Strategy of application of cell reproductive and DNA technologies in cattle breeding

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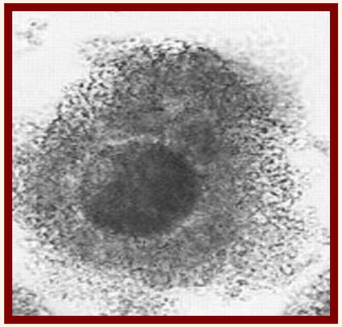
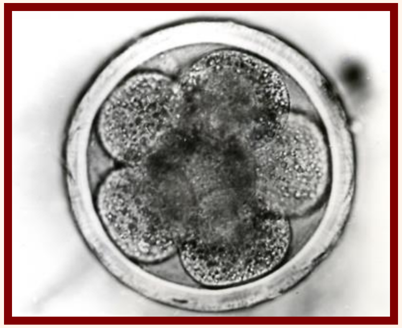
From



oocyte

to



embryo

S.Petersburg-Pushkin
2018

Cell reproductive technologies in cattle breeding

- Artificial insemination
- Sexed semen
- In vivo production of embryos (MOET)
- Ovum pick up technology
- IVM, IVF technology
- Cryopreservation of oocytes and embryos
- Cloning

“Omics” technologies

Investigate the genome and epigenome

Genotyping technologies

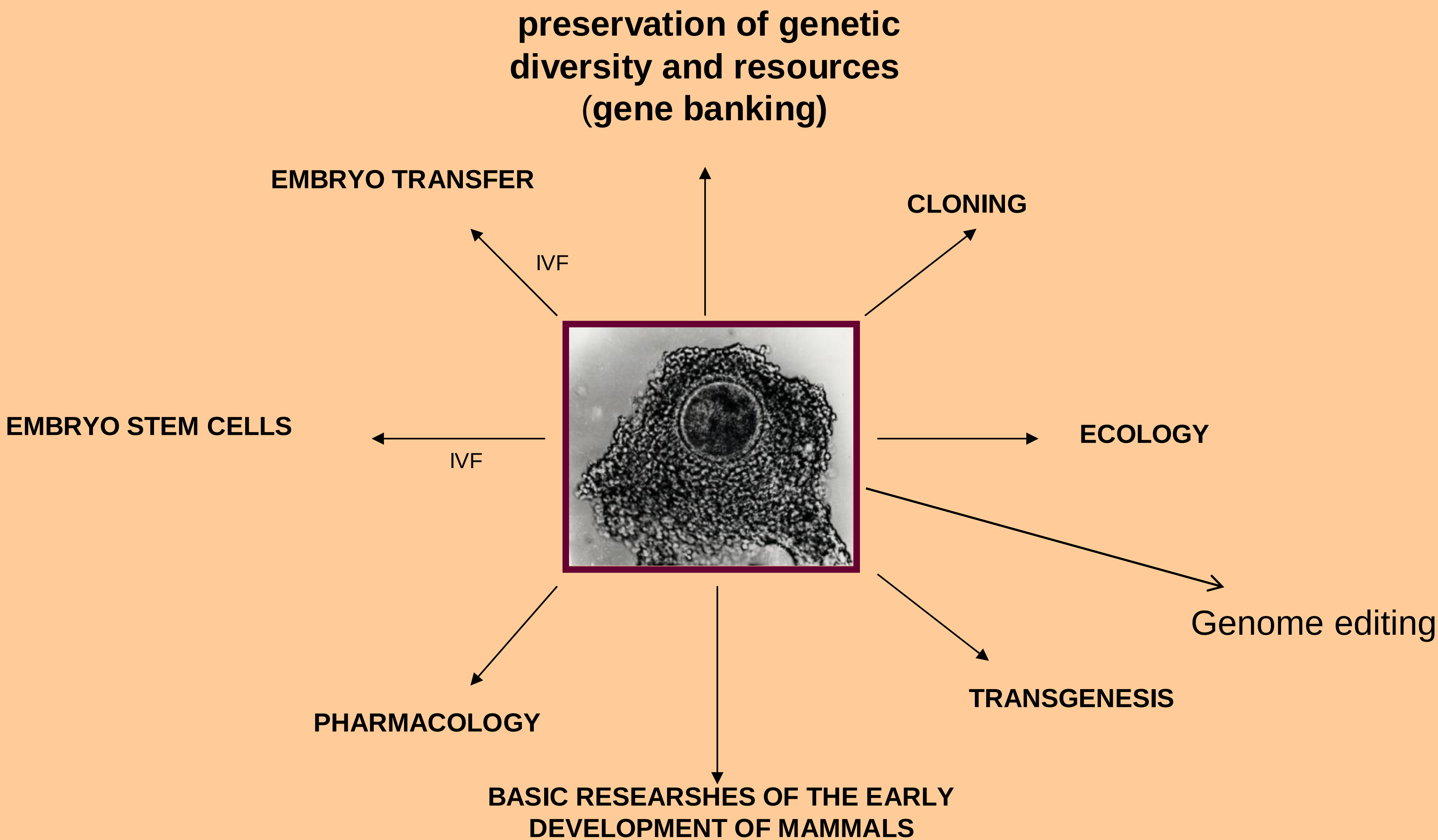
Marker-assisted/genomic selection

Methylation and gene expression analyses

