French avian cryobank: development of reproductive biotechnologies based on primordial germ cells (PGCs) and investigation of the impact of in vitro steps on PGCs integrity and reproductive capacity

Marina Govoroun, Sabine Alves, Laura Soler-Vasco, Sophie Fouchécourt, Nadine Gérard, Valérie Labas, Hervé Acloque, Bertrand Pain, Christophe Klopp, Maria Bernard, et al.

French avian cryobank: development of reproductive biotechnologies based on primordial germ cells (PGCs) and investigation of the impact of *in vitro* steps on PGCs integrity and reproductive capacity.

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INRAE
la science pour la vie, l'humain, la terre

Specificity of poultry genetic resources management

- Conservation of poultry genetic resources is largely based on the cryopreservation of sperm
- In France Avian National Cryobank (>15 years) until recently contained only sperm collections :
  Rare breeds, experimental lines, commercial lines

Sperm cryobanking limitations :

- Female specific chromosome and mithochondrial genome are not conserved

- Restoration of genotype at 98 % using frozen semen :
  more than 3years, 4 backcrosses *(Blesbois et al., 2007)*

- The egg is telolecithale, not freezable, the early embryo is not accessible

cryopreservation and cloning not possible

- freezing of the ovary of the day-old chick, transplantation of the ovary into the day-old chick. Mastered by 2 laboratories, technical and ethical limitations

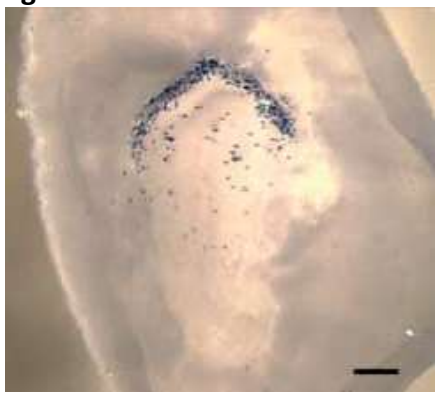
(*Liu et al., 2015; Liptói et al., 2020*)

Primordial germ cells (PGCs) cryobanking is a strategy of choice in the chicken

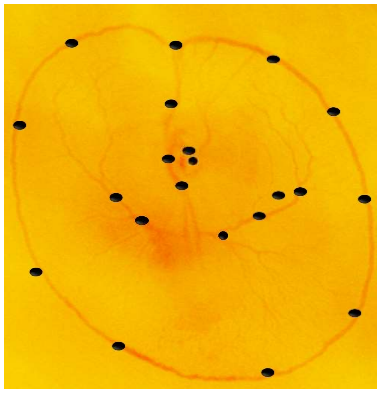
- PGCs are stem cells present in the embryo with at fate to develop into gametes

- Specificity of PGCs migration in avian embryo in comparison with other vertebrates

