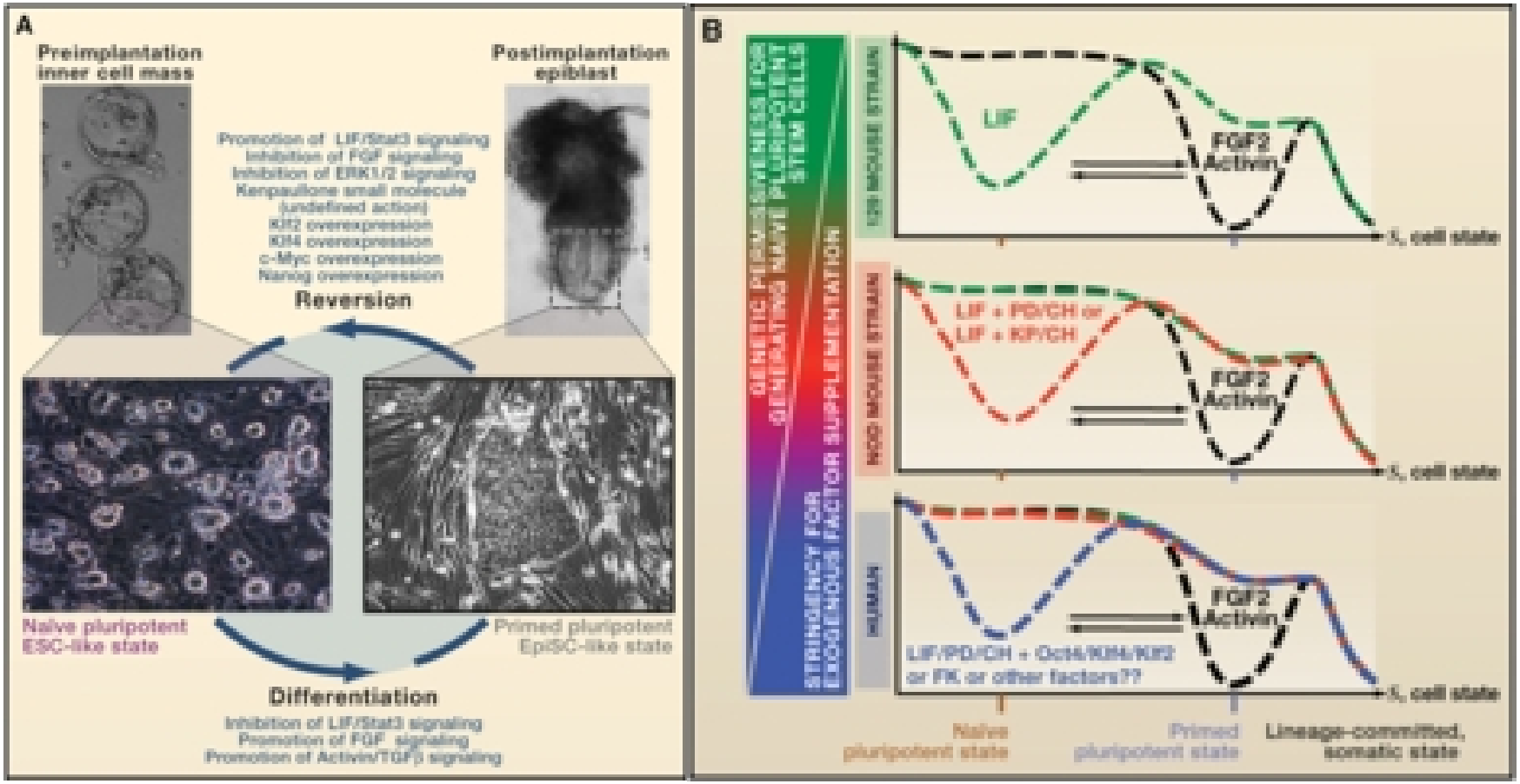
Pluripotent state of porcine embryonic germ cells is modulated by culture conditions

Seoul National University
Lab. of Animal Reproduction and Transgenesis.

