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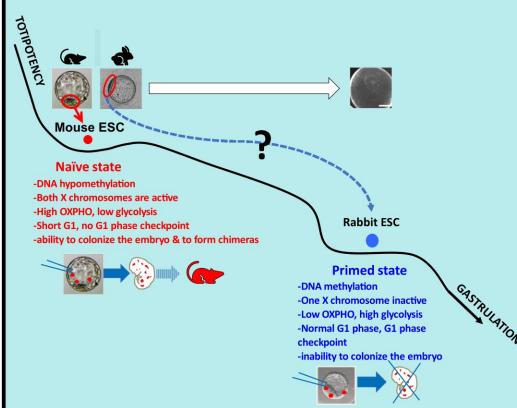
Deciphering the pluripotency continuum in rabbit pre-implantation embryos

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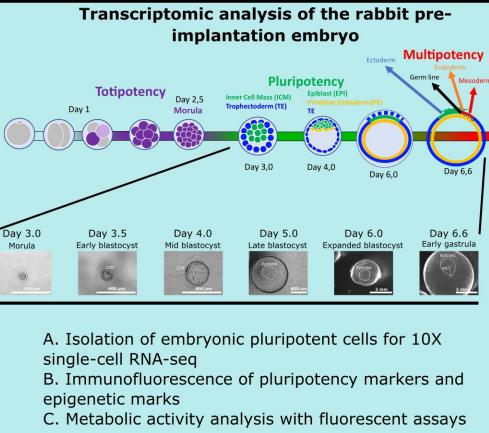
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Introduction

Pluripotency in mice versus rabbits

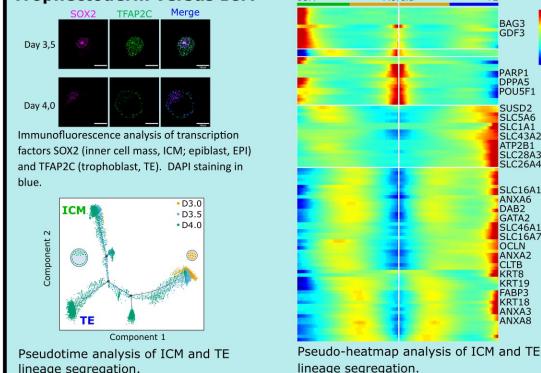


Transcriptomic analysis of the rabbit pre-implantation embryo

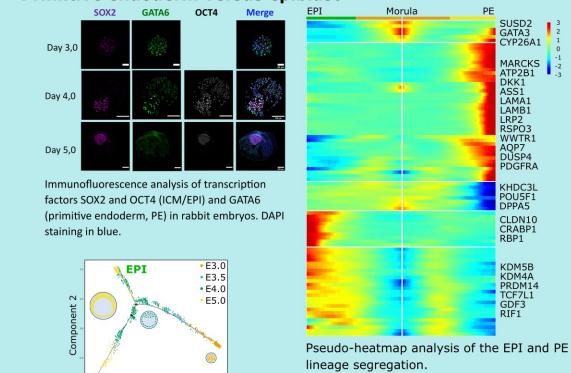


Early lineage segregation

Trophectoderm versus ICM



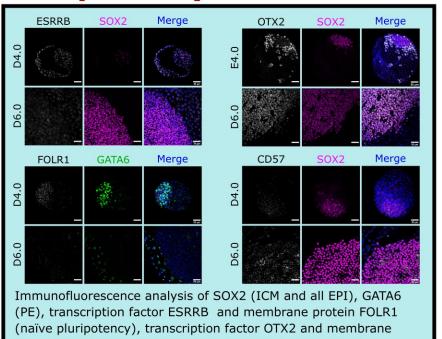
Primitive endoderm versus epiblast



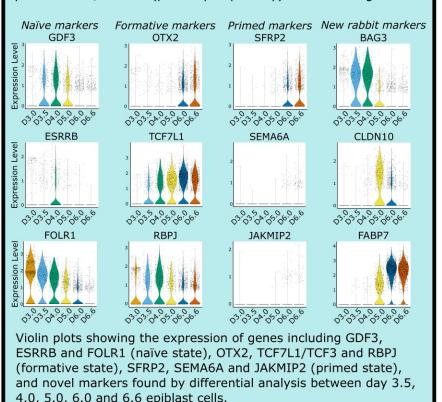
Pseudo-timeline analysis of the EPI and PE lineage segregation.

Based on these observations, we were able to determine the timing of early lineage segregation in rabbit embryos and delineate with certainty the ICM / EPI clusters that were studied in more details.