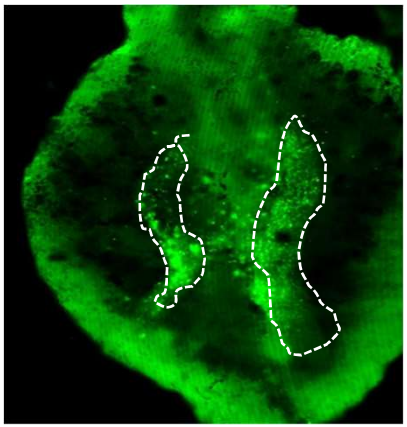
22h of incubation
germinal crescent HH5



52-62 h of incubation
HH 14-17



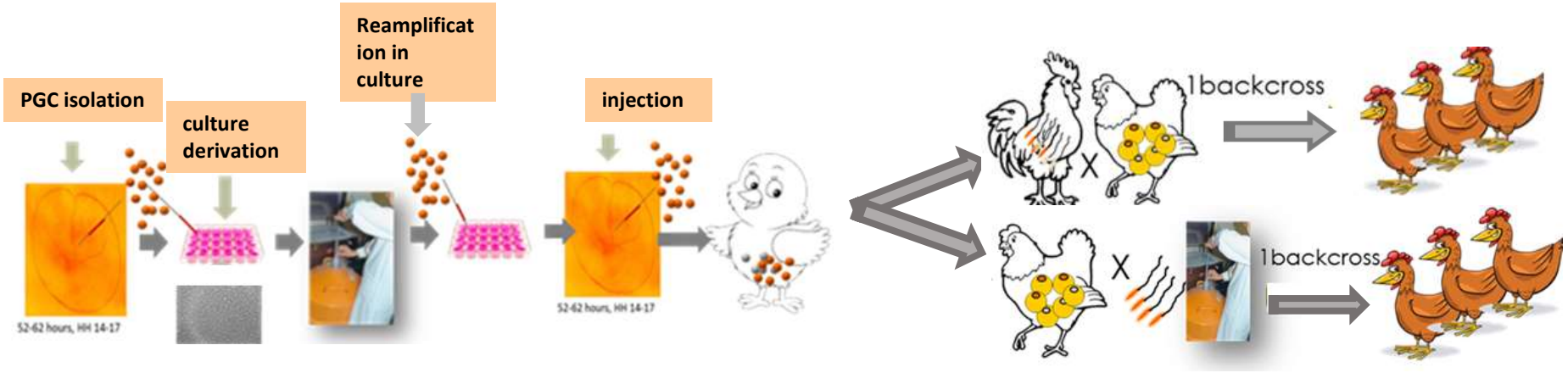
day 6-9 of incubation
embryonic gonadsGonades



Kang et al., 2015, Reproduction

Primordial germ cells (PGCs) cryobanking is a strategy of choice in the chicken

- Possibility of long-term PGCs culture in the chicken, use for the reproductive biotechnologies



van de Lavoir et al., 2006, Whyte et al., 2015, Woodcock et al., 2019

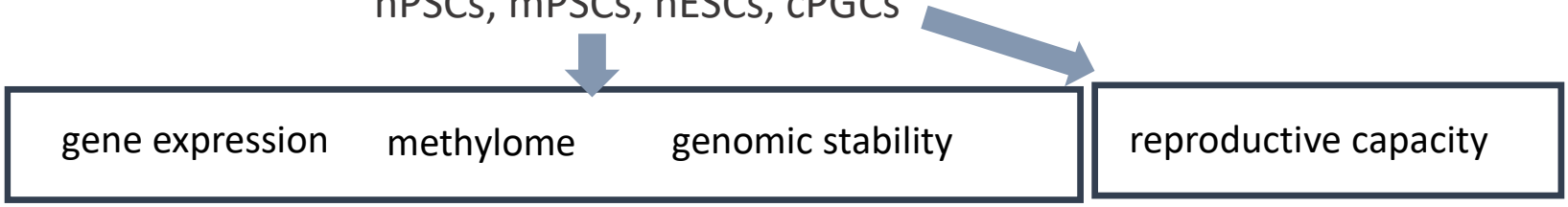
Variability of germline transmission rate

The impact of *in vitro* steps on PGCs?

- In vitro steps may affect molecular integrity of cells

In vitro culture duration, cryopreservation and culture conditions

hPSCs, mPSCs, hESCs, cPGCs



(Garitaonandia et al., 2015; McEwen et al., 2013 Wagh, 2011; Hawkins K, 2014) *(Woodcock et al., 2019).*

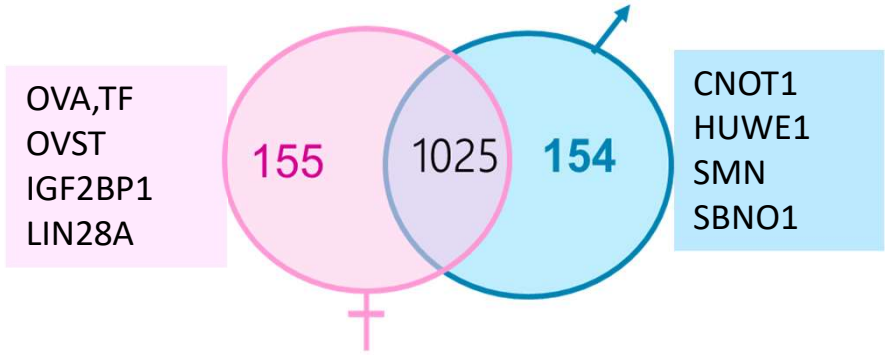
- Male and female PGCs can be differentially affected by *in vitro* environment

(Van de Lavoir et al, 2006; Song et al, 2013, Nandi et al.2016, Park and Han, 2013; Macdonald et al, 2010)

- Early sex:

  LC-MSMS proteomic study

  (Soler et al., 2022)



