Interleukin-6 inhibits the expression of luteinizing hormone receptor mRNA during the maturation of cultured rat granulosa cells

K Tamura, T Kawaguchi and H Kogo

Department of Pharmacology, Tokyo University of Pharmacy & Life Science, 1432-1, Hachioji-shi, 192-0392, Japan

(Requests for offprints should be addressed to K Tamura; Email: hiro@ps.toyaku.ac.jp)

Abstract

Interleukin-6 (IL-6) and its receptor components have been shown to be present in rat follicular granulosa cells. The present study was designed to examine the effect of this cytokine on changes in expression of the luteinizing hormone receptor (LHR) messenger RNA and of the steroidogenic enzyme, CYP11A1 (cytochrome P450 scc) in an *in vitro* model of granulosa cell maturation. Ovarian granulosa cells harvested from immature rats 2 days after treatment with equine chorionic gonadotropin were cultured for 48 h in media containing 10% fetal bovine serum. They were then transferred to a chemically defined serum-free medium and cultured for an additional 72 h. Within 24 h of transfer, the expressions of LHR and CYP11A1 mRNA increased significantly and remained increased for 72 h. The cells responded to exposure to

FSH, but not LH, by an increase in production of cAMP before the additional 72 h of culture. The cAMP response to LH was attained within 24 h and persisted for 72 h, whereas the response to FSH decreased continuously with time. Inclusion of IL-6 in the culture medium caused a dose-dependent decrease in expression of LHR mRNA, in addition to a decrease in the cAMP response to LH. Immunoneutralization of endogenous granulosa cell IL-6 resulted in an increase in expression of LHR mRNA, but not CYP11A1 mRNA. The results are consistent with the view that IL-6 may have a physiological role in the maturation of ovarian follicles by modulating the attainment of the LHR in granulosa cells.

Journal of Endocrinology (2001) **170**, 121–127

Introduction

Ovarian follicular development and ovulation are dependent upon proliferative and differentiative changes in the granulosa cells (Robker & Richards 1998). The gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), not only influence the morphological processes for production of preovulatory follicles, they also have a role in gaining expression of the enzymes needed for steroidogenesis by thecal cells and granulosa cells (Richards 1994). Particularly important for the latter are CYP11A1 (cytochrome P450 scc), the rate-limiting enzyme for conversion of cholesterol to pregnenolone, and CYP19 (cytochrome P450 aromatase (P450 arom)), the rate-limiting enzyme for synthesis of estradiol from androgen precursors (Goldring *et al.* 1987, Oonk *et al.* 1990, Tian *et al.* 1995). Associated with these enzymic changes, and essential for them, is the expression of LH receptors (LHR) on granulosa cells (Zelevnik *et al.* 1974, Erickson *et al.* 1979). Acquisition of these receptors is also a prerequisite for ovulation and differentiation of granulosa cells into luteal cells (Richards *et al.* 1979, Labarbera & Ryan 1981).

Recently, attention has focused upon secondary ovarian paracrine factors that influence follicular development (Erickson & Danforth. 1995). These include growth factors, mostly insulin-like growth factor I (IGF-I), and cytokines such as interleukin-1 β (IL-1 β) that can modulate gonadotropin receptor expression and ovulation. Their importance is exemplified by the finding that both male and female IGF-I null mice are infertile (Baker *et al.* 1996). Follicular development in these null mice is deficient, and even pharmacologic doses of gonadotropin fail to induce ovulation. Furthermore, *in vitro* studies have shown that IGF-I enhances FSH-stimulated expression of the LHR in rat granulosa cells (Kanzaki *et al.* 1994). Expression of these receptors in cultured rat granulosa cells was also markedly suppressed by IL-1 β (Gottschall *et al.* 1988, 1989).

Another cytokine, interleukin-6 (IL-6), usually associated with regulation of B and T cells in the immune system, has been found in the reproductive tract. Rat ovarian epithelium (Ziltener *et al.* 1993), granulosa cells (Gorospe *et al.* 1992) and luteal tissues (Telleria *et al.* 1998) have been shown to produce IL-6. Bioactive IL-6 was also found in the fluid of preovulatory human follicles

(Machelon *et al.* 1994). The IL-6 receptor consists of two parts, an α chain (CD126) that has affinity for IL-6 but no signalling capacity, and a β chain (gp130; CD130) that has no binding affinity for IL-6, but is responsible for signal transduction (Taga *et al.* 1989). The presence of both components of IL-6 receptor, in addition to IL-6, have been reported to be present in cultured rat granulosa cells (Tamura *et al.* 2000). The latter study showed that IL-6 inhibited the production of estrogen by granulosa cells that had been stimulated by FSH. An earlier study (Gorospe *et al.* 1992) found that IL-6 inhibited FSH-stimulated, but not basal, progesterone production in *in vitro* rat granulosa cells.

