Production of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in Primary Culture of Granulosa Cells from Mature Follicles of Estrous Cows

Shizuo KAWAKAMI and Hidemichi KOHMOTO¹⁾

Department of Theriogenology, School of Veterinary Medicine,
Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 229,
and ¹⁾ Futtu Branch Office, Central Animal Clinical Center,
Chiba Prefectural Federation of Agricultural Mutual Relief Associations,
267 Nakamasaishi, Sanuki, Futtu, Chiba 299-15, Japan

Abstract. Granulosa cells (GC) collected from follicular fluids of the mature follicles of 9 estrous cows were cultured for 6, 12 or 24 h at 37°C in a serum-free synthetic culture medium (SMF-101 "Nissui"). On completion of the culture periods, the concentrations of $PGF_{2\alpha}$ in the culture supernatants were determined by radioimmunoassay. The following results were obtained.

The $PGF_{2\alpha}$ concentrations in the culture supernatants were increased in 8 of the 9 samples as the culture elapsed. Statistic analysis was carried out by Tukey's q-test. Significant differences were found in the $PGF_{2\alpha}$ concentrations in the supernatants between 6 and 24 h culture. This suggested that GC derived from the mature follicles of the estrous stage of cows produced $PGF_{2\alpha}$ in the medium.

Key words: $PGF_{2\alpha}$, Primary Culture, Granulosa Cell, Mature Follicle, Estrous cow.

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Production of prostaglandin (PG) occurs during the estrous stage in various animals: Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations in the follicular fluid of matured follicles reach the maximum level immediately before ovulation in swine [1], rabbits [2, 3], and rats [4, 5]. Rabbit granulosa cells (GC) collected immediately before ovulation secreted $PGF_{2\alpha}$, PGF_2 [6] or PGF [7]. The secretion of PGF from bovine [8] and human [9] follicles has also been reported. There are many other reports describing the secretion of PG during the estrous stage. In an earlier study [10], we have found that the concentrations of $PGF_{2\alpha}$ in follicular fluids from matured follicles from 10 estrous dairy cows ranged from 346 to 11,700 pg/ml and that the follicular fluid contained 1.55 to 22.67 times higher $PGF_{2\alpha}$ concentration than

the serum. This study was undertaken to determine whether GC are involved as a productive source of $PGF_{2\alpha}$ which is contained at high concentration in follicular fluid of the mature follicle of estrous cows.

Materials and Methods

Materials

1) Estrous cows and GC: The cows used in this experiment consisted of 9 adult female Holsteins with regular estrous cycles. The estrus was confirmed by rectal palpation for those which manifested a sign of estrus. GC were collected when the animals were ready for mating. When the estrus was confirmed early in the morning (before 9:00 am), GC were collected in the afternoon of the day. When it was confirmed from 9:00 am till

noon, GC were collected in the evening of the day or early next morning. When the estrus was confirmed in the afternoon, GC were collected in the morning of the following day. GC were collected by aspirating follicular fluid from the mature follicles via the vaginal wall using the short-type aspirator which was developed for simultaneous collection of follicular fluid and GC from the mature follicles of live estrous cows [11].

2) Culture medium: A serum-free synthetic culture medium (SMF-101 "Nissui", Nissui Pharmaceutical Co., Ltd.) was used. The SMF-101 "Nissui" consisted of equal volumes of RPMI 1640 and Eagle's MEM. Other ingredients such as thymidine and serine were then added. Amino acid were readjusted. Using this mixture as a basic medium, insulin (bovine), transferrin and monoethanolamine were added [12, 13].

Experimental Methods

1) Primary culture of GC: The GC-containing follicular fluid was transferred into a sterile 10 ml-centrifugal tube immediately after collection, cooled to 4°C and brought back to the laboratory. It was then centrifuged at 1,000 rpm for 10 min. After discarding the supernatant, an appropriate volume of the culture medium was added. The sedimented cells were dispersed well with gentle stirring using a pipette. The number of cells was counted and adjusted to a concentration of 2×10^6 cells/5 ml with the medium. This cell suspension was divided into 3 portions and transferred into 3 plastic test tubes S "Nissui" (Nissui Pharmaceutical Co., Ltd.) in 5 ml. The tubes were then slanted laterally and the cells were cultured at 37°C in stationary condition.

