Università degli Studi di Bari Aldo Moro

Anno Accademico 2019/20

Scuola di Specializzazione in "Fisiopatologia della Riproduzione degli Animali Domestici"

Embryo quality assessment

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Summary (1)

- Historical aspects
- Physiology of embryo development in mammals
- Embryo metabolism
- Embryo genome activation (EGA)
- In vivo embryo culture systems
- In vitro embryo culture systems

Historical aspects

- Sreenan, 1968; Sreenan e Scanlon, 1968

(first reports of bovine in vitro embryo culture up to the blastocyst stage)

- Adams, 1968

(first report of bovine in vivo embryo culture in rabbit oviducts up to the hatching blastocyst stage)

- Gandolfi, 1986; Gandolfi and Moor, 1987

(first reports of bovine in vitro embryo culture up to the blastocyst stage on oviductal epithelial cell monolayers)

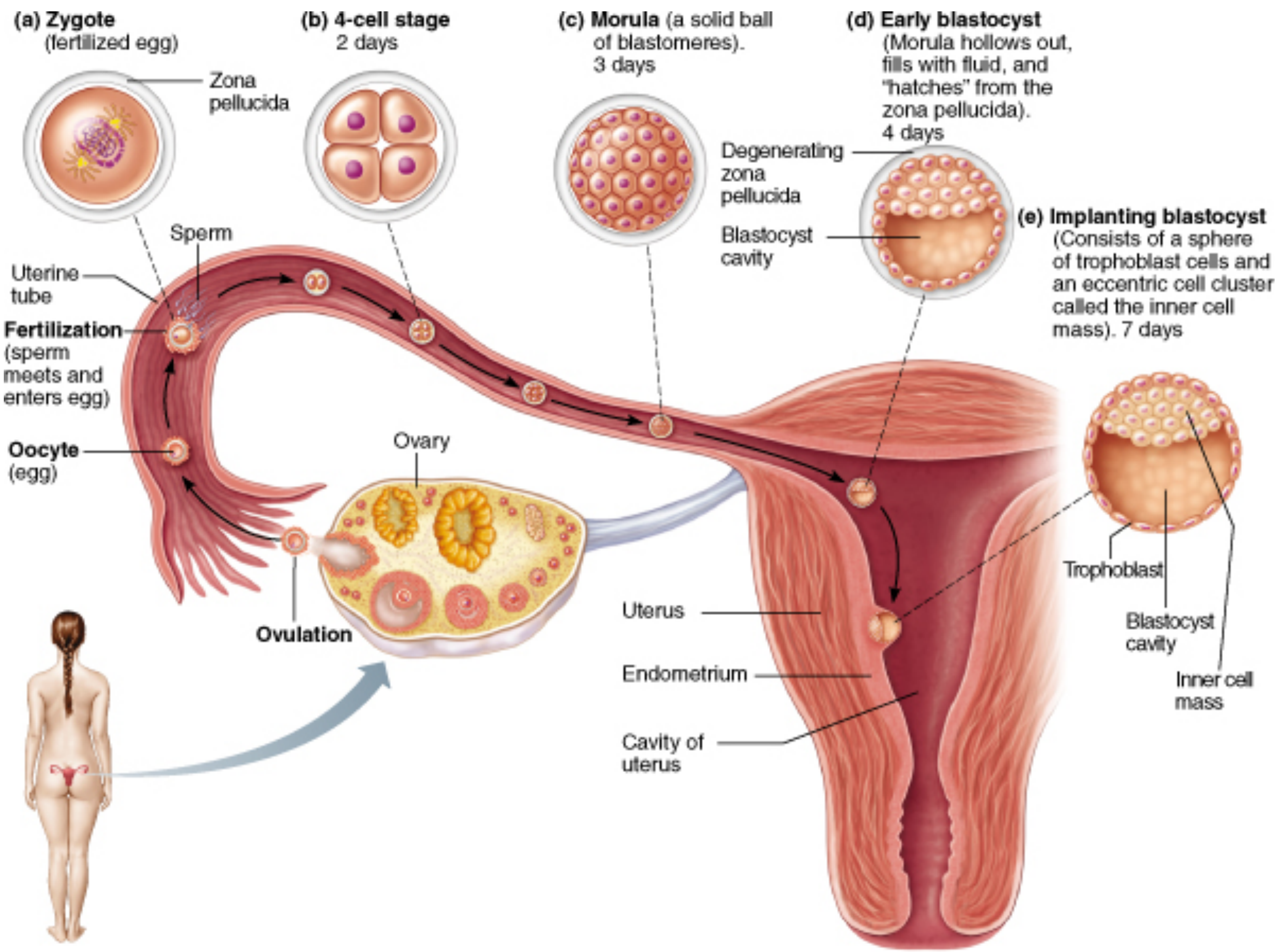
↓

Studies on embryo/oviduct biochemical interactions

Physiology of embryo development in the pre-implantation period

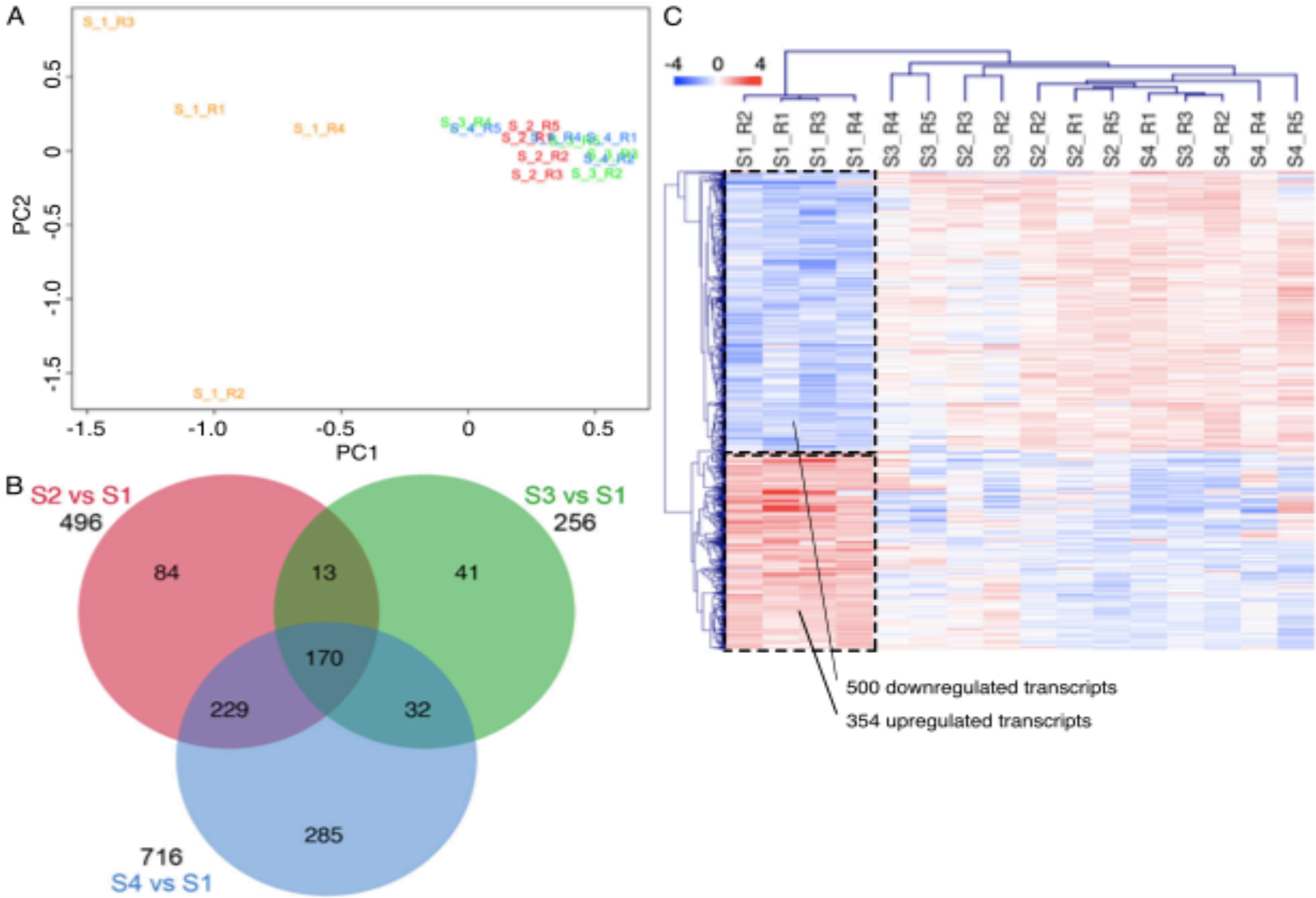
- Oviductal microenvironment
- Stages of embryo development
- Cleavages
- Morula formation and compaction
- Blastocyst formation
- Embryo mortality

Stages of embryo development within the oviduct



The oviductal environment

Recently decifered oviductal vesicles content in proteins, mRNA and small ncRNA across the estrous cycle in the cow (**13.197 genes**)



