

# mRNA expression pattern of insulin-like growth factor components of granulosa cells and cumulus cells in women with and without polycystic ovary syndrome according to oocyte maturity

The mRNA expression of insulin-like growth factor (IGF) components was investigated in granulosa cells (GCs) and cumulus cells (CCs) sampled from immature and mature follicles in women with and without polycystic ovary syndrome (PCOS). Gene expression for IGF-binding protein (IGFBP) 3 in GCs definitely differed between normal women and women with PCOS, whereas increase in IGF-I and IGF receptor (IGFR) 2 mRNA in mature-follicle GCs, increase in IGF-II, IGFR-1, and IGFBP-4 mRNA in immature-follicle GCs, increase in IGFR-2 and IGFBP-2 mRNA in mature-follicle CCs, and increase IGFBP-6 mRNA in immature-follicle CCs were observed in both normal women and women with PCOS. (*Fertil Steril*® 2010;94:2417–20. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** Granulosa cell, cumulus cell, IGF, IGF receptor, IGF binding protein

Insulin-like growth factor I (IGF-I) has been shown to stimulate estrogen synthesis of granulosa cells (GCs) and to act synergistically with FSH and LH in controlling granulosa aromatase levels (1, 2). The synthesis of IGF-II in the estrogen dominant follicle increases dramatically at the time of follicle selection (3). The biologic effects of IGFs are mediated through two types of cell membrane receptors, IGF receptor 1 (IGFR-1) and IGF receptor 2 (IGFR-2) (4). IGF-binding proteins (IGFBPs) 1, 2, 3, 4, 5, and 6 can both inhibit and potentiate IGF action at the target cells, depending on particular posttransductional changes, including phosphorylation, proteolytic degradation, and binding to the extracellular matrix (5). IGFBP-1 is expressed in GCs of the dominant follicle after the LH surge and in the corpus luteum (6, 7). IGF-II inhibits IGFBP-1 production, and IGFBP-1 inhibits IGF-II by luteinized

human GCs in vitro (8–11). Although one study reported higher IGFBP-3 concentrations in estrogen-dominant follicles compared with androgen-dominant follicles (12), that result was contradicted by another study (13). IGFBP-4 is the smallest among the six IGFBPs and has an inhibitory effect on both granulosa and theca cell steroidogenesis (14).

Human oocytes collected from follicles of similar size or volume during the maturation cycle cannot be assumed to have identical characteristics, because mature oocytes can be collected from very small follicles (15). For this reason, we classified follicles as immature or mature based on the morphologic characteristics of retrieved oocytes. Immature oocytes were defined as oocytes retrieved at the germinal vesicle (GV) stage, and mature oocytes were defined as oocytes retrieved at metaphase II (MII). According to the results of earlier studies, the IGF systems of women with polycystic ovary syndrome (PCOS) differ from those of women with normal ovaries (16–19). IGF system expression has been explored in mural GCs and thecal cells, but remains poorly studied in cumulus cells (CCs). Therefore, we divided our patient sample into women with and without PCOS. We then investigated differences in the mRNA expression patterns of IGF systems between the immature and mature follicles in GCs and CCs within each group.

The study protocol was approved by the Institutional Review Board of the Bundang CHA General Hospital, CHA University. Thirteen patients who visited Bundang CHA Fertility Center for IVF-ET procedures were registered. Each patient gave informed written consent. PCOS was defined according to the Rotterdam criteria (20).

All patients received recombinant FSH and hMG from day 3 after the onset of menstrual bleeding. Once the leading follicular size become 20 mm, hCG (250 µg subcutaneously; Ovidrel; Serono)

