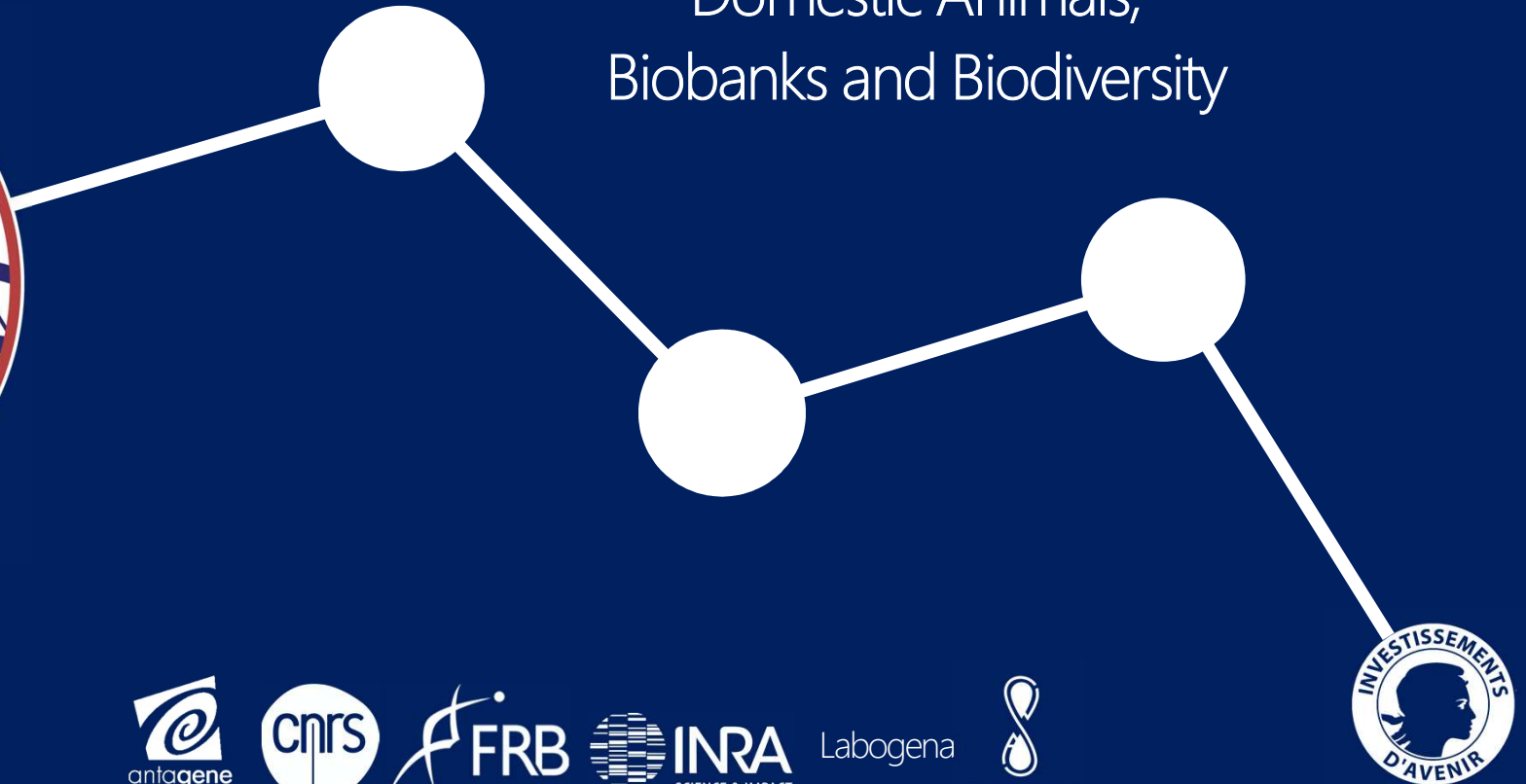


# CRB Anim

Centres de Ressources Biologiques

*3rd International Seminar of  
CRB-Anim Infrastructure*

Domestic Animals,  
Biobanks and Biodiversity



Agence Nationale de la Recherche  
**ANR**



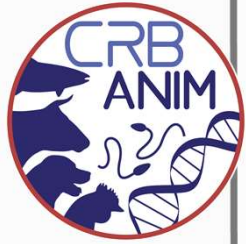
Labogena



Development of the tools for conservation of avian genetic resources based on primordial germ cells (PGCs).

Govoroun Marina, UMR PRC, INRA 37380 Nouzilly

# Management of reproductive cells to ensure the sustainability of poultry genetic diversity.

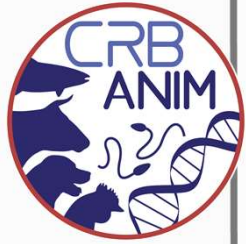


- Conservation of poultry genetic resources is largely based on cryopreservation of the sperm
- Avian National Cryobank :
  - Rare breeds, experimental lines, commercial lines
- Cryopreservation of chicken semen is well mastered in France

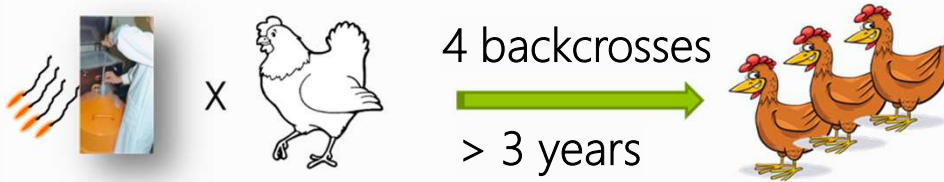
*(Blesbois et al, 2006; 2007; 2010, Thélie et al.,2019)*



