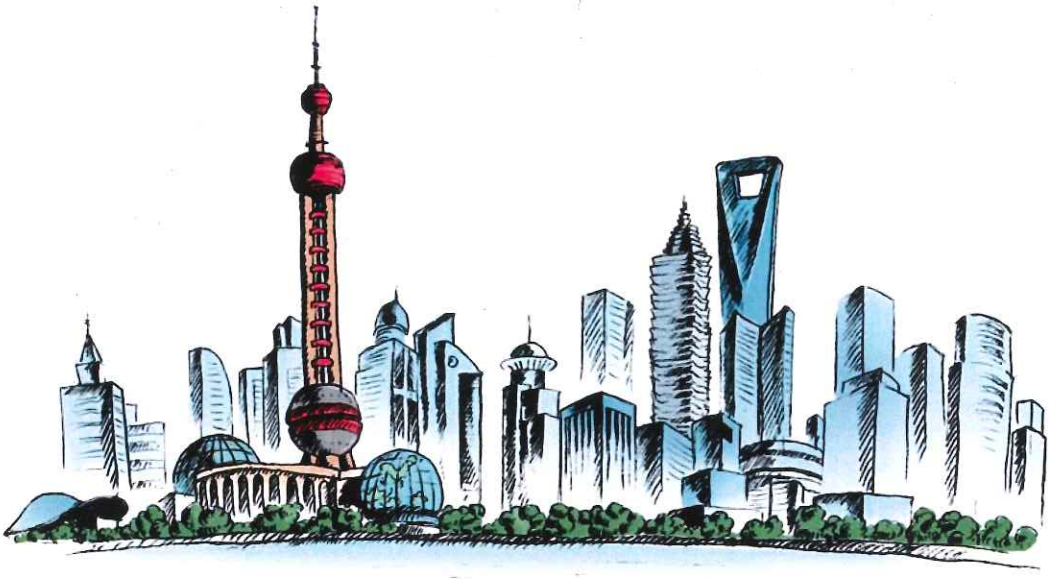
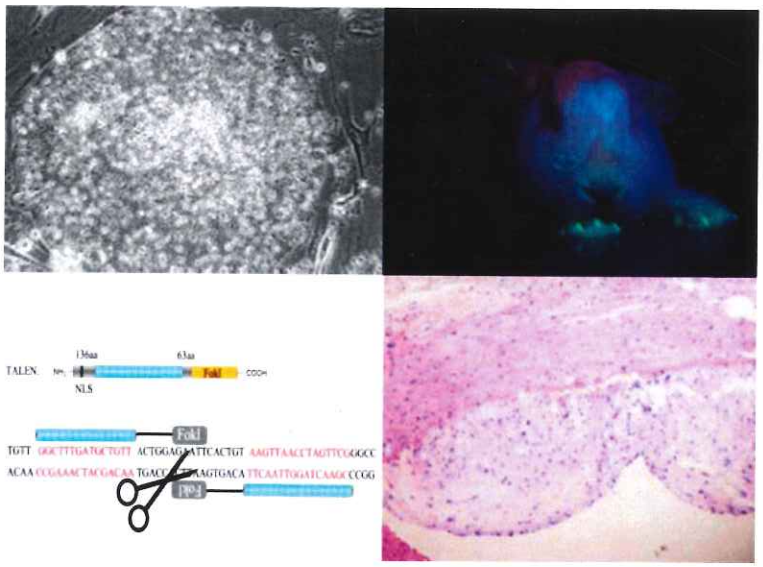
# The rabbit preimplantation embryo as a paradigm to explore naive pluripotent stem cell derivation in non rodent species

Pierre Osteil, Yann Tapponnier, Anaïs Moulin, Thierry Joly, Pierre Savatier, Marielle Afanassieff



