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Molecular biology of epididymal epithelium cultures *in vitro*: *de novo* synthesis of proteins

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Cultures of mammalian epididymal epithelium can be maintained in vitro for a considerable length if time (up to 35 days). These cultures are used in the identification of post testicular development changes of mammalian spermatozoa in detail since the microscopic nature of epididymal tubules do not allow close investigation of molecular events leading to spermatozoa maturation. However, maintenance of the epididymal epithelium cultures in vitro as well as establishment of smooth functioning of these cells with time are prerequisite for such studies.

This study therefore investigates protein synthetic and secretory activity of cultured hamster epididymal epithelium tissue. Cultures were maintained in CO2 incubator at 37 0C for 7 days. Cultured cells were treated with methionine free culture medium for 6 hours followed by the radiolabelling of the cultures with 35S Methionine for another 12 hours. Cells and the conditioned medium were separate and analyzed with SDS-PAGE electrophoresis under denaturing conditions. Amplified protein bands were detected by autoradiograhy. Several protein bands identified from the unlabelled epithelium plaques before culture under denatured conditions, including 172- 150 kDa, 150-100 kDa, 85-21 kDa.

After culture for 7 days and after radiolabelling several new protein bands were identified including 168, 151, 143, 130, 119, 104, 92- 37 kDa. Similar experiments were also carried out cells cultured without androgens in the medium and 119 and 104 kDa bands were missing. In the conditioned medium not all the protein synthesized were detected. Several explanations have been suggested such as modification of secreted proteins, aggregation or glycosylation, absorption or change by sperm (Cooper, 1986; Dacheux and Voglmayer, 1983). Similar to this experiment, several *in vivo* and *in vitro* labeling experiments have shown a fewer number of protein bands in epididymal fluid than in epididymal cells (Klinefelter and Hamilton, 1985; Vreeburg et al., 1992).

This presence of protein bands after 35S Methionine labeling is a good indication of the functional integrity of cultures. Therefore, functionally cultured epididymal epithelium cells are highly established for further investigations.

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