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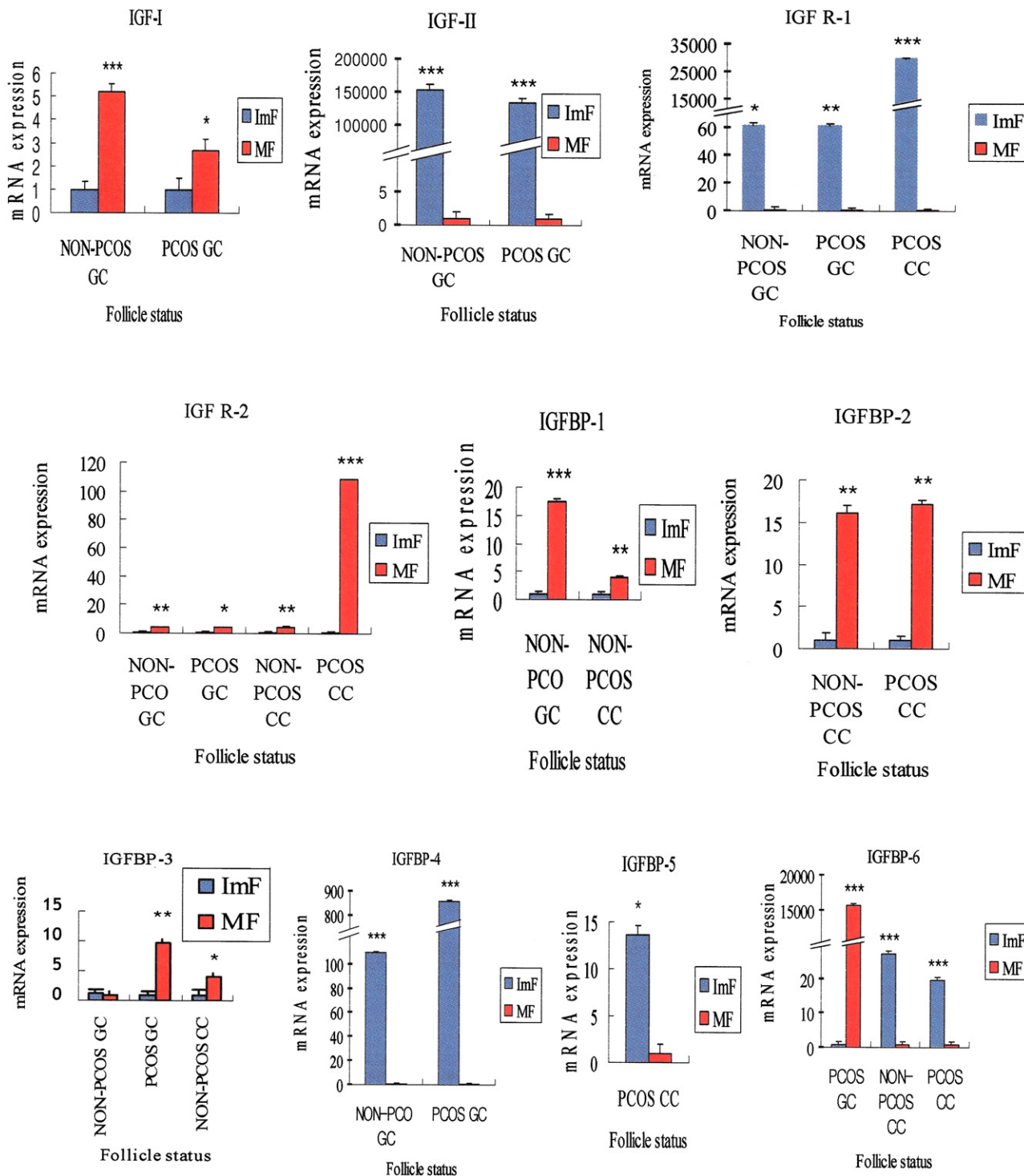
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# FIGURE 1

Messenger RNA expression of insulin-like growth factor (IGF) components in granulosa cells (GCs) of immature-follicular (ImF) non-polycystic ovary syndrome (PCOS) group, mature-follicular (MF) non-PCOS group, immature-follicular PCOS group, and mature-follicular PCOS group. Values are mean  $\pm$  SEM. \* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$ .



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was injected. Oocytes were retrieved 35 hours after hCG administration. For pituitary suppression, leuprolide acetate 1 mg subcutaneously (Lucrin; Abbott) was injected from 10 days before menstruation to day 2 of menstrual bleeding or cetrorelix 0.25 mg subcutaneously (Cetrotide; Serono, Korea) was administered from day 7 of menstruation to hCG injection. The number of GnRH agonist cycles was the same as the number of GnRH antagonist cycles in women with PCOS, and the number of GnRH antagonist cycles was one greater than the number of GnRH agonist cycles in normal women. We did not detect any bias due to different stimulation protocols between the two groups. Follicles were classified as mature or immature based on the morphologic characteristics of the retrieved oocytes. Aspirates in which immature oocytes were mixed with mature oocytes were excluded.