# The 5th International meeting on rabbit biotechnology

## Shanghai, China, 7-8 June 2013




**Department of Laboratory Animal Science, Shanghai Jiaotong University, School of Medicine**

**Shanghai Association for Laboratory Animal Science**

Poster session

## 5th International Rabbit Biotechnology Meeting: Shanghai 7-8/06/2013

# The rabbit pre-implantation embryo as a paradigm to explore naive embryonic stem cell derivation in non-rodent species

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Rabbit is a good model to improve embryonic stem cell (ESC) derivation in non-rodent species, as it can produce up to 30 embryos per super-ovulated females. All rabbit ESC line produced so far showed the characteristics of primed pluripotency. In this work, we investigated the conditions suitable for the generation of naïve ESCs in rabbits. A first experiment aimed to study the effect of the pharmacological inhibitor of MEK signaling (PD0325901) on ESC line derivation. ICMs were isolated by immunosurgery from 576 blastocysts and plated onto gelatin- or fibronectin-coated dishes in various media (DMEM/F12 + 20% KOSR, N2B27) supplemented with GSK3β inhibitor (CHIR99021), PD0325901, and LIF (2i/LIF), or not. 80% of the ICMs plated, but none could be expanded beyond passage 2. We concluded that inhibition of MEK signaling fails to prevent spontaneous differentiation of pluripotent stem cells in rabbit. A second experiment aimed to study the effect of LIF on ESC line derivation. We collected 262 ICMs, which were plated onto growth-inactivated mouse embryonic fibroblasts in DMEM/F12 supplemented with 20% KOSR (72), 20% KOSR + LIF (90), or 10% KOSR + 10% FCS + LIF (80). No outgrowth cultured in media lacking LIF could be expanded beyond passage 4. By contrast, 7 ESC lines were derived from outgrowths cultured and expanded in the presence of LIF, 2 in 20% KOSR + LIF, and 5 in 10% KOSR + 10% FCS + LIF. Two lines, designated rbES-LIF1 and rbES-LIF2, were expanded by gentle dissociation with collagenase until passage 40, and showed a normal karyotype. RbES-LIF1 and rbES-LIF2 displayed the cardinal features of pluripotent stem cells, *i.e.* expression of pluripotency markers, differentiation into derivatives of the 3 germ layers, and teratoma formation. However, they could not be cultured onto gelatin-coated dishes, they did not express markers (*Zfp42*, *Klf4*, *Pecam1*, *Dazl1*) associated with naïve pluripotency in rodents, and they did not survive in 2i/LIF medium. We concluded that LIF facilitates the derivation of ESCs but does not support naïve pluripotency in rabbits. When rbES-LIF1 and rbES-LIF2 cells were propagated for 20 passages in 10% KOSR + 10% FCS + LIF, and were enzymatically dissociated with Acutase into single cell suspensions, they acquired chromosomal abnormalities (43XX; 45XY). The same observation was made with 5 freshly derived ESC lines in 10% KOSR + 10% FCS + LIF with Acutase, of which 3 displayed abnormal chromosome numbers. We concluded that LIF-dependent rabbit ESCs cannot be propagated under stringent conditions without chromosomal rearrangement-based adaptation.