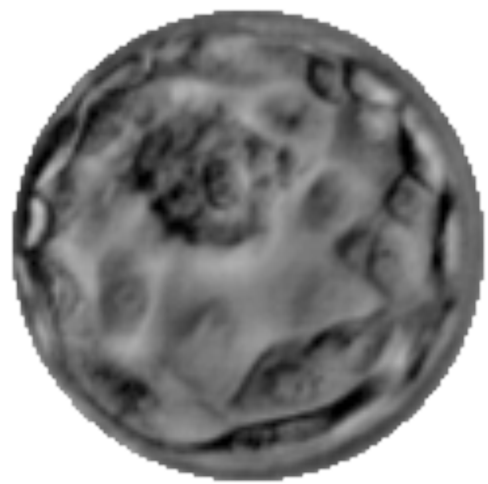
Stages of human embryo development

2PB 2PN

4 cell stage

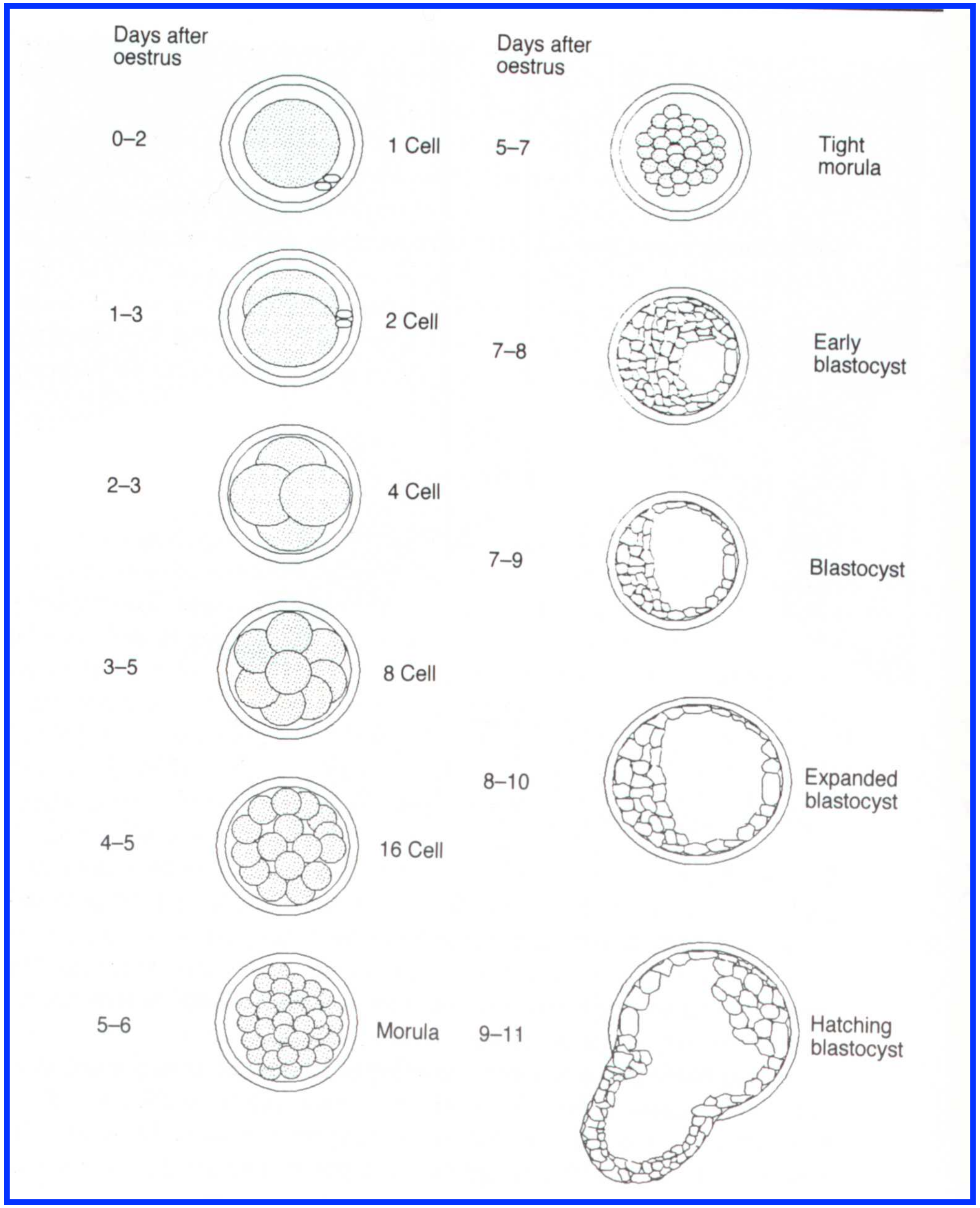
8 cell stage

morula

