The advantage of the aggregate culture of isolated ovarian cell types over the monolayer culture

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Abstract

Ovarian cells such as theca interna, granulosa and corpus luteum cells were isolated from pig ovaries and cultured in Erlenmayer flasks (25 ml) containing 3.5 ml of culture medium. The media were replaced every second day and frozen to -20°C for later steroid analysis. The reaggregation of cells was completed within 2–3 days and this was then followed by a period of cell migration. During the subsequent 5–6 day period the reaggregates became larger. The best results were obtained in cultures of isolated theca alone and in combination with granulosa cells, as well as of early corpus luteum cells. Granulosa cells did not aggregate as easily or as completely as the corpus luteum cells. All types of cells investigated were able to secrete progesterone into the culture medium. They secreted more progesterone and for a longer time than cells cultured as monolayers. The aggregate culture seems to be a good model to study the secretion of ovarian cells, by creating the tri-dimensional, and thus more physiological, culture system.

Introduction

Aggregate culture makes possible the tridimensional contact between cells which creates better physiological conditions for their function. The majority of ovarian cell types cultured as monolayers have a rather short secretory life span. This is specially true in cultures of theca interna and corpus luteum cells. Therefore it seemed important to look for a system in which these highly differentiated cells could secrete significant amounts of steroids for a longer time. This technique has been successfully used to obtain aggregate cultures of embryonic retina and cells from different areas of brain of chicken, mouse and rat embryos (Geyvai et al., 1978; Ishii, 1966; Lilien and Moscona, 1967).

So far, no data are available that demonstrate that isolated ovarian cells can aggregate and secrete steroid hormones in vitro.

The aim of the present investigation was: to compare aggregate cultures with their respective monolayers.

Materials and methods

The ovaries were collected from pigs in a slaughter-house. Ovaries in two stages of the sexual cycle were used:
1. proestrus ovaries containing only large follicles (1.0–1.2 cm in diameter). They were the source of theca interna and granulosa cells.
2. ovaries in early luteal phase containing corpora lutea with stigma still opened. They were the source of luteal cells.

The approximate stage of the estrous cycle of the ovaries was determined using criteria described by Akins and Morrisette (1968) and Channing and Ledwitz-Rigby (1975). For each culture 80 follicles were aseptically removed. Granulosa cells were isolated by the technique described by Channing and Ledwitz-Rigby (1975). Theca interna cells, from the same follicles from which granulosa cells were removed, were isolated by our own technique (Stoklosowa et al., 1978). Granulosa and theca cells were inoculated at a concentration of $1.5 \times 10^6$ and $4.5 \times 10^5$ cells/ml of culture medium respectively. For coculture the ratio of theca to granulosa cells in the inoculum was comparable to the level observed in vivo (1:4).

Corpus luteum cells from early corpora lutea were isolated by the technique described by Gregoraszczuk (1983). Luteal cells were inoculated at a concentration of $3.5–5.0 \times 10^5$ cells per ml medium. All types of ovarian cells investigated were suspended in medium M 199 supplemented with 10% calf serum. Half of the cultures were grown as monolayers in Leighton tubes for 8 days at 37°C. Another part of the cultures was grown in Erlenmeyer flasks (25 ml) containing 3.5 ml of medium. The flasks were incubated at 37°C with constant shaking at 70 rev/min, for 8 days. Culture medium was changed every second day, frozen and stored at −20°C for further steroid analysis. At the end of the culture period, monolayers and aggregates were stained histochemically for $\delta^5,\beta$-hydroxysteroid dehydrogenase ($\delta^5,\beta$-HSD) using the technique of Fischer and Kahn (1972).

**Steroid analysis**

Progesterone was analysed using the radioimmunoassay according to Abraham et al., (1971). In the progesterone assay a highly specific antibody was used. It was raised in sheep against $11\alpha$-progesterone hemisuccinate coupled to bovine serum albumin. Cross-reaction with pregnenolone was 2.9%. All related steroids that were tested exposed less than 1% cross-reaction. $[1,2,6,7-^3H]$ progesterone (Amersham, England; spec. activity 80 Ci/mmol) was used as a tracer. Progesterone was detected directly in the culture medium without extraction. The sensitivity of the assay was 50 pg. The coefficients of variation within and between assays were less than 2.5% and 15% respectively. The data were computed in nanogram, of progesterone per ml medium.

**Results and conclusion**

There are no data in the literature demonstrating that ovarian cells can aggregate and differentiate in vitro. The degree of the aggregation depends on the tissue that was taken for culture. George and Rao (1977) indicated that the degree of aggregation depended on the age of the embryos used. In addition, there was a difference in the ability to aggregate between cells of the different parts of the brain. Isolated granulosa cells do not aggregate easily, but form conglomerates of cells. Increase in the activity of $\delta^5,\beta$-HSD in granulosa cells during culture period in comparison to monolayers was observed (Figs. 1, 2).

The amount of progesterone released into the culture medium did not decrease during the 8 day culture in either monolayer or aggregate cultures (Fig. 9).

Theca cells cultured as aggregates secreted more progesterone than in the monolayer system. This type of cell aggregated more easily than granulosa cells. During 5–6 days the aggregates became larger and sometimes they became surrounded by a thin delicate membrane-like capsula (Figs. 3, 4). The amount of progesterone released into the culture medium did not decrease during 8 days in both types of culture, similar to cultures of granulosa cells alone (Fig. 9).

The best results in aggregate cell culture could