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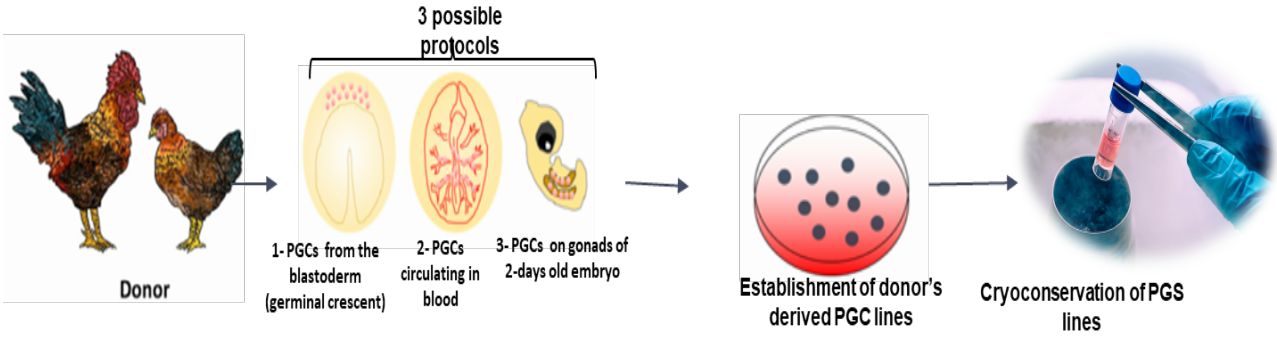
RESEARCH PROGRAM ON Livestock

CGIAR

Progress report on poultry biobanking activities in eastern (Kenya, Ethiopia, Tanzania) and central (Cameroon and DR Congo) Africa



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Centre for Tropical Livestock Genetics and Health – International Livestock Research Institute

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ILRI INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

ICARDA Science for resilient livelihoods in dry areas

CIAT International Center for Tropical Agriculture Since 1967 / Science to cultivate change

giz Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH

SLU

Activity information card

Title: **Progress of poultry biobanking activities in eastern and central Africa**

Activity Period: 2017–2021

Location – ILRI Nairobi/Kenya

Host Institutions: CTLGH/ILRI Lab3, Reproductive Technology lab

Supported by: CTLGH/ILRI, Reproductive Technology Programme, CRP Livestock Genetics, AU-IBAR and NARS (Kenya, Ethiopia, Tanzania, Cameroon, democratic Republic of Congo)

Types of activities: African indigenous chicken fertile eggs collection, laboratory biobanking experiments, poultry farm development at ILRI Nairobi and maintenance of experimental animals, training of NARS in collaboration with AU-IBAR and National Government.

Main staff involved: Steve Kemp, Yue Yaojing, Charity Muteti, Nabulindo Wilkister, Christian K. Tiambo, Pauline Kibui, Christine Kamidi Muhonja (KARLO)

Main participating institutions

- Centre for Tropical Livestock Genetics and Health (CTLGH)
- International Livestock Research Institute (ILRI)
- African Union – InterAfrican Bureau for Animal Resources (AU-IBAR)
- Agricultural and Livestock Research Organization (KALRO)

Background

The rationale/justification for the poultry biobanking activities:

Cryopreservation of animal germplasm enables sustainable and economical maintenance of genetic resources for the livestock industry and research. Cryopreservation of intact embryos is the simplest as well as most straightforward ex *situ* conservation strategy. However, in birds, this is impossible at present because of the large yolk-laden structure of their eggs (Nakamura, 2016). The cryopreservation of chicken male gametes can be achieved using traditional methods of semen preservation. There are inherent problems with using semen for reconstitution of chicken breeds (Blesbois et al., 2007). Semen viability after cryopreservation has proven variable between poultry breeds, and as the male chicken is the homogametic sex (ZZ) not containing the W sex chromosome, the entire avian genome cannot be conserved using semen preservation. Therefore, frozen semen collections can only be effectively used to safeguard and increase the genetic diversity of extant chicken breeds. The use of early germ cell precursors, the PGCs in avian species offers an innovative platform to reconstitute chicken breeds from frozen materials (Nandi et al., 2016). This technological development of avian PGC transplantation provides an insight into *ex situ* conservation because PGCs enable the capture of the entire genetics of the stock.

