

Developmental capacity of bovine oocytes following JC-1 staining

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Mitochondrial functions are vital for oocyte and embryo development. JC-1 staining is commonly used to assess mitochondrial activity. However, to our knowledge, JC-1 was never tested as a non-invasive marker for oocyte quality. We examined the effect of JC-1 when used at the end of in vitro oocytes maturation on subsequent embryo development. Bovine cumulus oocyte complexes (COCs, n=527, 3 replicates) were matured for 22h then randomly allocated to the following processing steps: Intact COCs (CUM), denuded oocytes (DEN), denuded and stained with JC-1 (5 µg/mL), with (CON) or without (JCE) confocal microscopy. All steps were done within 2h in HEPES-TALP media at 37°C. At 24h, oocytes from each processing step were fertilized (Day 0, Fert-TALP medium) and cultured (mSOF medium with 5% fetal bovine serum) in 384-well plates either individually (n=15-20/replicate, 30µl/well) or in a group of 20-25 (75µl/well). Embryo cleavage (Day 2) and blastocyst rates (D7.5) were recorded and analyzed by Wald-Chi square test and Bonferroni. In group fertilization and culture, cleavage rates in DEN, JCE, and CON were significantly ($P<0.05$) lower than CUM (59.2, 41.3, and 38.2 vs. 85.7%, respectively). Blastocyst rate was not affected in DEN (18.4%) but significantly reduced ($P<0.05$) in JCE (8.7%) and CON (8.8%) compared to CUM (31.0%). Similar effects were seen in individual culture; cleavage rates: 50.5*, 48.4*, and 40.5* vs. 84.1%, blastocyst rates: 15.2, 4.3*, 5.0* vs. 29.5%, respectively (* $P<0.05$). Therefore, in addition to the reduction in fertilization rates due to removal of cumulus cells, mitochondrial staining with JC-1 significantly reduces the oocyte's developmental capacity. W. Marei is supported by an FWO postdoctoral fellowship 1211417N.

	n	Cleavage (%)	4-cell embryos (%)	Blastocysts /total (%)	Blastocysts /Cleaved (%)
CUM group	88	85.7	69	31	36.1
DEN group	93	59.2	40.8	18.4	31
JCE group	91	41.3	26.1	8.7	21.1
CONFE group	84	38.2	23.5	8.82	23.1

	n	Cleavage (%)	4-cell embryos (%)	Blastocysts /total (%)	Blastocysts /Cleaved (%)
CUM Single	42	84.1	50	29.5	35.1
DEN Single	49	50.5	35.5	15.2	14.9
JCE Single	46	48.4	22	4.35	4.55
CONFE Single	34	40.5	22.6	5	5.88

Group culture	Cumulus enclosed (CUM)	Denuded		
		Not stained (DEN)	JC-1 Stained (JCE)	JC-1 + confocal CONFE
n	88	93	91	84
Cleavage rate (%)	85.7	59.2	41.3	38.2
4-cell embryos (%)	69.0	40.8	26.1	23.5
Blastocysts/total (%)	31.0	18.4	8.7	8.82
Blastocysts/cleaved (%)	36.1	31	21.1	23.1

Individual culture	Cumulus enclosed (CUM)	Denuded		
		Not stained (DEN)	JC-1 Stained (JCE)	JC-1 + confocal CONFE
n	42	49	46	34
Cleavage rate (%)	84.1	50.5	48.4	40.5
4-cell embryos (%)	50.0	35.5	22.0	22.6
Blastocysts/total (%)	29.5	15.2	4.35	5.0
Blastocysts/cleaved (%)	35.1	14.9	4.55	5.88