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LACTATE AND MALATE DEHYDROGENASE ACTIVITIES IN GRANULOSA CELLS OF COW FOLLICLES

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Granulosa cells from bovine ovarian follicles of the cows use carbohydrates and amino acids *in vivo* and *in vitro*, can be characterized by substrate oxidation, respiratory and restorative activity and intensity of ATP resynthesis. Ability to resynthesize ATP provides the physiological functions of cells and steroidogenesis. Among the enzymes that are able to characterize the intensity of metabolism in cells and provide a link between the processes of glycolysis in the cytoplasm and supplying substrates in mitochondrial citric acid cycle are lactate dehydrogenase and malate dehydrogenase.

The purpose of this study was to determine the lactate and malate dehydrogenase activity in granulosa cell culture of follicle layer in connection with the physiological state of cow ovaries.

After slaughtering of cows ovaries were collected with physiological state: "follicular growth" (without the corpus luteum); with "fresh ovulation" (on the site of follicle there was hole, the corpus luteum is missing or red, with a diameter of 0.5 cm); with "early corpus luteum" (red or brown color, diameter 1.0–2.0 cm); with "late luteum" (yellow, with a diameter of 0.5–1.5 cm). After the aspiration of follicles, follicular liquid was centrifuged at 2000 rev./min. The supernatant was removed and the remaining pellet with the granulosa cells was re-suspended in cultivation Basal Medium Eagle (BME) and then cultivated in RPMI-1640 supplemented with (in wt. %): estrus serum cows 8–12 %; follicular fluid 10–12 %, insulin (4 mg/ml), heparin (5 thousand units) — 0,001 U/100 ml. Granulose cells were cultivated in plates (hole diameter 3 cm) at 38.5 °C in 5.0 % CO_2 and 100 % humidity. Activities of lactate- (LDH) and malate dehydrogenases (MDH) were determined by the speed of oxidation of NADH (NADH mmol/min×mg of protein), the protein concentration (mg/ml) was measured by Lowry O. H. et al. (1951) method.

Granulosa cells were characterized by LDH activity — 2.0±0.19 mmol NADH/min×mg of protein and MDH activity — 1.0±0.06 mmol NADH/min×mg of protein. The LDH activity in the culture of granulosa ovarian follicles "early luteum" was higher (2.2±0.30 mmol NADH/min×mg of protein), in the "follicular growth" and "fresh ovulation" the activity were lower on 4.8–9.1 % and the lowest (at 22.8 %; 1.7±0.44 mmol NADH/min×mg of protein) in "late corpora luteum". The MDH activity in ovarian granulosa cell culture in physiological states "follicular growth" and "late luteal" were the lowest (1.0–1.1 mmol NADH/min×mg of protein) to 21.4 % ($P<0.01$) higher in "early corpus luteum" and the highest (1.9±0.20 mmol NADH/min×mg of protein) in "fresh ovulation". The difference between minimum and maximum values of the variables was 47.4 % ($P<0.001$).

In the culture of granulosa cells of ovarian follicles "fresh ovulation" MDH high activity indicates the activation of citric acid cylce and mitochondrial respiration and, in general, oxidative metabolism, and with "early luteum" it indicates the reducing energy needs of cells.