Avian reproductive biotechnology is the application of scientific techniques to modify, conserve and improve poultry genetic resources and to enhance their value. Therefore, avian

reproductive biotechnology can help African countries enhance chicken productivity while preserving the genetic resources.

Poultry genetic resource conservation using primordial germ cells is a part of research of the CTLGH program 3: Precision breeding through novel reproductive and germplasm technologies to achieve step changes in livestock productivity. It has been carried out under the support of CTLGH program 3 by a collaboration between Dr. Michael J Mcgrew, the Roslin Institute in Edinburgh UK, Dr Christian K. Tiambo and Professor Kemp at ILRI. The project objective is to establish primordial germ cell technology and infrastructure for bio-banking and for frozen indigenous chicken breed germplasm at ILRI, as the basis for successful conservation of economically and/or culturally important local populations of chicken in Africa.

On the other hands, transfer of the successful experience seems to be one of the most effective ways to solve the problems faced by the livestock sector. South-South knowledge exchange can be part of the solution for the lack of information, capacity building for both scientists and practitioners, as well as facilitate scaling up of successful solutions.

Following the successful piloting of biobanking African poultry genetic resources at ILRI, Training *of African scientist on Isolation and Cryopreservation of Primordial Germ Cells (PGCs) from indigenous chicken ecotypes* was the best approach on ensuring sustainability of the biobanking activities and transfer of skills in the Avian reproductive Biotechnology to African National Agricultural Research Systems (NARS) in Africa, in the framework of the partnership initiative with National Farmers Facing Programs

Relevance:

African poultry genetic resources are mostly maintained *in situ* in living populations either by smallholder farmers themselves, or in few cases in research stations. However, *in situ* conservation always carries the risk of loss owing to pathogen outbreaks, genetic problems, breeding cessation, or natural disasters. In addition to these risks, the periodic reproduction of in situ populations makes them costly to feed, and requires special facilities including an animal house and farm. On the other hands, cryobanking of germplasm in poultry has been limited to the use of semen, preventing conservation of the W chromosome and mitochondrial DNA. A further challenge is posed by the structure of avian eggs, which restricts the cryopreservation of ova and fertilized embryos, as contrarily done for mammalian species. As an alternative, avian Primordial Germ Cells, the first germ cell population established during early development, can be incorporated into the gonads (Yasuda et al., 1992) and differentiated into functional gametes following transplantation to recipient embryos (Tajima et al., 1993; Ono et al., 1998). This technological development of avian PGC transplantation provides insight into ex situ conservation because PGCs enable the capture of the entire genetics of the stock of the Kenyan indigenous chicken.

An efficient method for the propagation and conservation of chicken PGCs in vitro will not only provide a useful system for the study of PGC biology, mostly, this is an opportunity for conservation of the valuable Kenyan indigenous chicken genetic resources. This is also in line with the scenarios of operationalizing the five African regional gene banks created by the AU-IBAR. The chicken PGCs ability to form functional gametes after cryopreservation is useful to

develop a cell-based system for the genetic modification of the chicken genome. This is a valuable tool for the national research and poultry industry development in Africa.

Objectives:

The overall objective of the activities was to contribute to biobank indigenous chicken in Eastern and Central Africa, and train NARS scientist in reproductive technologies for poultry cryoconservation, including chicken Primordial Germ Cells (cPGC) isolation, long-term cultivation and re-injection for conservation of Kenya chicken Genetic Resources.

Activities carried out:

African indigenous chicken fertile eggs collection

Fertile eggs of African indigenous chicken were collected in Ethiopia, Tanzania and Kenya. Those eggs were transported to ILRI-Nairobi on successive batches. They were incubated according to the protocol (for PGCs harvesting from Blastodisc, Blood or Gonads.