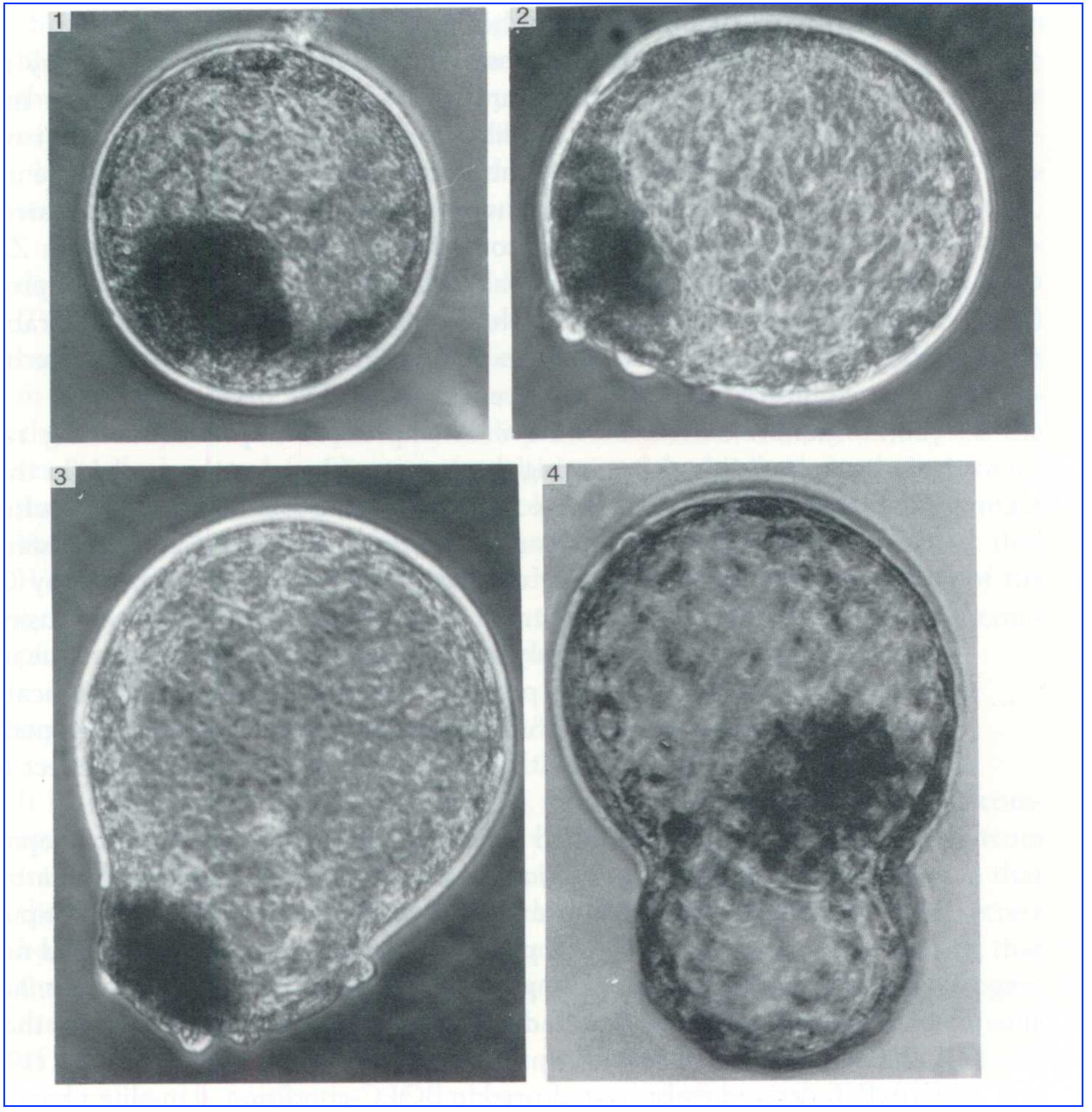


blastocyst

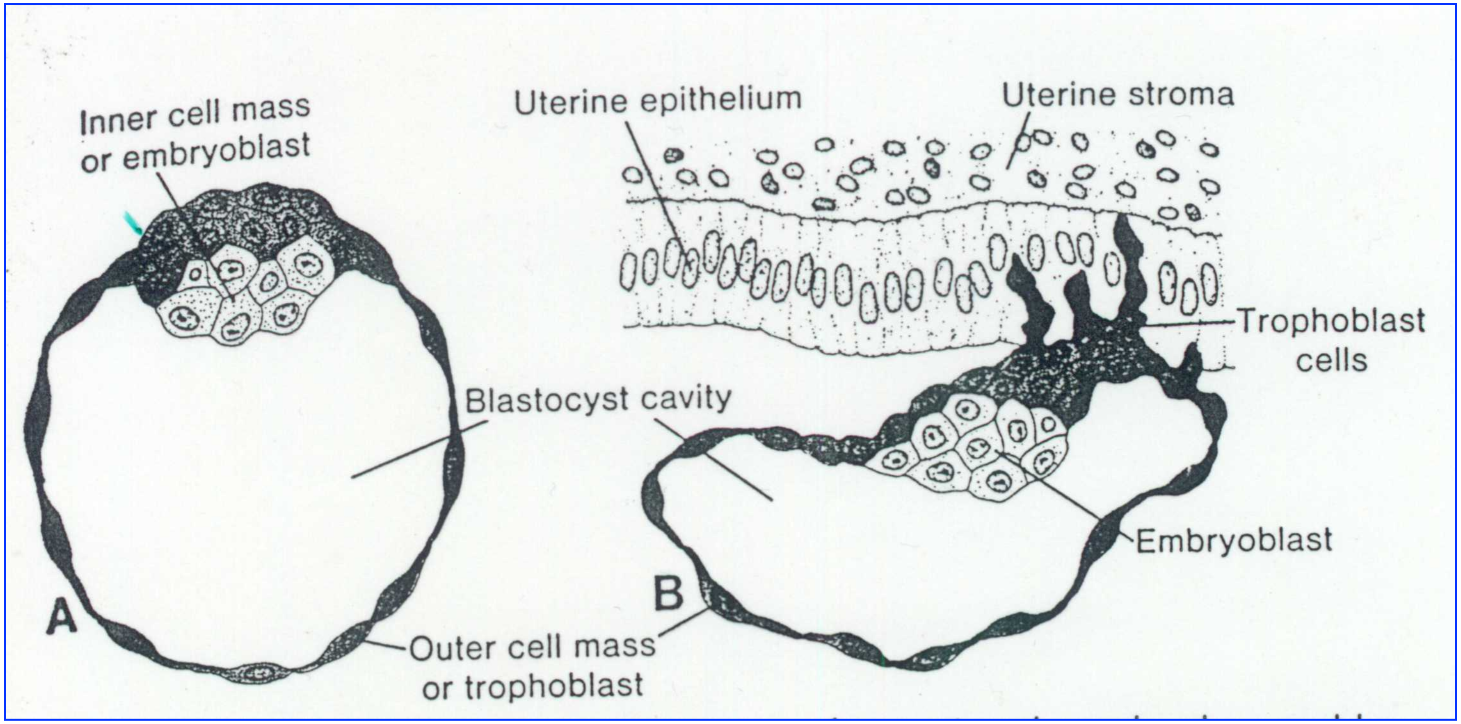
Assessment of bovine embryo quality as a model for human embryo development