The cells were cultured for 6, 12 or 24 h. On completion of the culture periods, 1 of the 3 plastic test tubes was centrifuged at 1,000 rpm for 10 min. The culture supernatant was transferred into an indomethacin-containing test tube for quantification of $\text{PGF}_{2\alpha}$. It was then frozen and stored until assay.

2) Quantification of $\text{PGF}_{2\alpha}$: The concentration of $\text{PGF}_{2\alpha}$ was determined at SRL Co., Ltd. 2-4-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo) by the original method of Bernards *et al.* [14] using a radioimmunoassay kit [^3H] $\text{PGF}_{2\alpha}$ Radioimmunoassay Kit, Dade, Baxter Travenol Diagnostics, Inc.).

Results

The $\text{PGF}_{2\alpha}$ concentrations in 6 (Nos. 1-6) of the 9 animals were increased as the culture elapsed.

In cow No. 7, $\text{PGF}_{2\alpha}$ was 520 pg/ml after 6 h culture and increased to 1,007 pg/ml by additional 6 hours culture. After 24 h culture, however, the value was reduced to 879 pg/ml.

In cow No. 8, almost the same concentrations of $\text{PGF}_{2\alpha}$ were obtained after 6 h (355 pg/ml) and 12 h (354 pg/ml) culture. After 24 h culture, the concentration was increased to 556 pg/ml.

In cow No. 9, the $\text{PGF}_{2\alpha}$ concentrations after 6, 12 and 24 h culture were 690 pg/ml, 594 pg/ml and 432 pg/ml, respectively. The concentration was gradually reduced with progress of culture.

Statistic analysis was carried out by Tukey's q-test. Significant difference was found between the mean 6 and 24 h value ($p < 0.05$).

Discussion

When GC derived from the mature follicles of the 9 estrous cows were cultured for 6, 12 or 24 h, the $\text{PGF}_{2\alpha}$ concentrations in the culture supernatants were increased with advance of culture in 6 of them. In one (No. 7) of the remaining 3 cows, the $\text{PGF}_{2\alpha}$ concentration was increased to 1.94 times after 12 h culture and increased to 1.73 times higher after 24 h culture as compared with the value of 6 h culture. However, the value of 12 h was slightly higher compared to that of 24 h. In another cow (No. 8), the $\text{PGF}_{2\alpha}$ concentrations

Table Production of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) in primary culture of granulosa cells ($2 \times 10^6/5$ ml) from mature follicles of estrous cows

Cow No.	Culture period		
	6 h	12 h	24 h
1	246 pg/ml	412 pg/ml	414 pg/ml
2	461	484	993
3	101	110	993
4	577	614	720
5	92	215	663
6	270	312	453
7	520	1007	897
8	355	354	556
9	690	594	432

after 6 and 12 h cultures showed almost the same values. After 24 h culture, however, the value was 1.57 times higher than the previous values. The results obtained indicate that the cultured GC produced PGF_{2α} in the medium in 8 of the 9 cows. It was found in this experiment that the number of cells cultured was slightly reduced after 24 h culture. Therefore, the PGF_{2α} increases in the culture supernatants after 12 and 24 h culture indicate further that the PGF_{2α} was produced by the cultured GC.

In one (No. 9) of the 9 cows, the PGF_{2α} concentration was slightly decreased with advance of culture. The decrease may attribute to the difference in susceptibility and physiological situation of individual cows.

Significant difference as demonstrated in the PGF_{2α} concentrations of the supernatants between 6 and 24 h culture by Tukey's q-test. This indicates that GC derived from the mature follicles of the estrous stage in cows produced PGF_{2α}.

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