Laboratory biobanking experiments

The procedures of ex situ conservation of chicken genetic resources consist of five steps

1) collection of embryonic tissues containing PGCs from target lines or breeds
2) purification or culture of PGCs,
3) PGC storage in liquid nitrogen,
4) PGC transplantation to recipient embryos, and
5) recovery of populations by successive mating of male and female recipients

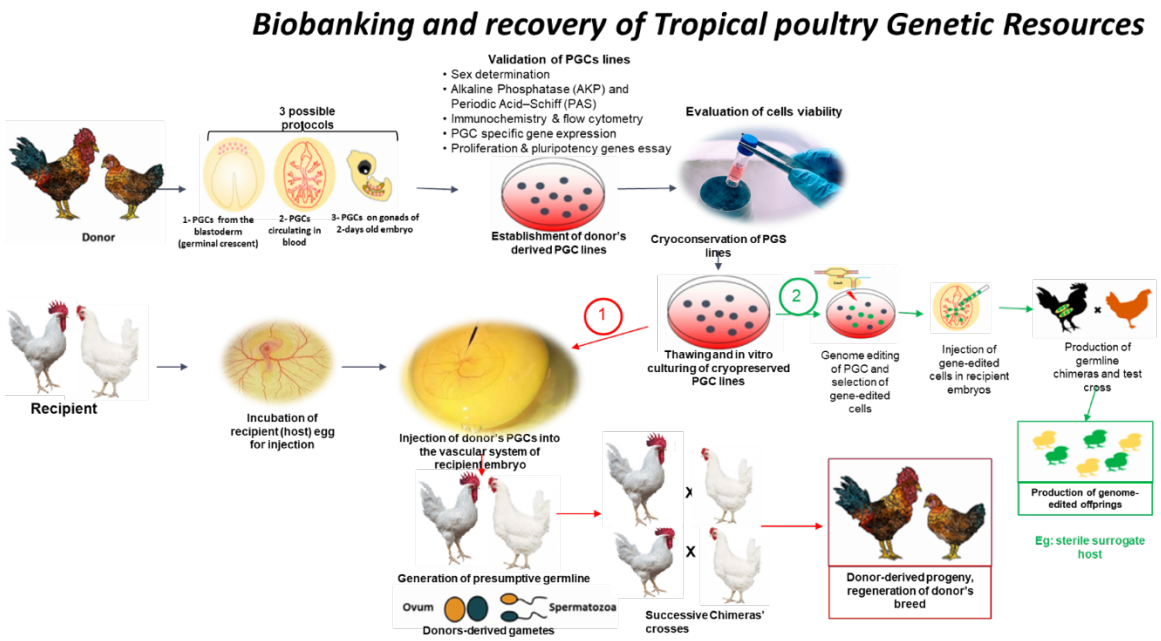
All the laboratory component of the activities were supported by the Centre for Tropical Livestock Genetic and Health (CTLGH) - ILRI, though their Poultry Genomics and reproductive technologies research components and the Livestock Genetics Flagship of the CGIAR Livestock CRP.

Figure 1 illustrates the outline of a chicken PGC-bank program at ILRI's reproductive technology lab and at the farm.

We have used three (03) protocols for biobanking of indigenous chicken primordial germ cells: extraction from blood, blastodisc and gonads. The last one is the one been the most recommended to African NARS, as presenting the advantage of avoiding the costly and time-consuming culturing phase.

The current process if PGC recovery utilizes pathway (1) through production of chimeras, however, the plan for adoption of the surrogate host technology (pathway 2) is ground breaking for accelerated restoration of the biobanked cells and rapide dissemination of elite breeds.

Figure 1. PGC biobanking activities at ILRI's reproductive technology lab and at the farm.