The hatching process in a bovine embryo



Embryo implantation to the uterine wall



Times of arrival in the uterine cavity and implantation in different species

Species	Arrival (d)	Implant (d)
Bovine	3-4	12
Bitch	5-8	13-17
Queen	5-8	13-17
Sheep	3	15
Mare	5-6	25-30
Sow	2	2
Woman	4	5-6

Causes of embryo mortality

- Genetic factors:
- chromosomal abnormalities
- single gene defects
- Maternal age
- Endocrine factors
- Nutritional factors
- Stress factors
- Internal environmental factors
- External environmental factors

Embryo metabolism

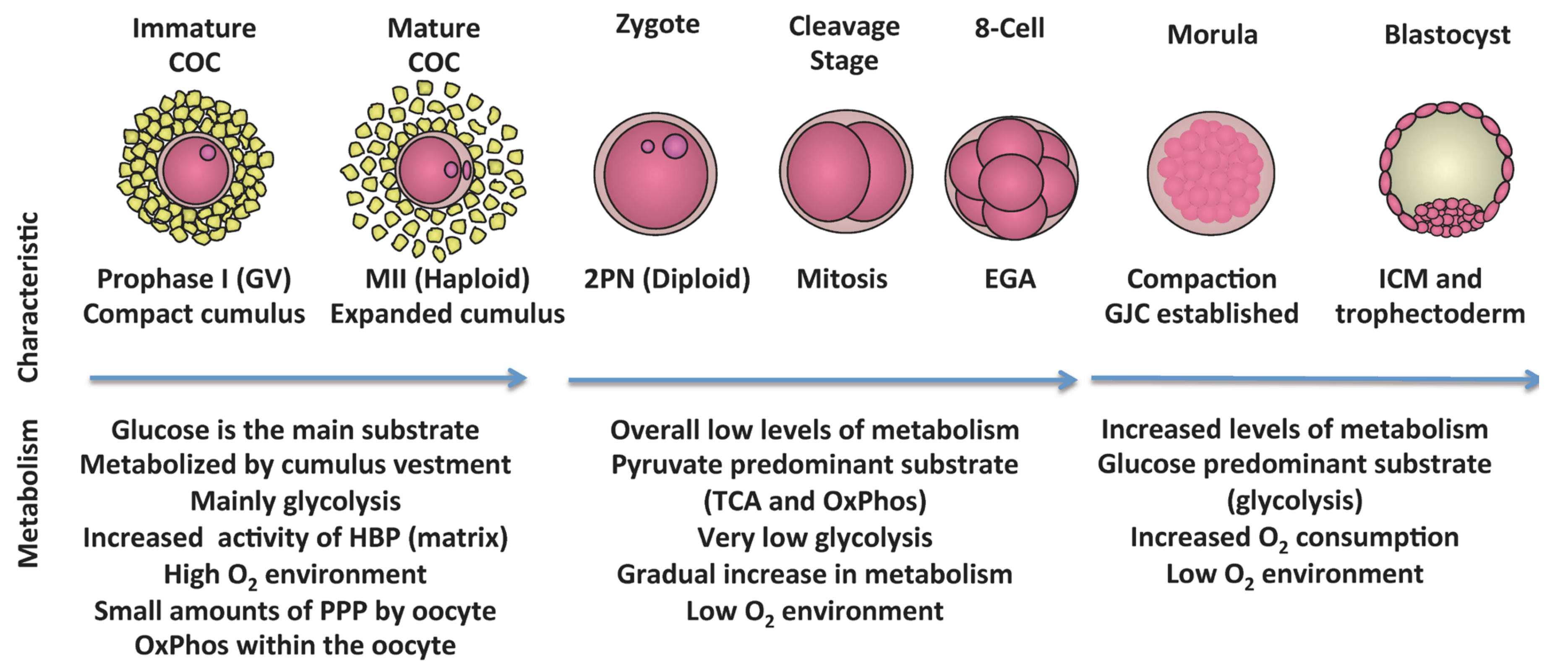
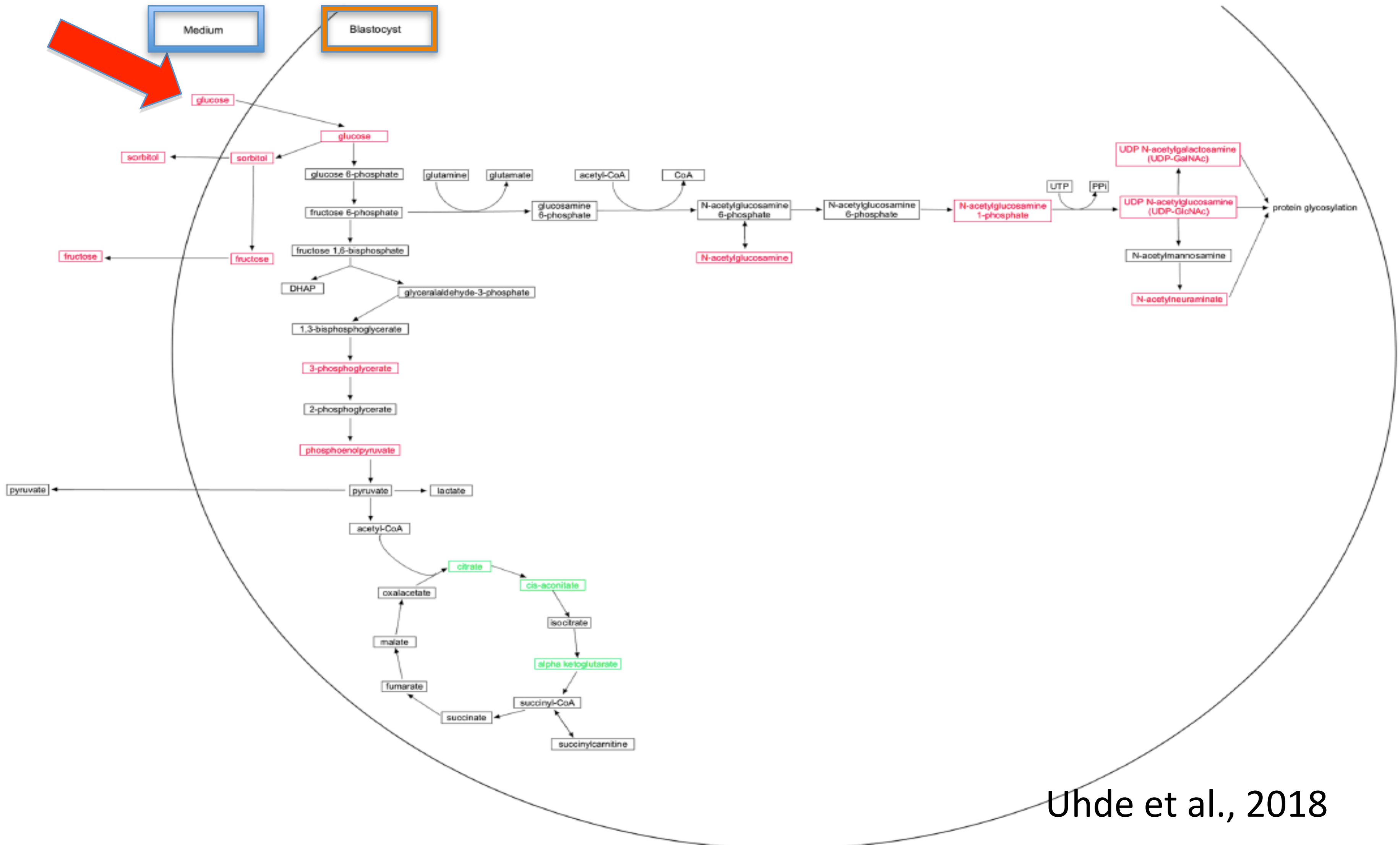