Choi, Kwang-Hwan

Naïve and Primed pluripotent state



Hanna et al., 2010

Naïve and Primed pluripotent state

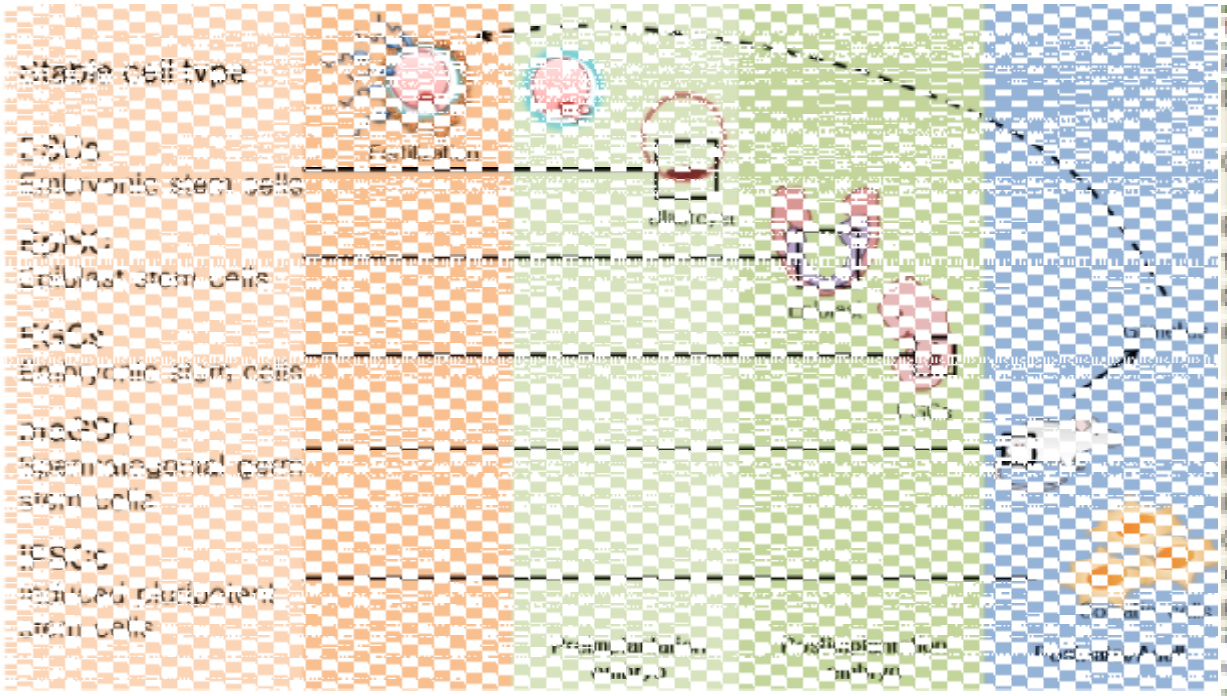


Table 1. Comparison of Naive and Primed Pluripotent States

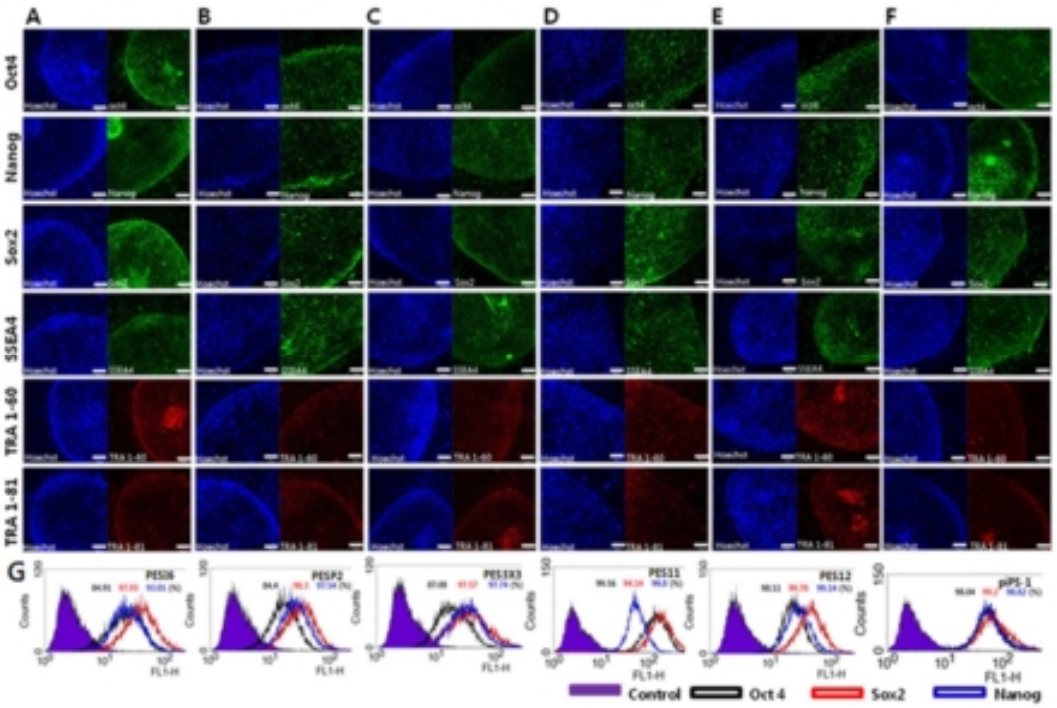
Property	Ground State	Primed State
Embryonic tissue	early epiblast	egg cylinder or embryonic disc
Culture stem cell	rodent ESCs	rodent EpiSCs; primate "ESCs"
Blastocyst chimaeras	yes	no[a]
Teratomas	yes	yes