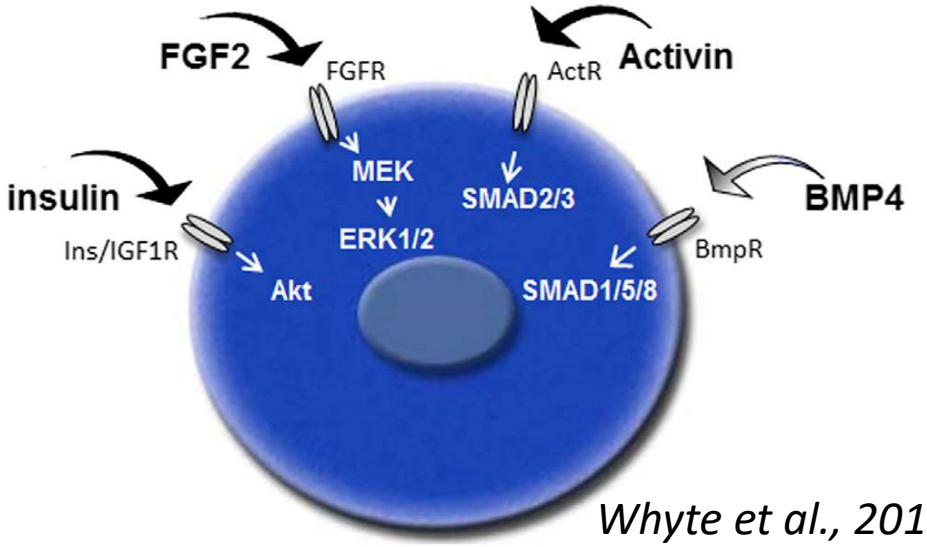
OBJECTIVES

- To understand the impact of *in vitro* culture duration and cryopreservation on the integrity of male and female PGCs using “omics” and *in vivo* approaches.

- To develop a comprehensive PGC-based system for the conservation and restoration of male and female chicken genetic resources, using a local breed "La Noire du Berry" (NB) as a model.

- To enrich French national avian cryobank with collections of male and female NB PGCs.

