# Session 9: THE EFFECT OF CHOLINE AND METHIONINE ON OXIDATIVE STRESS IN A BOVINE MAMMARY EPITHELIAL CELL LINE

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## State of the art

- The bovine mammary gland is a target tissue for choline both for secretion in milk and for the maintenance of tissue integrity.
  - A bovine mammary gland yielding 25l milk secretes 10±3g phospholipids per day, corresponding on average to 5% of the phospholipids of the mammary tissue.
- The above data suggest that choline is an important metabolite in lactating mammary tissue, that it is used avidly when available.
- Choline metabolism is closely linked to that of methionine.
- Studies also indicate that interaction of methyl group metabolism and choline with other essential nutrients and oxidative stress are incomplete.

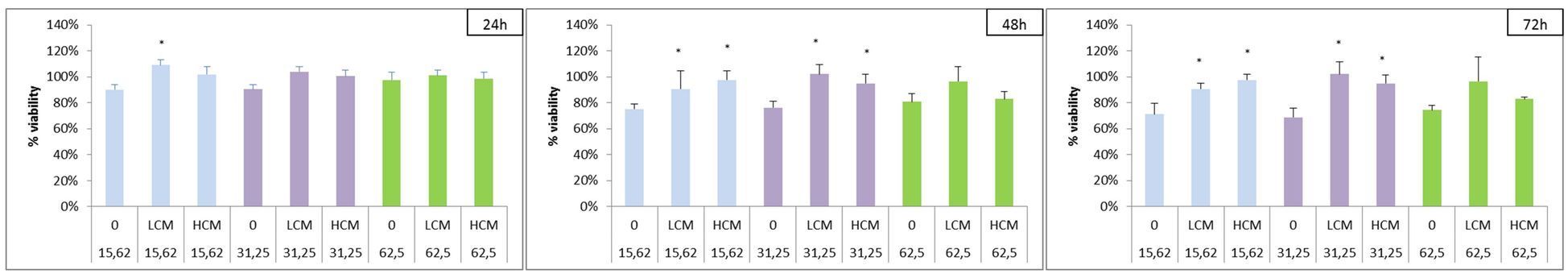
***Aim*** to evaluate the effect of hydrogen peroxide exposure on the survival and viability of bovine mammary epithelial cells in presence of choline and methionine.

## Materials & Methods

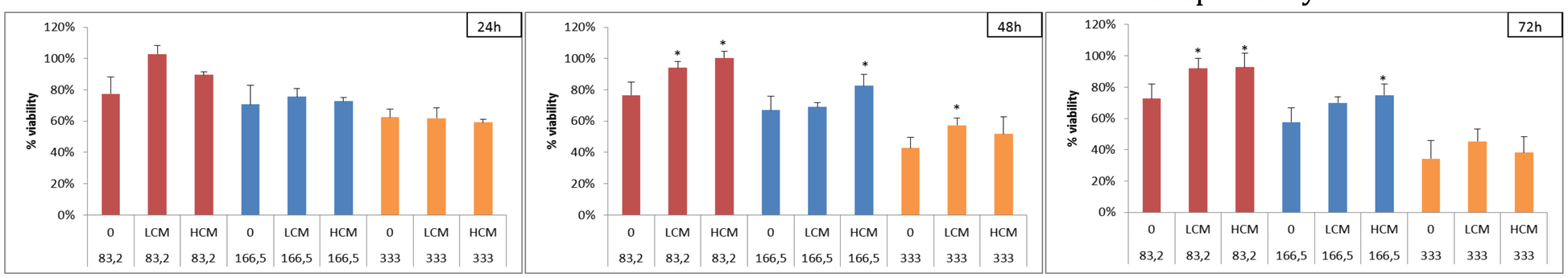
- The BME-UV1 cell line has been used as the in vitro model of the bovine mammary epithelium.
- Cells were incubated with choline and methionine at two different concentrations:
  - LCM: 500µM choline and 715µM methionine
  - HCM: 1000µM choline and 1430µM methionine
  - The ratio between Cho and Met has been established on molar basis and insulin (1µg/ml) has been included in order to support their uptake.
- The cells were exposed to low dosage (from 15.62 up to 62.5µM) and high dosage (from 83.2 up to 333µM) of hydrogen peroxide in presence or absence (0) of LCM and HCM .
- Membrane damage (LDH release), cell proliferation (MTT test) and apoptosis (TUNEL assay) were measured at different incubation times (24, 48 and 72h).

## Results

- The half lethal concentrations ($LC_{50}$) of hydrogen peroxide were 376.5µM, 249.9µM and 244.9µM after 24, 48 and 72h, respectively.
- The membrane damage (% LDH release) caused by hydrogen peroxide addition after 24 h was not significantly different to control (4% ) up to 333µM $H_2O_2$ (10%). After 48 and 72h LDH release was about 32-35% when $H_2O_2$ was present at the maximum concentration tested.

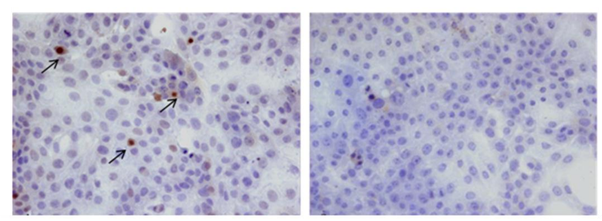



At the lowest range of $H_2O_2$ concentration tested (15.62 to 62.5µM) choline and methionine significantly ($P \leq 0.05$) enhanced cell viability on average by 19%, 21% and 25.8% after 24, 48 and 72h, respectively.




At the highest range of $H_2O_2$ concentration used (83.2 to 333µM) choline and methionine significantly ($P \leq 0.05$) enhanced cell viability on average by 15% and 17% after 48 and 72h, respectively.

When apoptosis (TUNEL assay) was considered, presence of supplemental choline and methionine in the medium exerted a dose dependent effect on BME-UV1 cells, reducing the cell death according to the nutrients concentration.




## Conclusions

Our results indicate that choline and methionine could play a role in counteracting oxidative damage induced by hydrogen peroxide in bovine mammary epithelial cells, even though the real mechanism merit further investigations.

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