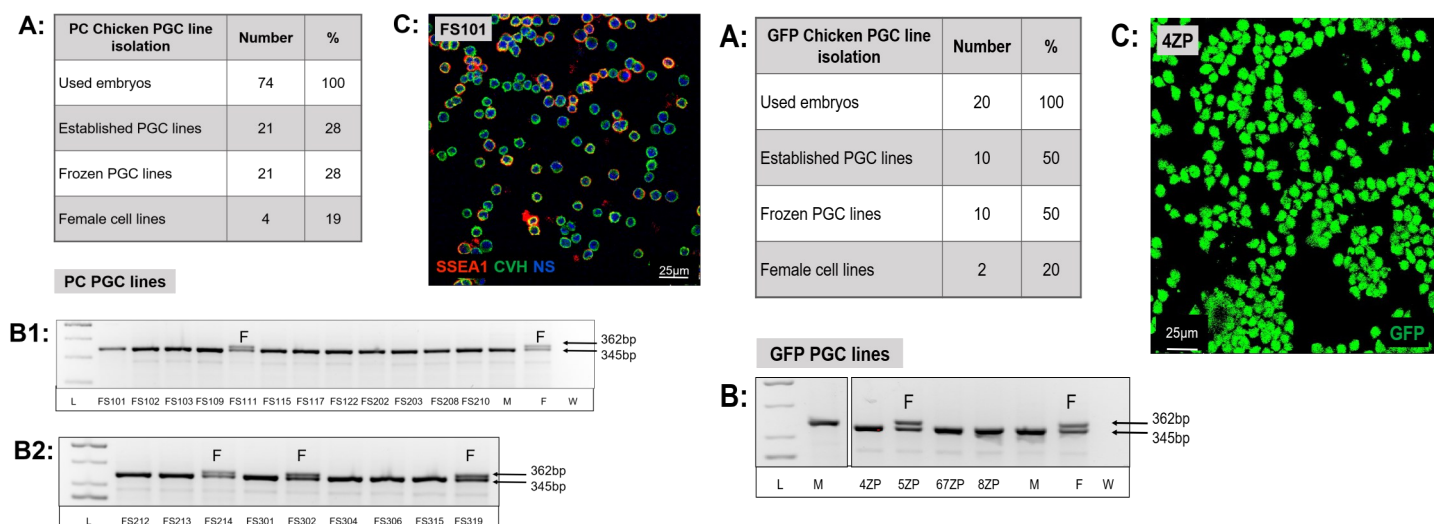


Image 1. PC Chicken PGC line

Image 2. GFP Chicken PGC Line

proliferating PGC lines compared to the gga-miR-302b-5p strand. We confirmed that the inhibition of miR-302b-5p significantly increases the doubling time of the examined PGC lines. In conclusion, we found that gga-miR-181-5p, gga-miR-2127, and members of the gga-miR-302/367 cluster have a dominant role in the regulation of avian primordial germ cell proliferation. (Image 3).

In-ovo Genotyping of Chicken using DNA Isolated from Allantoic Fluid

By Claudia Dierks
Friedrich-Loeffler-Institut

In TASK 6.3 of the IMAGE project, “From gene bank into breeding line — An animal model for introgression of blue egg shell color into a White Leghorn line”, we want to demonstrate how *ex situ* collections could efficiently be used to transfer a rare characteristic from a chicken gene bank collection into contemporary *in situ* populations by marker assisted introgression. This case study is a joint project between the Lohmann Tierzucht GmbH in Cuxhaven, the University of Göttingen and the Institute of Farm Animal Genetics — Friedrich-Loeffler-Institute in Mariensee.

As a model, the introduction of blue egg shell color as a single monogenic dominant trait from a gene bank population into a high performing, white egg layer chicken line will be carried out. Two marker-assisted backcross generations followed by an intercross-generation will be generated aiming at a high performing White Leghorn-like line which is homozygous for blue egg shell color.

“Our results suggest that DNA of sufficient amount and quality for genotyping can be obtained at day 10”

Since we only need males carrying a blue egg shell allele for further breeding of backcross generations and males and females carrying a blue egg shell allele for the intercross generations, we are focusing on in-ovo sorting of eggs in order to keep carriers of the blue egg allele, and hence reduce the number of chicks hatched. The objective of the present study is to prove the possibility of in-ovo sampling of allantoic fluid for blue egg shell genotyping and molecular sex determination in chicken. We developed competitive allele-specific PCR (KASP) assays for genotyping the blue egg shell mutation and

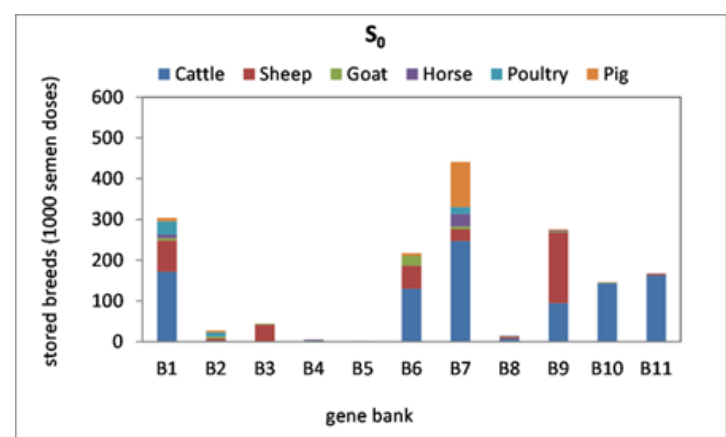
for sex determination, following a DNA isolation including a whole genome amplification procedure from allantoic fluid. Our results suggest that DNA of sufficient amount and quality for genotyping can be obtained at day 10, and to a lesser degree also at day 6 of incubation.