Development of NB PGCs cultures

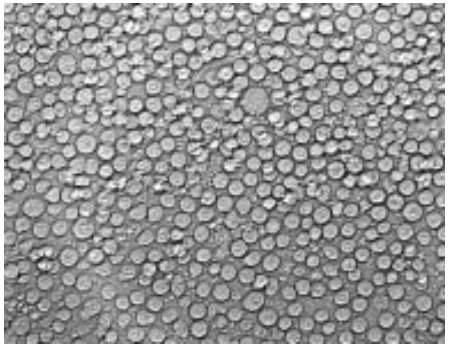


Whyte et al., 2015

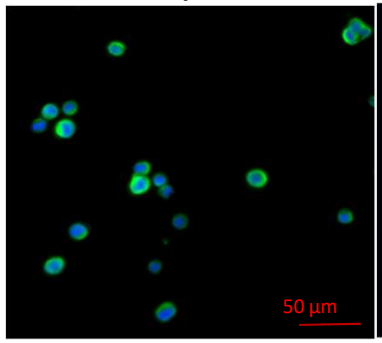
without serum and feeders

NB PGCs Cultures

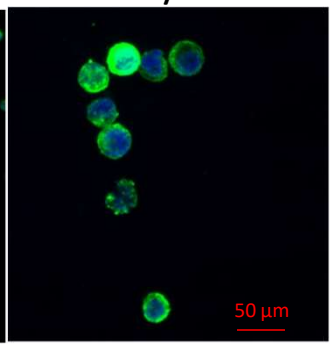
>90 % de dérivation



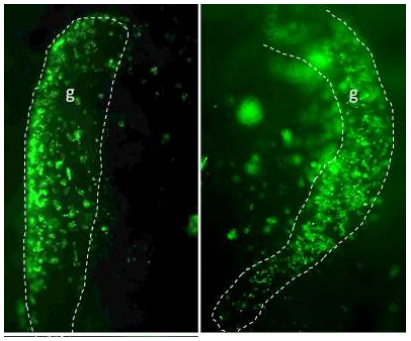
DDX4/DAPI



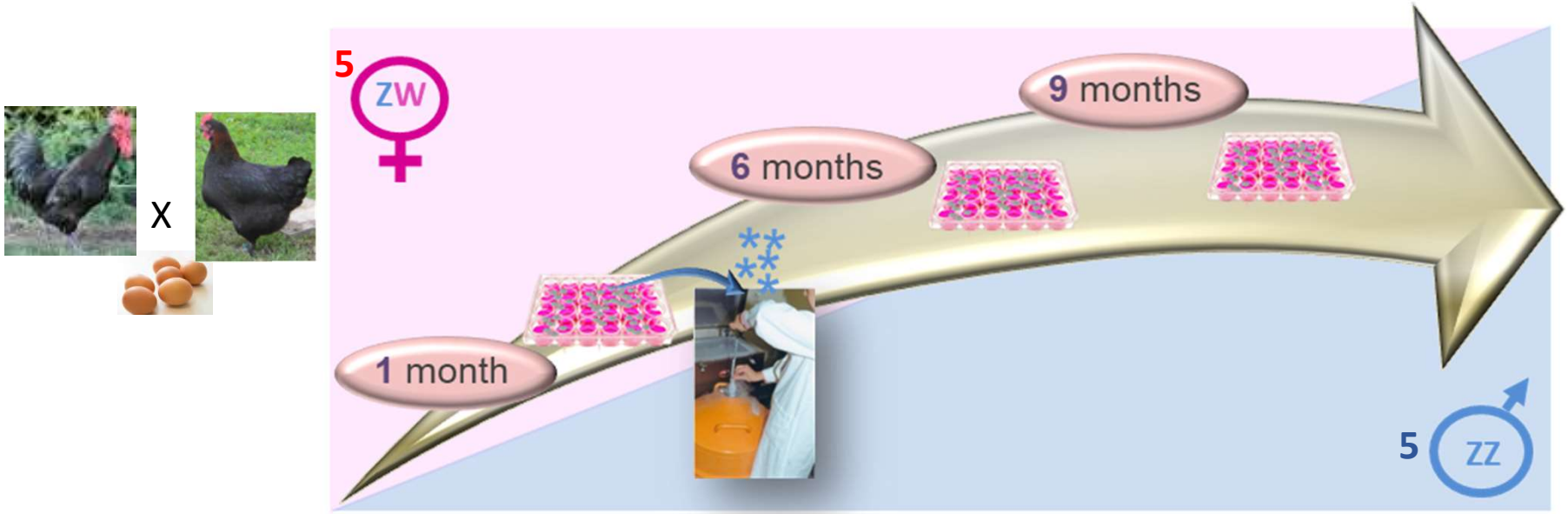
SSEA1/DAPI



gonade colonisation



Monitor chicken PGC integrity during in vitro amplification of cells and post-cryopreservation

