CYTOPLASMIC MATURATION OF IN VITRO CULTURED BOVINE OOCYTES AND THEIR DEHYDROGENASE ACTIVITY

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Supplementing morphological assessment of oocytes cultured in vitro by examination of their dehydrogenase activity will help to bring about control of the mechanisms of their normal development and subsequent fertilization. Succinate dehydrogenase (SDH) and glutamate dehydrogenase activity in dividing oocytes of laboratory animals, namely rats and rabbits [7], hamsters [3], and mice [5], have been studied by histochemical methods. Under these circumstances no appreciable differences have been found in SDH activity in hamster oocytes depending on age. Activity of SDH and glutamate dehydrogenase in unfertilized oocytes was low and was appreciably increased in the pronuclear stage. Granule formation in unfertilized oocytes was distributed irregularly, and in fertilized oocytes granules were formed in the cortical and perinuclear zones, leading to the appearance of def inite polarity.

Follicular bovine oocytes are characterized by active metabolism. Isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase (G6PDH), SDH, phosphorylase, and also glycogen and lipids have been found in them [4].

The aim of this investigation was to study activity of some dehydrogenase as parameters for evaluation of plasmatic maturation of bovine oocytes cultured in vitro.

EXPERIMENTAL METHOD

The ovaries of cows with preovulatory follicles but without corpora lutea and follicular cysts were obtained from slaughtered animals, not receiving hormonal treatment. The oocytes were removed from follicles 5-7 mm in diameter. The selected oocytes, with compact cumulus cells, were cultured in medium TCM-199 ("Sevac," Czechoslovakia) under mineral oil at 38°C, in an atmosphere of air with 5% CO_2 for 0-24 h. The oocytes were freed from cumulus cells with hyaluronidase ("Sevac," Czechoslovakia) and their quality was assessed under the phase-contrast microscope after fixation in acet-alcohol and staining with orcein.

Activity of the enzymes was determined in 300 oocytes (100 for each enzyme), by traditional methods described in a textbook of histochemical methods [6]. To media for determination of SDH (EC 1.3.99.1), glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8), G6PDH (EC 1.1.1.49) were added NADP (from "Serva," Germany), nitro-BT ("Lachema," Brno), and also the following substrates: sodium succinate (187 mg/ml), sodium α-glycerophosphate (315 mg/ml), and sodium glucose-6-phosphate (304 mg/ml).

The oocytes were cultured on watch glasses, on which 10 drops of incubation medium and oocytes free from cumulus cells were applied with a micropipet. The watch glasses were placed in a humid chamber and the oocytes incubated at 37°C for 30 min. After incubation the oocytes were transferred for fixation into 10% formaldehyde for 15 min, and after washing in distilled water, they were placed in glycerol-gelatin.

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TABLE 1. Maturation of Bovine Oocytes in Medium TCM-199 Depending on Duration of Their Culture

Duration of culture, h	Number of oocytes	Germinal vesicles	Dia-kinesis	Meta-phase I	Telophase I	Metaphase II		Number of divided oocytes
						abc.	%	
0	152	102	—	—	—	—	—	50
6	120	22	86	—	—	—	—	12
20	164	—	2	2	123	28	17	9
24	135	—	—	3	7	117	86	8
26	161	—	—	—	13	134	83	14

Legend. During culture of oocytes in medium TCM-199 with additives at pH 7.2-7.4 more than 80% developed to the metaphase II stage.