Genetic modification

Genetic engineering

Genome editing



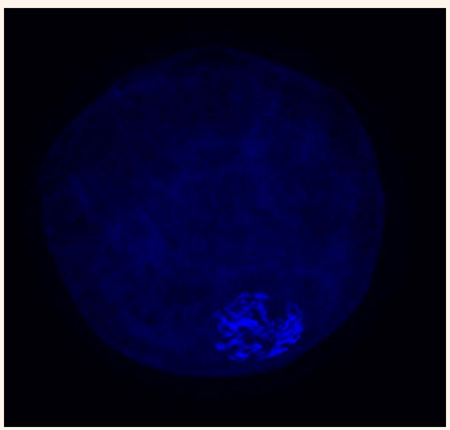
Maturation of donor's oocytes in vitro – basic method for in vitro embryo production, cloning, transgenesis, obtaining of embryo stem cells, genome editing

This topic will focus on the common limiting factors of the first step of these technologies: developmental competence of oocytes.

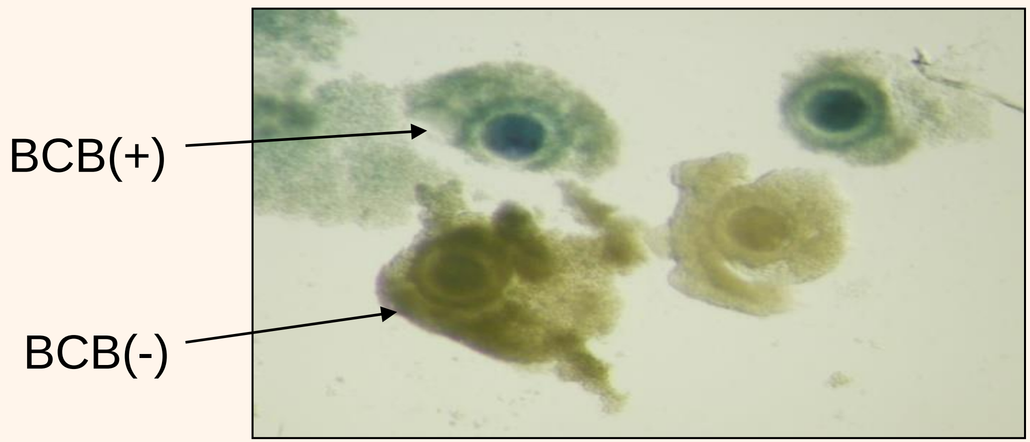
Table. BCB-test of bovine oocytes obtained from follicles depending on diameter

BCB test	Diameter of follicles		
	<3 mm n(%) oocytes	3-6 mm n(%) oocytes	6-8 mm n(%) oocytes
BCB(+)	**73 (58)[a]**	**23 (79)[b]**	**4 (69)[c]**
BCB(-)	**53 (42)**	**6 (21)**	**9 (31)**

