Rabbit Pluripotent Stem Cells : Why and How to Produce Them?

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Rabbit Pluripotent Stem Cells: Why and How to Produce Them?

Marielle AFANASSIEFF

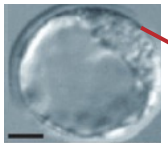
Stem-cell & Brain Research Institute

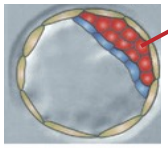
INSERM U1208 - INRA USC1361 – UCBL

2nd International Conference on Cell and Experimental Biology (CEB-2021), July 12-14, 2021

Pluripotent Stem Cells

Blastocyst



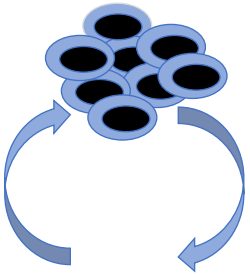


ICM

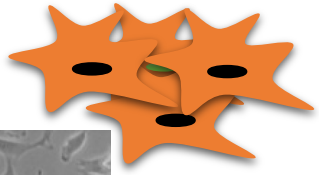
NAIVE STATE
LIF/BMP4

Embryonic Stem Cells: ESCs

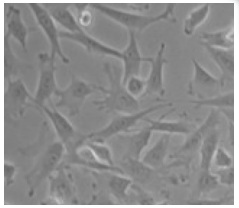
Self-renewal

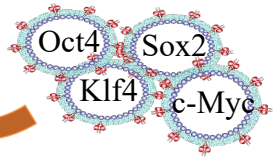


Differentiated cells



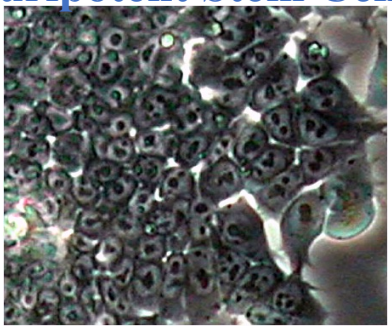
Reprogramming



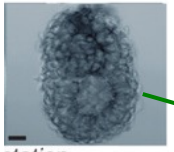


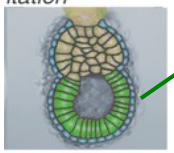
induced Pluripotent Stem Cells: iPSCs

Pluripotent Stem Cells



Egg cylinder





Epiblast

PRIMED STATE
Activin/FGF2

Epiblast Stem Cells: EpiSCs

Differentiation

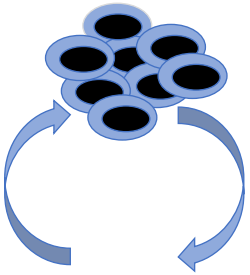
Endoderm

Mesoderm

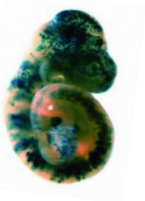
