# Increased Progesterone Secretion and 3β-Hydroxysteroid Dehydrogenase Activity in Human Cumulus Cells by Pregnenolone Is Limited to the High Steroidogenic Active Cumuli

SHALOM BAR-AMI[1–4] and HELA GITAY-GOREN[2]

*Submitted: March 10, 2000*
*Accepted: April 20, 2000*

***Purpose:*** *Several reports imply that lower progesterone secretion by cumulus–oocyte complexes (COCs) is associated with lower fertilization in the corresponding oocyte. The possible role of progesterone in oocyte fertilization in humans was studied using two approaches: (a) increasing the total progesterone secretion by culturing more than one COC per dish; and (b) increasing the cumulus cell progesterone secretion by providing pregnenolone as a substrate.*
***Methods:*** *Mature COCs were cultured individually or cocultured in groups. Oocyte fertilization and progesterone secretion were tested after 20 hr and 3 days in culture, respectively. The cumuli from individually plated COCs were cultured in the absence of oocyte for an additional 3 days in order to test the effects of pregnenolone on progesterone secretion and the 3β-hydroxysteroid dehydrogenase (3β-HSD) activity. A comparable study with pregnenolone was performed on the corresponding granulosa–lutein cells.*
***Results:*** *Increasing the number of COC to two instead of one led to a significant increase in both fertilization rate and progesterone secretion. The addition of pregnenolone during days 3–6 increased significantly both progesterone secretion and 3β-HSD activity. Comparable results were observed in granulosa-lutein cells subjected to pregnenolone treatment. Following the first 3 days culture, cumulus masses were categorized as secreting high or low progesterone levels. Adding pregnenolone had a greater effect on both progesterone secretion and 3β-HSD activity in the high-progesterone-secreting cumuli.*
***Conclusions:*** *Addition of pregnenolone increased progesterone secretion and 3β-HSD more efficiently in the higher-progesterone-secreting cumuli. Coculture of two COCs instead of one led to a higher fertilization rate and greater progesterone secretion.*

**KEY WORDS:** Cumulus cells; 3β-hydroxysteroid dehydrogenase; in vitro fertilization; progesterone; pregnenolone.

## INTRODUCTION

The mid-cycle surge of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) alters the steroidogenic pattern in the preovulatory follicle, yielding a significant increase of progesterone ($P_4$) secretion in several ovarian cell types. The increase in $P_4$ secretion is a consequence of a change in the activity of various steroidogenic enzymes such as cholesterol side chain cleavage cytochrome P-450 (P-$450_{scc}$), which catalyzes the first rate-limiting step of steroid biosynthesis (1) and the 3β-hydroxysteroid dehydrogenase (3β-HSD) (2). The increase in these steroidogenic enzymes has been noted in both granulosa and cumulus cells (3,4). In women undergoing in vitro fertilization (IVF), a significant increase in serum $P_4$ level is noted 12–14 hr after human chorionic gonadotropin (hCG) administration (5). At the time of retrieving the cumulus–oocyte complexes (COCs), 36 hr after hCG administration, the cumulus and granulosa–lutein (G-L) cells are highly active in $P_4$ secretion (6,7). This activity is sustained over 6–8 days in culture (8,9). By comparing the IVF outcomes in stimulated cycles, successful cycles appeared to be associated with elevated levels of serum $P_4$ and elevated rates of $P_4$ secretion in cultured G-L cells (10,11). Furthermore, the

[1] Department of Obstetrics and Gynecology, Rambam Medical Centre, P.O.B. 9602, Haifa 31096, Israel.
[2] The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, P.O.B. 9649, Haifa 31096, Israel.
[3] Assistant Research Professor, Department of Obstetrics and Gynecology, Boston City Hospital, Boston University School of Medicine, Boston Massachusetts 02118.
[4] To whom correspondence should be addressed at Department of Obstetrics and Gynecology, Poriya Hospital, Hagalil Hatachton 15208, Tiberias, Israel. (E-mail: sgyss@zahav.net.il)


1058-0468/00/0900-0437$18.00/0 

rate of $P_4$ secretion by the cumulus and G-L cells of individual follicles is positively correlated with the fertilizability and developmental competence of the corresponding oocytes (11–13). It has been also suggested that $P_4$ is involved in the process of the acrosome reaction in various mammals (14,15).

