

7. Summary

Aggregation and activity of mitochondria during in vitro maturation of cattle oocytes

The activity and the status of aggregation of mitochondria during in vitro maturation of cattle oocytes were examined in relation to cumulus-oocyte morphology and chromatin configuration of oocytes. In addition the rate of apoptosis was determined in oocytes and cumulus cells. The results were correlated with the developmental capacity of oocytes after IVM and IVF.

Freshly recovered cumulus-oocyte-complexes (COCs) were evaluated morphologically and classified into 4 groups: Compact cumulus oophorus (class I), slightly expanded cumulus oophorus (class II), expanded cumulus cells (class III) and denuded oocytes (class IV).

Mitochondrial parameters and chromatin configuration were determined in a total of 1473 COCs at 0, 4, 8, 12, 16, 20 and 24 hrs of IVM. The rate of apoptosis in cumulus cells was examined in 855 COCs. The activity of caspase-3 was used to determine apoptosis in 104 oocytes and their cumulus cells. 1104 COCs were subjected to IVM, IVF and IVC to test the developmental capacity of oocytes from different classes.

There was a clear relationship between chromatin configuration and morphology of freshly recovered COCs. Oocytes of class III and IV were in advanced stages of meiosis. More oocytes of class II showed diakinesis after 4 hrs of IVM when compared to class I oocytes. After 20 hrs of IVM, the percentage of oocytes in metaphase II was 16,2%, 43% and 70% in class I, II and III oocytes, respectively. The percentage of mature oocytes after 24 hrs of IVM was 56,5%. Signs of degeneration and activation could be observed in class III oocytes at the end of the IVM period. Meiosis was mostly irregular in class IV oocytes and these oocytes showed frequently signs of degeneration and activated chromatin.

Mitochondrial aggregation and activity was correlated with time of IVM, morphology of COCs and chromatin configuration. Oocytes from class I and II had evenly finely dispersed mitochondria which turned into a crystalline appearance during IVM, mostly notably at time when energy consuming events like MI-spindle formation or extrusion of the first polar body occurred. Only in class II oocytes these changes could be detected as early as 20 hrs after start of IVM. An increase of mitochondrial activity could be observed in the early phase of IVM in class I and II oocytes, the latter having the highest increase of activity. Diplotan, diakinesis and metaphase II were the stages of meiosis with the highest mitochondrial activity of oocytes.

Apoptosis of cumulus cells increased during IVM, especially in class III oocytes.

The active form of caspase-3 could be observed in cumulus cells of COCs from class I-IV. The highest activity was recorded at 12 h of IVM in class III COCs. In oocytes from all classes procaspase-3 (inactive form of caspase-3), but no active caspase-3, could be observed.

Oocytes of class I and II provided the highest rates of cleavage and blastocyst formation. The best results, though not statistically significant were obtained with class II oocytes. Oocytes from class IV did not develop to blastocysts.

Morphology of COCs was clearly correlated with chromatin configuration, mitochondrial status and developmental competence. Oocytes from class II showed strong mitochondrial activity early during IVM and developed a crystalline appearance of mitochondria already after 20 hrs of IVM which was accompanied by the extrusion of the first polar body (Metaphase II). These events might be correlated with the slightly better developmental competence of class II oocytes when compared to class I oocytes. The mitochondrial parameters investigated in this study may be helpful to assess the quality of oocytes in cattle.