Ectoderm

Pluripotent Stem Cells

Self-renewal



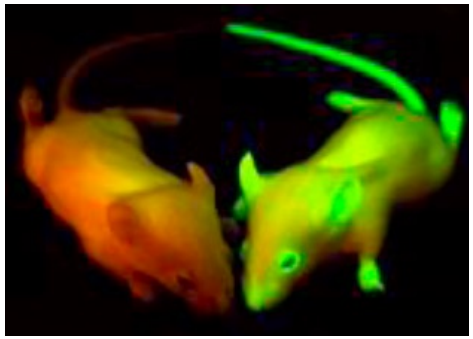
NAIVE STATE
LIF/BMP4



Colonisation capacity

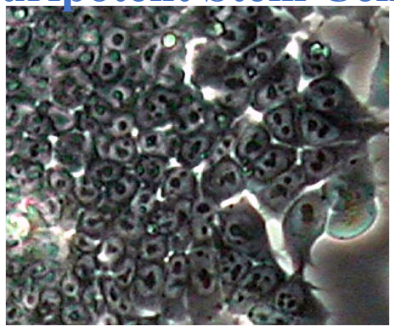
PRIMED STATE
Activin/FGF2

mESCs
miPSCs



mEpiSCs
miPSCs

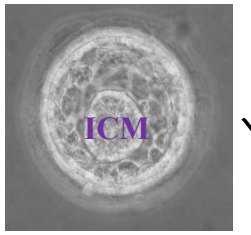
Pluripotent Stem Cells



Differentiation

Endoderm **Mesoderm** **Ectoderm**

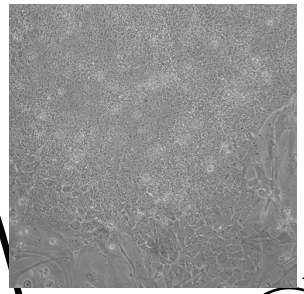
Non-rodent Pluripotent Stem Cells



Blastocyst

Derivation

Cell reprogramming
Oct4, Sox2, Klf4, c-Myc



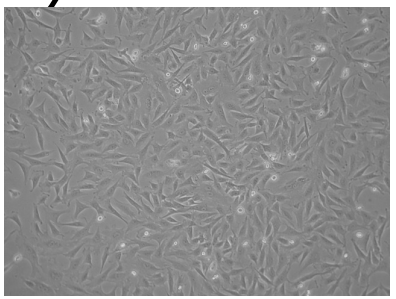
Pluripotent Stem Cells

PRIMED STATE
Activin/FGF2

Reprogramming

Multipotent
Stem Cells

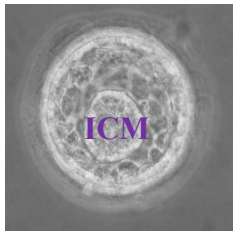
Fibroblasts



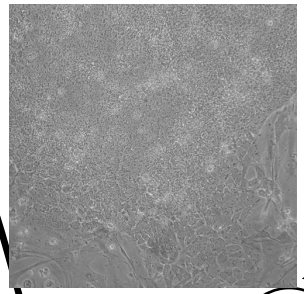
NAIVE STATE

Differentiated
Cells

Rabbit Pluripotent Stem Cells



Blastocyst



Derivation

Cell reprogramming
Oct4, Sox2, Klf4, c-Myc

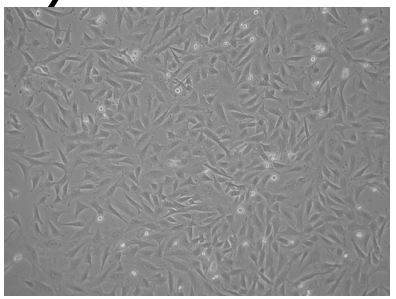
Pluripotent Stem Cells

NAIVE STATE
LIF/BMP4

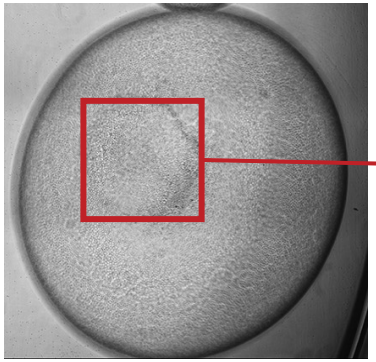
PRIMED STATE
Activin/FGF2

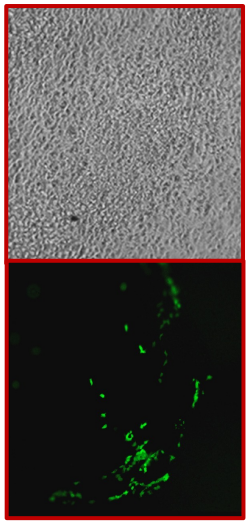
Our aim is to reprogramm rbPSCs toward the naive state