# Sperm Cryobanking: limitations

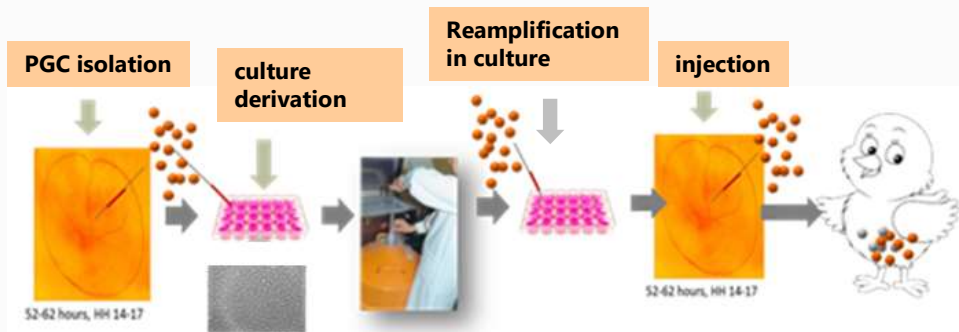


- Female genetic resources are not preserved  $z \text{ (W) } \text{♀} \quad zz \text{ ♂}$
- Time consuming
  - Restauration of genotype à 97 % using frozen semen

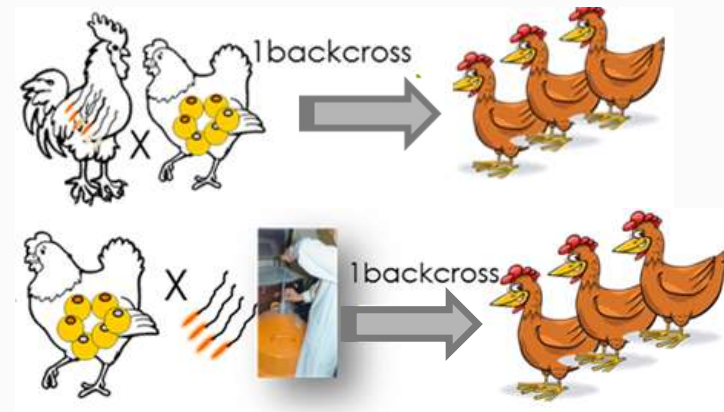


# Primordial germ cells cryobanking is complementary strategy of choice

- The avian egg is telolecithal containing high amount of yolk
  - cloning is impossible to develop
  - the embryos are not freezable
- Avian PGCs present great interest for reproductive biotechnologies



*van de Lavoir et al., 2006, Whyte et al., 2015*



*Woodcock et al., 2019*



# Scientific context



- In vitro steps may affect molecular integrity of cells
  - *In vitro* culture duration, cryopreservation and culture conditions  
hPSCs, mPSCs, hESCs.