The mechanism(s) of IL-6 action in the ovary, particularly regarding the maturation and differentiation of granulosa cells has not been investigated. The present study was therefore designed to investigate the role of IL-6 in regulating the expression of LH receptor mRNA, and the gonadotropic response of rat granulosa cells in an *in vitro* model.

Materials and Methods

Hormones and reagents

Ovine FSH (NIDK oFSH-19-SIAFP: AFP-4117A) and ovine LH (NISDK oLH26: AFP-5551B) were obtained from Dr A F Parlow of the National Hormone and Pituitary Program (Harbor/UCLA Medical Center, Torrance, CA, USA). Porcine collagen type I (Cellmatrix) and human extracellular matrix, which was comprised of laminin IV and heparan sulfate proteoglycan (HSPG), were purchased from Nitta Gelatin (Osaka, Japan) and Collaborative Biomedical Products (Bedford, MA, USA) respectively. Hydrocortisone, transferrin and fibronectin were acquired from Waco Pure Chemical Co. (Tokyo, Japan). Androstenedione, bovine serum albumin (BSA) and insulin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Murine IL-6 and IL-1 β were purchased from Genzyme (Cambridge, MA, USA). Goat anti-murine IL-6 antibody and normal goat IgG were obtained from R&D Systems (Minneapolis, MN, USA) and Vector Lab Inc. (Burlingame, CA, USA) respectively.

Animals and cell preparation

Immature (21-day-old) female Wistar strain rats were supplied by the Imamichi Institute for Animal Reproduction (Ibaraki-ken, Japan). Animals were maintained in quarters controlled for temperature ($23 \pm 1^\circ\text{C}$), humidity ($55 \pm 5\%$ relative humidity) and light (12 h light/day) and given free access to food and water. All procedures were performed in accordance with institutional guidelines for experimental animal care in the Tokyo University of

Pharmacy and Life Science. Animals (24-day-old) were injected s.c. with 20 IU equine chorionic gonadotropin (eCG) (Teikoku Hormone MFG Co., Tokyo, Japan) dissolved in saline. Twenty-four hours later, ovaries were removed and granulosa cells harvested as described previously (Davis *et al.* 1989, Tamura *et al.* 2000). Cultures were started in collagen Type I-coated dishes at a density of $1-2 \times 10^5$ cells/well and maintained for 2 days in 0.5 ml Dulbecco's Modified Eagle Medium (DMEM) (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) plus 100 $\mu\text{g}/\text{ml}$ gentamycin, and grown to subconfluency. For the induction of granulosa cell maturation and differentiation, a serum-free culture medium was used, supplemented with 5 $\mu\text{g}/\text{ml}$ transferin, 40 ng/ml insulin, 100 ng/ml FSH and 10 ng/ml LH. Granulosa cells maturation was monitored by the increase in cAMP production in response to LH or FSH, as described previously (Lindsey & Channing 1979, Asakai *et al.* 1995).

Cyclic AMP assay

After an incubation of 3 h in serum-free DMEM medium, phosphodiesterase activity was inhibited by replacing the medium with 150 μl DMEM containing either FSH (50 ng/ml) or LH (250 ng/ml) in the presence of 3-isobutyl-1-methylxanthine 400 μM . Incubation was continued for 1 h and the medium then collected for assay. The amount of cAMP released into the medium was determined using the Biotrak cAMP enzyme immunoassay (EIA) system (Amersham Pharmacia Biotech UK Limited, Amersham, Bucks, UK) as described before (Tamura *et al.* 2000).