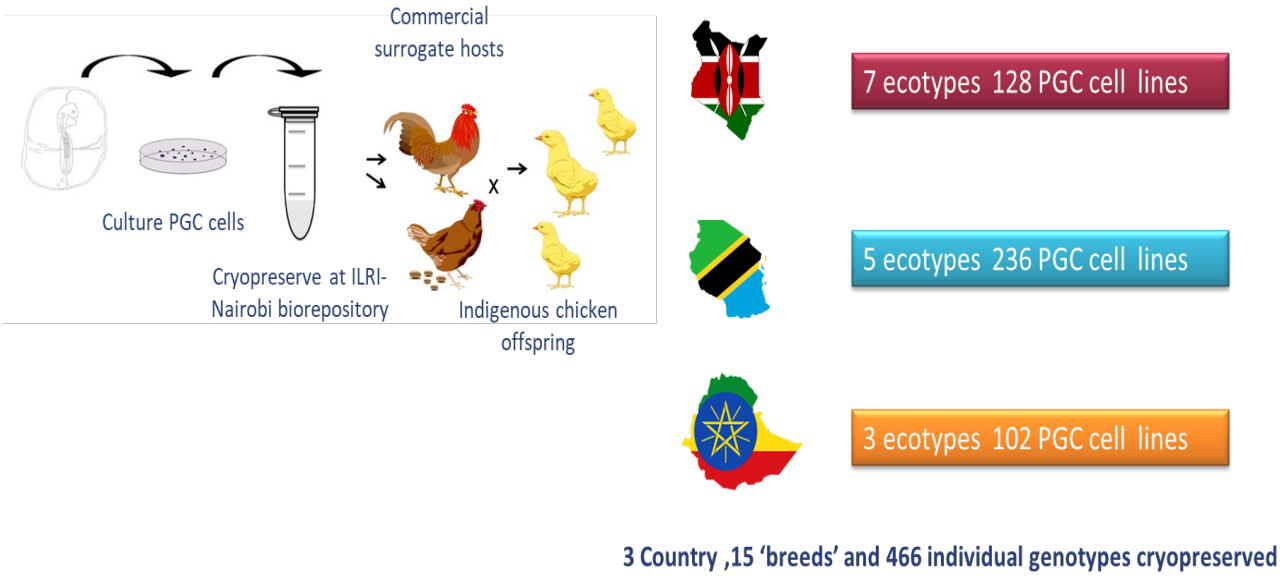


From the first phase of the biobanking activities in East Africa (Kenya, Ethiopia, and Tanzania), et total of 15 African indigenous chicken ecotypes have been cryopreserved, represented as follows in table 1 and illustrated by figure 2.

Table 1. Indigenous ecotypes cryopreserved per country

Country	Ecotype	Number cell lines
KENYA	White leghorn	43+
	Karen (Nairobi)	7
	Narok	14
	Bomet	42
	Siaya	23
	Migori	20
	Homabay	8
	Kakamega	13
Ethiopia	Arobe	59
	Horro	29
	Hawassa	32
Tanzania	Shinyanga	84
	Mwanza	11
	Mbeya	43
	Morogoro	69

Figure 2. Distribution if PGC sampling and biobanking during the piloting phase



Hence, this first phase of biobanking of African indigenous chicken genetic resources using the primordial germ cell technology has allow cryopreservation of 466 individual genotypes, representing 15 ecotypes of African chicken from 3 east African countries: Kenya, Ethiopia and Tanzania.

Poultry farm development at ILRI Nairobi and maintenance of experimental animals

The success of this phase would have not be a really without setting a small provisional poultry breeding farm at ILRI Nairobi (figure 3).

Setting this facility allowed the research team to move from the dependency of outsources on weekly basis fertile white leghorn eggs. Currently, the poultry breeding unit at ILRI Nairobi is keeping enough white leghorn animal of pure line to sustainable supply the laboratory for the amount of egg needed on weekly basis.

The farm is also keeping chimera chicken (table 2), obtained from the injection of indigenous chicken PGC in recipient white leghor embryos. Those chimeras are used for subsequent research toward recovery of the cryopreserved cells.

Figure 3. elements of the provisional poultry breeding unit at ILRI Nairobi for biobanking.