The possibility that artificially increasing the $P_4$ level in the proximity of low $P_4$-secreting COC may increase oocyte fertilizability is attractive. However, in the mature COC population it is impossible to determine prospectively whether the COCs secrete low or high $P_4$. Thus, blind addition of $P_4$ to the culture well is not recommended, since high $P_4$ levels could interfere with various oocyte activities (16,17). The alternative approach is to increase $P_4$ secretion by cumulus cells either by increasing substrate availability or by including a hormonal increase in steroidogenic enzyme activity. It was thought that addition of substrate for $P_4$ biosynthesis is more feasible, since it may drive the cumulus cells to secrete more $P_4$ in the short time that is available before insemination and with less involvement in the overall COC activity. The G-L cells were selected as a control cell population, since G-L cells in many ways display similar steroidogenic activity to that of cumulus cells. The aim of the present study was to test two options for increasing $P_4$ levels at IVF, either by coculturing more than one COC in the culture dish, or by adding pregnenolone, the direct substrate for $P_4$ biosynthesis.

## MATERIALS AND METHODS

### Subjects

The participants in this study were male with normal sperm parameters and regularly menstruating women who were infertile due to absent or blocked fallopian tubes and were undergoing IVF–embryo transfer (ET) treatment at the Rambam Medical Centre, Haifa, Israel. Multiple follicular growth was induced by treatment with FSH (150 IU/day) (Metrodin; Teva, Petah Tiqva, Israel) and human monopausal gonadotropin (hMG) (150 IU/day) (Pergonal; Teva). Ovulation processes were induced with one intramuscular injection of 10,000 U hCG (Chorigon; Teva).

### In Vitro Incubation

Human COCs were collected 36 hr after hCG administration, usually between 08:00 and 12:00 AM. Each individual COC was placed in a single organ culture dish (Falcon, Oxnard, CA), filled with 1-ml culture medium (see below), and incubated in a humidified 5% $CO_2$–95% air incubator. Occasionally, two or three COCs were placed in a single culture dish mostly because high unexpected numbers of COCs were retrieved. Twenty hours after addition of spermatozoa, the oocytes were mechanically denuded of their corona cells to view fertilization and transferred to new dishes filled with fresh culture medium for further embryonic development. Usually the remaining cumulus cells as well as G-L cells are discarded, since they have no clinical application. Permission was obtained to utilize these cells for the present study. Thus the dishes containing solely the corona and cumulus cells were cultured for 2 additional days (3 days total) in medium alone. Following the first 3 days' culture, the culture medium was collected and frozen for later determination of $P_4$ levels. After the first 3 days culture, the cumulus cells from individually plated COC were cultured for an additional 1, 2, or 3 days in a fresh culture medium alone (control) or in presence of $10^{-7}$ M pregnenolone.

The G-L cells of each woman were pooled and separated from follicular fluid, washed twice in plain Ham's F-10 medium, and loaded onto isotonic Percoll solution diluted to 50% of the original Percoll fluid (Pharmacia, Lund, Sweden). After centrifugation for 30 min at 500 *g*, the red blood cells were precipitated at the test tube tip, and the G-L cells were accumulated at the interphase between the medium and the Percoll solution. The residual Percoll in the collected G-L cells was removed by suspending the G-L cells in 12-ml culture medium and decanting the supernatant after centrifugation. Ten thousand viable G-L cells/200-μl well were cultured in 96-well plate (Falcon). Cell viability was assessed using 0.04% trypan blue (wt/vol) for the dye exclusion test (18). After an initial culture period of 3 days, fresh media were added and the G-L cells were cultured for 1, 2, or 3 additional days in the absence or presence of $10^{-7}$ M pregnenolone.

### Medium Preparation

COC were cultured during days 0–3 in culture medium that consisted of 9.8 g/Liter Ham's F-10 medium buffered with 25 mM $NaHCO_3$ (both from Gibco, Grand Island, NY). The medium was supplemented with 120 IU/ml penicillin, 60 IU/ml streptomycin (both from Sigma Chemical Co., St. Louis, MO), 2 mM calcium lactate (Calbiochem-Boehring, La Jolla, CA) and 10% (vol/vol) heat-inactivated human fetal

cord serum. Preparation of human cord serum was as previously described (12).

A slightly different culture medium was used during days 3–6 of the cumulus cell culture and during all days of G-L cell culture. The above medium was supplemented with 10 mM HEPES, 5% (vol/vol) heat-inactivated fetal calf serum and 2 mM L-glutamine (all from Gibco). Lactic acid and cord serum were omitted from this medium. The ingredients of both media were dissolved in purified water (BDH Chemicals, Poole, England) to achieve 280–290 mOsm/kg and then sterilized through 0.22-μm filters, 0.5-liter size (Nalge Co., Rochester, NY).

### Progesterone Radioimmunoassay (RIA)

The media collected on days 3, 4, 5, and 6 of culture were stored at −20°C for later determination of $P_4$ level. Steroid RIA was employed on the unextracted crude samples. The crude culture media were diluted in 50 mM Tris buffer, pH 8.0. The amount of $P_4$ was measured as previously described (12).