Fibroblasts



Multipotent Stem Cells





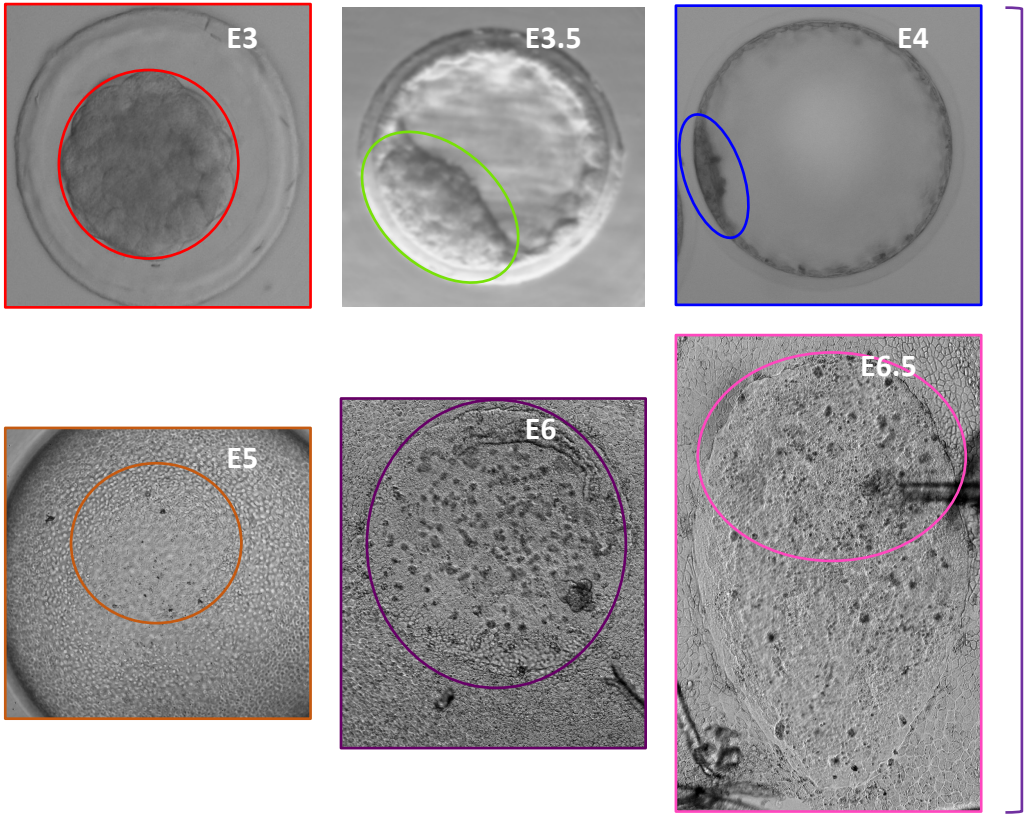
rbESCs/rbiPSCs

Differentiated Cells

Reprogramming of rbPSCs toward the naive state

We are developing four new strategies:

1. The definition of rabbit naïve pluripotency



Single cells
transcriptomic
with 10X Genomic technique

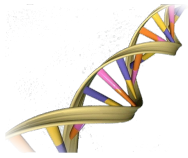
Complete characterisation of
rabbit pluripotency

Reprogramming of rbPSCs toward the naive state

We are developing four new strategies:

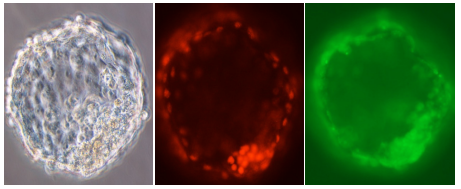
1. The definition of rabbit naïve pluripotency
2. The induction of naïve pluripotency

