

# Training KALRO visiting scientist on isolation and cryopreservation of primordial germ cells (PGCs) from Kenyan indigenous chicken ecotypes

Christian K. Tiambo

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*Patron: Professor Peter C Doherty AC, FAA, FRS*

*Animal scientist, Nobel Prize Laureate for Physiology or Medicine–1996*

Box 30709, Nairobi 00100 Kenya  
Phone +254 20 422 3000  
Fax +254 20 422 3001  
Email [ilri-kenya@cgiar.org](mailto:ilri-kenya@cgiar.org)

[ilri.org](http://ilri.org)  
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ILRI is a CGIAR research centre

Box 5689, Addis Ababa, Ethiopia  
Phone +251 11 617 2000  
Fax +251 11 667 6923  
Email [ilri-ethiopia@cgiar.org](mailto:ilri-ethiopia@cgiar.org)

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Training information:
<p><b>Title:</b> Training of KALRO visiting scientist on Isolation and Cryopreservation of Primordial Germ Cells (PGCs) from Kenyan indigenous chicken ecotypes.</p> <p><b>Training Period:</b> 6 April – 6 October 2021</p> <p><b>Venue</b> – ILRI Nairobi/Kenya</p> <p><b>Host Institutions:</b> CTLGH/ILRI Lab3, Reproductive Technology lab</p> <p><b>Supported by:</b> CTLGH/ILRI, Reproductive Technology Programme, CRP Livestock Genetics and KALRO Poultry Programme</p> <p><b>Format of the training course:</b> practical daily laboratory sessions on specific aspects of poultry reproductive biotechnology for bio-banking. This was followed by effective cryopreservation of the Kenya Indigenous chicken ecotypes.</p> <p><b>Visiting scientist trained:</b> Dr Christian Kamidi Muhonja</p>
Participating institution
<p><b>Agricultural and Livestock Research Organization (KALRO)</b></p> <p>Non-Ruminant Research Program</p> <p>P.O. Box 25 - 20117, Tel: + 020 2390930, NAIVASHA</p> <p>E-mail: <a href="mailto:Kalro.Poultry@kalro.org">Kalro.Poultry@kalro.org</a></p>
Background
<p><b>The rationale/justification for the workshop:</b></p> <p>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.</p> <p>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.</p> <p>The “Training of KALRO visiting scientist on Isolation and Cryopreservation of Primordial Germ Cells (PGCs) from Kenyan indigenous chicken ecotypes” is the first in a series planned by the Centre for Tropical Livestock Genetics and Health (CTLGH) – International Livestock Research Institute (ILRI) for the transfer of skills in the Avian reproductive Biotechnology to African National Agricultural Research Systems (NARS), in the framework of the partnership initiative with National Farmers Facing Programmes. All the laboratory component of the training was supported by the Centre for Tropical Livestock Genetic and Health (CTLGH) - ILRI, through their Poultry Genomics and reproductive technologies research components and the Livestock Genetics Flagship of the CGIAR Livestock CRP.</p> <p>The training was designed in respond to the demand from the NARS as a resolution from the pan African training workshop organized at ILRI in 2019 in collaboration with African Union – InterAfrican Bureau for Animal Resources (AU-IBAR) and the Kenya Animal Genetic Resources Centre (KAGRC).</p>

The training provided an opportunity for the visiting scientist from KALRO to improve her capacities and get acquainted with techniques in cryoconservation of poultry Genetic resources.