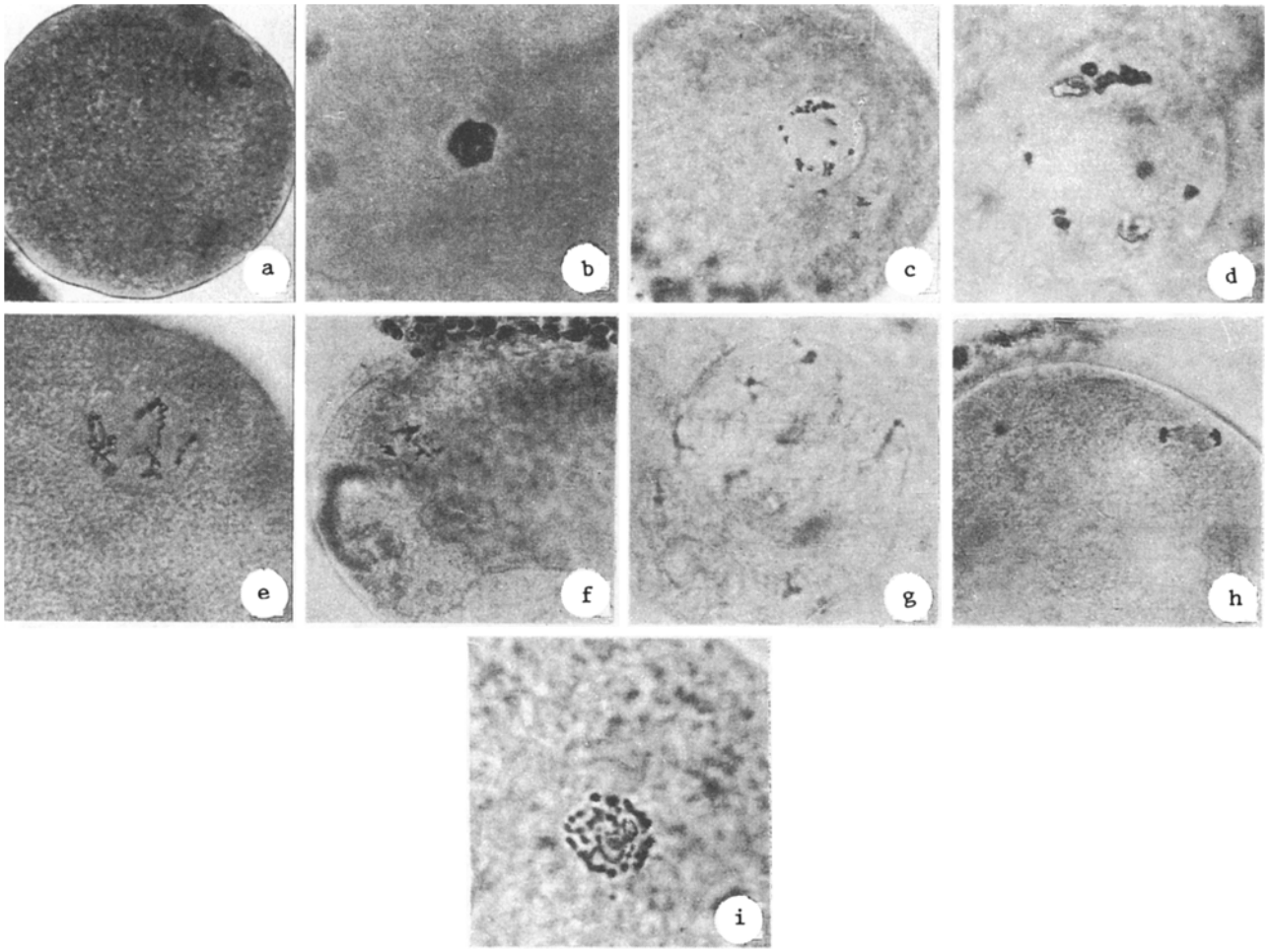

Fig. 1. Oocytes in germinal vesicle (GV1) stage. a) Control oocytes with readily observed nuclear membrane and condensed nucleolus, b) detained view of condensed nucleolus. 1500×, c) Oocyte in stage GV2, fixed after culture for 1 h; d) detailed view of germinal vesicle; e, f) oocyte in stage GV3 after 2 h in culture; 9) oocyte in stage GV4 after 5 h in culture; h, i) oocyte in stage telophase I (h) and metaphase II (i) after culture for 20-22 h. 400-1500×.

Dehydrogenase activity in the oocytes was estimated under the microscope immediately after the end of incubation. The response was recorded as the intensity of blue-violet staining of regions of the ooplasm of the oocyte. Active oocytes were distinguished from inactive by microscopic examination of the cytochemical reactions for the dehydrogenases, and they were divided according to their degree of activity into several groups. Depending on the number, size, and density of the