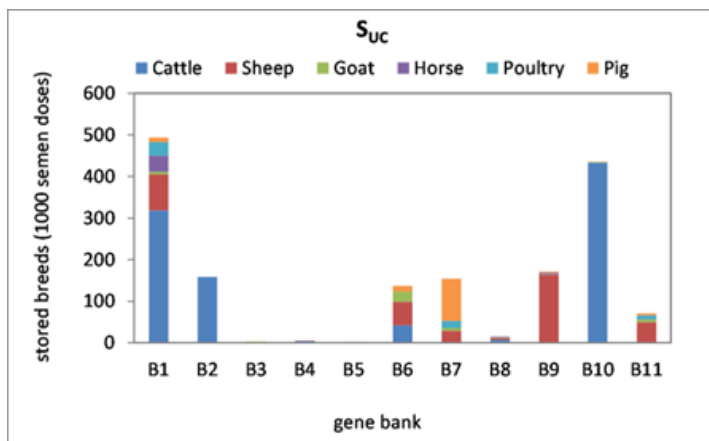
[Results of this part of the case study were presented as a poster at the EPC 2018.](#) I would like to acknowledge my co-authors of this project.

Optimising ex situ genetic resource collections for European livestock conservation

By Rafael de Oliveira Silva (University of Edinburgh),
Bouda Vosough Ahmadi (Scotland's Rural College), Sipke
Joost Hiemstra (Wageningen University & Research),
Dominic Moran (University of Edinburgh)

.Ex situ collections offer the potential to reduce extinction risks, affording option to society in maintaining future breeding opportunities for productivity and heritage traits. Gene banks complement in situ conservation, and include formal and informal use and exchange of genomic (e.g. DNA, blood, tissue) and reproductive germplasm (e.g. semen, embryos). Globally, there are many agricultural biobank collections, typically held for specialised productive purposes. There is general agreement that ex situ collections offer the value of preserving a back-up collection of threatened breeds, so that this genetic diversity might be available for use in the future. But there is clear duplication and redundancy in existing collections and this raises the question of how to optimise collection effort given global goals related to cost and diversity of material. We developed a mathematical model to optimise logistical decisions for breed conservation choices, and to evaluate alternative scenarios for efficiently re-allocating genetic materials currently stored in different European gene banks.





The analysis allowed for cross-country collections, and considered region-specific costs and cryogenic capacity differentials. Essentially the model finds alternative least cost collections relative to current breed allocations across eleven European gene banks. We show how alternative allocations could reduce overall estimated conservation costs of 23.2 million (M) EUR (around 20%) by selecting cryogenic banks that have relatively low fixed and collection costs, and that are geographically closer to collection regions. The figure below compares how breeds of different species, are distributed across the gene banks for the current allocation (So) and the hypothetical optimised collections (SUC).

The results show how optimisation can potentially inform policy decisions on efficient ex situ conservation.

Common Dissemination Booster

By Michèle Tixier-Boichard

Institut National De La Recherche Agronomique (INRA)

IMAGE is a member of the Common Dissemination Booster CDBo4-GenTORE coordinated by the GenTORE H2020 project, which gathers five other EU projects dealing with livestock : Feed-a-Gene, SAPHIR, GPlusE, IMAGE, SmartCow. The CDB is a group of experts in exploitation of research products who was chosen by the EU commission to help the EU funded projects to increase their impact. The CDB has identified as a common objective for the CDBo4-GenTORE the promotion of "European Strategic Actions in Genomics for Efficient Animal Agriculture". To support these actions, CDB has organized five services: project description and targeted results to identify the portfolio for dissemination, stakeholder mapping, dissemination development plan, dissemination capacity buildings, and dissemination actions in practice. IMAGE has completed the surveys associated to the services 1 to 4. Data provided by all projects made possible to define a

dissemination portfolio of the group and to identify possible synergies between projects regarding dissemination and exploitation of results of EU funded projects.