Pluripotency continuum

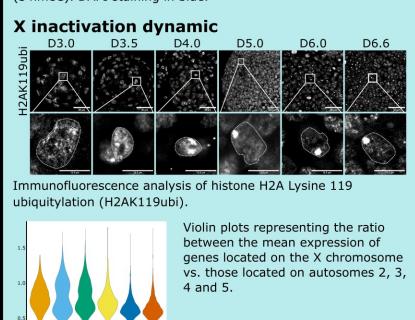
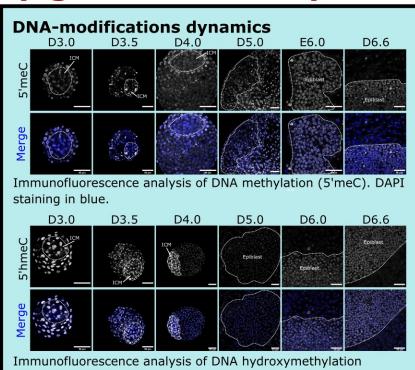


Immunofluorescence analysis of SOX2 (ICM and all EPI), GATA6 (PE), transcription factor ESRB and membrane protein FOLR1 (naive pluripotency), transcription factor OTX2 and membrane protein CD57/B3GAT1 (primed pluripotency). DAPI staining in blue.



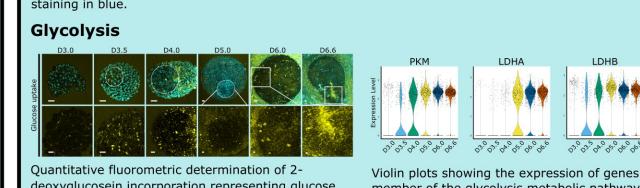
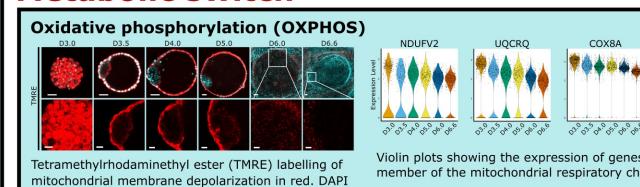
These data demonstrate that the transition from the naïve state of pluripotency toward the primed state occurs between day 3.5-4.0 and day 6.0-6.6 in the rabbit ICM/EPI cells.

Epigenetic landscape



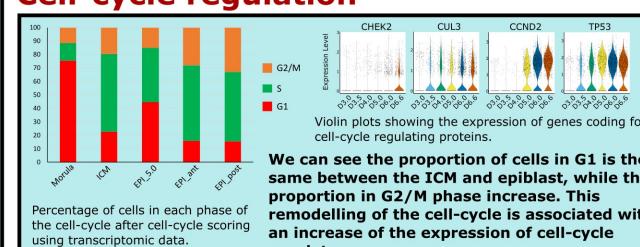
We can see an increase of heterochromatin associated markers (DNA methylation and X inactivation) between the ICM (day 3.5) and epiblast (day 5.0) cells.

Metabolic switch



The gradual decrease of mitochondrial activity is associated with a decrease of expression for OXPHOS related genes in the epiblast. On the other hand, glucose intake increases as well as the expression of genes involved in glucose metabolism.

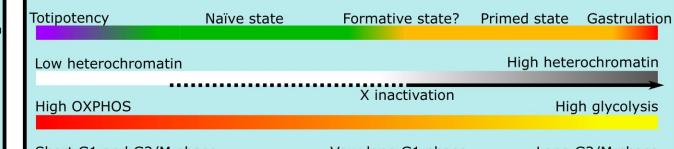
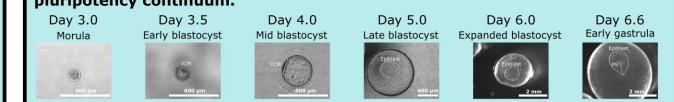
Cell-cycle regulation



We can see the proportion of cells in G1 is the same between the ICM and epiblast, while the proportion in G2/M phase increase. This remodelling of the cell-cycle is associated with an increase of the expression of cell-cycle regulators.

Conclusion

Schematic representation of the gradual progression through the pluripotency continuum.



The rabbit embryo progresses through several transient states and ICM/EPI cells *in vivo* should be considered as a continuum of pluripotency. Indeed, rabbit embryonic development is characterized by:

- Gradual progression of pluripotent embryonic cells from the naïve to primed-like state. Expression of formative markers highlight the switch from the naïve to primed state at day 5.0 epiblast.
- Gradual increase of heterochromatin fraction in the nuclei, with the day 5.0 being in an intermediate state.
- Gradual switch from an OXPHOS dependent metabolism toward a glycolysis dependent metabolism.
- Switch from an unregulated cell-cycle to a controlled cell-cycle, with extended G2/M phase.

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