Microinjection of plasmid expressing V5-hStat3-ER^{T2}



Transgenic Rabbit V5-hStat3-ER^{T2}

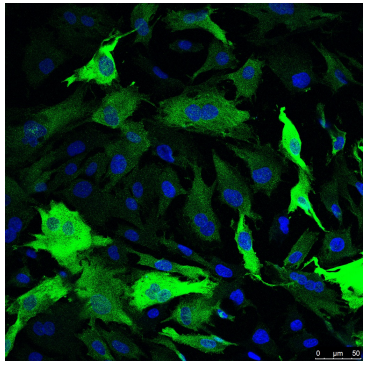
Phase | Anti-Oct4 | Anti-V5

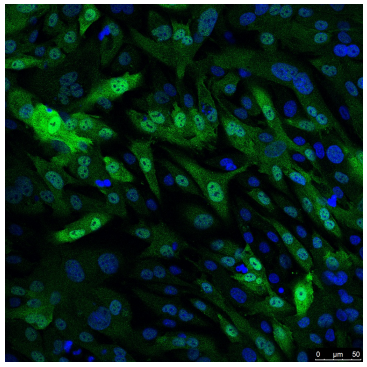


Transgenic embryo expressing V5-hStat3-ER^{T2}

Anti-V5 + Hoechst

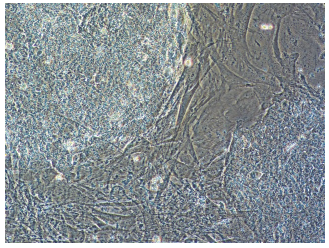
- Tamoxifen





+ Tamoxifen

Rabbit fibroblasts



naive cells

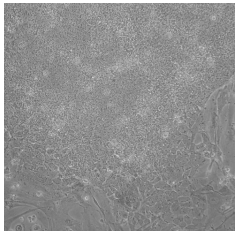
Inducible overexpression of Stat3 → rbESCs derivation with LIF and Tamoxifen

Reprogramming of rbPSCs toward the naive state

We are developing four new strategies:

1. The definition of rabbit naïve pluripotency
2. The induction of naïve pluripotency
3. The genetic selection of naïve pluripotency