For service 1, IMAGE selected five of its deliverables with impact ranging from TRL3 to TRL7:

D2.5 Report on costs and potential values/benefits of genetic collections. M36. TRL3

D3.1 SPINK2, a new quality marker in chicken sperm. M24. TRL5

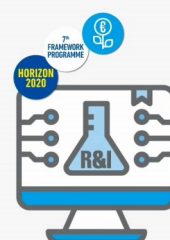
D5.3 Web portal for Europe wide gene bank material and its diversity browser M48 TRL7

D6.3 Novel methods and software to optimise conservation and introgression schemes. M36. TRL6

D7.13 Guidelines for management of gene banks. M47. TRL6

For service 2, the project has identified five categories of stakeholders which are relevant for all 6 projects of CDBo4-GenTORE 1) Policy makers (2) livestock breeders (3) Farming Industry (4) Technology and service providers (5) Veterinaries.

IMAGE appeared to be the project with the best connection with policy makers, thanks to its dialogue forum held jointly with ERFP each year. The participation of FAO in IMAGE also made possible to raise issues regarding quality management and ethics of gene banks in front of national coordinators of animal genetic resources.



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Services 3 and 4 will help us refine our dissemination tools particularly in the view of setting common actions with the other projects. One option for service 5 is to associate several projects, members of CDBo4-GenTORE, in a common stakeholder event. For instance, monitoring and preservation of animal health is critical for gene banks as well as central to SAPHIR project devoted to vaccination strategies. Another example can be found with landscape genetics analysis planned within IMAGE and studies of genotype x environment interactions undertaken by GPlusE and GenTORE projects.

profiles



Alessandra Stella, IBBA

WP5 Leader— Information system for data integration and data sharing

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Dr. Stella is a research scientist at the National Research Council of Italy and a research associate at UCSC. She holds a M.Sc. from the University of Milan, Italy, and a Ph.D. from the University of Guelph, Canada. Her skills span from the statistical and bioinformatic analysis of biological processes, genetics and genomics, biotechnologies, and technology transfer. She has 20 years of experience in the application of statistical and bioinformatic methods to the analysis of plant and animal genetic data. Her main research interests are the application of novel models and algorithms to the analysis and integration of -omics data and, in particular, to the application to analysis and conservation of biodiversity. Moreover, she's developed methods and tools for the integration of genomic, phenotypic and production system information. Her work is represented in (co-)authorship of more than 100 peer reviewed papers. H-index is 22. She was actively involved in the scientific management of the Business Incubator Alimenta for 4 years. She has coordinated several National projects and has served as WP leader in several FP7 and H2020 projects.



Prof. Luis T. Gama, University of Lisbon

WP7 Leader— Outreach

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Luis T. Gama is Professor of Animal Breeding and Genetics at the Faculty of Veterinary Medicine, University of Lisbon, Portugal. For several years, Luis was involved in the design and implementation of conservation and genetic improvement programs for various livestock species in Portugal. Over time, he established close cooperation ties with research groups in several European and Latin American countries, and he has taught post-graduate courses and conducted joint research projects in many of these countries. In the last few years, his research activities have essentially focused on gaining a better understanding of how Iberian livestock have contributed to the establishment and development of Creole populations of different species, and how these have diverged and adapted to different environmental challenges.

publications

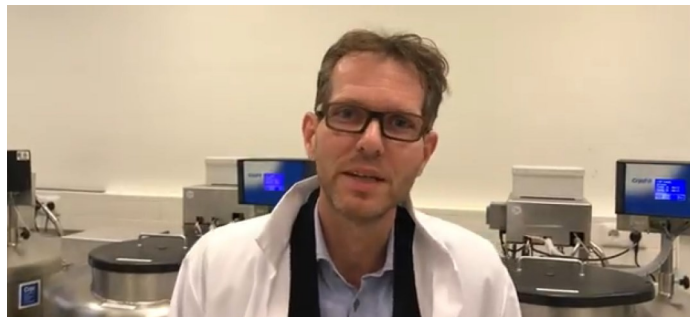
An overview of all IMAGE publications can be found on the website: www.imageh2020.eu. The newest publications are listed below:

[B. Lázár, M. Anand, R. Tóth, E. P. Várkonyi, K. Liptói, and E. Gócza. "Comparison of the MicroRNA Expression Profiles of Male and Female Avian Primordial Germ Cell Lines" *Stem cells international*. 2018.](#)

[De Oliveira Silva et al. "Optimising ex situ genetic resource collections for European livestock conservation". *J Anim Breed Genet.* \(forthcoming\). 2018.](#)

videos

IMAGE has its own [YouTube channel](#). Every now and then we will publish a video from an IMAGE partner, explaining the impact of their contributions to the IMAGE project. We use this videos for informing a wide range of individuals — from lay people to animal breeders or gene bank managers.



Sipke Joost Hiemstra, CGN/WUR
IMAGE WP2 Leader on "Gene bank functioning"



The IMAGE WP2 Leader on "Gene bank functioning", **Sipke Joost Hiemstra**, explains the importance of improving the quality and access transparency of gene banks. Click the picture above to see the video.



Elisabeth Blesbois, INRA
IMAGE WP3 Leader on "Reproductive technologies for gene banking"



Elisabeth Blesbois, is WP3 Leader on "Reproductive technologies for gene banking". She explains why it is important to improve reproductive technologies for gene banking. Click the picture above to see the video.

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