### Cell Harvest and Counting

After the culture media were removed for RIA, cumulus or G-L cells were detached by incubation for 20 min in a buffer consisting of 0.05 M phosphate-buffered saline, pH 7.4, with 1% (wt/vol) disodium EDTA and 5% (vol/vol) dimethyl sulfoxide (both from Sigma). Cumulus cells corresponding to each individual COC were collected into a separate Eppendorf test tube. Similarly, the G-L cells corresponding to a single woman were collected into a single Eppendorf test tube. After one wash, cells (cumulus or G-L cells) were divided equally into three Eppendorf test tubes. Two Eppendorf test tubes were used to determine the basal and total 3β-HSD values (detailed below). The third Eppendorf test tube was used for cell nucleus staining. Cell nuclei were stained by adding 50 μl crystal violet (50-mg/100 ml in 0.1-M trisodium citrate) for 10–20 min at 37°C and then counted in a hemocytometer. This method was reproducible and produced results similar to those obtained by counting intact granulosa cells from rats, pigs, and humans. The advantage of this approach was the ability to count accurately cells aggregated in clumps.

### Assessment of 3β-HSD Activity

3β-HSD activity was assessed by a histochemical approach as previously described (13). Briefly, the cells harvested into the other two Eppendorf test tubes (see above) were suspended in plain medium, and after centrifugation at 300 *g* for 10 min the supernatant was decanted. The cell pellet in both Eppendorf test tubes was suspended in the reaction solution (50 μl/test tube). The reaction solution contained nicotinamide–adenine dinucleotide, nitroblue tetrazolium, sodium cyanide, and $MgCl_2$ (all from Sigma) in Tris buffer. In one test tube the reaction solution was supplemented with dehydroepiandrosterone [(+)DHEA]. The second Eppendorf test tube served as control, and therefore the reaction solution was not supplemented with DHEA [(−)DHEA]. Following incubation for 1 hr at 37°C, the cells were visualized under a light microscope in a hemocytometer. Cells containing 3β-HSD activity were stained blue violet. The degree of 3β-HSD activity was expressed as percentage of stained cells per total cell count.

In the absence of DHEA in the reaction solution [(−)DHEA], the percentage of 3β-HSD-positive cells (violet-blue-stained cells) is dependent on the enzyme level (3β-HSD) in the cells and on the intracellular levels of substrate that is available for 3β-HSD activity. Therefore, the observed value of 3β-HSD-positive cells in the absence of DHEA [(−)DHEA] is defined as basal 3β-HSD activity. On the other hand, in the presence of saturated levels of DHEA [(+)DHEA], values of 3β-HSD activity manifest the total enzyme level, since maximal substrate is available for the enzyme activity and the only limiting factor for cell staining is the presence of the enzyme. Low number of 3β-HSD-positive cells in the basal test tube [(−)DHEA] and high levels of 3β-HSD in the total test tube [(+)DHEA] may indicate that the cells contain high levels of enzyme activity but low levels of endogenous substrate available for 3β-HSD.

### Statistical Analyses

The experimental data were subjected to one-way analysis of variance (ANOVA), followed by unpaired Student's *t* test for individual comparisons between means. $P < 0.05$ was considered statistically significant. The results are expressed as mean ± SEM.

## RESULTS

### Effect of Number of COC in Culture Dish on Fertilization and $P_4$ Secretion

The percentage of fertilized oocytes (2 pronuclei) increased by 1.3-fold ($P < 0.001$) when two COCs

versus one were cultured together in a single culture dish (Table I). The increase in number of COCs per culture is associated with an increase in $P_4$ secretion during days 0–3 of culture. The total amount of the secreted $P_4$ per culture dish at two versus one COCs increased by 1.8 fold ($P < 0.001$) (Table I).

### Effect of Added Pregnenolone on Cumulus and G-L Cell $P_4$ Secretion

The calculated daily amounts of $P_4$ that were secreted during days 0–3 of culture varied among the individual cumulus masses (when each cumulus mass was plated alone in the culture dish) from 13.5 ng to about 370 ng/cumulus mass. In this population of cumulus masses, about 44% of them secrete less than 100 ng $P_4$/cumulus mass, while about 25% secrete more than 200 ng $P_4$/cumulus mass. Compared to the large differences in $P_4$ secretion in days 0–3, a decrease in $P_4$ secretion was noted during days 3–6 that minimize these differences. About 90% of the cumulus masses secreted less than 100 ng $P_4$/cumulus mass and none of them secreted more than 200 ng $P_4$/cumulus mass.

Addition of pregnenolone on day 3 of culture to individually cultured cumulus masses did not alter significantly the rate of $P_4$ secretion during days 3–4 and 3–5 of culture. However, a 1.8-fold increase in $P_4$ secretion ($P < 0.01$) was noted in cumulus mass exposed to pregnenolone during days 3–6 of the culture period (Fig. 1). In G-L cells, $P_4$ secretion increased by about 1.4-fold ($P < 0.05$) and 1.3-fold ($P < 0.01$) when cultured in the presence of pregnenolone during days 3–5 and 3–6, respectively, but not during days 3–4 of culture (Fig. 1).