Analyses of LH receptor mRNAs by RT-PCR

Poly(A) RNA was isolated using a Quick Prep Micro mRNA purification kit (Amersham Pharmacia Biotech, Piscataway NJ, USA). The rat cDNA clone for the LHR was kindly provided by Dr Deborah L Segaloff (University of Iowa College of Medicine, Iowa City, IA, USA). A digoxigenin-labeled LHR cDNA probe corresponding to bases 10–622 was prepared for Southern blot using a labeling kit (Boehringer Mannheim GmbH, Mannheim, Germany) and following the manufacturer's instructions. RT-PCR with LHR mRNA was performed using a one-step kit (AMV, TaKaRa, Shiga, Japan) with 30 cycles (30 s at 94°C , 30 s at 55°C and 2 min at 72°C). Oligonucleotide primers that were successfully used for the RT-PCR reaction (Zhang *et al.* 1994) were synthesized (sense primer: 5'-CGAGTCCCAGTCTGAGAA-3', antisense primer: 5'-ACAGCGCACTGAGGAGAACT-3'). The predicted lengths of the LHR mRNA fragments amplified by RT-PCR are 953 bp. A similar analysis was performed to examine CYP11A1 mRNA levels (sense primer: 5'-CTGGAAGGTGTAGCTCAGG-3' and

antisense: 5'-GTACTTGCTGAAGTCTCGCT-3'). RT-PCR data for β -actin were used for standardization as an internal control, as described before (Tamura *et al.* 2000). For Southern blot analysis for LHR, the PCR products were subjected to electrophoresis on a 1.2% agarose gel, transferred to nylon membranes, and fixed by baking. After the membranes had been prehybridized for 3 h at 45 °C in high-SDS buffer, hybridization was performed for 18 h at 45 °C with high-SDS buffer containing the digoxigenin-labelled cDNA probes (25 ng/ml). The membranes were washed twice at 65 °C for 15 min with 0.1% SDS-2 × SCC and then incubated with alkaline phosphatase-labeled anti-digoxigenin antibody (75 mU/ml; Boehringer Mannheim) followed by detection with a chemiluminescent substrate (CDP-Star; New England Nuclear, Boston, MA, USA).

Statistics

The results of EIA and densitometric analysis are presented as the means $\pm$ S.E.M. and examined by one-way ANOVA with Fisher's protected least significant difference test. Differences with $P < 0.05$ are considered significant.

Results

Effect of the chemically defined medium on gonadotropin-stimulated cAMP production and on expression of mRNAs for LHR and CYP11A1 (P450 scc)

To characterize the *in vitro* granulosa cell maturation model, we first examined the changes in cAMP production induced by exposure to FSH or LH during 72 h of culture in a chemically defined serum-free medium. Subconfluent cells, which had been cultured for 2 days in DMEM containing 10% FBS, responded to FSH with a significant increase in cAMP production, but there was no response to LH (Fig. 1A, 0 h). However, an enhanced response to LH was obtained within 24 h of transfer to the serum-free medium and this was maintained for 72 h. Concomitant with the attainment of sensitivity to LH, there was a loss in response to FSH, so that by 72 h it was barely above the control value (Fig. 1A, 72 h). The serum-starved cells quickly increased expression of LHR and CYP11A1 (P450 scc) mRNAs (Fig. 1B). Increased expression in both was at or near maximum by 24 h and remained increased for 72 h. A similar pattern of changes was observed in the expression of CYP 11A1 (P450 arom) mRNA.

Effect of IL-6 on LHR mRNA expression in the *in vitro* granulosa cell maturation model

Subconfluent cells were incubated in the chemically defined medium in the presence or absence of IL-6 (10 ng/ml) for 0–72 h and the level of LHR mRNA

Figure 1 Time-course of effects of the chemically defined medium (CDM) on FSH- or LH-induced cAMP production (A) and on the expressions of mRNAs for LHR, CYP11A1 (P450 scc), and CYP19 (P450 arom) (B) in rat granulosa cells. Subconfluent cells were cultured for 0–72 h in CDM. (A) Cyclic AMP concentrations were determined 1 h after exposure to 50 ng/ml FSH or 250 ng/ml LH as described in Materials and Methods. Results are the means $\pm$ S.E.M. ($n=4-6$) of four experiments. [#] $P < 0.05$, ^{##} $P < 0.01$ compared with the respective control. (B) Poly(A) RNA (0.1 μ g) was subjected to RT-PCR analysis. Each value obtained by densitometric analysis was normalized to the value at 0 h. Left and right axes show the ratio of LHR and steroidogenic P450 mRNAs as compared with that of 0 h respectively.

examined (Fig. 2A). LHR mRNA expression, which was increased by removal of serum from the medium (Fig. 1B) was markedly reduced by IL-6. Figure 2B shows representative data for the effect of increasing doses of IL-6 on LHR mRNA after 72 h of culture. Quantitation of the dose-dependent inhibition by IL-6 is shown in Table 1. Inhibitory effects of IL-6 on the expression of LHR were confirmed by northern analysis using cRNA probe (data not shown).

