Simplified Method for Trypsinization and Establishment of Fibroblastic Tissue Cultures from Placentas

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The trypsinization of a placenta and the establishment of a fibroblastic line by existing methodology is a tedious and time-consuming process (1-4). The purpose of this report is to describe a simplified method for the establishment of fibroblastic cultures from placentas.

The placenta is collected under sterile conditions at the time of delivery. Large 10-g chunks of placenta are collected and passed through three individual baths composed of phosphate-buffered saline (pH 7.0). The chunks are then minced until cubes of approximately 0.3 cm are obtained. These are then washed thoroughly in phosphate-buffered saline, and 200 ml of preheated (37 C) 0.25% trypsin is then added to approximately 25 g of tissue. Trypsinization is allowed to progress for 1 to 2 hr, at which time the eluate is filtered through a fine gauze filter. It is then centrifuged at 900 x g for 15 min in a refrigerated International centrifuge (PR-2 with 253 head). A cell count is performed and the cells are suspended at 5 x 10^4 to 6 x 10^4 cells/ml in Eagle's Basal Medium with Earle's Salts and 15 to 20% fetal calf serum plus 0.5% lactalbumin, 0.1% yeast extract, and (per ml) 50 μg of kanamycin or 400 μg of penicillin and 200 μg of streptomycin.

With this method, fibroblastic tissue culture lines were successfully established and maintained for a minimum period of 2 months from 65% of the placentas processed in our laboratory.

Once the placenta is minced and placed in the trypsinization flask, no further technical manipulation is required. The trypsinization chamber is never again opened until the trypsin and cells are decanted. Because of the volume of trypsin used, rigid monitoring of the trypsinization flask temperature is not necessarily a mandatory feature. Although the time of trypsinization can be shortened by rigid maintenance of the reaction temperature at 33 to 37 C, the time saved does not appear to outweigh the advantage of freeing trained personnel for other tasks.

The prime advantages of this system are a consistent high yield of viable cells and the negligible possibility of contamination.

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