The GCs were sampled separately from immature and mature follicular fluids. The CCs were stripped and sampled separately from around immature and mature oocytes. GCs and CCs were washed by phosphate-buffered saline solution (PBS) treated with diethyl pyrocarbonate (Sigma, St. Louis, MO), frozen with liquid nitrogen immediately, and preserved in deep freezer at  $-80^{\circ}\text{C}$ . The total RNA of GCs and CCs was extracted using Trizol (Invitrogen, Calsbad, CA). Reverse transcription was performed using the Superscript first strand synthesis system (Invitrogen, Calsbad, CA) as suggested by the manufacturer. Quantitative real-time polymerase chain reaction (PCR) was performed with Sybr Green PCR kit (Qiagen, Valencia, CA) As an internal control for real-time PCR, glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used. PCR was performed on a Rotor-Gene RG-3000 (Corbett Life Science, Sydney, Australia). After initial denaturing at  $95^{\circ}\text{C}$  for 15 minutes, 40 thermal cycles of denaturing at  $95^{\circ}\text{C}$  for 10 seconds, annealing at  $55^{\circ}\text{C}$  for 10 seconds, and extension at  $72^{\circ}\text{C}$  for 10 seconds were performed. Data acquisition was conducted on the Fam/Sybr channel at  $80^{\circ}\text{C}$  for 10 seconds. Melting curve analysis was carried out at  $75^{\circ}\text{C}$ – $90^{\circ}\text{C}$  with  $0.5^{\circ}\text{C}$  increments, and values of threshold cycle (Ct) were determined. The expression levels of mRNA of each gene were calculated as  $2^{-\Delta\Delta\text{Ct}}$  and the values (mean  $\pm$  SEM) were expressed considering the lowest mRNA level among groups as one. Statistical comparison was performed by ANOVA test and Fischer least standard test. A  $P$  value of  $<.05$  was considered to be statistically significant.

IGFBP-2 and IGFBP-6 mRNA was not detected in immature-follicle GCs from normal women, and it was not possible to compare this result with results from mature-follicle GCs. IGFBP-1, IGFBP-2, and IGFBP-5 mRNA was not detected in immature-follicle GCs of women with PCOS. We did not detect IGF-I, IGFR-2, and IGFBP-5 mRNA in immature-follicle CCs of normal women and IGF-I, IGFBP-1, IGFBP-3, and IGFBP-5 mRNA were not detected in immature-follicle CCs of women with PCOS.

In the GCs of normal women, mRNA levels of IGF-II ( $P<.001$ ), IGFR-1 ( $P<.05$ ), and IGFBP-4 ( $P<.001$ ) in immature follicles were significantly higher than those in mature follicles, while mRNA levels of IGF-I ( $P<.001$ ), IGFR-2 ( $P<.05$ ), and IGFBP-1 ( $P<.001$ ) in mature follicles were higher than those in immature follicles. In the GCs of women with PCOS, mRNA levels of IGF-II ( $P<.001$ ), IGFR-1 ( $P<.01$ ), and IGFBP-4 ( $P<.001$ ) in immature follicles were significantly greater than those in mature follicles, while mRNA levels of IGF-I ( $P<.05$ ), IGFR-2 ( $P<.05$ ), IGFBP-3 ( $P<.01$ ), and IGFBP-6 ( $P<.001$ ) in mature follicles were

significantly higher than those in immature follicles. No difference of IGFBP-3 in GCs of normal women was found, but an increment of IGFBP-3 in mature-follicle GCs of women with PCOS was observed. An increment of IGFBP-6 in mature-follicle GCs was observed only in women with PCOS.