gene expression      methylome      genomic stability

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

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

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



# Objectives

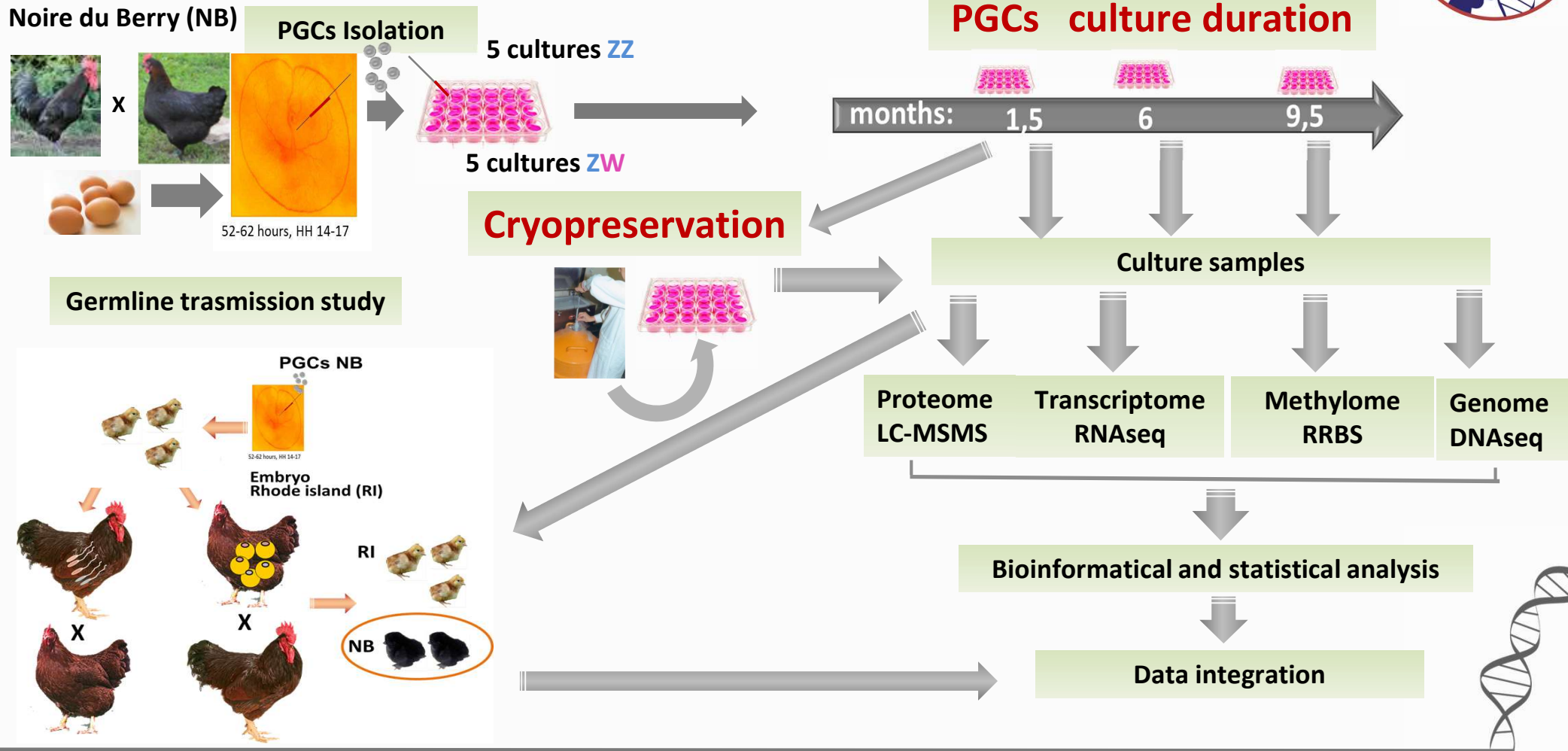


- To develop a complete system for the conservation and restoration of male and female chicken genetic resources based on PGCs, using as a model a local endangered breed "La Noire du Berry" (NB)
- To understand the impact of the *in vitro* steps (culture duration and cryopreservation) on the integrity of male and female PGCs by « omics » and *in vivo* approaches
- To enrich the collection of national avian cryobank by male and female NB PGCs samples.



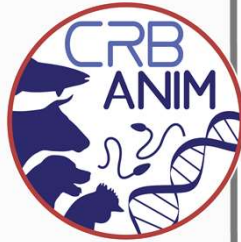


# Experimental Design



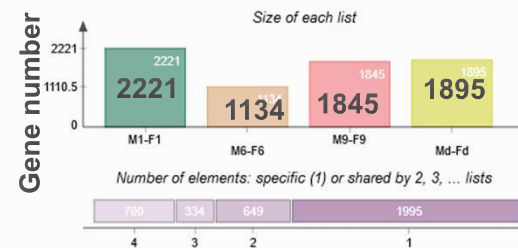
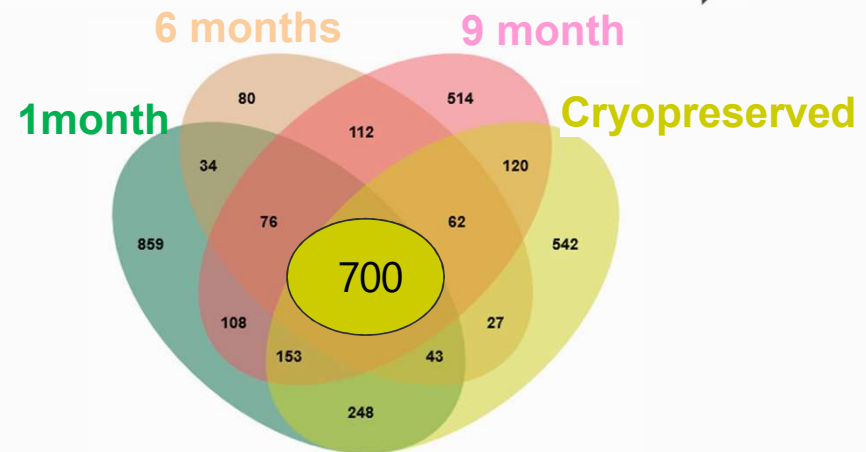
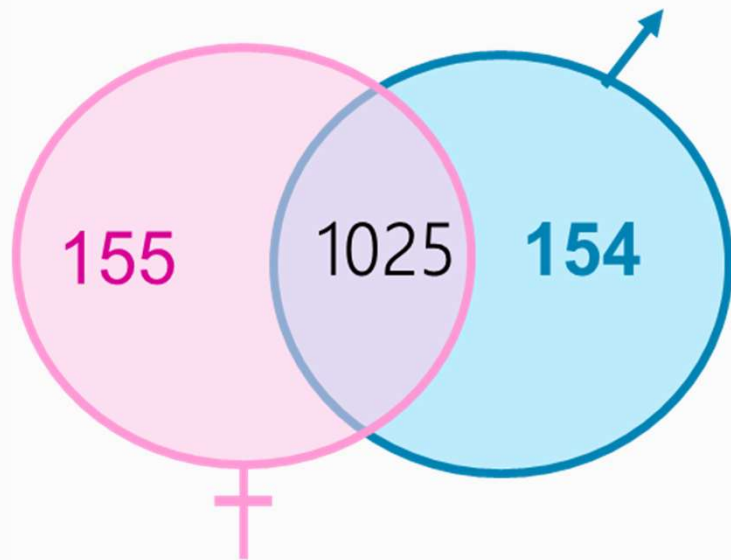