# THE RABBIT PRE IMPLANTATION EMBRYO AS A PARADIGM TO EXPLORE NAIVE EMBRYONIC STEM CELL DERIVATION IN NON RODENT SPECIES

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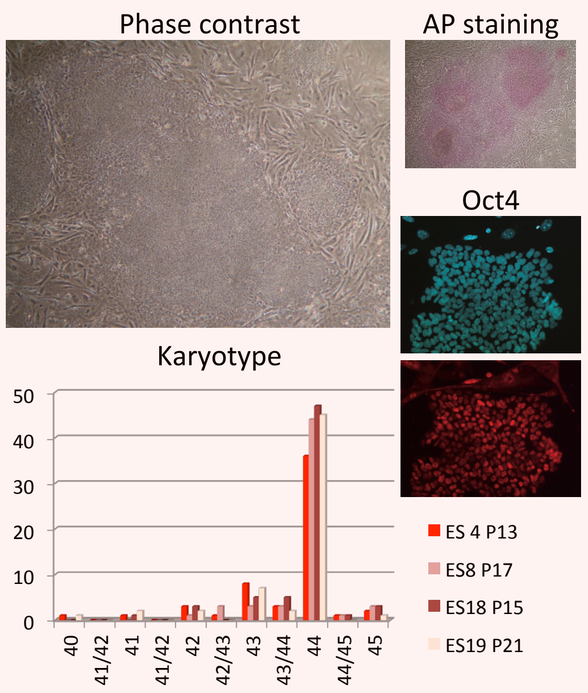
**INTRODUCTION:** Rabbit is a good model to improve embryonic stem cell (ESC) derivation in non-rodent species, as it can produce up to 30 embryos per super-ovulated females. All rabbit ESC line produced so far showed the characteristics of primed pluripotency. In this work, we investigated the conditions suitable for the generation of naïve ESCs in rabbits.

## II-RbESC-FGF

### A) Culture conditions

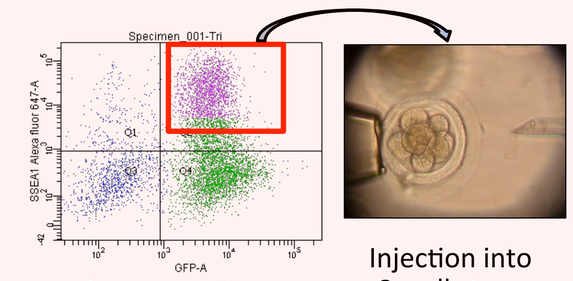
*Serum:* 20% KOSR
*Growth factor:* FGF2
*Feeders:* MEF (from OF1 mice)
*Passaging method:* Clumps (Collagenase)

### B) Characterization




RbESC-FGF cells display the cardinal features of embryonic stem cells.
*Osteil, Tapponnier, Markossian et al. Induced pluripotent stem cells derived from rabbits exhibit some characteristics of naïve pluripotency (2013) Biology Open doi: 10.1242/bio.20134242*

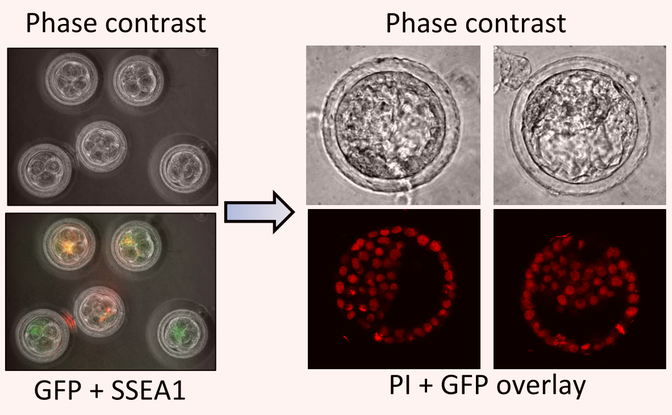
### C) ICM colonization after injection into 8-cell stage embryos