Figure 2 Effects of IL-6 LHR mRNA levels in rat granulosa cells. (A) Time-course of the effect of IL-6 (10 ng/ml) on the expression of LHR mRNA. Subconfluent cells were cultured for 0–72 h with the chemically defined medium in the presence or absence of IL-6 (10 ng/ml). (B) Dose-related effects of IL-6 on the expression of LHR mRNA at 24 h of culture.

Effect of IL-6 on LH-induced cAMP production

In order to examine whether the LHR was influenced functionally by IL-6 treatment, the FSH- or LH-induced cAMP response was examined (Fig. 3). After subconfluent cells were cultured for 72 h in the chemically defined serum-free medium, LH-induced, but not FSH-induced, cAMP production was significantly enhanced (Fig. 1). Addition of IL-6 into the medium had no effect upon basal production, but significantly decreased the LH-stimulated cAMP production after 72 h of culture (Fig. 3A). This inhibition of LH response by IL-6 was dose-dependent (Fig. 3B). Included in Fig. 3B is the result of exposing the

cells to 10 ng/ml of IL-1β. An inhibitory effect on cAMP concentrations similar to that seen with IL-6 was observed with this cytokine, which also has been shown to decrease the number of LHR in granulosa cells (Gottschall *et al.* 1989).

Effect of immunoneutralization of endogenous IL-6 secreted from cultured granulosa cells

To examine the role of endogenous IL-6 (which has been shown (Gorospe *et al.* 1992, Tamura *et al.* 2000) to be secreted by granulosa cells) in the expression of LHR, granulosa cells were cultured for 72 h in the absence or presence of anti-IL-6 IgG. Treatment with the anti-IL-6 IgG (10 µg/ml) enhanced LHR mRNA expression by 72 h, compared with cells incubated with nonimmune IgG, although no such effect was observed after 24 h of culture (Fig. 4). However, anti-IL-6 IgG did not affect the expression of CYP11A1 (P450 scc) mRNA levels (Fig. 4), which was different from the inhibitory effect of this antibody on CYP19 (P450 arom) (Tamura *et al.* 2000).

Table 1 Densitometric analysis of the levels of LH receptor mRNA detected by RT-PCR analysis. Band density was measured using a densitometer. Results are from three different experiments, including data presented in Fig. 2B. Values are expressed as a ratio to the density of the band on control, which was represented as 1.00 (no treatment with IL-6) and corrected using β-actin as an internal mRNA standard

	LHR mRNA
IL-6 (ng/ml)	
0	1.00 ± 0.004
1	1.05 ± 0.127
3	0.79 ± 0.159
10	0.39 ± 0.110*

*P<0.05 compared with IL-6 (0 ng/ml).

Discussion

The effect of IL-6 on the acquisition of LHR expression by developing granulosa cells was examined using an *in vitro* model of granulosa cell maturation. We show

Figure 3 Effects of IL-6 on LH-stimulated production of cAMP in rat granulosa cells. Subconfluent cells were cultured for 72 h with the chemically defined medium in the presence (striped bar) or absence (solid bar) of IL-6 (10 ng/ml) (A) and various doses of IL-6 (1–10 ng/ml) or IL-1 β (10 ng/ml) (B). Cyclic AMP concentrations were determined by stimulation of the cells with FSH or LH for 1 h as described in Materials and Methods. Results are the means $\pm$ S.E.M. ($n=4-6$) of three separate experiments. # $P<0.05$, ## $P<0.01$ compared with none (A) or IL-6 (0 ng/ml) (B).

that IL-6 attenuates the expression of LHR mRNA, in addition to the LH responsiveness for cAMP production in cells cultured in a serum-free medium. We have recently confirmed (Tamura *et al.* 2000) that, in addition to IL-6 mRNA, cultured granulosa cells express mRNAs encoding the components of the IL-6 receptor complex – that is, the ligand-binding α subunit and the signal transducing gp130 chain. These findings prompted us to investigate IL-6 as a possible autocrine/paracrine regulator of LHR expression. In the *in vitro* culture system, granulosa cells secrete about 2 ng/ml of bioactive IL-6 per 24 h, which implies a possible physiological role for this cytokine in ovarian function.