Table 2. Chimeras chicken present at the ILRI farm

Batch number	Date hatched	Number of birds	Males (ID No.)	Females (ID No.)	Remarks	Total Dead	Total live
B9	26–28.03.2021	2	1 (B9.2)	1 (B9.1)		0	2
B10	30.03.2021	2	2 (B10.1, B10.2)			0	2
B11	05.04.2021	3	1 (B11.3)	1 (B11.2)	1 (B11.1 euthanized)	1	2
B12	10.04.2021	2	1 (B12.1)	1 (B12.2)		0	2
B13	12.04.2021	1	1 (B13.1)			0	1
B16	14.05.2021	2	1	1		1	1
B18	21.05.2021	3	1	1	1 Euthanized	1	2
B20	04.06.2021	3	1	1	1 Dead	1	2
B21	16.06.2021	1	0	0	1 Euthanized	1	0
B22	19.06.2021	1	0	0	1 Dead	1	0

B25	09-11.07.2021	6			1 (B25.15, Batch25-02 dead)	2	4
B26	20.07.2021	1 (B26-12)			1 Euthanized	1	0
B29	17.08.2021	1	1	0	1 Dead (20.08.2021)	1	0
Total		**28**				**10**	**18**

Training of NARS in collaboration with AU-IBAR and national government

To successfully propagate avian PGCs it is useful to understand the mechanisms how they arise and the genetic pathways that regulate germ cell survival, proliferation, and migration in the early embryo. The trainings intended to refresh the NARS scientists and other actors of the local poultry value chain on these fundamentals of chicken embryology, and transfer to then the skill of using these techniques for conservation and improvement of indigenous chicken genetic resources.

The first training on reproduction technologies for cryo-conservation of African Animal Genetic Resources was organized at ILRI Nairobi from 16-20 July 2019 in collaboration with the African Union – InterAfrican Bureau for Animal Resources (AU-IBAR) and the Kenya Animal Genetic Resources Centre (KAGRC), and technical support from the trainers, collaborators from ARIS (the Roslin Institute). The 5-day residential training included lectures and practical sessions on specific aspects of mammals and poultry reproductive biotechnology for biobanking. From various perspectives, the workshop was a success on which to building future livestock genetic actions for Africa, a special request came from northern Africa wishing to have more interaction and collaboration with ILRI for the livestock development programmes and capacity building.

Figure 4 illustrate a section of the team attending the ToT on Reproduction technologies for cryopreservation of African animal genetic resources at ILRI Nairobi-Kenya.