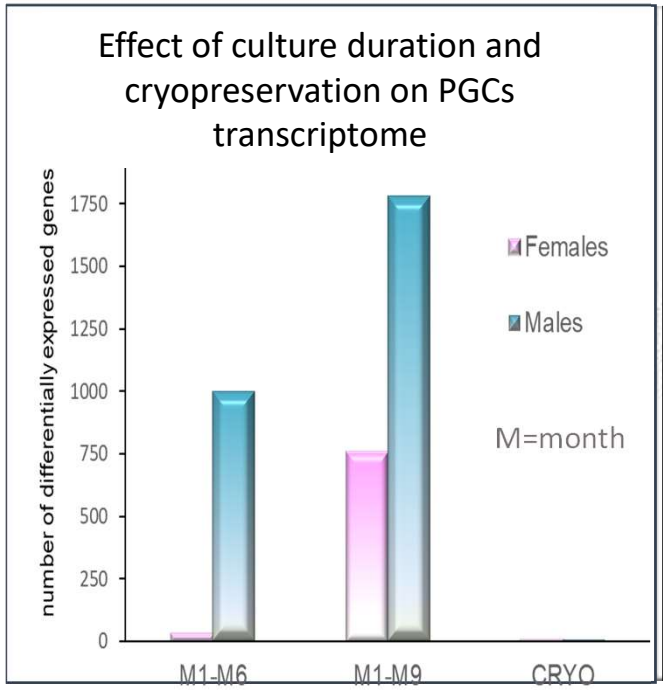


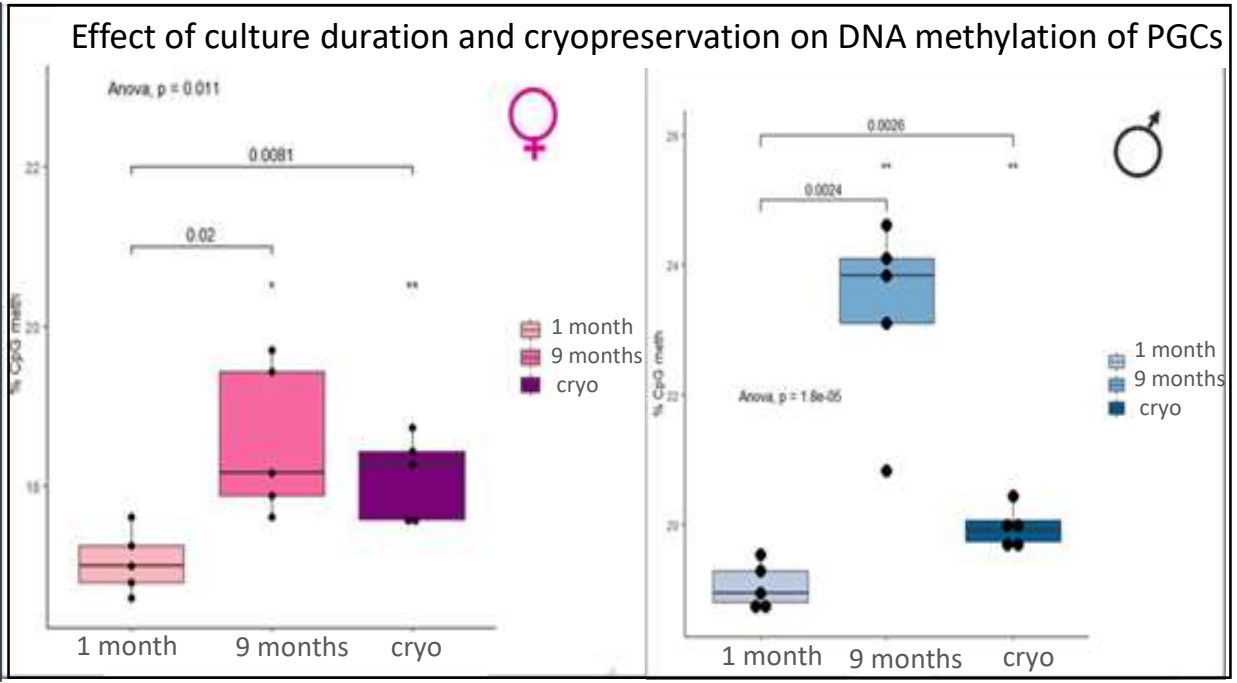
- transcriptome
- DNA methylation
- germline transmission

Monitor chicken PGC integrity during in vitro amplification of cells and post-cryopreservation

RNAseq Study

RRBS study (Reduced Representation Bisulfite Sequencing)