Differentiation bias	none	variable
Pluripotency factors	Oct4, Nanog, Sox2, Klf2, Klf4	Oct4, Sox2, Nanog
Naive markers[b]	Rex1, Nr0b1, Fgf4	absent
Specification markers	absent	Fgf5, T
Response to Lif/Stat3	self-renewal	none
Response to Fgf/Erk	differentiation	self-renewal
Clonogenicity	high	low
XX status	XaXa	Xa0
Response to 2i	self-renewal	differentiation/death

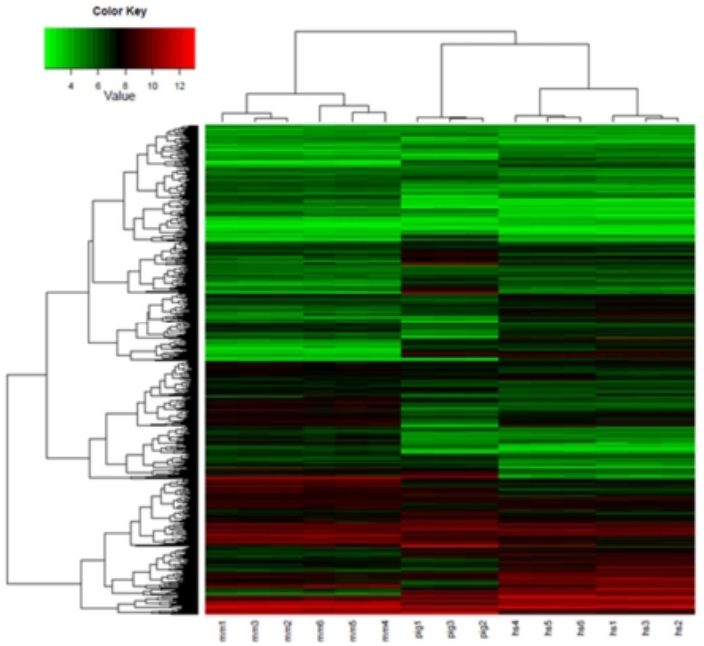
[a] Not applied to primate cells.
[b] Representative examples.