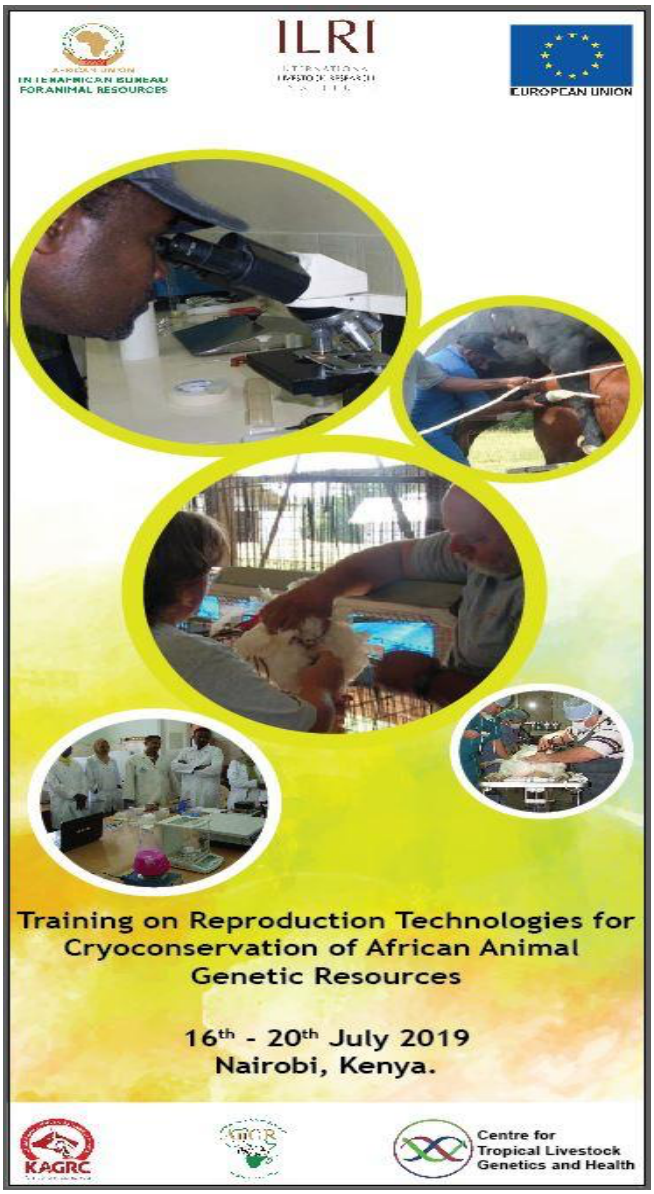


Figure 4. Moses Ogugo and a section of the team attending the ToT on Reproduction technologies for cryopreservation of African animal genetic resources at ILRI Nairobi-Kenya.

This training was followed by targeted training and field experiential learning in the NARS (Cameroon and DRC) with technical support from local poultry experts.

Training in Cameroon*:* From 16th to 20th of November 2020, a workshop on **'Training of Trainers on technologies for the promotion of local poultry value chain in Africa'** took place in Douala-Cameroon supported AU-IBAR which main objective is to support the transformation of the African poultry sector in the quest for environmentally sustainable, climate resilient, socio-economic development and equitable growth. The 5 days working group (figure 5), sharing various experiences, field visits and learning resulted in propositions of priority innovative technologies for the development of the Cameroon local poultry value chain, with emphasis on genetic and breeding aspects, eggs and meat production and transformation

source of added value in local poultry products and by-products, and marketing channels development to be supported by information technology innovators also present and represented by JANGOLO start-up (https://www.jangolo.cm/).

Figure 5: a working session under the supervision of one of the facilitators, Christian K. Tiambo.

Training in DRC: The '**Training of Trainers (ToT) on technologies for the promotion of local poultry value chain in Africa'** workshop was held in Kinshasa-DRC, at the Centre Theresianum from the 06th to 10th of April 2021. The training workshop was supported by Live2Africa, a project of AU-IBAR funded by the European Union (EU). The training workshop was conducted over 5 days with PowerPoint presentations, plenary discussion sessions deliberating on issues and sharing various experiences, focussed group discussions, field visits and hands-on learning.

The five days meeting were attended by governmental officials (Figure 6), enlightening participants of the national vision and strategies where and when need was.

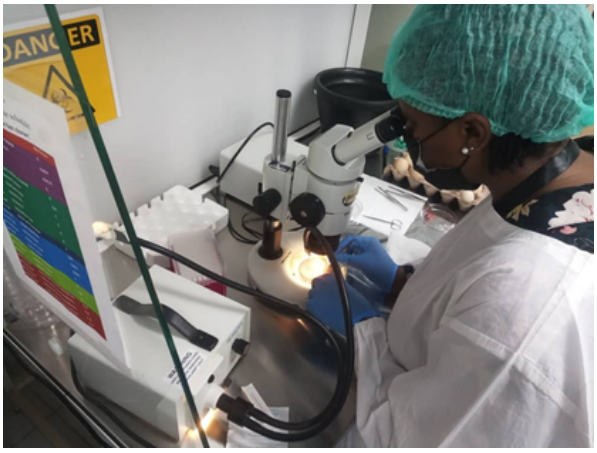
Figure 6. View of workshop participants at Centre THERESIANUM conference hall KINSHASA, being addressed by Dr Bertin Matumo, National Director of Livestock Production and Development.