**Table I.** Fertilization Rate and $P_4$ Secretion in Correlation with Number of COCs in Culture Dish[a]

| Number of cocultured COCs | Fertilization rate (%) | $P_4$ (ng/ml) |
|---|---|---|
| 1 | 65 ± 4.6*[b] | 166.7 ± 13.1* |
| 2 | 83 ± 3.4** | 304.8 ± 34.4** |
| 3 | 76 ± 7.4*,** | 520.2 ± 101.4** |

[a] Results are expressed as means ± SEM. Data were evaluated by one-way analysis of variance (ANOVA). $P < 0.05$ indicated a significant difference of the specific measurment. Significant difference between two individual subgroups was determined by student's *t* test. For fertilization rate, total number of women is 30 and the following statistical parameters were observed by ANOVA: $F = 7.07$ and $P < 0.05$. For $P_4$ secretion, the total number of replicates is 78 and the following statistical parameters were observed by ANOVA: $F = 23.75$ and $P < 0.001$.

[b] Values not sharing common symbol (* or **) in the same column are significantly different ($P < 0.05$) by student's *t* test.

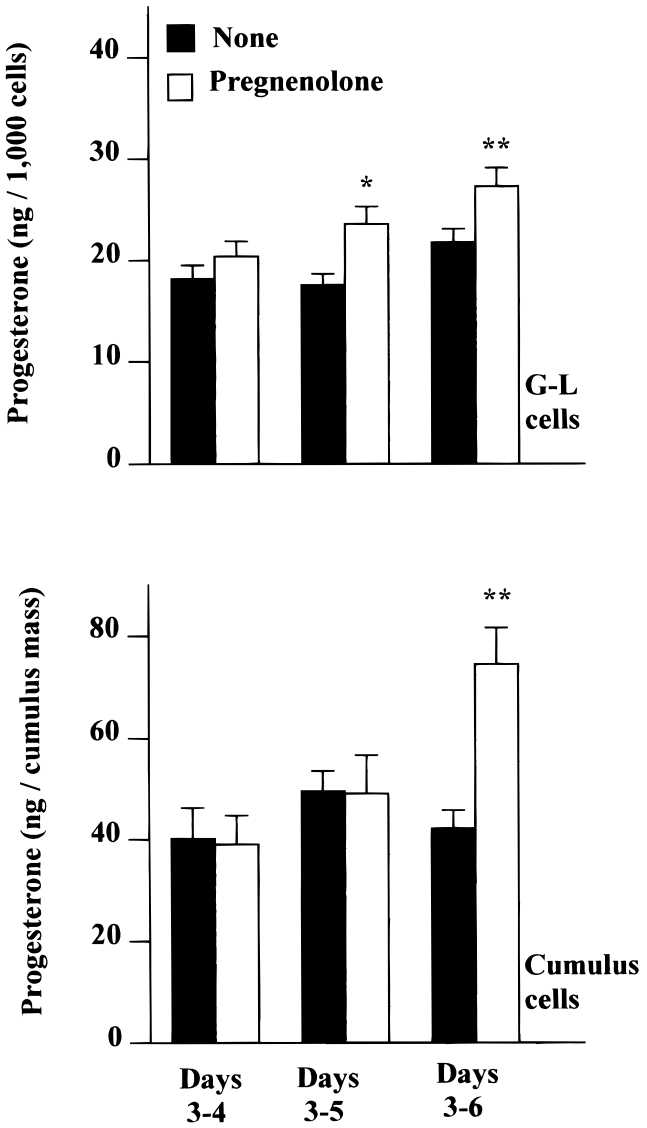


**Fig. 1.** Levels of daily $P_4$ that accumulated during days 3–4, 3–5, and 3–6 in cumuli (total number of cumuli is 187) and G-L cells (total number of women is 12) cultured in the absence (■) or presence of pregnenolone (□). *$P < 0.05$, ** $P < 0.01$, compared to corresponding cell type cultured in the absence of pregnenolone during the same culture period (Student's *t* test).