Pluripotent stem cells in pigs

References	Embryo Source	Passage Number	Remarks
Piedrahita et al. (1990)	In vivo blastocysts day 7–8	>32 passages	ES- and epithelial-like morphology; epithelial-like cells expressed cytokeratin 18; EB formation; differentiation into vesicular structures
Anderson et al. (1994)	In vivo blastocysts day 6–10	10 passages	ES-like cells; establishment of ES-like cell lines greater for day 10 embryos, but they readily differentiated into epithelial-like cells, fibroblasts and cardiac muscle; no Chimera
Moore and Piedrahita (1996, 1997)	In vivo blastocysts day 7	4 day culture	Two morphologies: nonepithelial and epithelial-like cells; AP positive; cytokeratin negative
Li et al. (2003)	In vivo blastocysts day 7–9 minipigs	6 passages	Mouse ES-like cells; AP positive; EB formation; differentiation into neuron-like, smooth muscle, and epithelium-like Cells
Li et al. (2004)	In vitro 4-cells to blastocysts	4 passages	ES-like cells derived only from blastocysts; AP positive; EB formation; differentiation into fibroblasts and neurons
Brevini et al. (2005)	In vitro parthenogenetic blastocysts day 6	32 passages	Circular colonies with distinct margins of small round cells; EB formation; three colonies expressed OCT4, NANOG, and were negative for differentiation markers
Vackova and Madrova (2006)	In vivo blastocysts day 6–7	19 passages	Colonies expressed OCT4 and NANOG
Kim et al. (2007)	In vitro, parthenogenetic, and NT blastocyst	5 passages	Colonies with typical morphology of mouse ES-cells; expressed AP; EB formation; spontaneous differentiation; more primary colonies were formed from IVF and NT than parthenogenetic blastocysts
Alberio et al. (2010)	In vivo blastocyst day 10.5–12	12 passages	Flat, compact colonies; SSEA-1 expression; normal karyotype; OCT4, NANOG, SOX2, and NODAL expression; AP and REX1 negative; EB formation; spontaneous differentiation into cells of three somatic germ layers
Vassiliev et al. (2010)	In vitro blastocysts day 7 and in vivo blastocysts day 6	14 passages	Polygonal shape morphology, two cell lines from in vivo blastocysts and four from in vitro blastocysts; expressed OCT4, NANOG, and SSEA-1; stable karyotype; EB formation; differentiation into cell types representative of all thee germ layers