GFP+/SSEA1+ Cell sorting

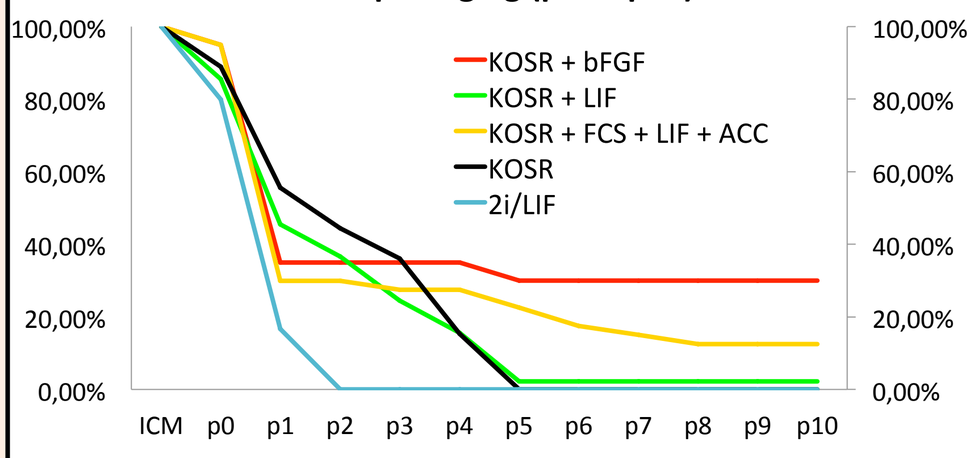


Injection into 8- cell stage embryos




One hundred and twelve 8-cell stage embryos were injected with 10-15 cells, and further cultured to the blastocyst stage. No blastocyst with GFP positive cells in the ICM could be observed.

**Percentage of expanding ICM outgrowths during serial passaging (p0 to p10)**

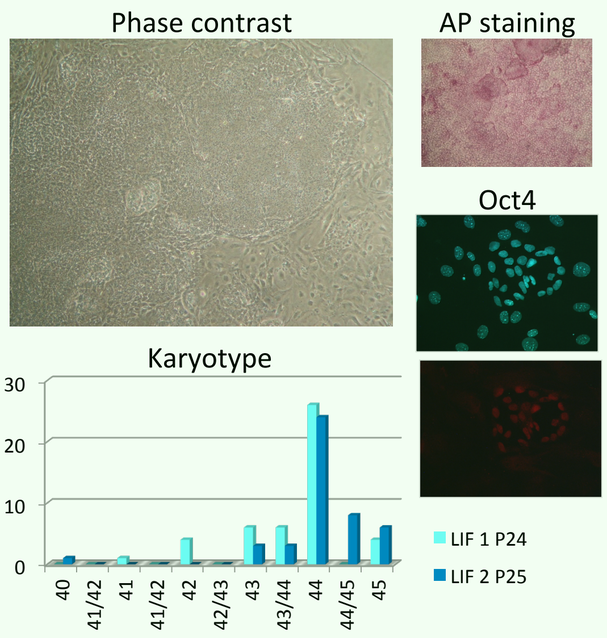



## III-RbESC-LIF

### A) Culture conditions

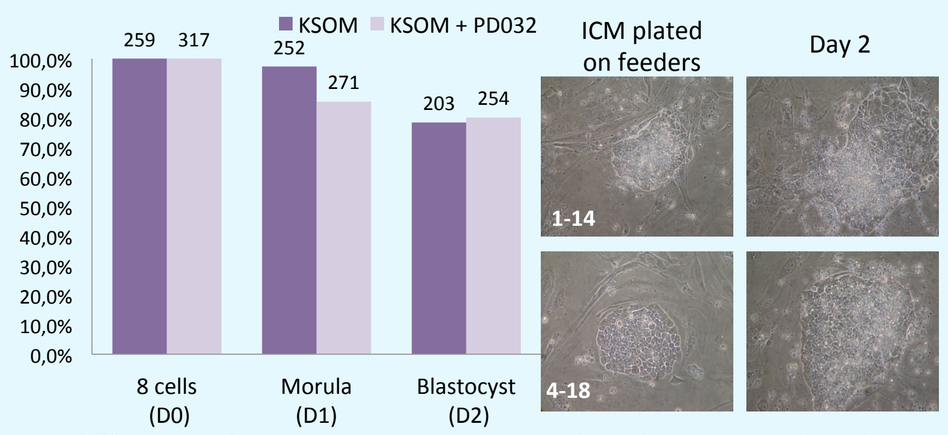
*Serum:* 20% KOSR
*Growth factor:* LIF
*Feeders:* MEF (from OF1 mice)
*Passaging method:* Clumps (Collagenase)

### B) Characterization




RbESC-LIF cells display the cardinal features of embryonic stem cells. They express pluripotency markers, they form teratomas, and they have a stable karyotype (44XY).