# Sexual dimorphism of chicken PGCs

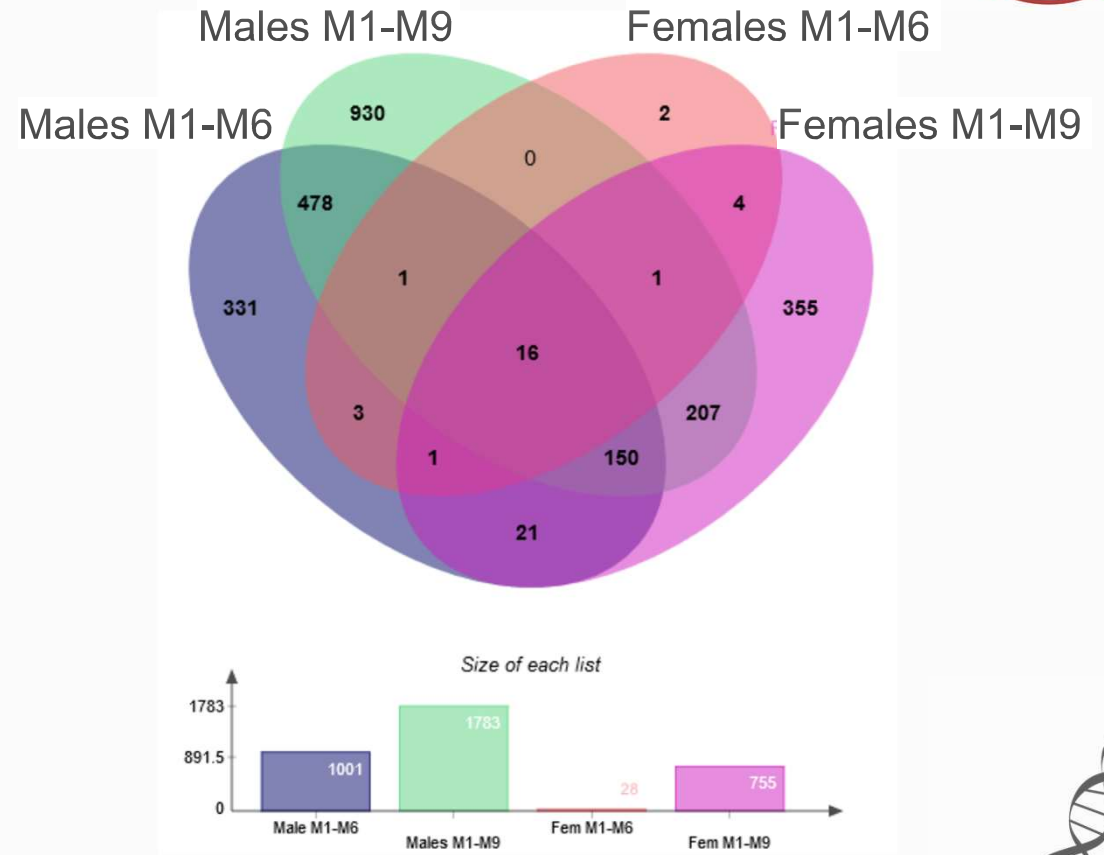
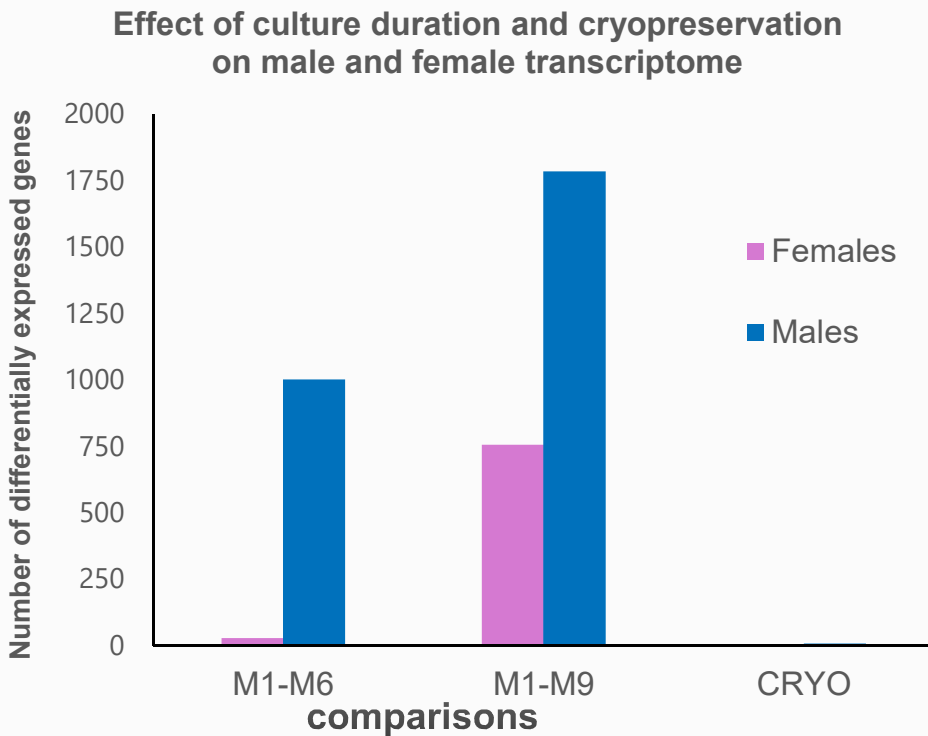
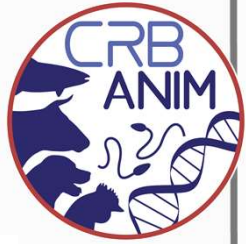


Proteomic LC-MSMS study of short term PGCs cultures (<1.5 month of culture)

