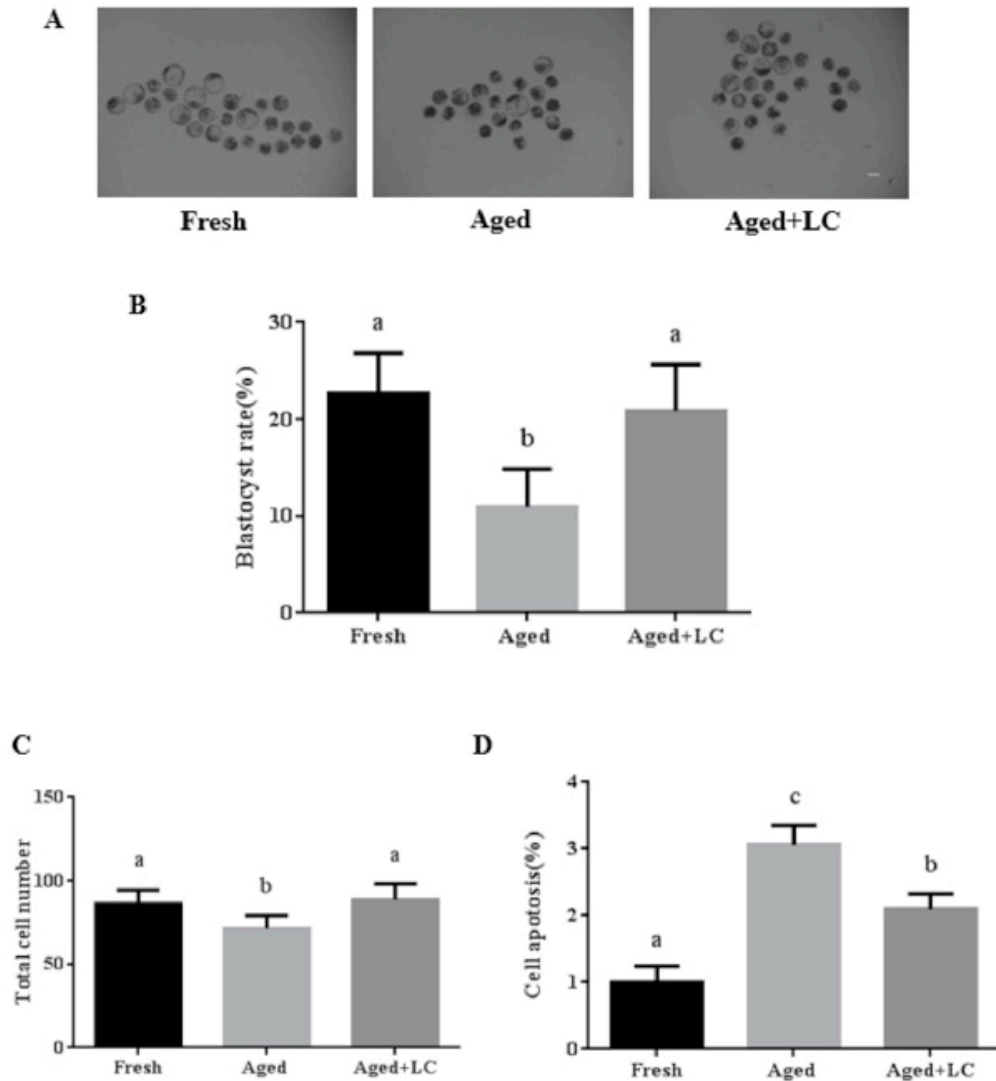


Supplementary materials (Parthenogenetic activation)

Parthenogenetic activation (PA) and embryo culture

After the fresh group, aged group, and aged + LC group were cultured for 24 h, 30 h, and 30 h, respectively, the oocytes were denuded by 0.1% hyaluronidase and activated in activation medium which comprised of CRI medium supplemented with 0.4% BSA (fatty acid-free BSA, Sigma A8806) (BSA-CRI) and Ionomycin (0.5 $\mu\text{mol/l}$) for 5 min. After activation, the oocytes were cultured in BSA-CRI medium contain 6-(Dimethylamino) purine (2 mmol/l) and overlaid by mineral oil for 4 h. After PA (day 0), the presumptive zygotes of the three groups were washed three times with 0.4% BSA-CRI droplets, placed in BSA-CRI medium and cultured to the 8-cell stage (72 h). Subsequently, the three groups were placed in 10% FBS-CRI medium and cultured to the blastocyst stage.



Effect of LC on the development and quality of aged bovine oocytes in vitro. (A) Blastocyst formation on day 7. Scale bar: 100 μ m. (B) Blastocyst rate. R = 4. (C) Total cell number in each day-7 blastocyst. R = 3. (D) The rate of cell apoptosis in the day-7 blastocysts, R = 3. Statistically significant differences are represented with different letters ($p < 0.05$).