Figure 1. Changes in the metabolism of cumulus oocyte complexes (COCs) and preimplantation embryos. 2PN = 2 pronuclei; GJC = gap junction communication; GV = germinal vesicle; HBP = hexosamine biosynthetic pathway; ICM = inner cell mass; OxPhos = oxidative phosphorylation and TCA cycle = tricarboxylic acid cycle.

Sutton-McDowall & Thompson, 2015

Embryo metabolism:

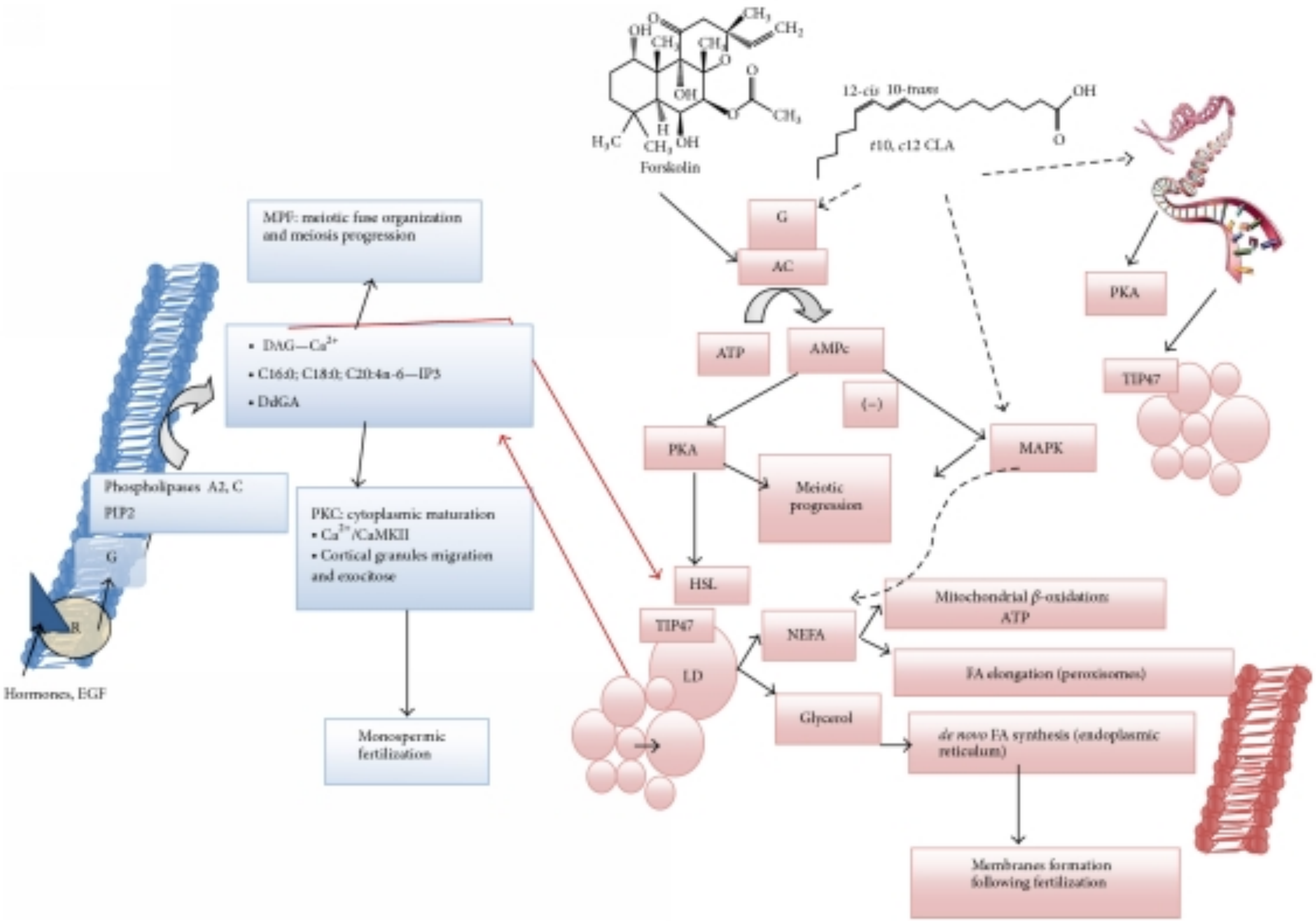
the importance of glucose levels



Uhde et al., 2018

Embryo metabolism:

Fatty acid metabolis (Prates et al., 2014)



Embryo genome activation (EGA) and the developmental arrest

- Embryo genome activation, in different species, occurs at different developmental stages

- Mouse, Goat, Hamster: 2 cell stage

- Cow, Sheep: 8-16 cell stage

- Pig: 4 cell stage

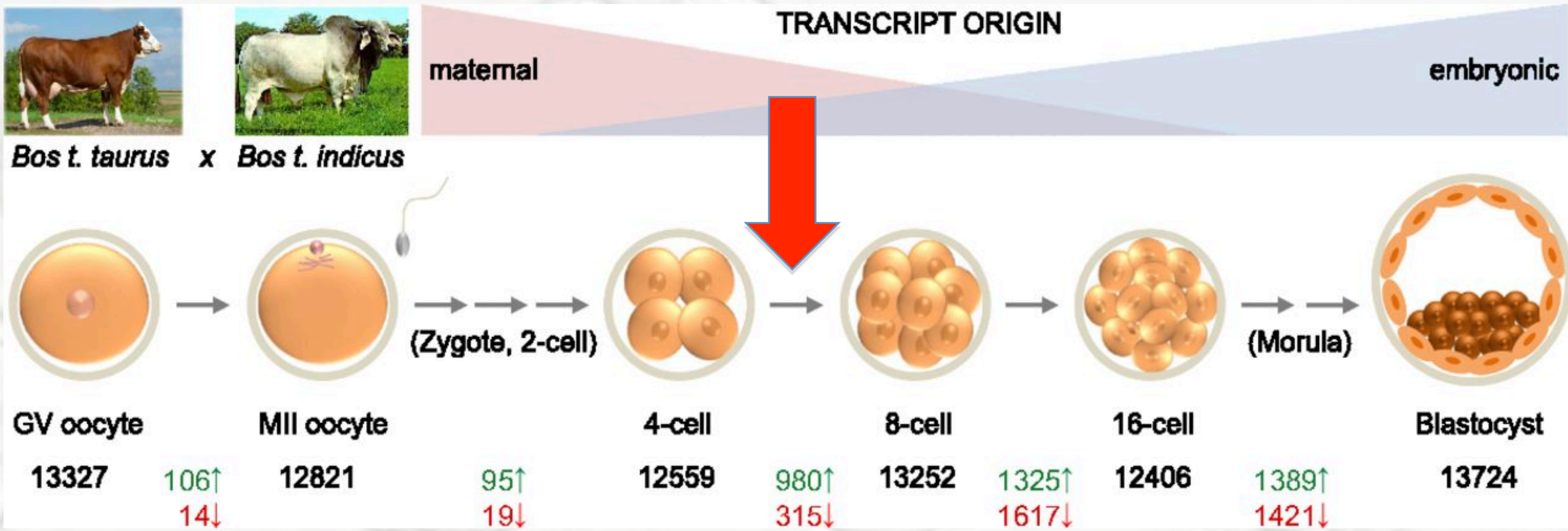
- Human: 4-8 cell stage

Embryo differentiation

Hierarchic organization of genes coding for factors involved in the differentiation processes:

- **Maternal effects genes** (expressed in the stages comprised from oocyte maturation to initial embryo cleavages)
- **Gap genes** (expressed in subsequent stages and coding for basic cell functions, such as nucleus functions, cytoskeleton elements, membrane channels, ion transporters, surphace or secretory proteins, growth factors, receptors, …)
- **Genes for segmentation and embryo polarity** (genes controlling the formation pattern of the embryo antero-posterior axis)
- **Homeotic genes** (homeo-box containing genes, for segment-specific genes)
- **Tissue-specific genes**

Embryo genome activation and developmental arrest



Dean J, NIH Research Images

PNAS

Fine mapping of genome activation in bovine embryos by RNA sequencing

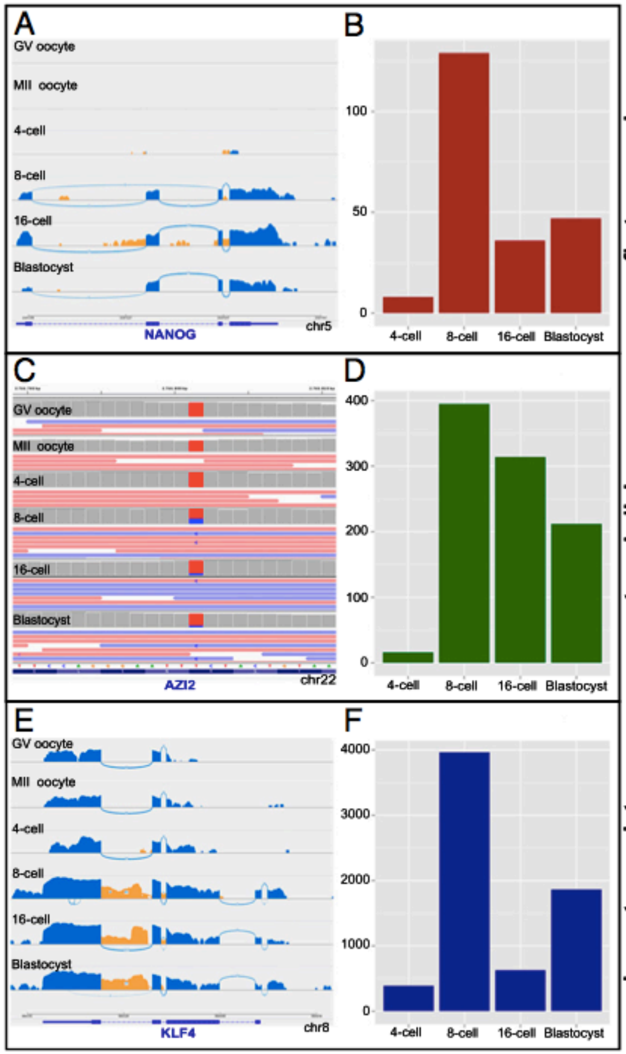
Alexander Graf[a], Stefan Krebs[a], Valeri Zakhartchenko[b], Björn Schwalb[c], Helmut Blum[a,1,2], and Eckhard Wolf[a,b,1,2]