## I- Pre-implantation embryo development, and ICM growth after plating onto feeders (+/- MEK inhibition with PD032)



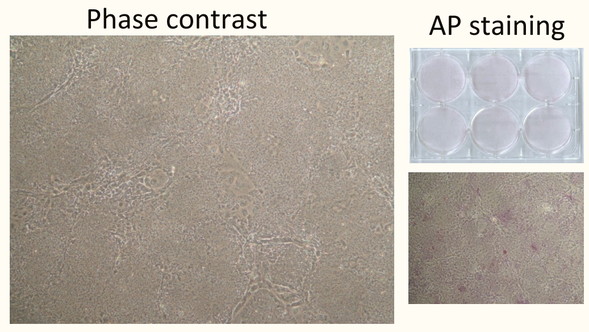

No difference between PD032-treated and untreated embryo was observed in the rate of successful development. ICM cultured in PD032 failed to generate ESC lines.

## IV-RbESC-ALF

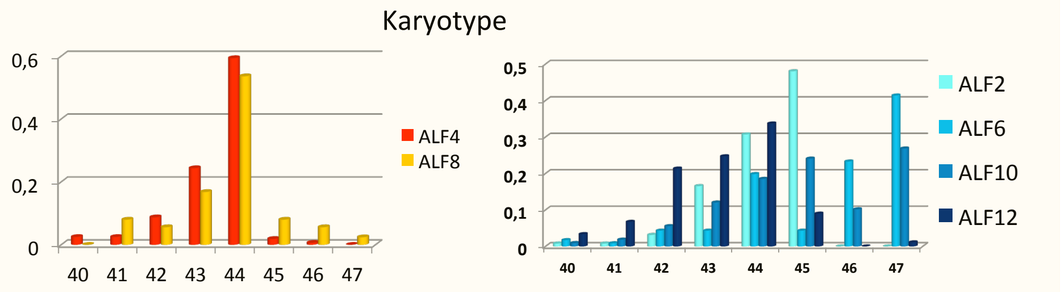
### A) Culture conditions

*Serum:* 10% KOSR + 10% FCS
*Growth factor:* LIF
*Feeders:* MEF (from OF1 mice)
*Passaging method:* Single cell suspension (Accutase)

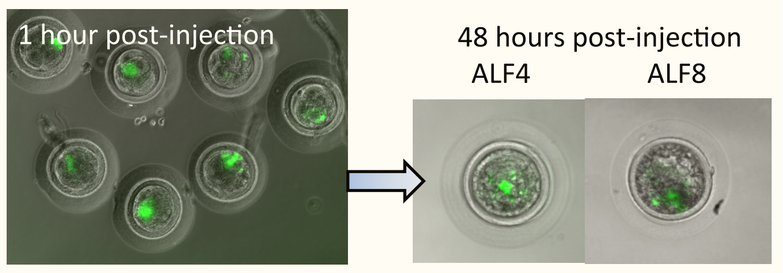
### B) Characterization




RbESC-ALF cells are pluripotent (characterization is ongoing). However, four lines out of six acquired chromosomal abnormalities during passaging (43XX, 45XY).