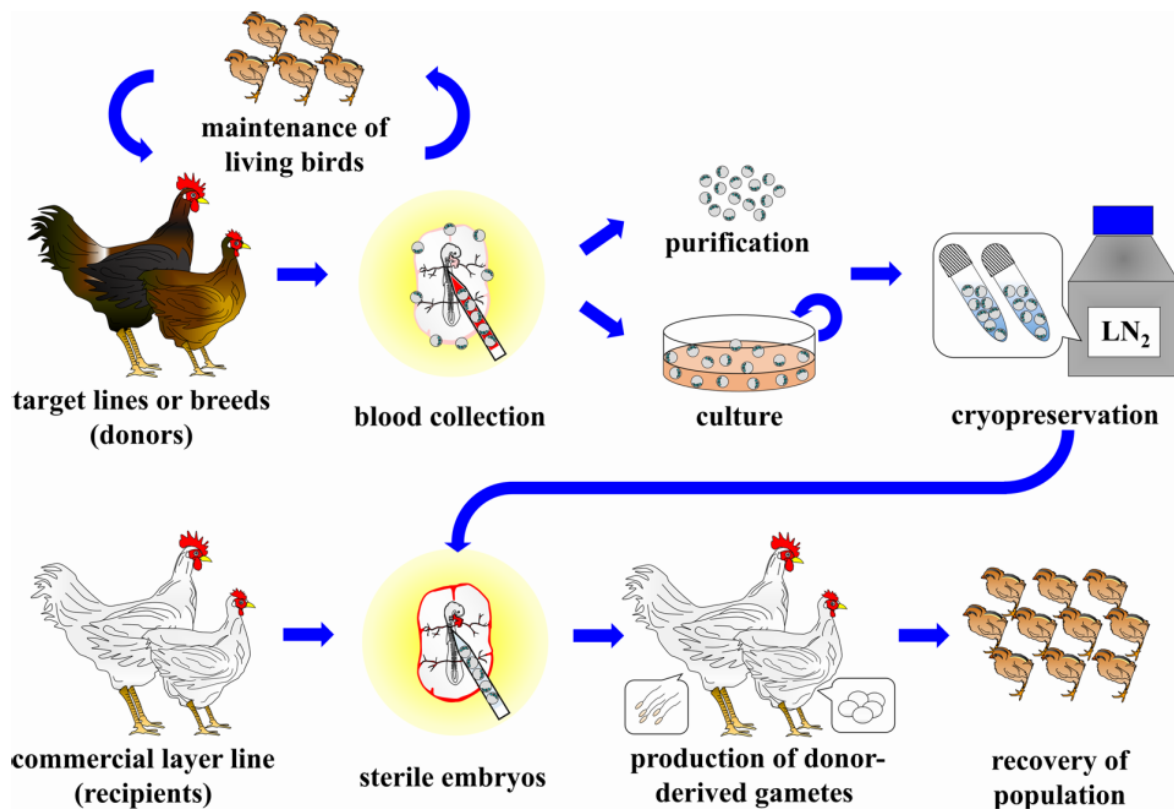
The production of an efficient method for the propagation of chicken PGCs in vitro represents a useful system for the study of PGCs biology. The chicken PGCs ability to form functional gametes after long-term culture is useful to develop a cell-based system for the conservation and genetic improvement of the chicken genome. 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. Avian stem cells have been a promising tool for biotechnology. The long-term PGC cultures are presently seen as one of the most promising tools for maintaining the avian genetic biodiversity across Africa without moving the genetic material from their regions of origin.

In the frame 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

#### **Relevance:**

Kenyan poultry genetic resources are maintained in situ in living populations at KALRO Poultry in Naivasha. 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 Kenya. 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 training intended to refresh the visiting scientist on these fundamentals of chicken embryology, and transfer to her the skill of using these techniques for conservation and improvement of chicken genetic resources. The figure below illustrates the outline of a chicken PGC-bank program.



#### Outline Illustration of a chicken PGC-bank program

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 sterilized recipient embryos, and
- 5) recovery of populations by mating of male and female recipients

#### OBJECTIVES:

The overall objective of the training was to equip the KALRO visiting scientist with knowledge and skills in reproductive technologies for poultry cryoconservation, in chicken Primordial Germ Cells (cPGC) isolation, long-term cultivation and re-injection for conservation of Kenya chicken Genetic Resources.

#### Topics covered

- PGC isolation from chicken Blastodisc, blood and gonads,
- Long-term culture technique for cPGCs.
- cPGC freezing, thawing
- cPGC chimera production
- Safe handling, storage, labeling,
- Recording and transport of samples

## Activities carried out

### Collection of fertile eggs and isolation of gonads from chick embryos

The first phase of the training consisted of getting acquainted with the various techniques mentioned above.

A total of 250 fertile eggs were collected from KALRO - Naivasha and were transported to ILRI on successive batches during the training period. They were incubated up to day 9 after which gonads were isolated. After isolation the gonads were stored at -80°C freezer overnight and transferred to liquid nitrogen the next day.

### Training achievements and impacts

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.

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 training, the conservation actions of the Kenyan chicken genetic resources will be launched and successfully ran by KALRO in collaboration with CTLGH-ILRI.

### Training results

Below is Table 1 summarizing the activities and outputs for the week. While Table 2 gives a summary of the gonads isolated per ecotype.