RNA seq transcriptomic study : Comparison of male and female PGCs cultures



# Effect of culture duration and cryopreservation on PGCs Transcriptome : RNAseq study

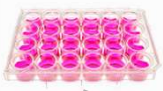


# Effect of culture duration and cryopreservation on DNA Methylation of PGCs : RRBS study

EPIGERMCHICK



## Cryopreservation



30 RRBS libraries (5 female and 5 males PGC)

months: 1,5 9,5

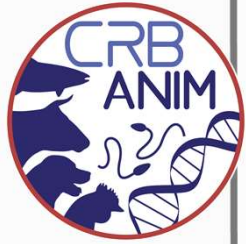


Sequencing Depth : 30M reads/library

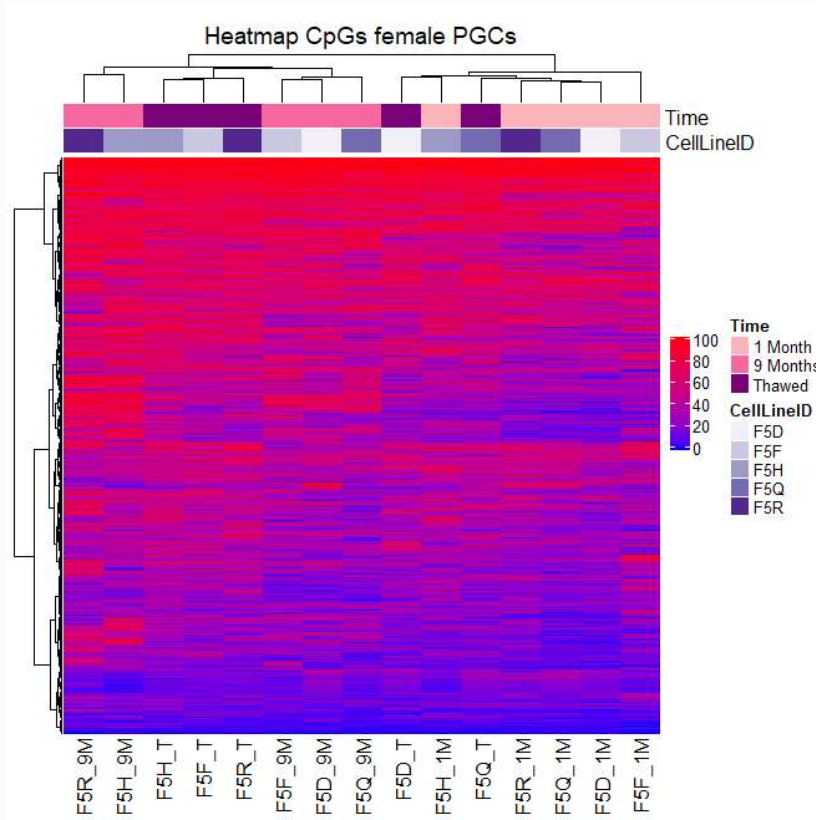
- **Female: mean number of detected CpG** with a coverage > 5 : **1.85M**
- **Male : mean number of detected CpG** with a coverage > 5 : **1.87M**
- **Female: number of common CpG** between conditions : **829,032**
- **Male: number of common CpG** between conditions : **995,128**