According to the amount of $P_4$ that was secreted during days 0–3 of culture, cumulus masses were categorized as secreting high $P_4$ (>200 ng/cumulus mass) or low $P_4$ levels (<100 ng/cumulus mass). It appeared that in cumulus masses designated as secreting high $P_4$ levels, addition of pregnenolone significantly increased $P_4$ secretion during days 3–5 and 3–6 of culture (Fig. 2). However, in cumulus masses designated as secreting low $P_4$ levels, addition of pregneno-

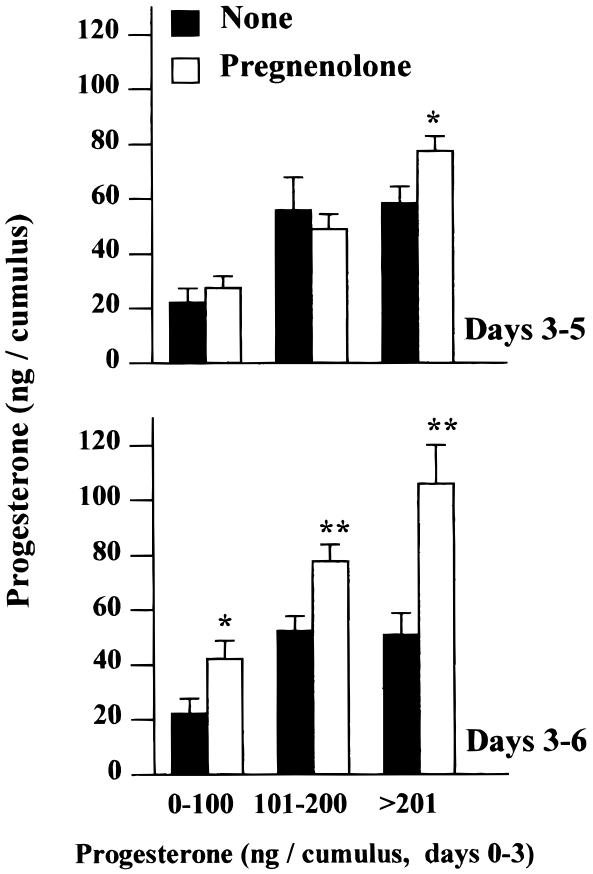


**Fig. 2.** Relationship between $P_4$ secretion (0–201 ng/cumulus) by cumulus masses during days 0–3 of culture (X axis) and $P_4$ secretion during days 3–5 and 3–6 of culture (Y axis). Cumulus masses (total number of cumuli is 144) were cultured in the absence (■) and presence (□) of pregnenolone. When data were subjected to one-way ANOVA, the following statistical values were observed for $P_4$ secretion during: days 3–5, $F = 8.18$, $P < 0.001$; days 3–6, $F = 6.34$, $P < 0.001$. Other details are as described in Fig. 2.

lone significantly increased $P_4$ during days 3–6 but not in shorter incubation time (Fig. 2).

## Effect of Added Pregnenolone on Cumulus and G-L Cell 3β-HSD Activity

The level of 3β-HSD was always examined on cells harvested after completion of the culture period. The basal level of 3β-HSD activity was measured in the absence of exogenous substrate in the reaction solution [(−) DHEA] and total 3β-HSD activity was measured in the presence of exogenous substrate [(+) DHEA].

Basal 3β-HSD activity was significantly low in the cumulus cells that were exposed to pregnenolone versus medium alone during days 3–6 of culture (Fig. 3). The low values of 3β-HSD activity could be a consequence of an actual decrease in the level of 3β-HSD activity in cumulus cells or may be associated with a decrease in the endogenous available substrate. However, the total level of 3β-HSD activity significantly increased in the 3 day (days 3–6) pregnenolone-treated cumulus cells compared to cells cultured in medium alone (Fig. 3). A 48-hr (days 3–5) culture of cumuli in the presence of pregnenolone did not alter either the basal or total 3β-HSD activity. In the pregnenolone-treated G-L cells, a significant increase in the total 3β-HSD-activity was observed during both days 3–5 and 3–6 of culture compared to G-L cells cultured in medium alone (Fig. 3). No significant difference in the basal or total of 3β-HSD-activity was seen during days 3–4 in either cumulus or G-L cells, whether treated or not with pregnenolone (data not shown).