rbiPSC

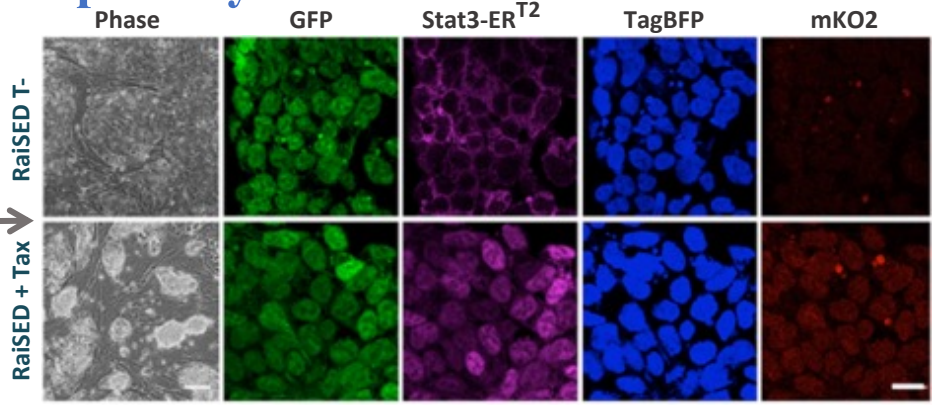


CAG-GFP

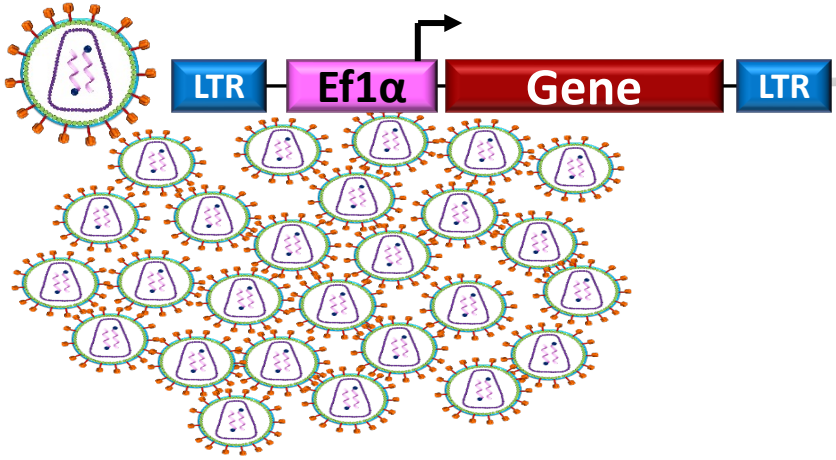
CAG-hStat3-ER^{T2}

Dppa2^{mKO2}

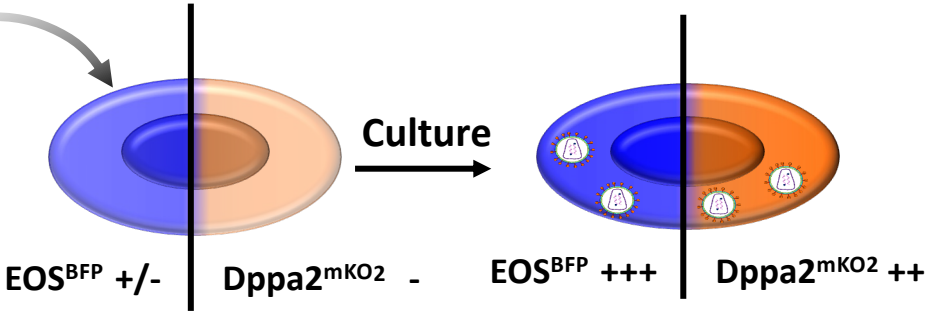
Etn/Oct4DE-TagBFP (EOS)



Production of a pluripotency-reporter rbiPSC line = RaiSED



Easely testing effect of different factor overexpression alone or by combination

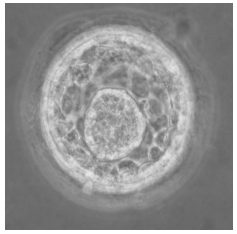


Reprogramming of rbPSCs toward the naive state

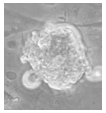
We are developing four new strategies:

1. The definition of rabbit naïve pluripotency
2. The induction of naïve pluripotency
3. The genetic selection of naïve pluripotency
4. **The direct capture of naïve pluripotency**

Growth factors	LIF	Yes
	IL6/IL6R	Yes
	Activin A	Yes
	Vitamin C	Yes
Inhibitors of signaling pathways	GSK3bi	No
	MEKi	No
	P38i	Yes
	JNKi	Yes
	PKCi	Yes
	AXINi	Yes
	ROCKi	No
	SRCi	No
	BRAFi	Yes
	Notchi	?
	YAPi	?
	BMPi	?

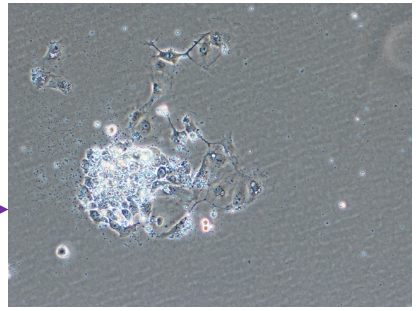


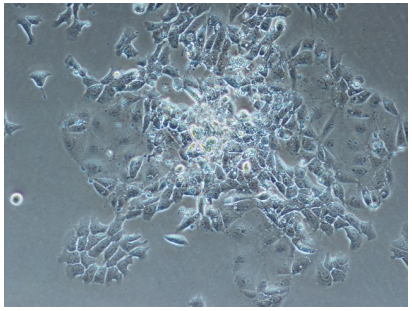
E3.5 Blastocyst



Isolated ICM

On Matrigel with MEF-conditioned N2B27 medium with tested molecules, alone or in combination





Read-out: growth of ICM cells & rbESC derivation

Conclusion

Our aim is to reprogram rbPSC
toward the naïve state of pluripotency
in order to produce rabbit germline chimaera
and transgenic models

↓

We are developing four new strategies:

1. **The definition of rabbit naïve pluripotency**
2. **The induction of naïve pluripotency**
3. **The genetic selection of naïve pluripotency**
4. **The direct capture of naïve pluripotency**

↓

All together, these strategies should help us to reach our goals

Acknowledgements

SBRI Stem-cell & Brain Research Institute Lyon

Pluripotency in Mammals

Pierre Savatier

Nathalie Beaujean

Irène Aksoy

Pierre-Yves Bourillot

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Florence Perold

Wilhelm Bouchereau

Hong Thu Pham

Synara Moreira

Etienne Masfaraud

PROJET FINANCÉ PAR L'ANR – PROJECT FUNDED BY THE ANR

ANR

INVESTISSEMENTS D'AVENIR

isaralyon

Une école d'ingénieurs au cœur de la vie

Rabbit National Cryobank

Thierry Joly

INRAE

BDR Jouy-en-Josas

Véronique Duranthon

Thanks for your attention

Questions?