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Preservation of Primary Bovine Embryonic Kidney Cells with Dimethyl Sulfoxide

NORMA J. LINDSEY AND T. L. CHOW
Department of Microbiology, Colorado State University, Fort Collins, Colorado 80521

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Primary bovine embryonic kidney cells frozen with dimethyl sulfoxide can be recultured with satisfactory results.

Primary bovine embryonic kindey (BEK) cells are widely used for the propagation of viruses, especially in research and diagnostic laboratories studying the viruses of veterinary medicine. The preservation of these cells for long periods of time would be a convenience and an economy. Dimethyl sulfoxide (DMSO) is reported to be a practical preservative in the freezing and storing of other primary and line cells (1, 3). This report describes the successful use of DMSO in the freezing and storing of primary BEK cells.

Kidneys from fetuses collected shortly after death from local abattoirs were prepared for culture by the following modification of the method of Youngner (4). Cortical tissue from the decapsulated kidney was minced with scissors and washed in cold Dulbecco's salt solution containing streptomycin and penicillin. The minced tissue was agitated with a magnetic stirrer in 50 volumes of warm trypsin solution (0.25% trypsin in magnesium-free Dulbecco's salt solution) for 10 min, and the supernatant fluid was discarded. Successive digestions, each consisting of agitation of washed, minced tissue in 50 volumes of warm trypsin solution for 20 to 30 min, were performed until the desired quantity of dispersed cells was recovered in the supernatant fluids. Each harvested fluid was centrifuged for 10 min at 1,000 rev/min (about 225 × *g*) and washed in warm LE medium (Earle's balanced salt solution containing 0.45% enzymatic lactalbumin hydrolysate and antibiotics); the cells were resuspended in LE medium and counted in a Neubauer hemocytometer. Cells were then packed by centrifugation and resuspended to a concentration of 8 × 10^6 cells per ml in freezing solution (37 C, *p*H 7.2), consisting of 82.5% LE medium, 10% inactivated fetal calf serum, and 7.5% redistilled DMSO. The suspended cells were dispensed into 5- or 10-ml ampoules, 4 or 8 ml per ampoule. The ampoules were flame-sealed, wrapped in cotton batting, and transferred directly to a freezer at −70 C. The packing was designed to permit slow decrease in the temperature of the cell suspension. Because of the known toxicity of DMSO for cells in vitro (2), all manipulations of cells in freezing solution were completed as rapidly as practical.

TABLE 1. *Growth of primary BEK cells frozen in DMSO*

Age of fetus	No. of days cells stored	Length of incubation for monolayer formation
months		*days*
4	0	8
	6	6
	10	10
	18	10
	51	9
5	0	6
	18	6
	42	7
6	0	6
	54	7
6	0	6
	31	11
7	0	5
	25	4
	75	9
8	0	5
	17	5
	51	5
	69	7

For the planting of cells, ampoules were thawed by rapid swirling in a water bath at 37 C for 30 to 60 sec, and the cells were withdrawn, centrifuged, and washed with LE medium containing 10% calf serum (TC medium). Cells were planted in Roux bottles, each containing 80 to 90 ml of TC medium, at a concentration of 32 × 10^6 cells per

bottle. The bottles were incubated for 48 to 72 hr at 37 C, TC medium was exchanged, and the bottles were reincubated until a cell monolayer was formed.

Table 1 shows the incubation periods required for growth of these cells to monolayer in this initial culture (BEK-1). Cultured frozen cells presented no microscopically detectable morphological or population variations from cells of the same fetuses cultured without prior freezing, when both groups were examined as BEK-2 (first subculture). Moreover, differences in susceptibility to viral infection or rate of viral replication were not detected when BEK-2 progeny of frozen cells were employed in tube culture and plaque assay of infectious bovine rhinotracheitis virus. It was concluded that this is a satisfactory method for preserving BEK cells and that the advantage in convenience and economy is not negated by undesirable deviations in the cell progeny, at least in the systems evaluated.

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