The ToT workshop was an opportunity for experts and local poultry value chain stakeholders, including farmers associations, Livestock extension agents, poultry technology innovation platforms and poultry breeders from the Economic Community of Central African States (ECCAS countries) to improve their understanding of the local poultry value chain development and get acquainted with new skills and technologies in poultry production. The long-term conservation of poultry stem cells is one of the most promising tools/technologies for maintaining the avian genetic biodiversity across Africa without moving the genetic material from their regions of origin, and in the context of power shortage to maintain the cryobanks.

Wet lab training of the KALRO visiting scientist and biobanking of Kenyan indigenous chicken: The training provided an opportunity for the visiting scientist from KALRO (Dr Christine Kamidi Muhonja, figure 7) to improve her capacities and get acquainted with techniques in cryoconservation of poultry Genetic resources.

No.	Ecotype	Male	Female
1	Laikipia (LP)	44	43
2	Bungoma (BG)	9	18
3	Kilifi (KF)	4	1
4	Kakamega (KK)	14	13
5	Bomet (BM)	1	1
6	Homabay (HB)	21	19
7	Siaya (SY)	13	5
8	Kwale (KW)	0	1
	Total	**106**	**101**

Figure 7. Dr Kamidi after PGC cryopreservation and injection holds her first chimera chick from recovered.



In the framework of this training, the KALRO staff was acquainted with technique on isolating of primordial germ cells (PGCs) from blastodisc, blood and chicken embryos, cryopreserving and thawing of cPGCs with serum-free medium, checking the viability of the cultured PGCs, their re-injection back to the blood circulation of a developing embryo and evaluation of the integration ratio of the injected cells in the gonad.

The visiting scientist (Dr Christine Kamidi Muhonja) got insight into a new technology, which allows the preservation of Poultry Genetic biodiversity. The training raised her awareness on modern biotechnology approaches in poultry development, and she acquired practical knowledge on applying specific technics for ensuring preservation of chicken Genetic Resources.

Potential impacts

The long-term PGC cryopreservation is presently seen as one of the most promising tools for maintaining the African chicken genetic resources. There is a tremendous opportunity for the use of the regional gene banks established by AU-IBAR to conserve poultry and mammals' genetic material across all African countries.

It is now expected that from this preliminary phase, the conservation actions of the African chicken genetic resources will be launched at larger scale and successfully ran by national research systems like KALRO, in collaboration with CTLGH-ILRI and farmers facing programmes like ACGG/TPGS.

Conclusion and way forward

From various perspectives, the piloting phase of the African indigenous chicken biobanking, as well as the trainings have been a success on which to building future collaboration with NARS

across Africa for the conservation of indigenous poultry genetic diversity, development programmes and capacity building.

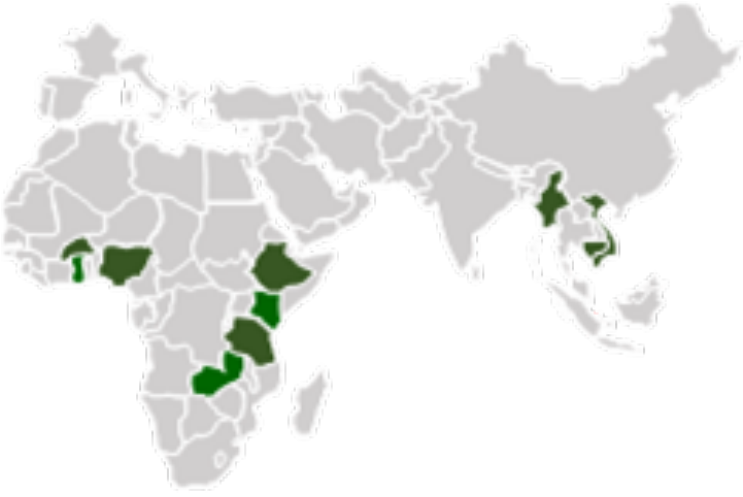
The detailed results of the laboratory activities were presented on 8th September 2021 to a panel of collaborators and scientists from ILRI (Nairobi & Addis Ababa), The Roslin Institute, and KALRO. A subsequent follow up meeting two weeks later at KALRO Naivasha allowed to review the progress and plan for the way forward. The KALRO management very satisfy by the achievement is open to extended collaboration on chicken cryopreservation, as well as the application of the stem cell technologies to other livestock species on interest.