Pluripotent stem cells in pigs





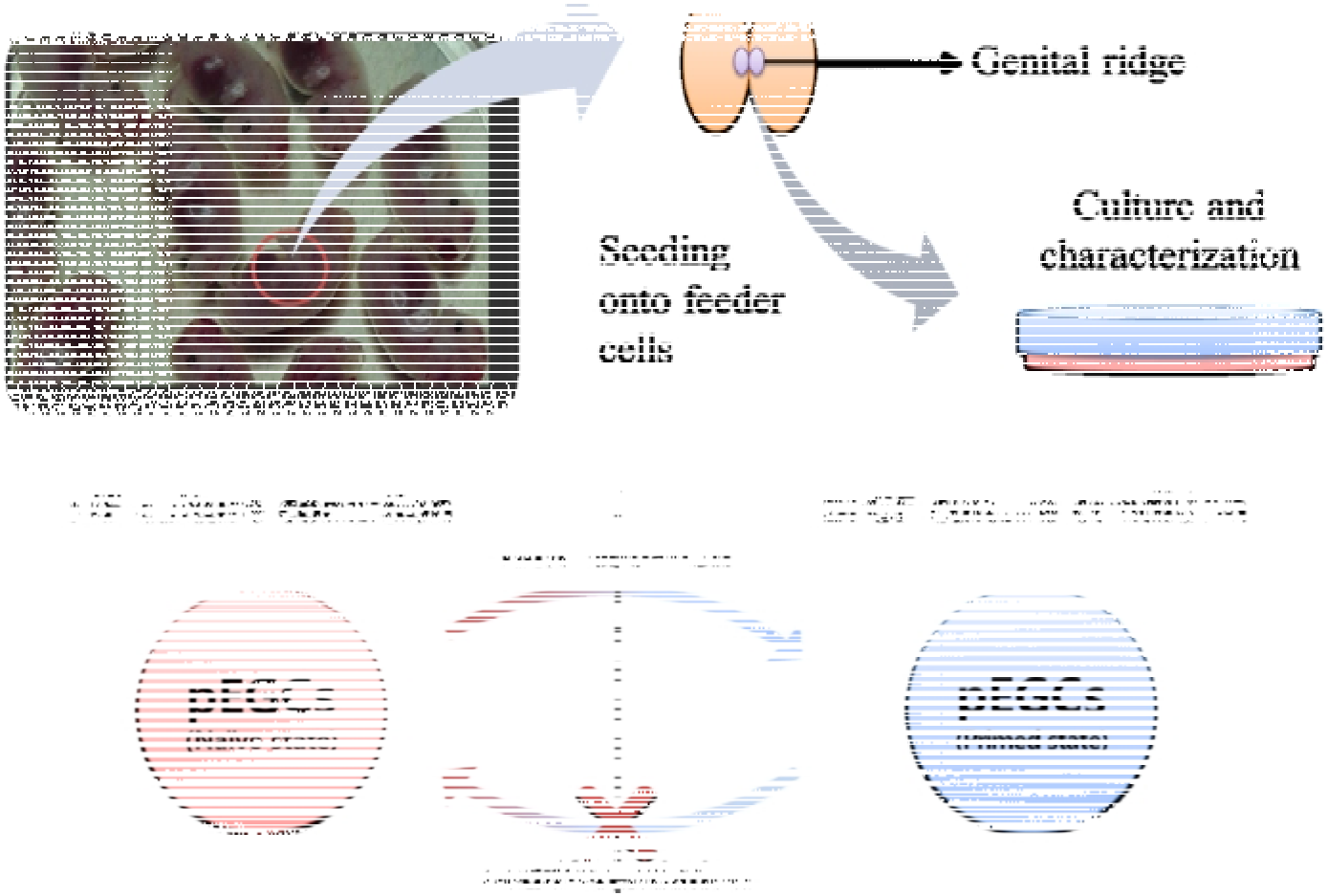
Park et al., 2013

This Study aims to...

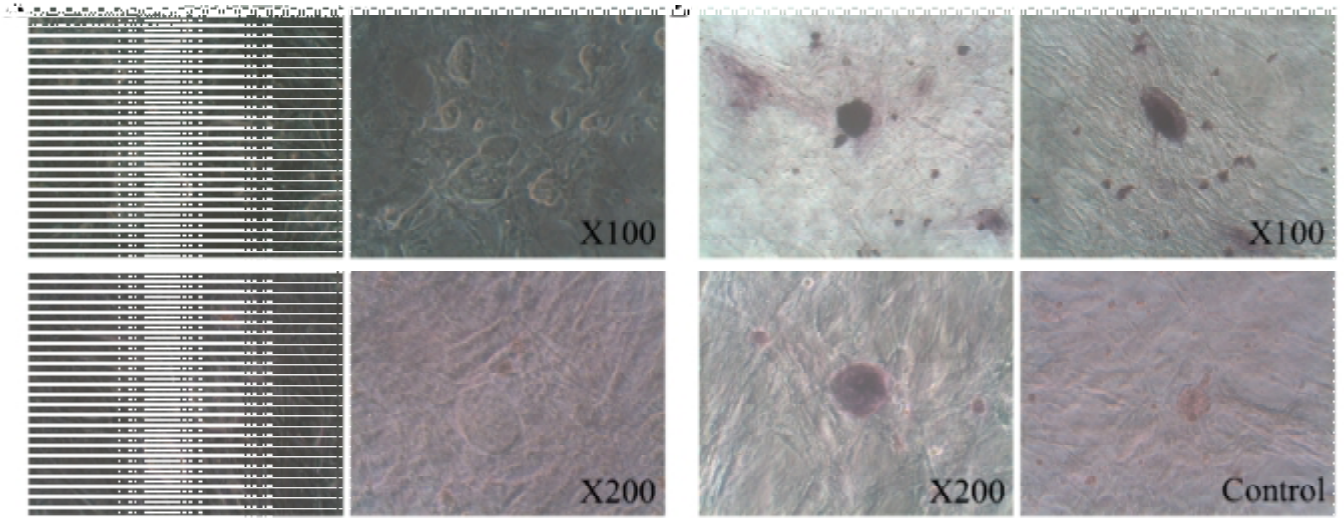
1. Derive a porcine embryonic germ cell line.