**Table 2: Summary of gonads isolated per ecotype**

	Male	Female
Laikipia	44	43
Bungoma	9	18
Kilifi	4	1
Kakamega	14	13
Bomet	1	1
Homabay	17	18
Siaya	13	5
Kwale	0	1
7226	4	1
<b>Total</b>	<b>106</b>	<b>101</b>

## Conclusions and way forward

From various perspectives, the training is a success on which to building future collaboration with KALRO and other NARS across Africa for the livestock development programmes and capacity building.

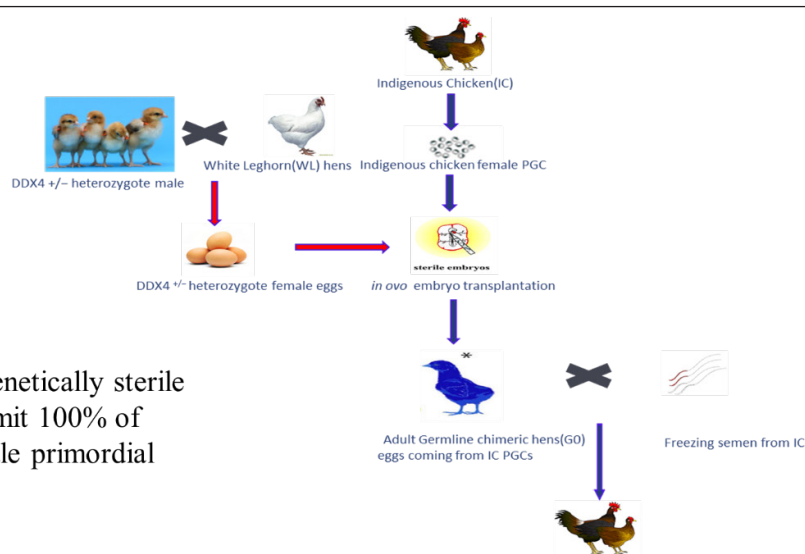
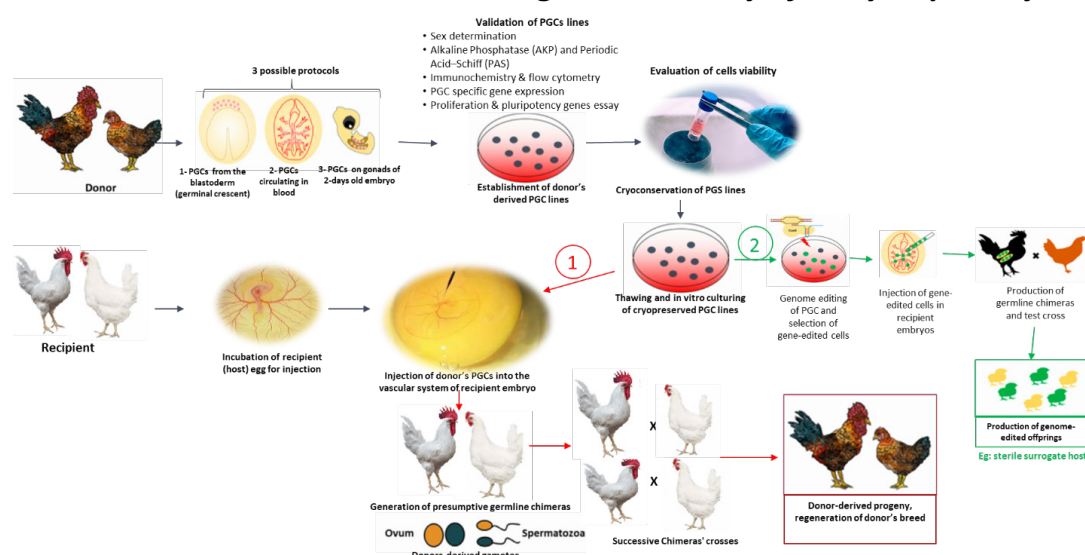
A total of 207 gonads were isolated and cryopreserved in all the ecotypes mentioned above with 106 males and 101 females. Laikipia still had the highest number of gonads isolated.

The detailed results of the laboratory activities were presented on 8<sup>th</sup> September 2021 to 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, and open to extended collaboration on chicken cryopreservation

The poultry biobanking activities with continues to the FI 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.

## Biobanking and recovery of Kenyan poultry



The **DDX4** KO line is a genetically sterile female line, that can transmit 100% of offspring from donor female primordial germ cells (PGCs).

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.

Pluripotent stem cells Biobanking and super dad