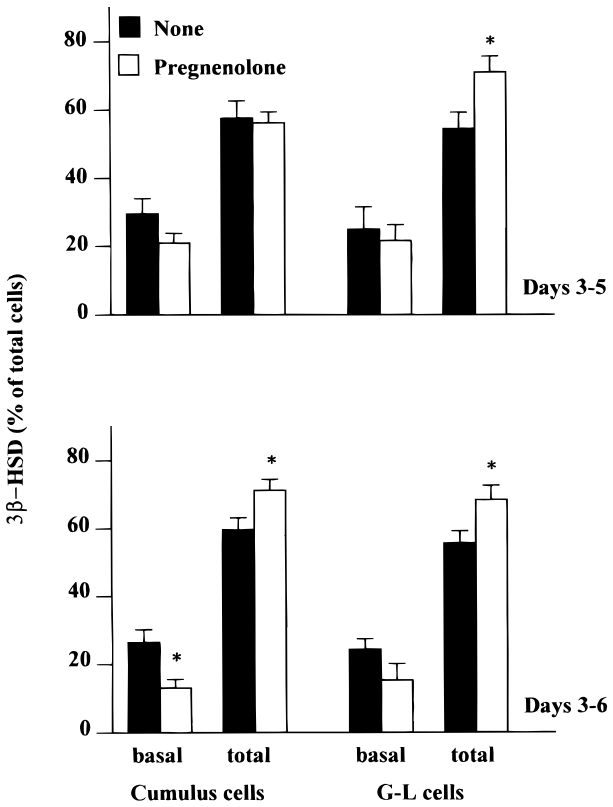


**Fig. 3.** Degree of 3β-HSD activity during days 3–5 and days 3–6 of culture in cumulus (total number of cumuli is 67) and G-L cells (total number is 12). Cells were cultured in medium alone (■) or in medium supplemented with pregnenolone (□). After termination of culture the levels of basal and total 3β-HSD activity were measured as detailed in the materials and methods. *$P < 0.05$, compared to cells cultured in the absence of pregnenolone in the culture medium.

## DISCUSSION

The retrieved COCs are not synchronized to the same degree of maturation (5,12,19). The possibility that a less mature COC is less fertilizable had been demonstrated by many studies (20,21). The present study indicates that coculture of two COCs versus one creates a more beneficial microenvironment to enhance fertilization. COCs were cocultured in combinations of two or three together when the number of retrieved COCs was higher than expected. Thus, this may indicate that there was an advantage of coculturing two COCs versus one at least in the population of women who produce larger number of oocytes in stimulating cycles. The mechanism that underlies these results was not the aim of the present study. However, it is more likely that in the two cocultured COCs there is a greater chance that one of the COCs is more mature and fertilizable and this COC may facilitate in some way the fertilization of the other cocultured COC. It had been suggested that cumulus cells facilitate activities that are involved in the processes of oocyte maturation and fertilization (22,23). Several studies had indicated that specific factors that are secreted by cumulus cells (24,25) and oocyte (26) are involved in oocyte maturation and/or fertilization.

The possible involvement of $P_4$ in enhancing fertilization is supported by recent studies (27). It appears that $P_4$ enhances acrosome reaction by stimulating $Ca^{2+}$ influx in sperm plasma membrane via nongenomic $P_4$ receptors (28). Exposure of the spermatozoa to $P_4$ sensitizes them to undergo an acrosome reaction upon interaction with the zona pellucida, thereby enhancing spermatozoal penetration through the zona pellucida (14,15). Furthermore, it has been suggested that $P_4$ affects the pattern of ciliary motility in the fallopian tube (29); thus, cumulus cell $P_4$ secretion by the ovulated COC may be regulating COC movement within the fallopian tube.

The present study, as well as other studies (8,12), indicates that the cumulus and G-L cell $P_4$ secretions in stimulated cycles are highly variable among the individual follicles. In vitro, $P_4$ secretion can be increased in both cell types either by hormonal stimulation (7,32) or by adding the appropriate substrate (8,33). Addition of pregnenolone increased cumulus and G-L cell $P_4$ secretion only within 5 days after follicular aspiration. This may indicate that following this period the cell substrate storage for $P_4$ biosynthesis is critically depleted to satisfy the steroidogenic capacity of these cells. Alternatively, the significant increase in steroidogenic activity within 5 days after follicular aspiration also may be involved in an increased capacity to utilize the exogenously provided pregnenolone for $P_4$ biosynthesis. Actually, the maximal serum $P_4$ level during the luteal phase of the menstrual cycle is seen within the 6–8 days after ovulation. At this time there is a peak in the expression of 3β-HSD and P-450scc (34). In cumuli that were designated as secreting high levels of $P_4$, the significant increase in $P_4$ secretion was already observed by 48 hr of continuous exposure to pregnenolone. The cumulus cells manifesting higher active steroidogenic machinery may utilize the provided pregnenolone at a significantly higher rate for $P_4$ biosynthesis and secretion.

The present study assay to determine 3β-HSD activity depends on the presence of saturated levels of the enzyme substrate. Performing this assay in the absence of both exogenous and endogenous enzyme substrate yields low 3β-HSD activity, which may not necessarily indicate the absence of 3β-HSD. Therefore a low percentage of 3β-HSD positive cells in the absence of exogenous saturated levels of the enzyme substrate (basal levels) may indicate either a significant depletion in the available endogenous substrate or a decrease in 3β-HSD expression.

The present study indicates that the basal 3β-HSD-activity is significantly decreased in the 72-hr pregnenolone-treated cumuli compared with cumulus masses cultured in medium alone (Fig 3). This finding could be explained as either indicating that addition of pregnenolone induced either a major depletion in the intercellular enzyme substrate or a decrease in the 3β-HSD expression. Total 3β-HSD activity, however, is significantly increased in the pregnenolone-treated cumuli. This latter finding may support the option that addition of pregnenolone induced an increase in the 3β-HSD.