Analysis of the 5' region of the rat *LHR* gene revealed that rat *LHR* gene seems to be constitutively inhibited by 5' upstream sequences and that the promoter activity is regulated by at least three additional DNA domains that bind multiple *trans*-factors in a tissue-specific manner (Dufau *et al.* 1995). Competition between inhibitory and neutral DNA binding factors within upstream and promoter domains (C-box) appears to be important for the regulation of *LHR* gene expression. It is generally accepted that homodimerization of gp130 induced by IL-6 activates two signal transduction pathways: the STAT3/APRF pathway and the MAP kinase cascade (Kishimoto *et al.* 1995). Activation of JAK kinase induces tyrosine-specific phosphorylation of APRF/STAT3 and its nuclear translocation. Activation of an unidentified tyrosine kinase triggers the activation of Ras-MAP kinase cascades followed by the activation of NF-IL-6, which is a

Figure 4 Effects of anti-IL-6 antibody on LHR mRNA levels in rat granulosa cells. Subconfluent cells were cultured for 24 or 72 h in the chemically defined medium in the presence or absence of different doses of anti-IL-6 antibody (A/IL-6). Representative RT-PCR analyses are shown.

transcription factor, highly homologous to C/EBP (Akira *et al.* 1990, 1995). Although there is no information regarding NF-IL-6 expression in the ovary, C/EBP β mRNA is abundant in the granulosa cells of preovulatory follicles. Furthermore, the ovaries of C/EBP β -deficient mice lack corpora lutea (Sterneck *et al.* 1997), suggesting that this transcription factor is essential for the final differentiation of granulosa cells into luteal cells. We have not examined whether IL-6 influences transcription rates, the stability of transcripts or the possible mechanism(s) by which it could do so. Attenuation of LHR mRNA levels by IL-6 in chemically defined medium-treated cells may be due to decreased gene transcription or mRNA stability, or both. There is no clue regarding direct interactions between the 5'-flanking region of LHR and IL-6-inducible transcription factor NF-IL-6. There certainly is evidence for regulation of mRNA stability by hormones or cytokines (Nielsen & Shapiro. 1990). Zhang *et al.* (1998) have suggested that the increase in LHR mRNA by IGF-I in murine Leydig tumor cells is mainly due to increased mRNA stability. Lu *et al.* (1993) have observed that the decrease in steady-state LHR mRNA levels by human chorionic gonadotropin (hCG) in pseudopregnant rats was not associated with a decrease in the transcription rate of the gene, but rather to a significant decrease in the half-life of the receptor transcripts. Therefore IL-6 might decrease the synthesis of protein(s) that stabilize the transcripts or increase the synthesis of those that destabilize them.

IL-6 is produced by many different cell types and has a wide variety of physiological functions. Increasing evidence has emerged indicating that IL-6 plays an autocrine or paracrine role in the regulation of several reproductively important functions. For example, IL-6 stimulates the release of anterior pituitary hormones such as adrenocorticotrophin, prolactin, growth hormone and LH in rats (Spangelo *et al.* 1989, 1995) and regulates the secretions of placental lactogen (Stephanou & Handwerger 1994) and hCG (Nishino *et al.* 1990) in human trophoblast cells. Inhibitory effects of IL-6 on ovarian steroidogenesis (Gorospe *et al.* 1992, Tamura *et al.* 2000) have been reported for rats. This cytokine also may be involved in endometrial proliferation and differentiation (Tabbibzadeh *et al.* 1989). In previous studies in our laboratory, IL-6 was shown to reduce the expression of CYP19 mRNA during granulosa cell maturation (Tamura *et al.* 2000). Ample evidence has been presented to show that expression of this enzyme, in addition to the LHR, are essential for the preovulatory maturation of the follicle and its ovulation (Richards 1994). The present results provide evidence that IL-6 down-regulates the expression of LHR in the process of *in vitro* granulosa cell maturation. This cytokine may have an important inhibitory role in the regulation of follicular maturation that is related to final stages of granulosa cell differentiation in preovulatory follicles.

Acknowledgements

The authors are grateful to Professor D C Johnson at the University of Kansas-Medical Center for his critical reading of the manuscript, and to Professor D L Segaloff at the University of Iowa College of Medicine for providing rat LH receptor cDNA. This work was supported in part by the US-Japan Cooperative Research Grant from the Japan Society for Promotion of Science.