In the CCs of normal patients, the IGFBP-6 ( $P<.001$ ) mRNA levels in immature follicles were significantly higher than those in mature follicles, and IGFR-1 ( $P<.05$ ), IGFR-2 ( $P<.01$ ), IGFBP-1 ( $P<.010$ ), IGFBP-2 ( $P<.01$ ), and IGFBP-3 ( $P<.05$ ) mRNA levels in mature follicles were significantly greater than those in immature follicles. In the CCs of women with PCOS, the IGFR-1 ( $P<.001$ ), IGFBP-5 ( $P<.05$ ), and IGFBP-6 ( $P<.001$ ) mRNA levels in immature follicles were significantly higher than those in mature follicles, and the IGFR-2 ( $P<.001$ ) and IGFBP-2 ( $P<.001$ ) mRNA levels in mature follicles were significantly above the levels observed in immature follicles (Fig. 1).

This study is the first to assess whether there are differences in the levels of IGF components in CCs when women with and without PCOS are compared. Elevation of IGF-I production in luteinized GCs of mature follicles implies that IGF-I plays an important role in steroid synthesis at the time immediately preceding ovulation. The high levels of IGF-II mRNA in immature-follicle GCs irrespective of PCOS status is consistent with results of previous studies about IGF-II (3, 21). The high levels of IGFR-1 mRNA expression in immature-follicle GCs in this study were similar to expression patterns of IGF-II mRNA, regardless of PCOS status. Because IGF-II acts via IGFR-1 (and not IGFR-2) in the ovary (22), the similarity of IGF-II and IGFR-1 mRNA expression seems to be relevant. The increase in IGFR-2 levels in mature-follicle GCs regardless of PCOS status is difficult to explain, but this pattern was similar to the expression pattern of IGF-I.

The present study affirms findings related to paracrine feedback cycles between IGFBP-1 and IGF-II reported in earlier studies (8–11), because IGFBP-1 was found in higher levels in mature-follicle GCs in normal women, in contrast to the lower levels of IGF-II found in mature-follicle GCs in normal women. In addition, the increment of IGFBP-1 expression observed in mature-follicle CCs of normal women was no different from the IGFBP-1 expression in GCs. An increase in IGFR-2 and IGFBP-2 mRNA in mature-follicle CCs of normal women and women with PCOS could be related to favorable factors in oocyte maturation.

Although no significant difference of IGFBP-3 mRNA levels was detected between mature-follicle GCs and immature-follicle GCs in normal women, the observed increase in IGFBP-3 mRNA in mature-follicle GCs of women with PCOS is consistent with an earlier study demonstrating that IGFBP-3 levels increase during stimulation in PCOS patients (23). We observed higher levels of IGFBP-3 in mature-follicle CCs in normal women only, which agrees with an earlier study suggesting that high IGFBP-3 levels in follicular fluid during oocyte retrieval correlate with better oocyte maturation (24). The observed increment of IGFBP-4 mRNA expression in immature-follicle GCs regardless of PCOS in the present study is consistent with earlier reports that IGFBP-4 inhibits steroid synthesis in GCs and thecal cells (14). The expression pattern of IGFBP-4 mRNA in GCs from women with PCOS in the present study also agrees with the increase in IGFBP-4 in small follicles of women with PCOS observed in previous reports (25–27). The increment of IGFBP-5

mRNA in immature-follicle CCs in women with PCOS observed in this study may inhibit oocyte maturation in women with PCOS by decreasing IGF availability. A decrease in IGFBP-6 observed in mature-follicle CCs in both normal women and women with PCOS suggests that IGFBP-6 may be related to inhibit oocyte maturation.

In conclusion, gene expression for IGFBP-3 in GCs definitely differed between normal women and women with PCOS, whereas increase in IGF-I and IGFR-2 mRNA in mature-follicle GCs, increase in IGF-II, IGFR-1, and IGFBP-4 mRNA in immature-follicle GCs, increase in IGFR-2 and IGFBP-2 mRNA in

mature-follicle CCs, and increase in IGFBP-6 mRNA in immature-follicle CCs were observed in both normal women and women with PCOS. In addition to our results being consistent with earlier reports in GCs, we have shown for the first time the patterns of mRNA expression for all IGF components in CCs of the human ovary regarding oocyte maturity and PCOS. It is difficult to determine the role of IGF components related to follicular growth and oocyte maturation based on differences in mRNA expression in this study. Further studies are required to investigate which ones of these IGF components positively affect follicular growth and oocyte maturation.

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