What caused the increase in the 3β-HSD activity and $P_4$ secretion in the pregnenolone-treated cells? Evidence for a significant role of $P_4$ in the regulation of $P_4$ secretion has been reported. In rats, $P_4$ enhances 3β-HSD activity in granulosa cells at the time of ovulation and later (35). In humans, G-L cell $P_4$ plays an autocrine role in regulating cell proliferation (36). In primates, $P_4$ receptors and 3β-HSD have been codetected in luteal cells, and inhibition of 3β-HSD resulted in luteolysis without affecting $P_4$ receptor density (37). This reported evidence implies that, in the present study, the parallel increase in 3β-HSD and $P_4$ in the presence of pregnenolone might be facilitated by the biosynthesized $P_4$.

Our previous studies indicated that a lower fertilization rate is associated with lower $P_4$ secretion (12,13).

The present study suggests that in cumulus masses defined as secreting low $P_4$ levels, addition of pregnenolone increases $P_4$ secretion during 72 hr of culture, but not earlier. This increase cannot assist in enhancing fertilization, which should take place within a few hours after COCs pickup (38). The alternative approach, in which cumulus cell $P_4$ secretion is increased by culturing two COCs, is simple and more successful. Whether the increase in fertilization rate in the two cocultured COCs is due the associated increase in $P_4$ secretion was not assessed in the present study.

## ACKNOWLEDGMENTS

The secretarial work of Cibina Stanton and the editorial assistance of Miss Ruth Singer are gratefully acknowledged. The authors thank Prof. Raymond Coleman for a critical reading of the manuscript.

## REFERENCES

1. Zlotkin T, Farkash Y, Orly J: Cell-specific expression of immunoreactive cholesterol side-chain cleavage cytochrome P-450 during follicular development in the rat ovary. Endocrinology 1986;119:2809–2820
2. Pupkin M, Bratt H, Weisz J, Lloyd CW, Balogh K, Jr: Dehydrogenases in the rat ovary. I. A histochemical study of $\Delta^5$-3β- and 20α-hydroxysteroid dehydrogenases and enzymes of carbohydrate oxidation during the estrous cycle. Endocrinology 1966;79:316–327
3. Goldschmit D, Kraicer P, Orly J: Periovulatory expression of cholesterol side-chain cleavage cytochrome P-450 in cumulus cells. Endocrinology 1989;124:369–378
4. Gore-Langton RE, Armstrong DT: Follicular steroidogenesis and its control. *In* The Physiology of Reproduction, vol 1, 2nd ed, E Knobil, JD Neill, GS Greenwald, CL Markert, DW Pfaff (eds). New York, Raven Press, 1994, pp 571–627
5. Bar-Ami S, Zlotkin E, Brandes JM, Itskovitz-Eldor J: Failure of meiotic competence in human oocytes. Biol Reprod 1994;50:1100–1107.
6. Wickings EJ, Hillier SG, Reichert LE, Jr: Gonadotrophic control of steroidogenesis in human granulosa-lutein cells. J. Reprod Fertil 1986;76:677–684
7. Bar-Ami S, Khoury C: Effect of cell-plating density on the steroidogenic activity of human cumulus cells. J Reprod Fertil 1994;101:729–735
8. Gitay-Goren H, Brandes JM, Bar-Ami S: Altered steroidogenic pattern of human granulosa-lutein cells in relation to cumulus cell culture morphology. J Steroid Biochem 1990;36:457–464
9. Bar-Ami S, Khoury C, Zlotkin E, Brandes JM: Increasing progesterone secretion in human granulosa-lutein cells induced by human follicular fluid. Hum Reprod 1993;8:46–53
10. Yovich JL, McColm SC, Yovich JM, Matson PL: Early luteal serum progesterone concentrations are higher in pregnancy cycles. Fertil Steril 1985;44:185–189
11. Hill GA, Herbert CM, III, Wentz AC, Osteen KG: Use of individual human follicles to compare oocyte in vitro fertilization to granulosa cell in vitro luteinization. Fertil Steril 1987;48:258–264
12. Bar-Ami S, Gitay-Goren H, Brandes JM: Different morphological and steroidogenic patterns in oocyte/cumulus-corona cell complexes aspirated at in vitro fertilization. Biol Reprod 1989;41:761–770.
13. Bar-Ami S: Increasing progesterone secretion and 3β-hydroxysteroid dehydrogenase activity of human cumulus cells and granulosa-lutein cells concurrent with successful fertilization of the corresponding oocyte. J Steroid Biochem Mol Biol 1994;51:299–305
14. Parinaud J, Labal B, Vieitez G: High progesterone concentrations induce acrosome reaction with a low cytotoxic effect. Fertil Steril 1992;58:599–602
15. Roldan ERS, MuraseT, Shi Q-X: Exocytosis in spermatozoa in response to progesterone and zona pellucida. Science 1994;266:1578–1581
16. Tyler JP, Smith DM, Biggers JD: Effect of steroids on oocyte maturation and atresia in mouse ovarian fragments in vitro. J Reprod Fertil 1980;58:203–212
17. Barrett CB, Power RD: Progestins inhibit murine oocyte meiotic maturation in vitro. J Exp Zool 1993;265:231–239
18. Tennant JR: Evaluation of the trypan blue technique for determination of cell viability. Transplantation 1964;2:685–694
19. Bar-Ami S, Regev A, Gitay-Goren H: Effect of androgen substrates on the steroidogenic pattern of cumulus cells: Correlation with cumulus culture morphology. J Assist Reprod Genet 1997;14:270–276
20. De Vos A, Van de Velde H, Joris H, Van Steirteghem A: In-vitro matured metaphase-I oocytes have a lower fertilization rate but similar embryo quality as mature metaphase-II oocytes after intracytoplasmic sperm injection. Hum Reprod 1999;14:1859–1863
21. Beckers NG, Pieters MH, Ramos L, Zeilmaker GH, Fauser BC, Braat DD: Retrieval, maturation, and fertilization of immature oocytes obtained from unstimulated patients with polycystic ovary syndrome. Assist Reprod Genet 1999;16:81–86
22. Cecconi S, D'Aurizio R, Colonna R: Role of antral follicle development and cumulus cells on in vitro fertilization of mouse oocytes. J Reprod Fertil 1996;107:207–214
23. Ka HH, Sawai K, Wang WH, Im KS, Niwa K: Amino acids in maturation medium and presence of cumulus cells at fertilization promote male pronuclear formation in porcine oocytes matured and penetrated in vitro. Biol Reprod 1997;57:1478–1483
24. Illera MJ, Lorenzo PL, Illera JC, Petters RM: Developmental competence of immature pig oocytes under the influence of EGF, IGF-I, follicular fluid and gonadotropins during IVM-IVF processes. Int J Dev Biol 1998;42:1169–1172
25. Silva CC, Knight PG: Modulatory actions of activin-A and follistatin on the developmental competence of in vitro-matured bovine oocytes. Biol Reprod 1998;58:558–565
26. Elvin JA, Clark AT, Wang P, Wolfman NM, Matzuk MM: Paracrine actions of growth differentiation factor-9 in the mammalian ovary. Mol Endocrinol 1999;13:1035–1048
27. Meizel S: Amino acid neurotransmitter receptor/chloride channels of mammalian sperm and the acrosome reaction. Biol Reprod 1997;56:569–574