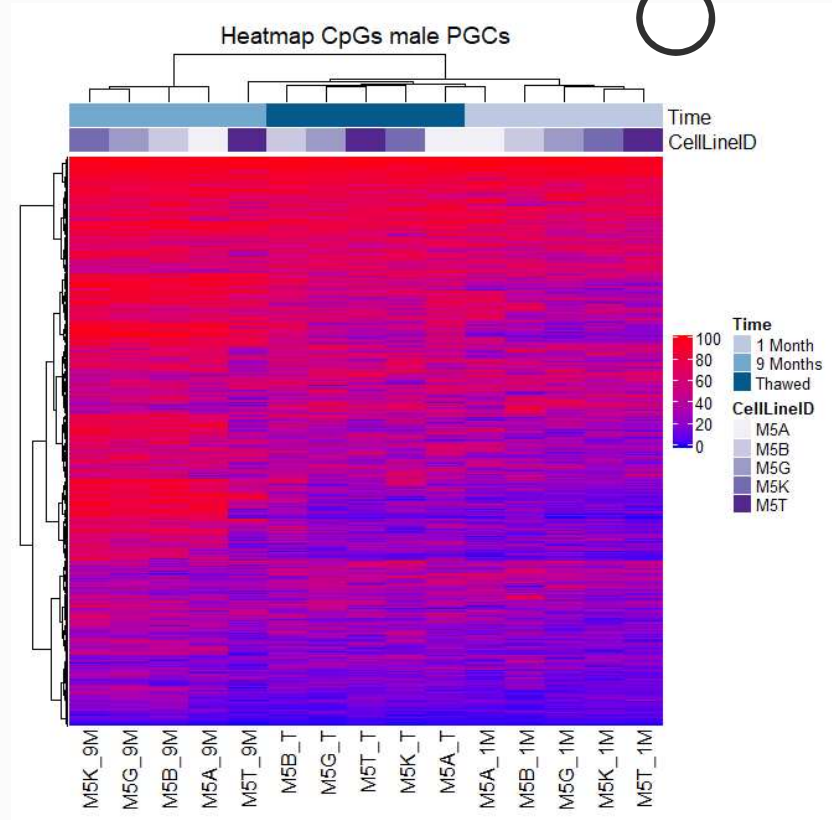
# DNA methylation study: Heatmaps



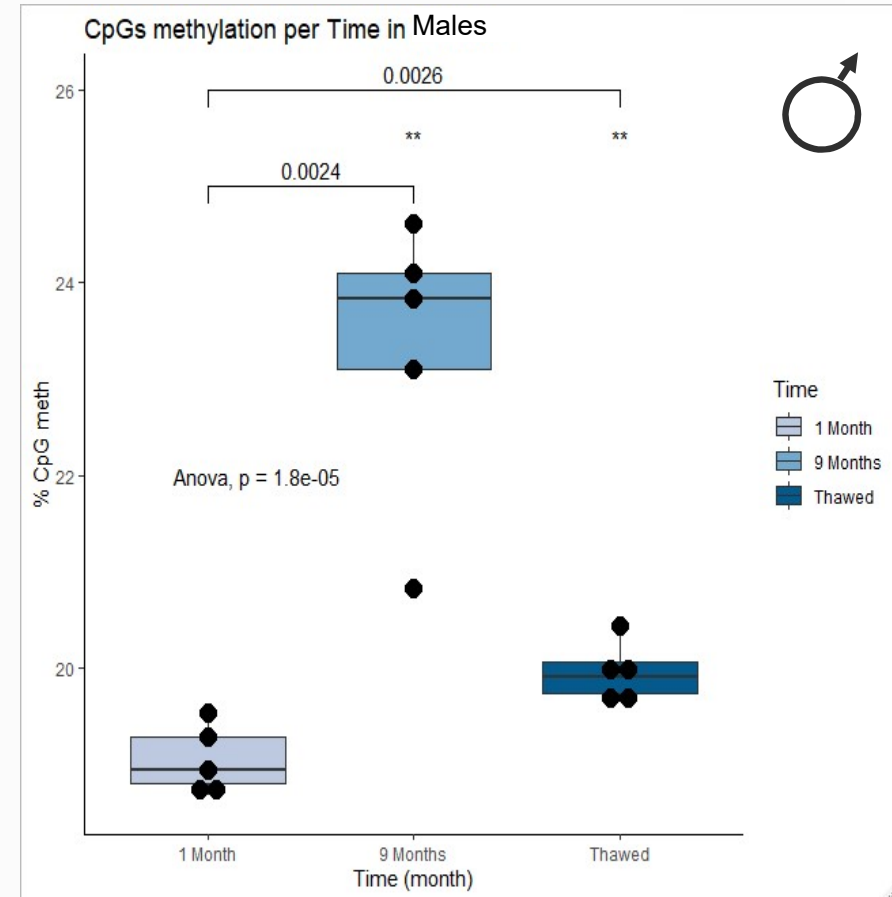
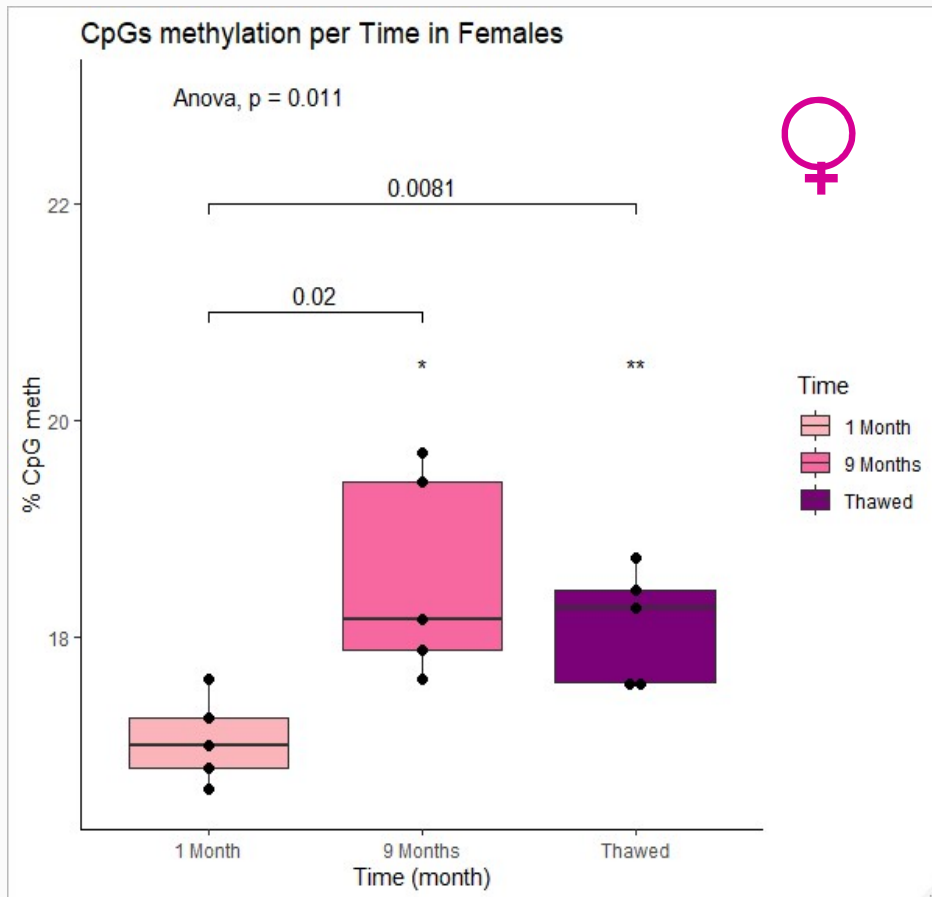
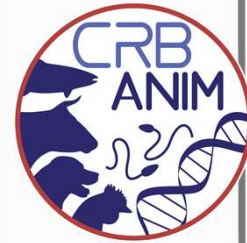
■ 9 months   
 ■ Frozen/thawed 1 month   
 ■ 1 month