2. Characterize a pluripotent state of pEGCs.

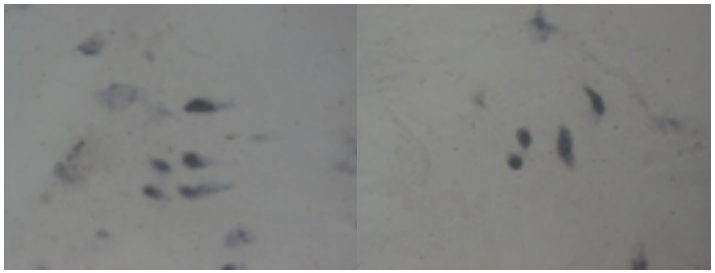
Experimental scheme and Hypothesis



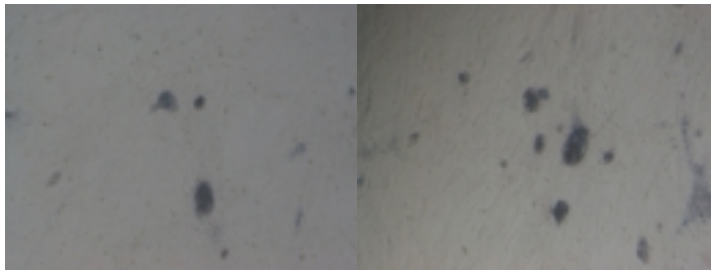
Porcine embryonic germ cells



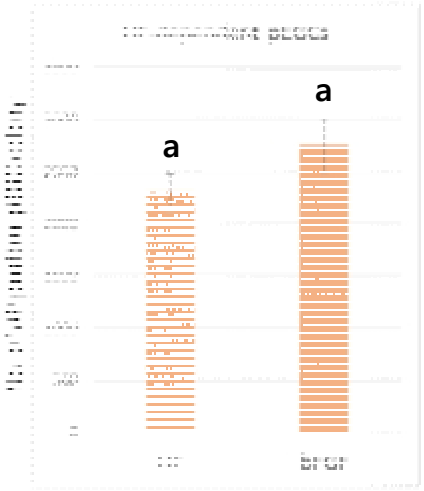
+LIF

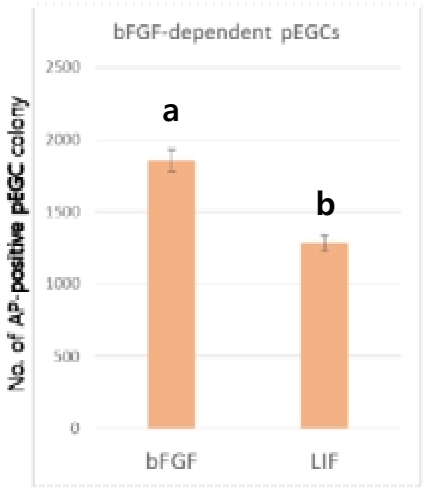


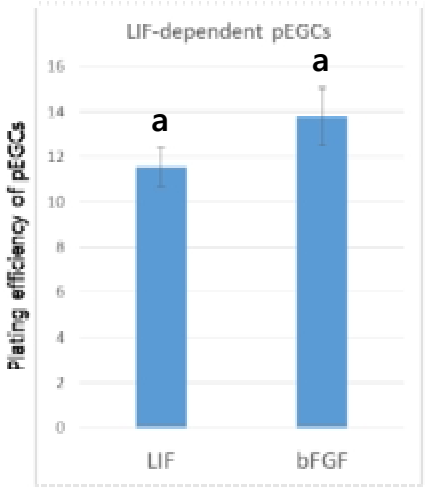
+bFGF

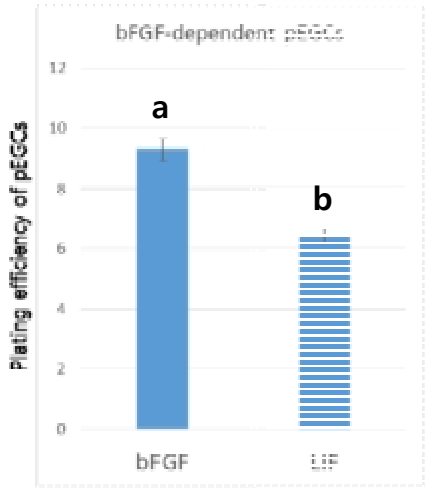