References

- Akira S, Isshiki H, Sugita T, Tanabe O, Kinoshita S, Nishio Y, Nakajima T, Hirano T & Kishimoto T 1990 A nuclear factor for IL-6 expression (NF-IL-6) is a member of a C/EBP family. *EMBO Journal* **9** 1897-1906.
- Akira S, Yoshida K, Tanaka T, Taga T & Kishimoto T 1995 Targeted disruption of the IL-6 related genes: gp130 and NF-IL-6. *Immunological Reviews* **48** 221-253.
- Asakai R, Akita Y, Tamura K, Kenmotsu N & Aoyama Y 1995 Protein kinase C-dependent down-regulation of basic fibroblast growth factor (FGF-2) receptor by phorbol ester and epidermal growth factor in porcine granulosa cells. *Endocrinology* **136** 3470-3479.
- Baker J, Hardy MP, Zhou J, Bondy C, Lupu F, Bellve AR & Efstratiadis A 1996 Effect of an Igfl gene null mutation on mouse reproduction. *Molecular Endocrinology* **10** 903-918.
- Davis JS, Weakland LL, Coffey RG & West LA 1989 Acute effects of phorbol esters on receptor-mediated IP₃, cAMP, and progesterone levels in rat granulosa cells. *American Journal of Physiology* **256** E368-E374.
- Dufau ML, Tsai-Morris CH, Hu ZZ & Buczko E 1995 Structure and regulation of the luteinizing hormone receptor gene. *Journal of Steroid Biochemistry and Molecular Biology* **53** 283-291.
- Erickson GF & Danforth DR 1995 Ovarian control of follicle development. *American Journal of Obstetrics and Gynecology* **172** 736-747.
- Erickson GF, Wang C & Hsueh AJW 1979 FSH induction of functional LH receptors in granulosa cells cultured in a chemically defined medium. *Nature* **279** 336-338.
- Gorospe WC, Hughes Jr FM & Spangelo BL 1992 Interleukin-6: effects on and production by rat granulosa cells *in vitro*. *Endocrinology* **130** 1750-1752.
- Goldring NB, Durica JM, Lifka J, Hedin L, Ratoosh SL, Miller WL & Orly J 1987 Hormonal regulation of cholesterol side-chain cleavage P450 messenger RNA in rat ovarian follicles and constitutive expression in corpora lutea. *Endocrinology* **120** 1942-1950.
- Gottschall PE, Katuura G, Hoffman ST & Arimura A 1988 Interleukin 1: an inhibitor of luteinizing hormone receptor formation in cultured rat granulosa cells. *FASEB Journal* **2** 2492-2496.
- Gottschall PE, Uehara A, Hoffman ST & Arimura A 1989 Interleukin-1 inhibits follicle stimulating hormone-induced differentiation in rat granulosa cells *in vitro*. *Biochemical and Biophysical Research Communications* **149** 502-509.
- Kanzaki M, Hattori M-A, Horiuchi R & Kojima I 1994 Co-ordinate actions of FSH and insulin-like growth factor-I on LH receptor expression in rat granulosa cells. *Journal of Endocrinology* **141** 301-308.
- Kishimoto T, Akira S, Narazaki M & Taga T 1995 Interleukin-6 family of cytokines and gp130. *Blood* **86** 1243-1254.
- Labarbera AR & Ryan RJ 1981 Porcine granulosa cells in suspension culture. Follicle-stimulating hormone induction of human chorionic gonadotropin-binding sites on cells from small follicles. *Endocrinology* **108** 1561-1570.