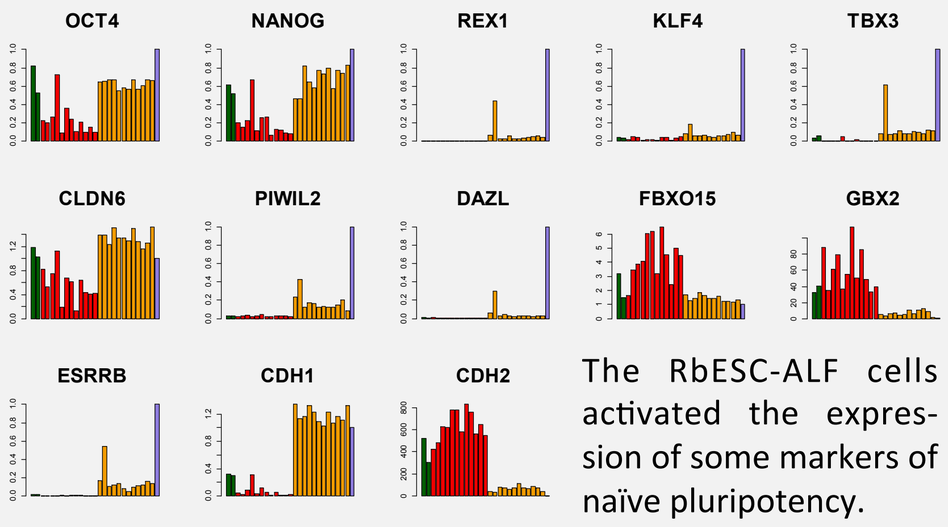

### C) ICM colonization after injection into 8-cell stage embryos




Of 303 embryos that developed to the blastocyst stage, 9 had GFP positive cells in the ICM.

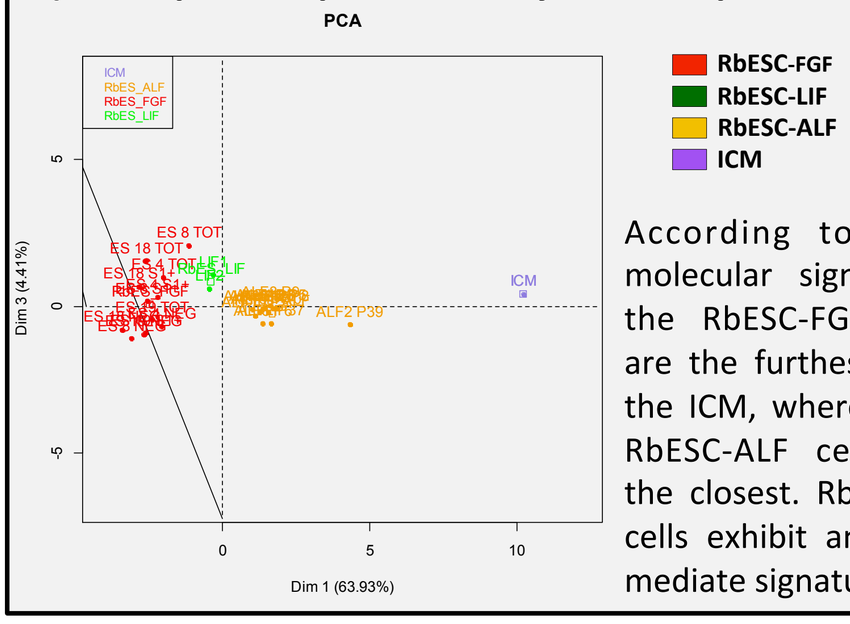
## V- Gene expression analysis

### A) Barplots from qPCR data




The RbESC-ALF cells activated the expression of some markers of naïve pluripotency.

### B) Principal Component Analysis from qPCR data




According to their molecular signatures, the RbESC-FGF cells are the furthest from the ICM, whereas the RbESC-ALF cells are the closest. RbESC-LIF cells exhibit an intermediate signature.

## CONCLUSIONS:

1/ Inhibition of MEK signaling prevents ICM outgrowths from generating ESCs in rabbit.
2/ Rabbit ESCs derived in FCS on feeders, and dissociated to single cell suspension during passaging (RbESC-ALF), exhibit an elevated expression of genes whose expression is associated with naive pluripotency in rodents.
3/ RbESC-ALF cells can colonize the ICM of the rabbit blastocyst following injection into 8-cell stage embryos.

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