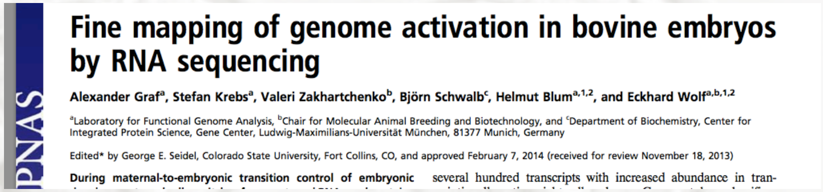
[a]Laboratory for Functional Genome Analysis, [b]Chair for Molecular Animal Breeding and Biotechnology, and [c]Department of Biochemistry, Center for Integrated Protein Science, Gene Center, Ludwig-Maximilians-Universität München, 81377 Munich, Germany

Edited* by George E. Seidel, Colorado State University, Fort Collins, CO, and approved February 7, 2014 (received for review November 18, 2013)

During maternal-to-embryonic transition control of embryonic several hundred transcripts with increased abundance in tran-

Early embryo genome activation and developmental arrest

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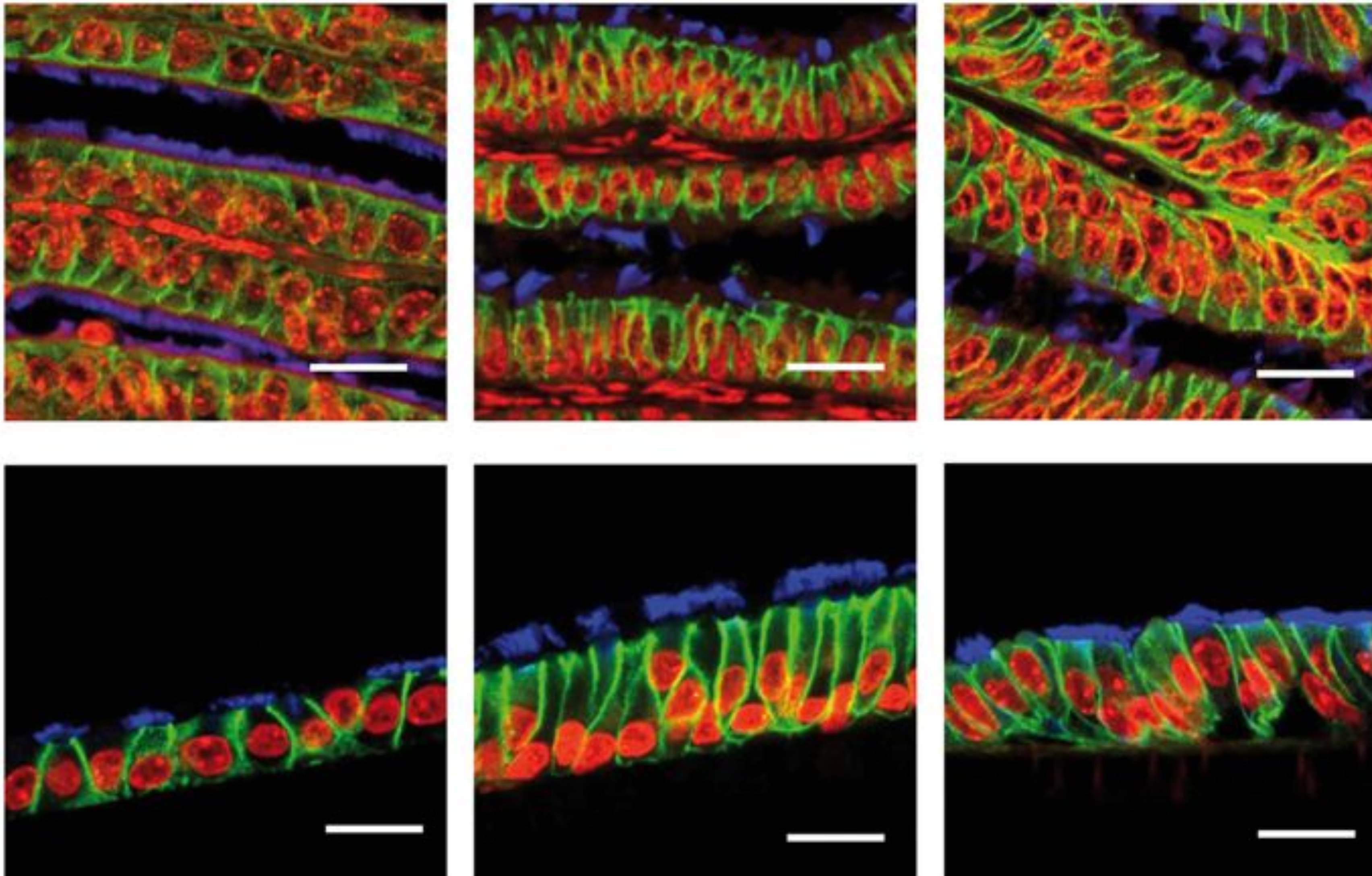
Epithelial markers in murine, porcine and bovine OECs



(Chen et al., 2017)

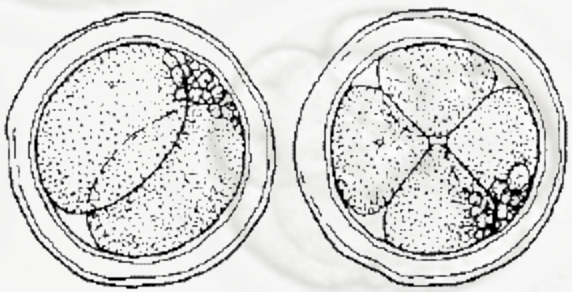
Embryo quality assessment

- Morphologic and morphometric analysis
- Correspondence between the culture time and the embryo developmental stage (time course)
- Correspondence between the number of blastomeres and nuclei
- Blastocyst expansion and hatching
- Metabolic tests
- Genomics
- Transcriptomics
- Proteomics
- Metabolomics

Conventional embryo morphology assessment (CMA)