28. Meizel S, Turner KO, Nuccitelli R: Progesterone triggers a wave of increased free calcium during the human sperm acrosome reaction. Dev Biol 1997;182:67–65
29. Paltieli Y, Weichselbaum A, Eibschitz I, Ziskind G, Silbermann M: The effects of estrogen and progesterone on the ciliary activity in the human fallopian tubes. J Assist Reprod Genet (Suppl) 1995;12:112S
30. Bar-Ami S, Gitay-Goren H: Altered steroidogenic activity of human granulosa-lutein cells at different cell densities in culture. Mol Cell Endocrinol 1993;90:157–164
31. Khoury C, Itskovitz-Eldor J, Bar-Ami S: Induction of maturation of cumulus–oocyte complex by gonadotropin-releasing hormone analog is associated with lower progesterone secretion. J Clin Endocrinol Metab 1994;79:1001–1006
32. Doody KJ, Lorence MC, Mason JI, Simpson ER: Expression of messenger ribonucleic acid species encoding steroidogenic enzymes in human follicles and corpora lutea throughout the menstrual cycle. J Clin Endocrinol Metab 1990;70:1041–1045
33. Tanaka N, Iwamasa J, Matsuura K, Okamura H: Effects of progesterone and antiprogesterone RU486 on ovarian 3β-hydroxysteroid dehydrogenase activity during ovulation in the gonodotrophin-primed immature rat. J Reprod Fertil 1993; 97:167–172
34. Chaffkin LM, Luciano AA, Peluso JJ: Progesterone as an autocrine/paracrine regulator of human granulosa cell proliferation. J Clin Endocrinol Metab 1992;75:1404–1408
35. Duffy DM, Hess DL, Stouffer RL: Acute administration of a 3β-hydroxysteroid dehydrogenase inhibitor to rhesus monkeys at the midluteal phase of the menstrual cycle: Evidence for possible autocrine regulation of the primate corpus luteum by progesterone. J Clin Endocrinol Metab 1994;79:1587–1594
36. Ben-Rafael Z, Kopf GS, Blasco L, Tureck RW, Mastroianni L, Jr: Fertilization and cleavage after reinsemination of human oocytes in vitro. Fertil Steril 1986;45:58–62