[a:b, a:c] $P < 0.05$; (***Mann-Whitney*** U-test)



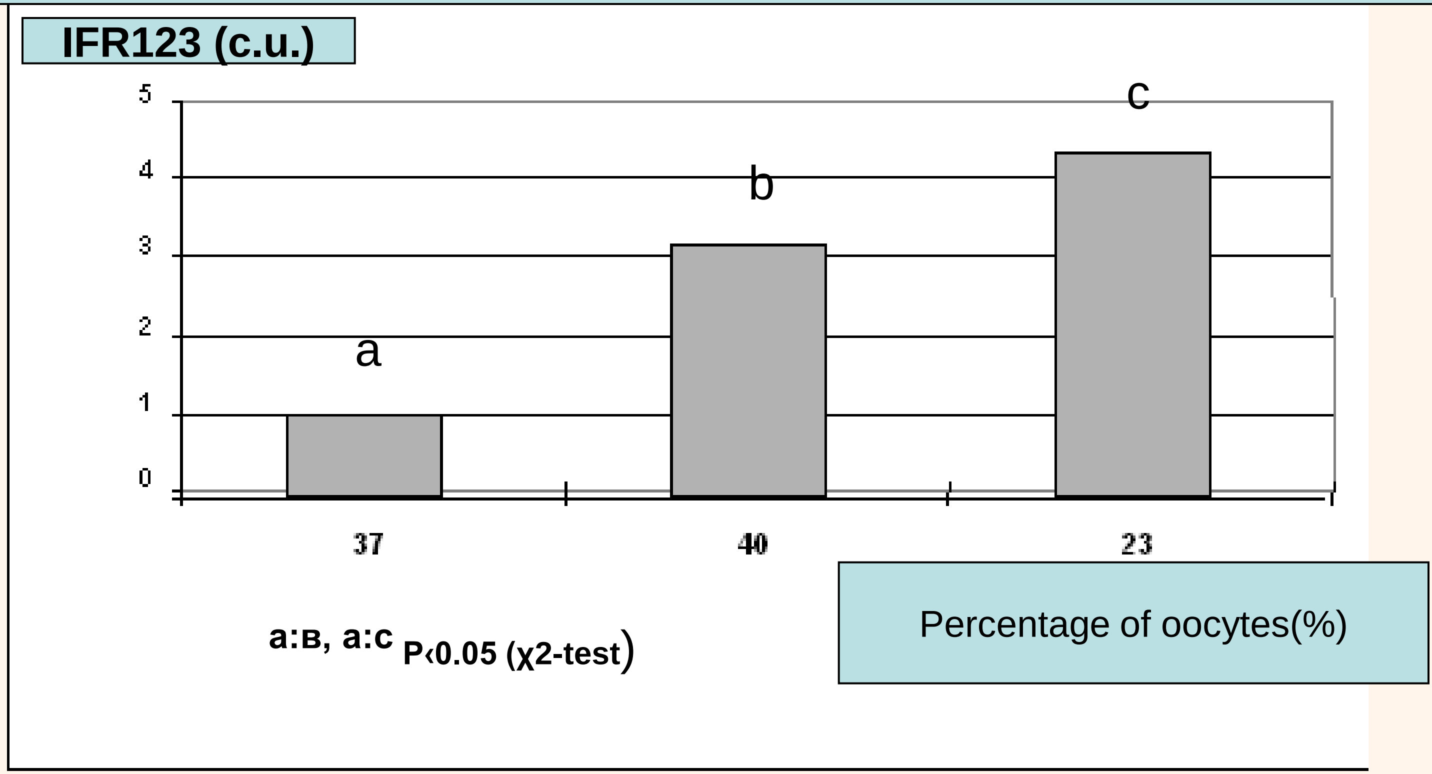
Chromatin of oocyte on diplotene stage

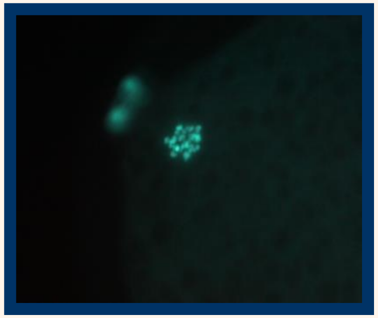


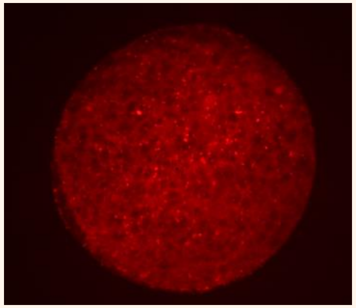
BCB(brillant cresyl blue) test of bovine oocytes

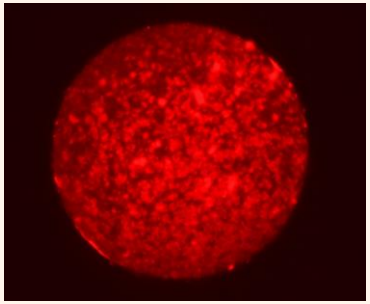
Oocyte selection based on glucose-6-phosphate dehydrogenase (G6PDH) activity has been successfully used to differentiate between competent and incompetent bovine oocytes (X.Alm et al., 2005, T.Kuzmina et al., 2009). 79% of bovine oocytes evaluated as competent were found in follicles (diameter 3-5 mm) after tested by this marker.

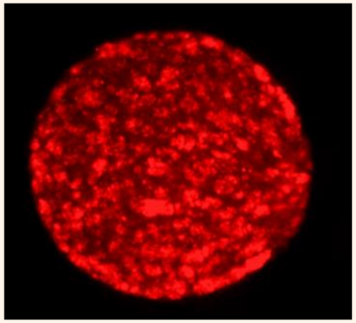
Intensity of fluorescence of rhodamine 123(IFR123) in bovine oocytes matured in vitro (n=278)