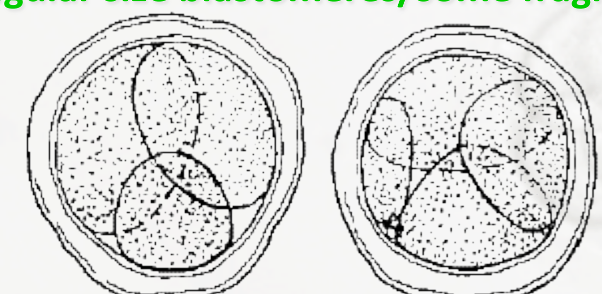
Grade 1

Regular size blastomeres/no fragments



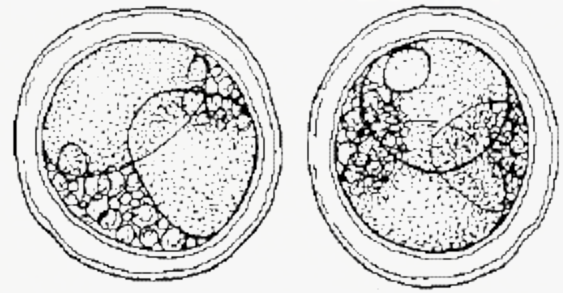
Grade 2

Regular size blastomeres/some fragments



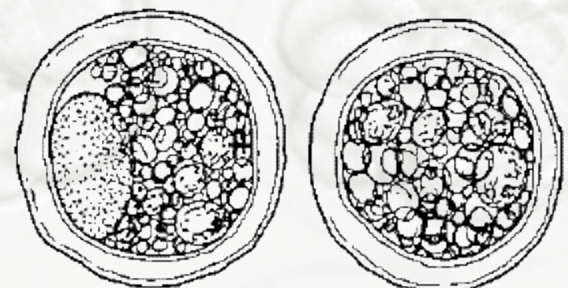
Grade 3

Irregular size blastomeres



Grade 4

Reg-irreg blastomeres/many fragments



Grade 5

Few blastomeres/severe fragmentation

Non invasive pre-implantation genetic screening (NIPGS)

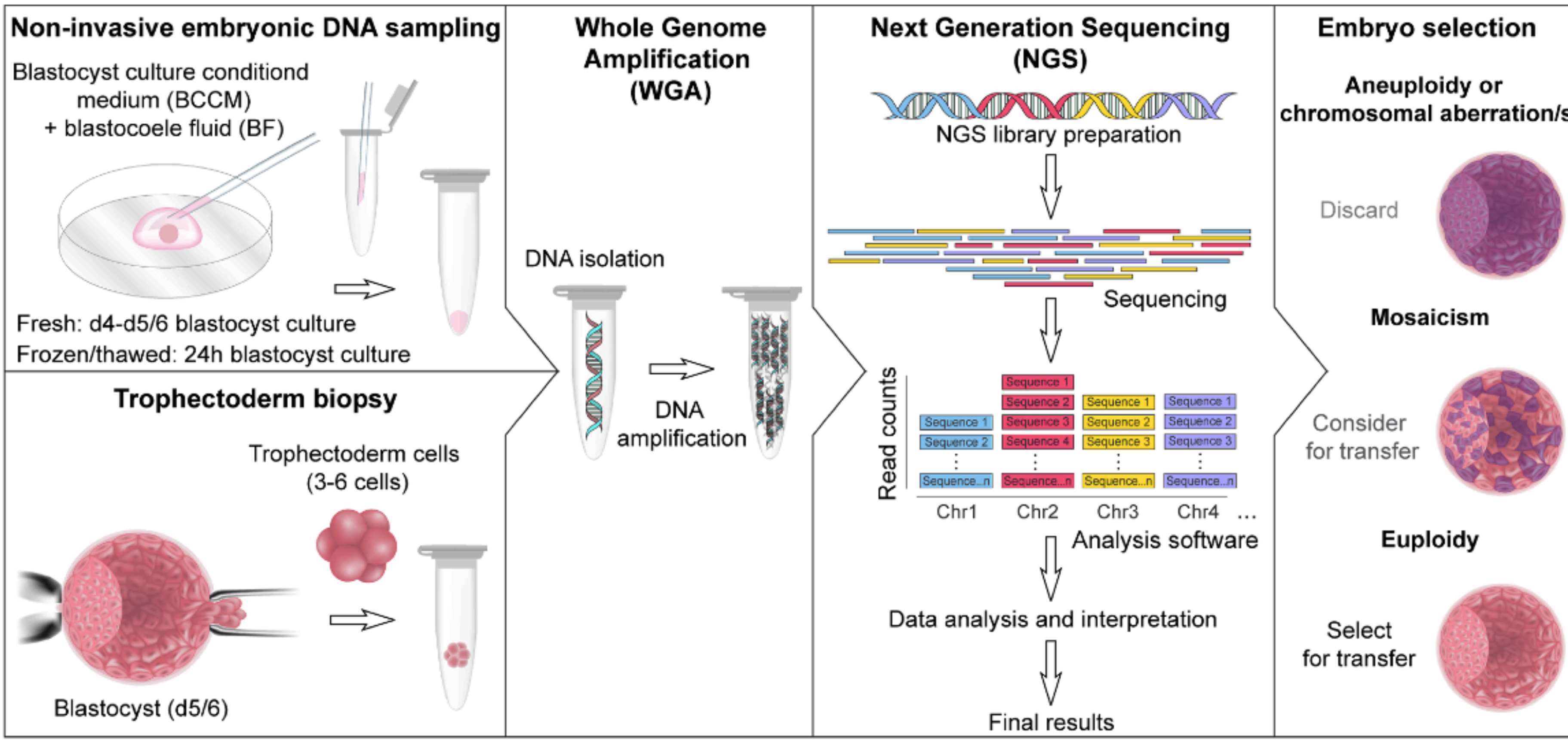


Fig 1. Non-invasive and invasive preimplantation genetic testing workflow.

https://doi.org/10.1371/journal.pone.0197262.g001

Metabolic tests to evaluate embryo viability

- Lactic dehydrogenase (LDH) activity
- Glucose, Glutamine, Pyruvate uptake
- Oxygen uptake

References

- “Culturing and evaluating the early embryo” in: Gordon I. Laboratory production of cattle embryo. CABI publications 2003, Chapter 7;
- “Assisted reproductive technologies and embryo culture methods for farm animals” in : Pinkert CA. Transgenic Animal Technology – A laboratory handbook. 2002. Chap 20.