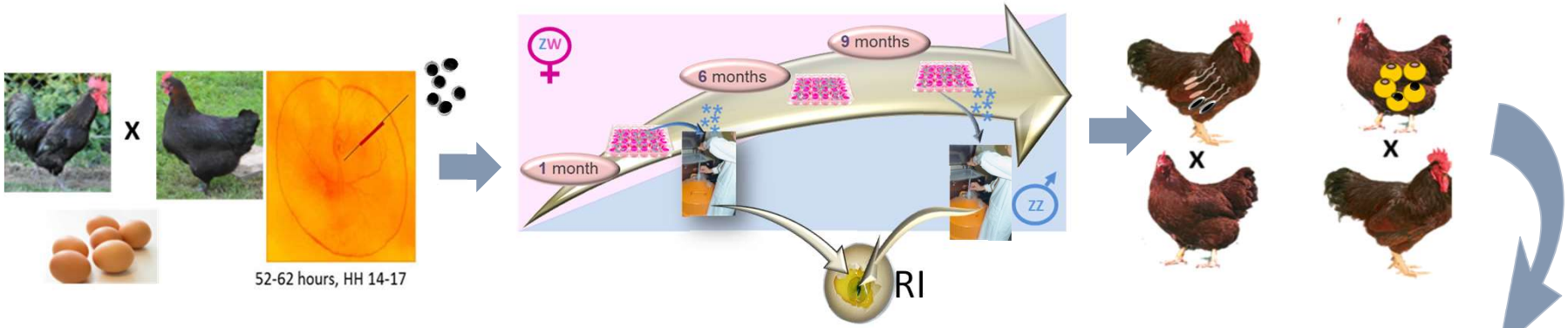


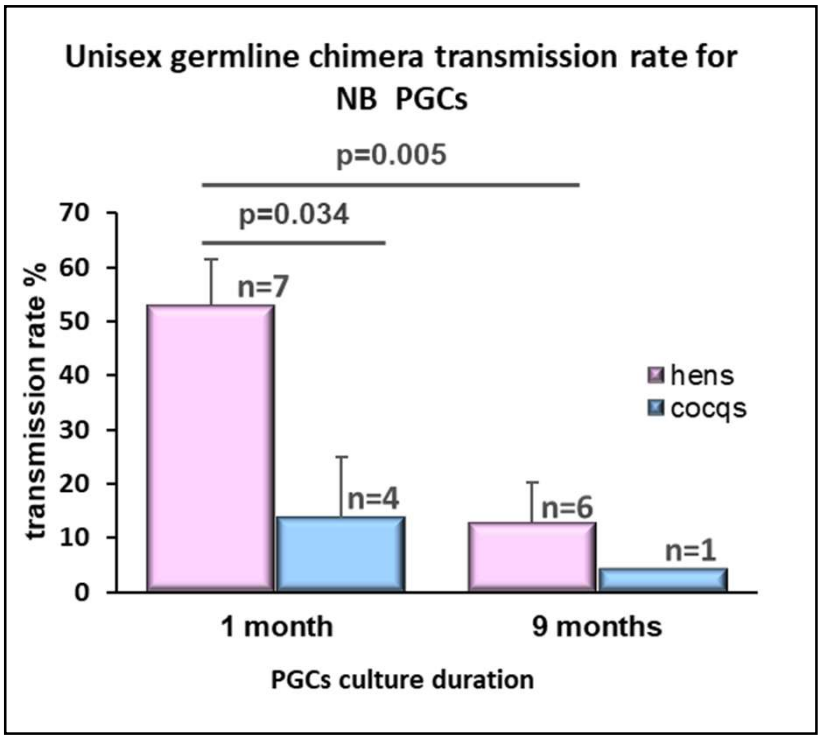


Culture duration
Cryopreservation

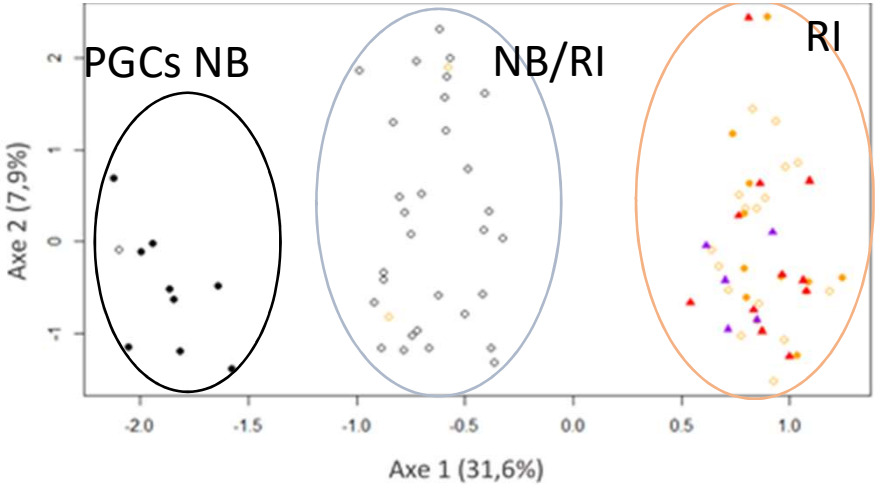
Molecular integrity of PGCs

Germline transmission of NB PGCs cultured *in vitro*

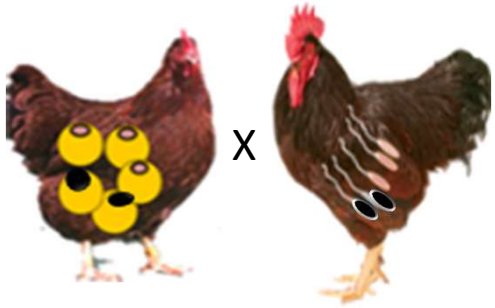




PCA on the basis of allelic frequencies of 87 individuals analysed (KASPar technology)