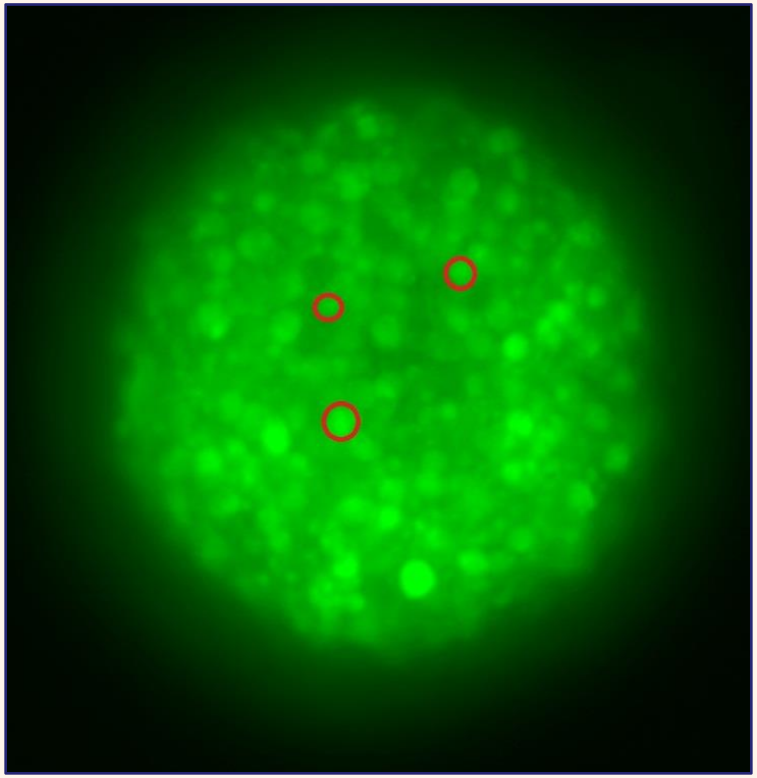




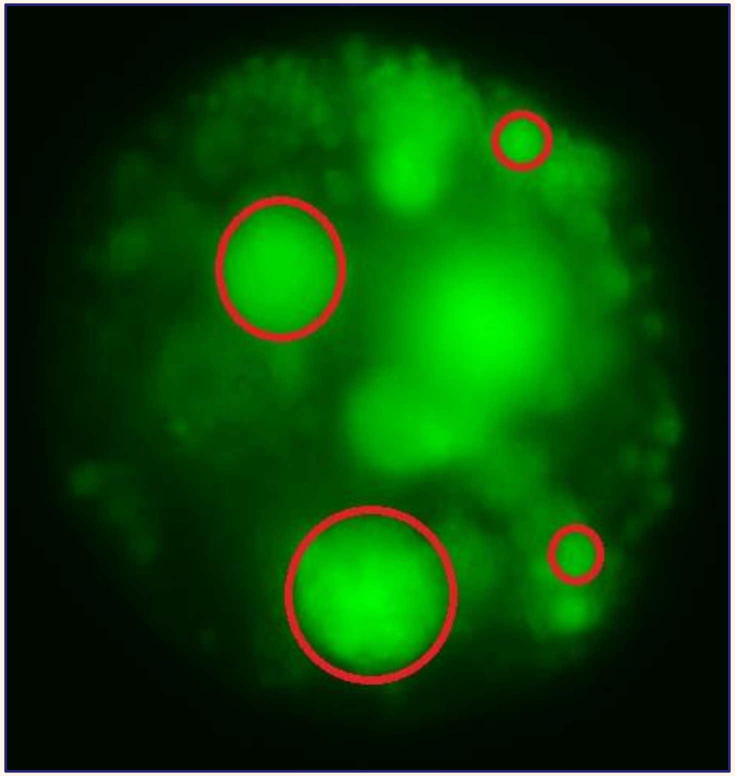


Morphology of intracytoplasmic lipid droplets in oocytes (Nile red visualization)

Granules



Clusters



The scale is 200 μm. Magnification - 10x20

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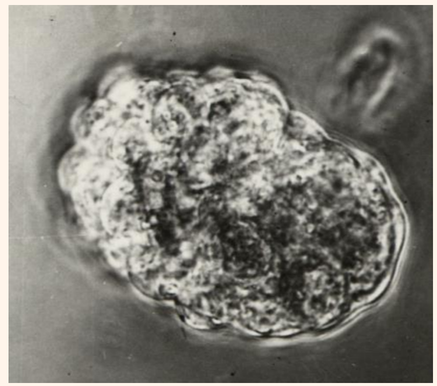
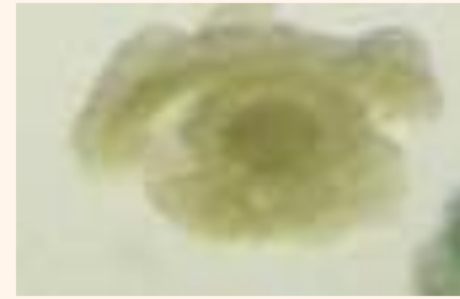
Modeling of in vitro maturation system

BCB-test



BCB(+) oocytes that have finished growth phase in vivo
(colored cytoplasm)



BCB(-) growing oocytes
(uncolored cytoplasm)

Table 1: Effect of prolactin on developmental competence of bovine oocytes selected by brilliant cresyl blue staining

Treatment during IVM	BCB	N. oocytes	8-16 cells embryo n (%)	Blastrocyst n (%)
TCM 199 + 10% FCS + 10^6 GC	+	141	89(63.1)[a]	19 (21.3)[a]
TCM 199 + 10% FCS + 10^6 GC	-	137	66(48.2)[b]	3 (4.5)[b]
TCM 199 + 10% FCS + 10^6 GC + 50 ng/ml PRL	+	133	101 (75.9)[c]	38 (37.6)[c]
TCM 199 + 10% FCS + 10^6 GC + 50 ng/ml PRL	-	139	87 (62.6)[d]	17 (19.5)[d]

a:b, a:c, b:d, c:d $P<0.05$

Thanks for your attention

БЛАГОДАРЮ ЗА ВНИМАНИЕ

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