

3D visualization of early bovine embryogenesis by multicolour confocal microscopy

Felix A. Habermann¹, Sandra Leidenfrost¹, Marc Boelhauve², Eckhard Wolf², Fred Sinowatz¹

¹Institute of Veterinary Anatomy, Histology and Embryology, LMU Munich

²Institute of Molecular Animal Breeding and Biotechnology, Gene Center, LMU Munich

Aims

- 3-dimensional visualization of early bovine development from the oocyte to the blastocyst stage
- Description and distinction of normal and abnormal patterns of cell divisions, cell lineaging and cell death
- Identification and unravelling of critical steps and developmental checkpoints at the cellular level
- Analysis of the temporal and spatial expression of key genes and proteins

Experimental approach

- Bovine embryos produced by *in vitro* fertilization (IVF) and *in vitro* culture (IVC), collected at defined time points 24 h – 9 days after fertilization
- Multiple fluorescent staining of whole-mount specimen
- Optical sectioning by confocal laser scanning microscopy (CLSM):
 - large-scale image stacks encompassing the entire specimen (voxel size = 125 x 125 x 1.000 nm)
 - high resolution stacks of individual blastomeres of interest (voxel size = 50 x 50 x 200 nm)
- Digital image restoration by iterative MLE (maximum likelihood estimation) deconvolution algorithms using the measured point spread function (PSF) of the microscope
- CLSM: Zeiss LSM 510, laser lines 364, 488, 543, 633 nm, 40 x Plan-Neofluar, oil immersion objective, NA 1,3.

Results and Conclusions

We demonstrate how confocal laser scanning microscopy can be used to visualize mammalian embryos up to the hatching stage *in toto* to analyze the developmental and functional status at the blastomere level.

Early blastomere arrest and loss prior to/around major embryonic genome activation

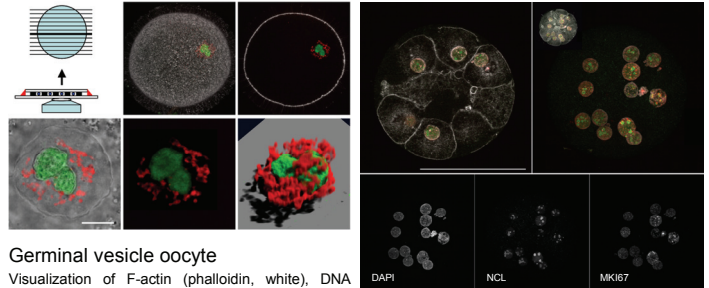
- is a frequent finding in bovine IVF embryos
- is a critical determinant for
 - the cell division tree and the cell number increase
 - normal cell lineage specification
 - the embryo fate

Acknowledgements

This work is supported by the DFG (FOR 478 and GRK 1029).

Isolation and *in vitro* maturation of bovine oocytes, *in vitro* fertilization and embryo culture was performed by Tuna Guengoer (Institute of Molecular Animal Breeding and Biotechnology).

Staining of oocytes and embryos was performed by Melanie Pansa und Monica Settles (Institute of Veterinary Anatomy, Histology and Embryology).



Germinal vesicle oocyte

Visualization of F-actin (phalloidin, white), DNA (DAPI, red) and MKI67 (green).

Upper row: Z-projection (120 optical sections) and single section.

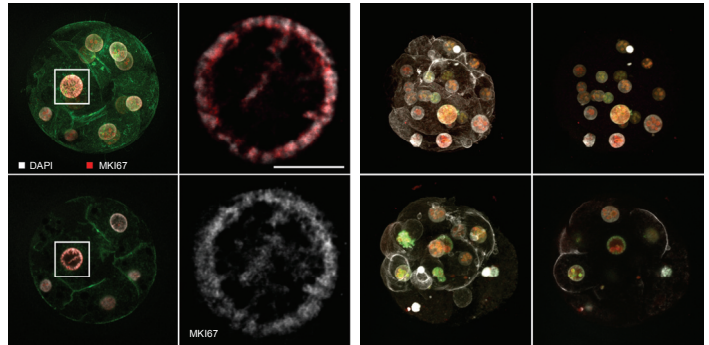
Lower row: High resolution scan of the germinal vesicle processed by iterative (MLE) deconvolution. Single optical section and 3D rendering.

Scale bar = 10 µm.

Day 3 IVF embryo

Visualization of F-actin (phalloidin, white), DNA (DAPI, white), NCL (nucleolin, green) and MKI67 (red). Upper left: Central optical section

Upper right and lower row: Z-projections from 115 serial optical sections. Scale bar = 100 µm.



Day 5 IVF embryo

Staining of F-actin (phalloidin, green), DNA (DAPI, white) MKI67 (red).

Left column: Z-projection (top) and central optical section (bottom) from the embryo.

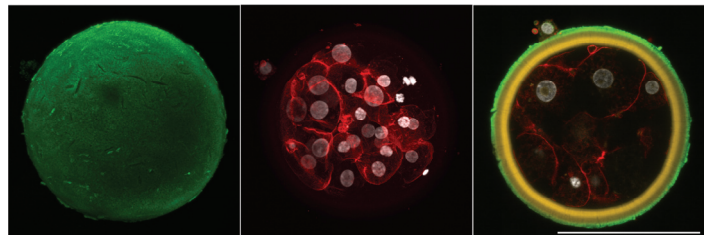
Right column: High resolution scan of an arrested(?) blastomere processed by iterative (MLE) deconvolution. Single optical section. Scale bar = 10 µm.

Day 5 IVF embryos

Early blastomeres arrested at different cell cycle stages (see arrows). Staining of F-actin (phalloidin, white), MKI67 (green) and NCL (nucleolin, red)

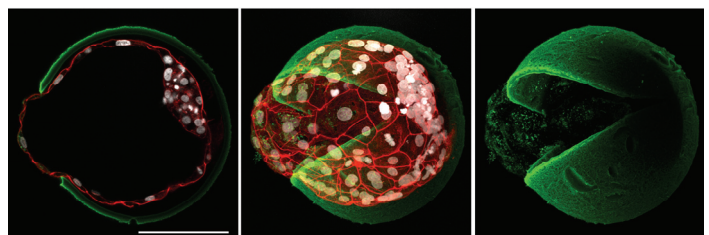
Upper row: Z-projections from an embryo with/without F-actin staining.

Lower row: Z-projection and central optical section from another embryo. Scale bar = 100 µm.



Day 5 IVF embryo at the late morula stage

Staining with phalloidin (red), DAPI (white), Wheat germ agglutinin (WGA) and Sambucus nigra agglutinin (SNA) (green). Maximum intensity Z-projections (113 optical sections) and central optical section. Scale bar = 100 µm.



Hatching bovine blastocyst

Staining with phalloidin (red), DAPI (white) and Sambucus nigra agglutinin (SNA) (green). Central optical section and maximum intensity Z-projections (170 optical sections). Scale bar = 100 µm.