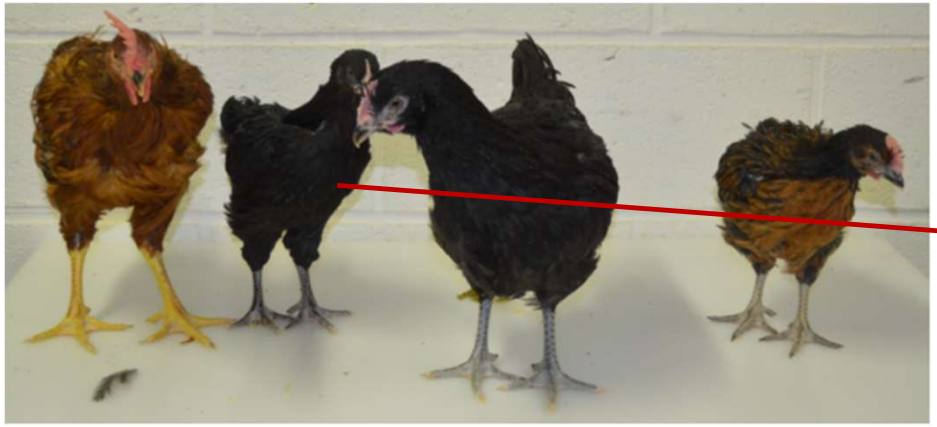


Restoration of genotype

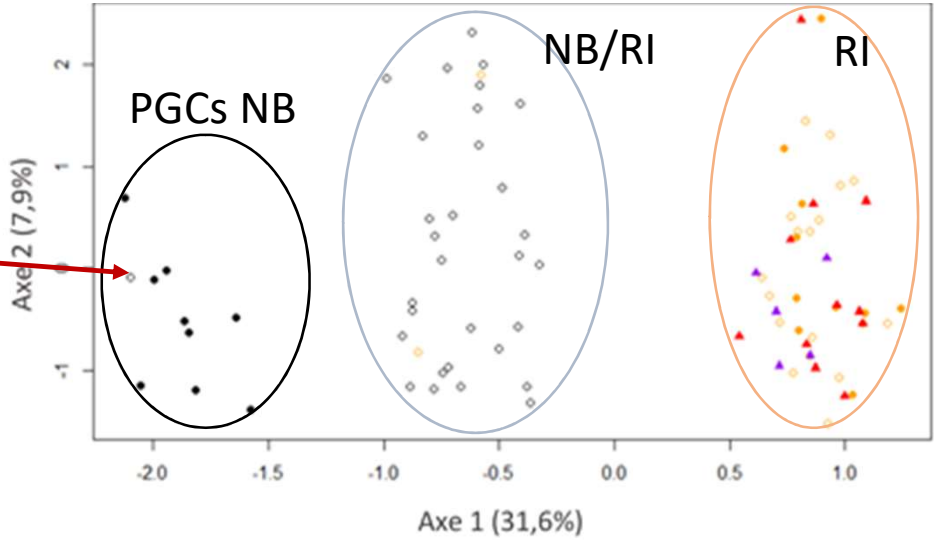


X

9 chiks/28 hatched



PCA on the basis of 87 individuals analysed (KASPar technology)



CONCLUSIONS

- Chicken PGCs cryobanking in France takes flight
- We demonstrated the restoration of male and female genotypes on the endangered breed “La Noire du Berry” using cryopreserved and stored for more than 1 year *in vitro* derived PGCs and for the first time one pure donor PGC derived individual was obtained in one generation using non sterile host chickens.
- Germline transmission rate was sexually dimorphic with female PGCs presenting higher germline transmission rate than male PGCs
- Negative sexually dimorphic effect of long term cultures on the molecular integrity of PGCs and their germline transmission. Weak effect of cryopreservation.
- Adaptation of culture conditions to the sex of PGCs may be usefull to improve PGCs germ line transmission

Thank you for your attention

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IMAGE
Innovative Management of Animal Genetic Resources

CRB ANIM

Région Centre

INRAE
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Cryobanking NB PGCs

Strategy 1

- Carried out on NB chickens raised at INRA for 6 generations
- Conservation des PGCs des embryons des couples bien identifiés

PGCs from 24 embryos, 6 different hen-cock pairs

Strategy 2

- Realized on the animals rented from Plume Cane Farm for 2 months
- Natural matings, pairs were not identified

- Returned to the farm after storage of the PGCs

PGCs from 24 embryons