The poultry biobanking activities with continues to the F1 and the newly collected ecotypes by KALRO from northern corridor, and in collaboration with ACGG/TPGS, with association of metadata and genomic data. It is envisaged pursuing the collaboration in using the surrogate host technologies in recovery and dissemination of the Kenya chicken ecotypes.

A larger collaboration to other livestock species is also envisaged, to apply the stem cell technologies to the conservation and development of other livestock species of interest for Kenya.

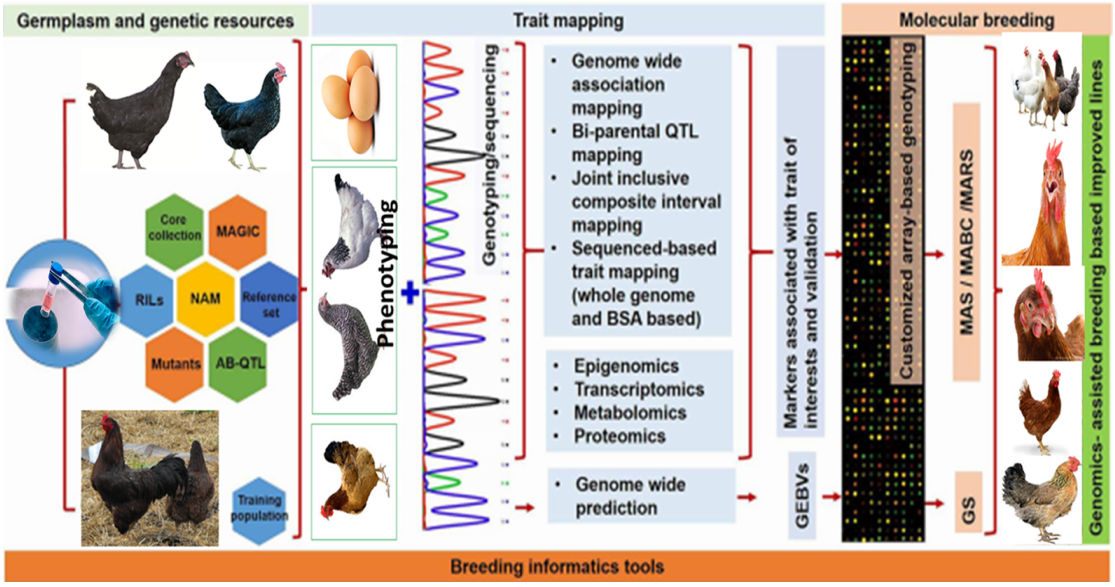
It is expected that the biobanking activity will be extended to west and southern Africa, and to south east Asia (figure 8) with support for the African Chicken Genetic Gain/ Tropical poultry genetic solution (ACGG/TPGS) project.

Figure 8. Potential areas of extension of the biobanking activities in collaboration with ACGG/TPGS project.



Further, scaling up/out poultry biobanking activities with be a powerful tool connecting the PGC biobanking technology to national poultry breeding programmes, and developing a battery of genomic studies (figure 9), building on that diversity available to identify candidate genes of importance to support and sustain the tropical poultry industry.

Figure 9. Proposed subsequent genomic activities to support the development of tropical poultry breeding industry.



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Special recognition is made the to CTLGH's reproductive technology and precision breeding program:

Christian K. Tiambo Steve Kemp Yao-Jing Yue Charity Muteti Nakami Wilkister Moses Ogugo Pauline Kibui Christine Kamidi (KALRO)	Mike McGrew Bruce Whitelaw Simon Lillico Maeve Ballantyne Tuan Jun Hu