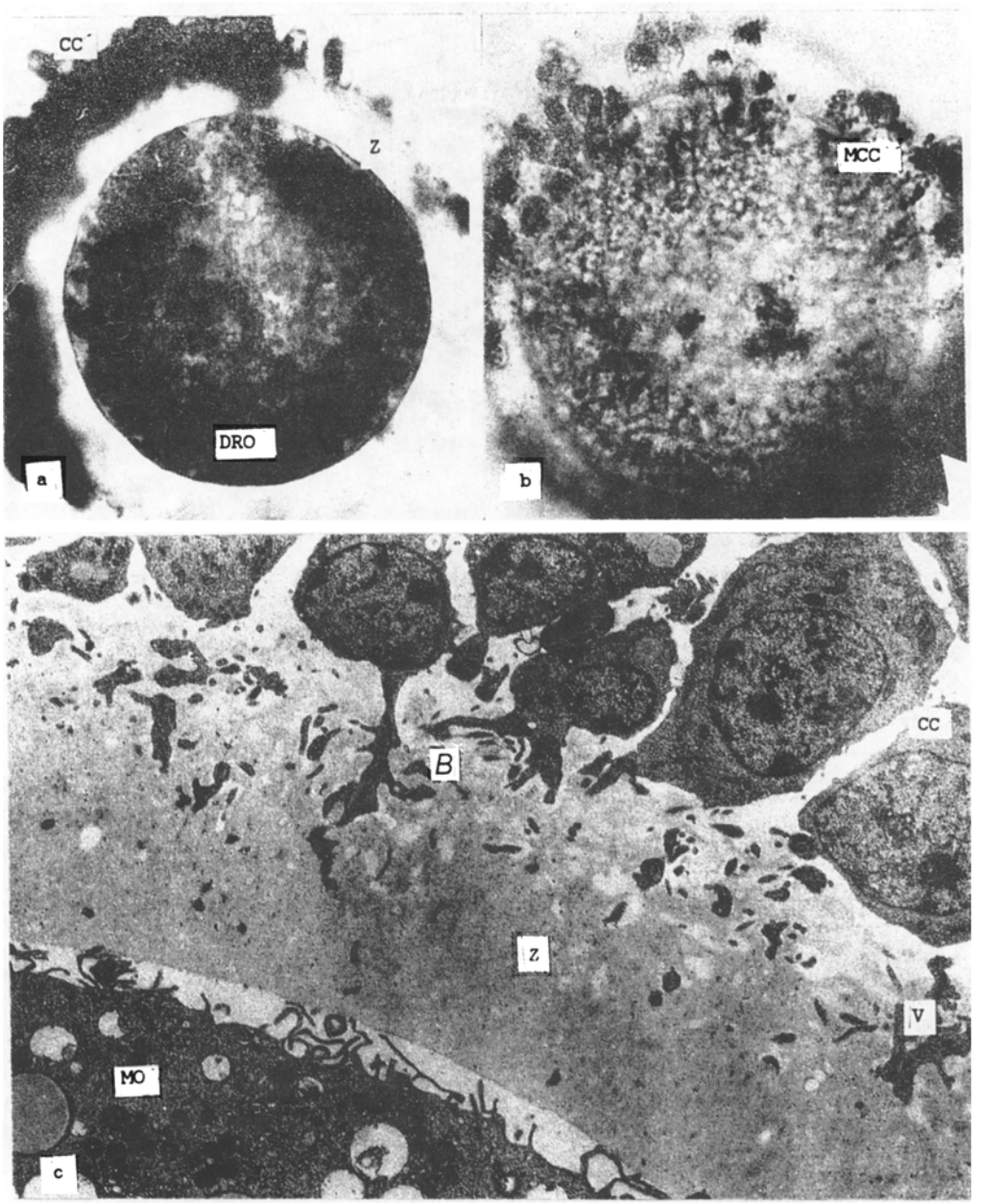

Fig. 2. Cytochemical reaction for GPDH and SDH in bovine oocytes after culture for 24 h in medium TCM-199 with additives. Ultrastructure of cultured oocyte. a) Diffuse reaction of ooplasm (DRO), due to GPDH. Very strong reaction of cumulus cells (CC), penetrating into zona pellucida (Z). 350×; b) Reaction due to SDH. Mitochondria (MCC) can be seen in cytoplasm of cumulus cells. Granular reaction of SDH indicates mitochondrial localization of the enzyme. 350×; c) Electron micrograph of oocyte cultured in vitro in an environment of cumulus cells (CC) with cytoplasmic projections (P), penetrating into zona pellucida (Z) and maintaining a connection with the ooplasm. Many diffusely distributed mitochondria (MO) can be seen in ooplasm. (2500×).

formazan granules the functional state of each oocyte was determined. To correspond with the enzyme activity the oocytes were divided into four groups: with high (+++), average (++), and low (+) enzyme activity and without enzyme activity (O).

The intensity of the reaction was estimated in the ooplasm, its polarity was noted, and the presence of mitochondrial and extramitochondrial-diffuse reactions was determined.

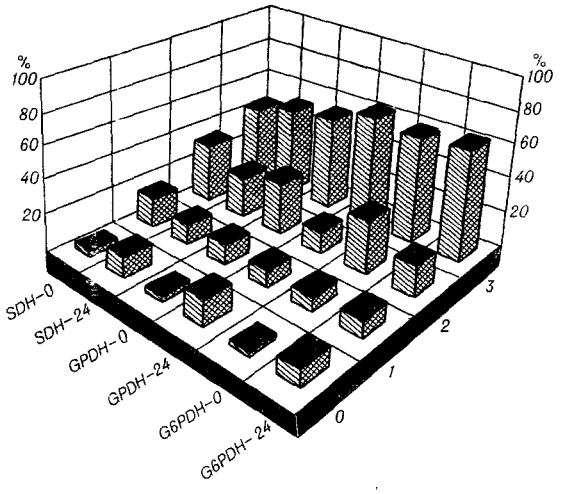

Fig. 3. Distribution of oocytes depending on their dehydrogenase activity before and after culture for 24 h.