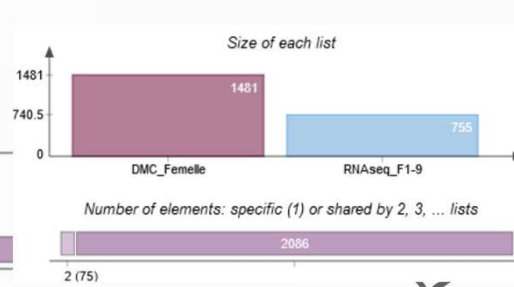
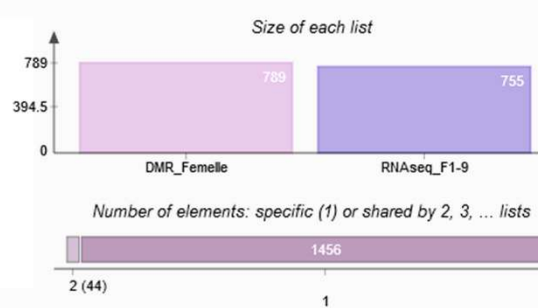
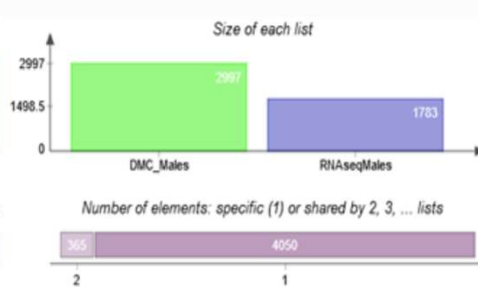
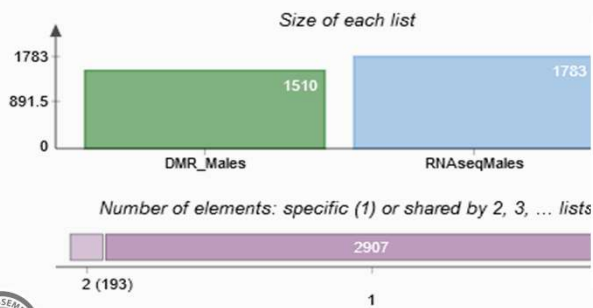
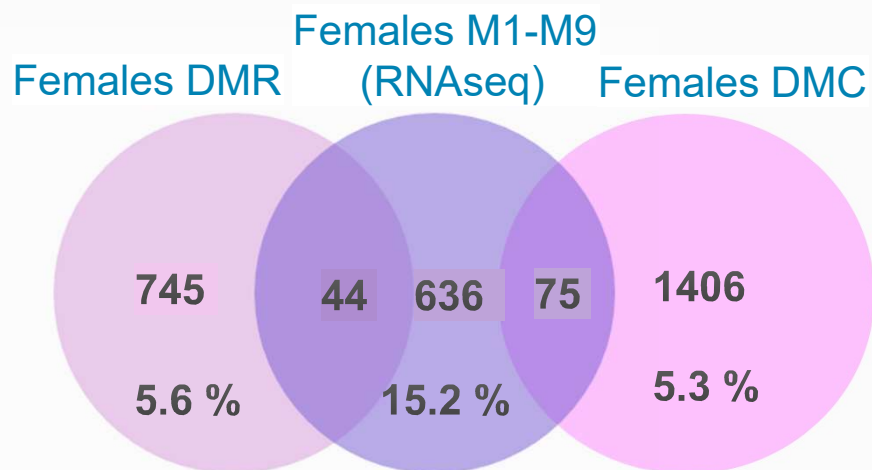
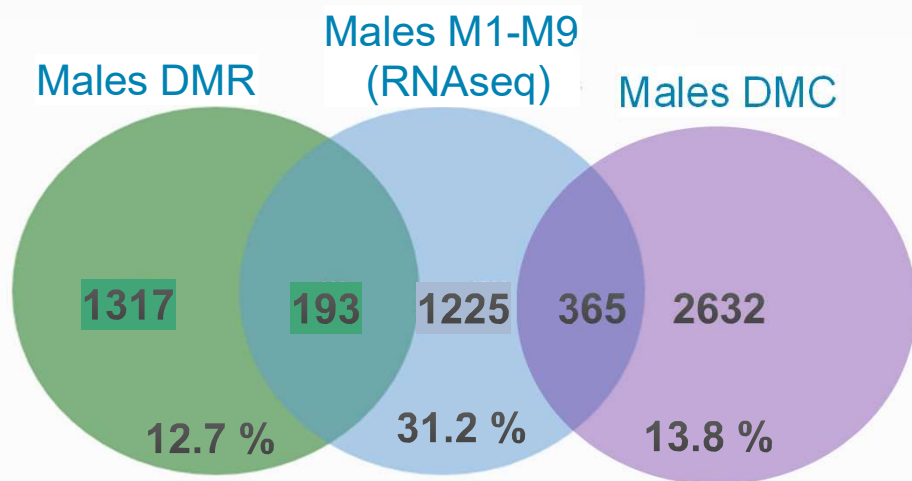
■ 9 months   
 ■ Frozen/thawed 1 month   
 ■ 1 month