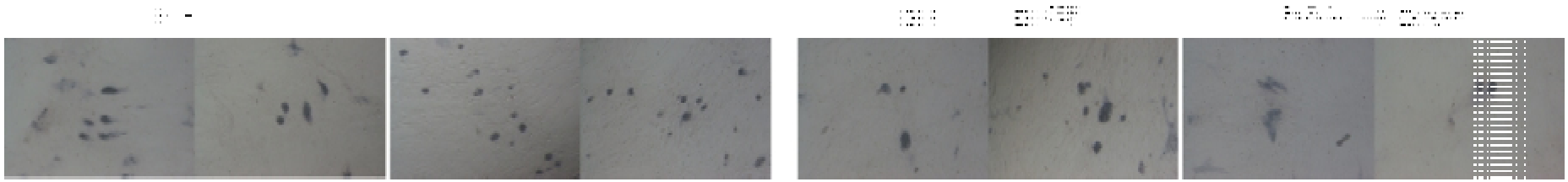
Effects of culture conditions

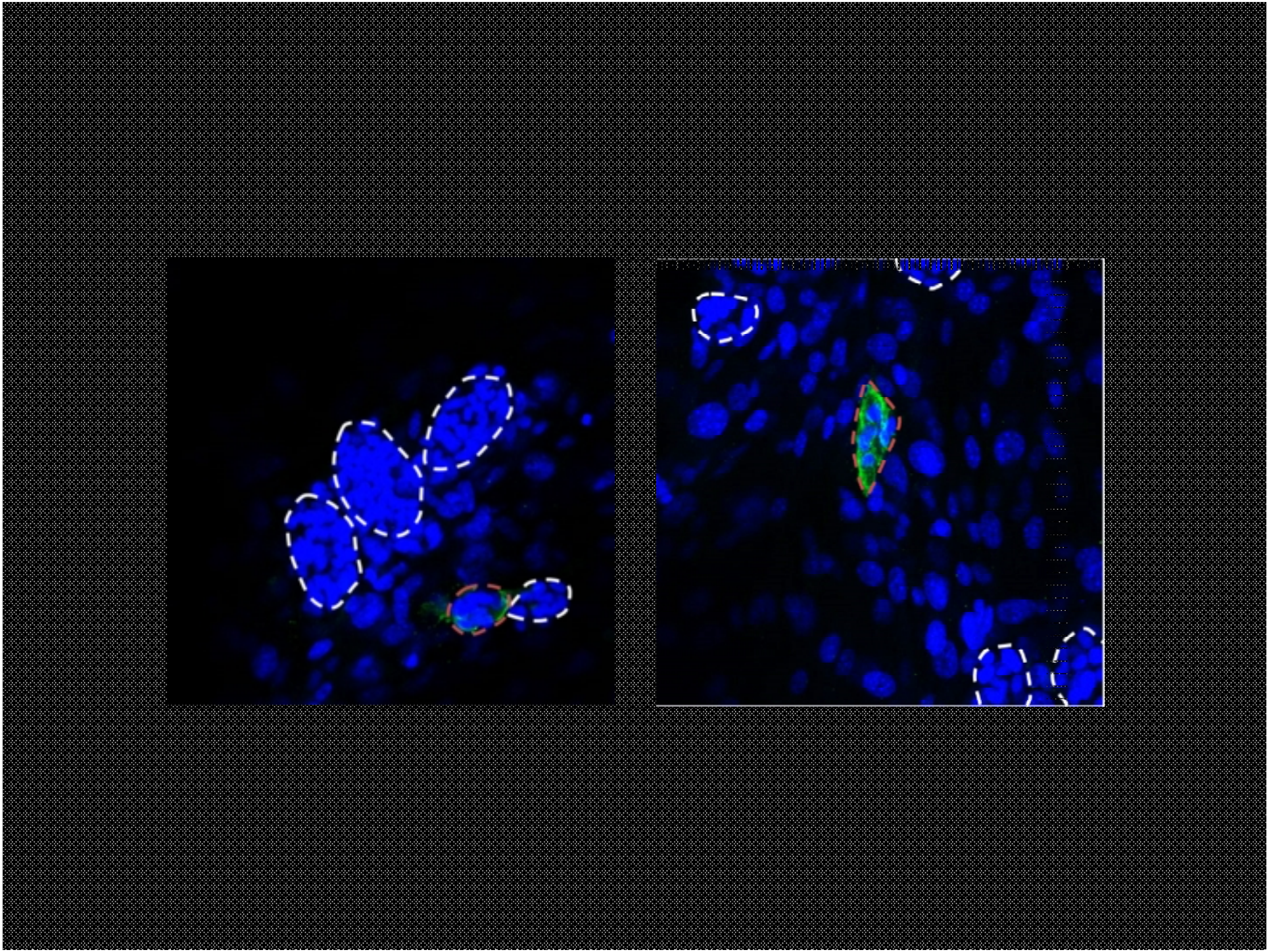












X chromosome inactivation

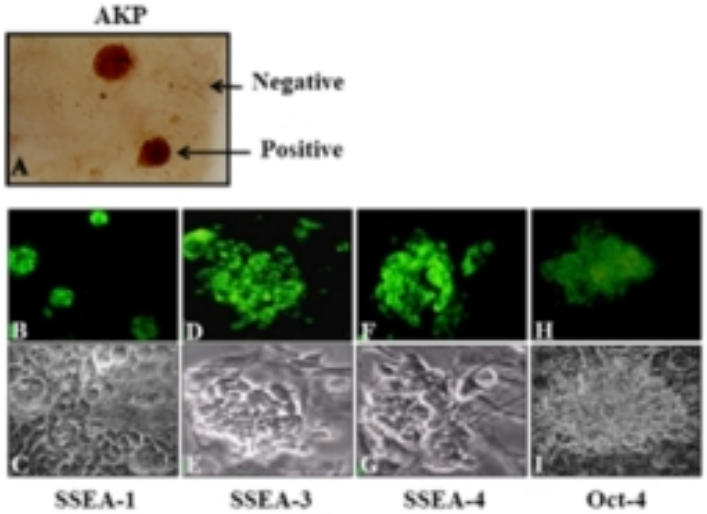
Conclusion

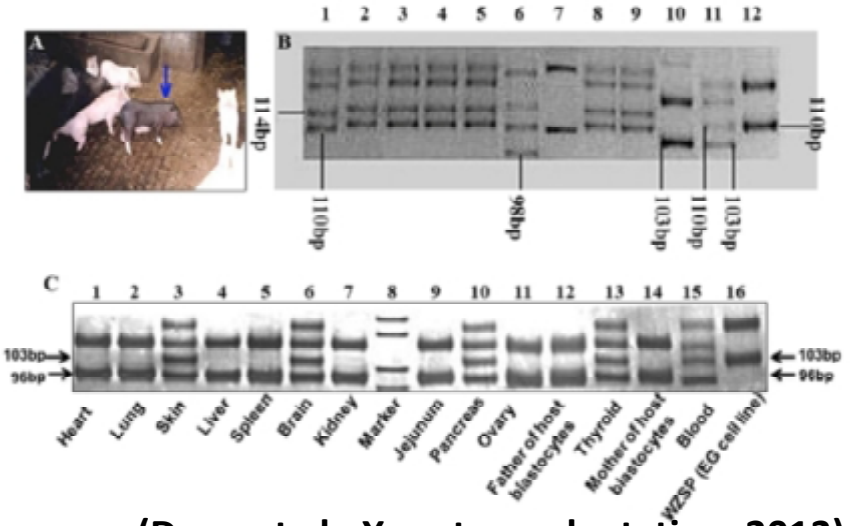
1. we were able to successfully derive embryonic germ cells from genital ridges of a porcine fetus.

2. In LIF supplement, pEGCs showed naive-pluripotency expressing SSEA1, while pEGCs show primed-pluripotency expressing SSEA4 in bFGF condition.

3. two cell lines showed fully methylated pattern similarly in XIST promoter regions.

Perspectives





(Dong et al., Xenotransplantation, 2013)

Standard of Naïve pluripotent state in pig study.

Generation of transgenic animals

- Bioreactor, Xenotransplantation, disease model, disease-resistance animal

Thank You !