For electron-microscopic investigation the oocytes were fixed with 2% glutaraldehyde in Dulbecco's balanced salt solution (PBS), and then with 1% osmic acid in PBS. After dehydration in alcohols the oocytes were embedded in Epon. Sections were cut on the "LKB Nova" ultramicrotome and stained with uranyl acetate and lead citrate. Electron micrographs were obtained on the TEM-100 CX11 electron microscope.

EXPERIMENTAL RESULTS

Investigation of 632 bovine oocytes revealed that destruction of the germinal vesicle in most cases was complete during culture for 6 h and the oocytes passed into the stage of diakinesis. Stages of the first meiotic division (metaphase I, anaphase I, and telophase I) were then observed, and after culture for 24 h about 80% of oocytes were in metaphase of the second meiotic division — metaphase II (Table 1, Fig. 1).

The test dehydrogenases (SDH, GPDH) were located around the yolk drops in the mitochondria of the ooplasm of the oocyte, in the cumulus cells, and in their cytoplasmic outgrowths, penetrating through the zona pellucida into the perivitelline space of the oocyte (Fig. 2c). In most oocytes the reaction was exhibited uniformly throughout the cytoplasm, but some of them showed polarity in their reaction. In a few oocytes an extramitochondrial diffuse reaction was found.

G6PDH exhibited an extramitochondrial reaction, uniformly distributed throughout the ooplasm, the cytoplasm of the cumulus cells, and their cytoplasmic processes. The greatest intensity of reaction in the oocytes and their cumulus cells after culture for 24 h was shown by GPDH (Fig. 2a). The intensity of the reaction of all three dehydrogenases rose 24 h after culture. About 10-15% of oocytes did not exhibit dehydrogenase activity (Table 2, Fig. 3). Cytochemical detection of dehydrogenase activity is proof of the functioning of the glycolytic pathway in the oocytes, and the level of their activity evidently correlates positively with the degree of cytoplasmic maturation. In experiments in which dehydrogenase activity was not recorded, cytoplasmic or nuclear maturation did not take place. The cytochemical reaction for GPDH was exhibited in the form of a diffuse reaction of the ooplasm. The highly intensive reaction of the cumulus cells points to their important role in energy processes, maintaining development of the oocyte (Fig. 2a).

The granular reaction of SDH indicates the mitochondrial location of the enzyme. Cumulus cells, rich in mitochondria, also exhibit an intensive reaction (Fig. 2b).

Electron-microscopic investigation also revealed many mitochondria diffusely scattered in the ooplasm of the oocytes. The ultrastructural organization of the cumulus cells confirmed their role in the cytoplasmic maturation of the oocyte. Cumulus cells penetrate with their cytoplasmic projections into the zona pellucida, and maintain a connection with the ooplasm (Fig. 2c).

It will be clear from Fig. 3 that activity of all three dehydrogenases increased during culture. This was manifested to a greater degree as an increase in the proportion of oocytes giving a positive reaction for GPDH and SDH.

TABLE 2. Distribution of Oocytes in Accordance with Their Enzyme Activity before Beginning of Culture and after Culture for 24 h

Duration of culture, h	Number of oocytes and corresponding activity of test enzymes, %			Enzyme activity, points
	SDH	GPDH	G6PDH	
0	45	55	60	3
24	52	63	63	3
0	35	30	30	2
24	24	13	17	2
0	17	12	8	1
24	12	9	10	1
0	3	3	2	0
24	12	15	10	0

Proof of the active involvement of enzymes in energy metabolism of the cultured oocytes may also be given by the results of a study of glucose uptake by oocytes in preimplantation embryos [1, 2]. It has been shown that hexokinase (EC 2.7.1.1) has low activity in oocytes and in the early stages of development of mouse embryos, but by the time of compaction and blastocyst formation its activity rises from 2.3 pmoles NADP/h in oocytes to 26.1 pmoles NADP/h in the blastocysts. Comparison of hexokinase activity and the level of glucose uptake shows that besides hexokinase, an important role in the carbohydrate metabolism of oocytes and embryos is played by other mechanisms of control of metabolism.

Dehydrogenase activity can provide an indicator of cytoplasmic maturation of oocytes, and they can be used to characterize the group and individual features of their metabolism.

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