# Effect of culture duration and cryopreservation on DNA Methylation of PGCs : RRBS study

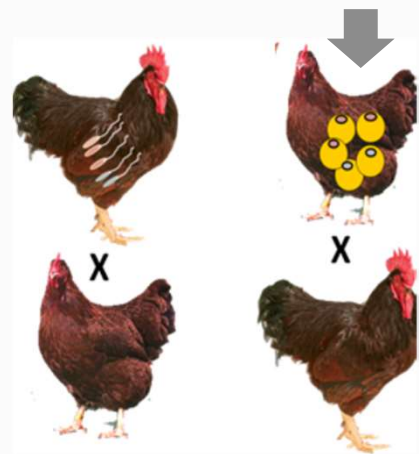
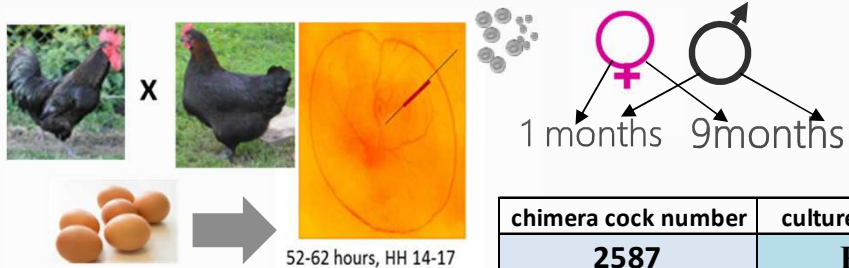


# Effect of culture duration and cryopreservation : DNA Methylation and gene expression





# Germ line transmission



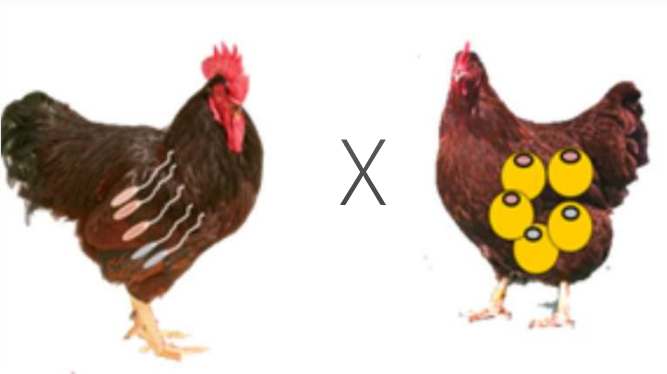
chimera cock number	culture name	months in culture	number of black chicks	% black chicks/hatched	germline transmission rate
2587	K	1	6	21.4	42,8
2592	K	1	2	2.4	4.8
2589	K	1	2	1.9	3.8
2584	K	1	1	1,9	3
2626	K	9	0	0	0
2623	K	9	0	0	0
chimera hen number	culture name	months in culture	number of black chicks	% black chicks/hatched	germline transmission rate
2599	R	9	2	1,9	3,8
2612	R	9	10	30,3	60,6
2643	R	1	0	0	0

First French *in vitro* derived PGCs derived progeny





# Germ line transmission : restoration of genotype in one generation



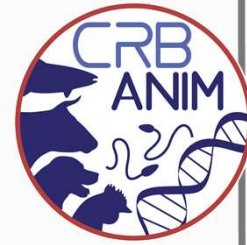
→ 9 black chicks/28 hatched



Homozygous?



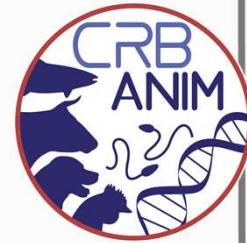
# Conclusions.



- 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 NB with good germline transmission rate.
- Primordial germ cells derived *in vitro* exhibit molecular and physiological sexual dimorphism that has to be taken in account when developing PGCs based biotechnologies
- Long term *in vitro* culture and cryopreservation affected PGCs methylome. This effect was stronger in the male PGCs.
- Long term *in vitro* culture but not cryopreservation affected PGCs transcriptome and this effect was stronger in male PGCs
- National avian cryobank was enriched by the collections of PGCs NB



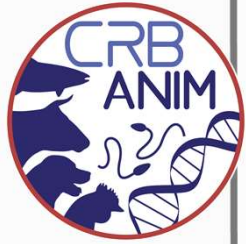
# Perspectives.



- To verify whether the effect of long term *in vitro* culture on germ line transmission is gender specific.
- To study the effect of culture duration on genome stability (is currently developed)
- To integrate data and publish obtained results
- To verify whether the effect of *in vitro* steps on DNA methylation in PGCs is transmitted to the progeny.
- To understand the factors that are involved in the variability of the germline transmission rate.
- Using sterile edited host for 100% germline transmission?



# Many thanks



## Technical assistance

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Aurore Jacques  
Vanessa Guérin

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Valérie Labas

## DNA methylation Study

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Vincent Coustham

## Statistical and bioinformatic analysis

Christophe Klopp  
Maria Bernard  
Christelle Hennequet-Antier  
Aurélien Brionne

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Collaboration: Mike McGrew, Roslin Institute  
Bertrand Pain

## WP3 coordinator

Elisabeth Blesbois

## Eggs production and animals care

Joel Delaveau  
Christophe Rat  
Philippe Didier



THANK YOU

