A Study on Bovine Ovarian Function by Cell Culture

- With Special Reference to Response of Granulosa Cells and Luteal Cells to Gonadotropin and Prostaglandin $F_{2\alpha}$ -

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Abstract

Cell culture initiated in 1907 by Harrison's nerve fiber culture has achieved so tremendous a progress as to enable detailed in vitro studies using cultured cells.

In this study, culture of granulosa cells (GC) and luteal cells (LC) from a cow ovary was undertaken to investigate the in vitro behavior of these cells free from the biological control mechanism which is presumed to regulate their behavior in vivo to a certain extent under the control of cerebroneural system, and also their behavior in the presence of gonadotropin (GTH) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The behavioral aspects mainly studied were the multiplication and morphological characteristics of these cells together with secretion of progesterone and estrogen into the culture fluid.

Materials and Methods

GC was collected from the follicles of slaughtered animals and from those of live animals of the estrous stage using an injector. LC of the almost functional luteal stage was collected from slaughtered animals. For both the cells, subculture and primary culture cells were used in the experiment. Used in addition were slices of the corpus luteum.

The culture medium used was an Eagle MEM (Nissui) (1). After dissolution of this medium to a desired concentration,

10% cow and newborn calf sera were added. Culture was carried out by stationary incubation at 37°C.

GTH consisted of human chorionic gonadotropin (HCG), pregnant mare serum gonadotropin (PMS), prolactin, follicle stimulating hormone (FSH) and luteinizing hormone (LH). They were added to the medium at the commencement of culture. The cell multiplication was determined when the cell growth was microscopically found to be spread over the entire bottom of the culture vessel. The supernatant was freeze-preserved for quantification of hormones. The cells adhered to the culture vessel were counted after the required treatment.

The cells grown on the bottom of the culture vessel was immediately examined using an inverted phase-contrast microscope. Furthermore, the cells adhered onto the cover glass placed in the culture vessel were, when necessary, stained by Giemsa or May-Gruenwald Giema to examine the cell morphology.

Results

1. Cell culture of GC and LC

Multiplication and morphology: GC and LC achieved smooth multiplication in vitro. The cells grown showed an epithelium-like morphology and indicated a possibility of subculture.

Hormone production: Hormone production in the culture fluid seemed to be most affected by the ovarian cycle at the time

of cell collection.

In culture of GC from animals of the estrous stage, the maximum productins of estradiol (E_2) and progesterone were considerably high, indicating significant differences compared to culture of GC from slaughtered animals. High production of progesterone suggested a functional shift of GC into LC.

In culture of LC, estrogen production was barely detected. Although progesterone production was large, remarkable variations such as seen in GC were not found.

2. Response of GTH to GC and LC

Single mixture of hormones: HCG, PMS, prolactin, FSH or LH generally accelerated multiplication of GC and LC. Especially prolactin demonstrated a higher accelerative effect than HCG. However, PMS had obviously an inhibitory action on multiplication of GC and LC.

No demonstrable results were obtained in relation to hormone production.

Combined mixture of hormones: Within the concentrations used in this experiment, no synergic effect was demonstrated on multiplication of GC and LC. No distinct results were also obtained on hormone production.

3. Response of $PGF_{2\alpha}$ to GC and LC

Cell multiplication: $PGF_{2\alpha}$ seemed to somewhat inhibit multiplication of GC and stimulate that of LC.

Hormone production: Progesterone production was markedly

accelerated in GC from slaughtered animals. However, no specific tendency in hormone production could be defined in GC from the follicles of animals at their estrous stage. Estrogen production seemed to be slightly accelerated in 5 of 8 culture vessels of GC from animals of the estrous stage.

Progesterone production tended to increase in LC. Estrogen production showed no distinct tendency. Almost the identical results were obtained in the experiment of the sliced corpus luteum.

The above findings confirmed that the phenomena occurring in vitro do not necessarily correspond with biological reactions occurring in vivo and suggested that the sexual function is governed in vivo by a complexity of factors. Thus the experiment with the cultured cells of follicular granulose cells and luteal cells seemed to be a helpful technique that allows simple analysis of the reproductive phenomena occurring in vivo under complicated control mechanisms.