- Lindsey AM & Channing CP 1979 Influence of follicular maturation upon the effect of ovine follicle stimulating hormone and luteinizing hormone upon cyclic AMP accumulation by isolated porcine granulosa cells. *Biology of Reproduction* **20** 473–482.
- Lu D, Peezel H, Mosier MS & Menon KML 1993 Loss of leutropin/human choriongonadotropin receptor messenger ribonucleic acid during ligand-induced down-regulation occurs post-transcriptionally. *Endocrinology* **132** 235–240.
- Machelon V, Emilie D, Lefevre A, Nome F, Durand-Gasselin I & Testart J 1994 Interleukin-6 biosynthesis in human preovulatory follicles: some of its potential roles at ovulation. *Journal of Clinical Endocrinology and Metabolism* **79** 633–642.
- Nielsen DA & Shapiro 1990 Insight into hormonal control of messenger RNA stability. *Molecular Endocrinology* **4** 953–957.
- Nishino E, Matsuzaki N, Masuhiro K, Kameda T, Taniguchi T, Takagi T, Saji F & Tanizawa O 1990 Trophoblast-derived interleukin-6 (IL-6) regulates human chorionic gonadotropin release through IL-6 receptor on human trophoblasts. *Journal of Clinical Endocrinology and Metabolism* **71** 436–441.
- Onok RB, Parker KL, Gibson JL & Richards JS 1990 Rat cholesterol side-chain cleavage cytochrome P450 (P450 scc) gene. Structure and regulation by cAMP *in vitro*. *Journal of Biological Chemistry* **65** 22392–22401.
- Richards JS 1994 Hormonal control of gene expression in the ovary. *Endocrine Review* **15** 725–751.
- Richards JS, Jonassen JA, Rolfes AI, Kersey K & Reichert LE Jr 1979 Adenosine 3', 5'-monophosphate, luteinizing hormone receptor and progesterone during granulosa cell differentiation: effects of estradiol and follicle-stimulating hormone. *Endocrinology* **104** 765–770.
- Robker RL & Richards JS 1998 Hormone-induced proliferation and differentiation of granulosa cells: a coordinated balance of the cell cycle regulators cyclin D2 and p27 Kip1. *Molecular Endocrinology* **12** 924–940.
- Spangelo BL, Judo AM, Isakson PC & Macleod RM 1989 Interleukin-6 stimulates anterior hormone release *in vitro*. *Endocrinology* **125** 575–577.
- Spangelo BL, Judd AM, Call GB, Zumwalt J & Gorospe WC 1995 Role of the cytokines in the hypothalamic–pituitary–adrenal and gonadal axes. *Neuroimmunomodulation* **2** 299–312.
- Stephanou A & Handwerger S 1994 Interleukin-6 stimulates placental lactogen expression by human trophoblast cells. *Endocrinology* **135** 719–723.
- Sterneck E, Tessarollo L & Johnson PF 1997 An essential role for C/EBP β in female reproduction. *Genes and Development* **1** 2153–2162.
- Tabbibzadeh SS, Santhanann U, Sehgal PB & May LT 1989 Cytokine-induced production of IFN- β /IL-6 by freshly explanted human stromal cells. *Journal of Immunology* **143** 3134–3139.
- Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, Hirano T & Kishimoto T 1989 Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell* **58** 573–581.
- Tamura K, Kawaguchi T, Hara T, Sakamoto T, Tohei A, Miyajima A, Seishi T & Kogo H 2000 Interleukin-6 decreases estrogen production and messenger ribonucleic acid expression encoding aromatase during *in vitro* cytodifferentiation of rat granulosa cell. *Molecular and Cellular Endocrinology* **170** 103–111.
- Telleria CM, Ou J, Sugino N, Ferguson S & Gibori G 1998 The expression of interleukin-6 in the pregnant rat corpus luteum and its regulation by progesterone and glucocorticoid. *Endocrinology* **139** 3597–3605.
- Tian XC, Berndtson AK & Fortune JE 1995 Differentiation of bovine preovulatory follicles during the follicular phase is associated with increases in messenger ribonucleic acid for cytochrome P450 side-chain cleavage, 3 beta-hydroxysteroid dehydrogenase, and P450 17 alpha-hydroxylase, but not P450 aromatase. *Endocrinology* **136** 5102–5110.
- Zelevnik AJ, Midgley Jr AR & Reichert Jr LE 1974 Granulosa cell maturation in the rat: increased binding of human chorionic gonadotropin following treatment with follicle-stimulating hormone *in vivo*. *Endocrinology* **95** 818–825.
- Zhang F-P, Hamalainen T, Kaipia A, Pakarinen P & Huhtaniemi I 1994 Ontogeny of luteinizing hormone receptor gene expression in rat testis. *Endocrinology* **134** 2206–2213.
- Zhang F-P, El-Hafnawy T & Huhtaniemi I 1998 Regulation of luteinizing hormone receptor gene expression by insulin-like growth factor-I in an immortalized murine Leydig tumor cell line (BLT-1). *Biology of Reproduction* **59** 1116–1123.
- Ziltener HJ, Maines-Bandiera S, Schrader JW & Auersperg N 1993 Secretion of bioactive interleukin-1, interleukin-6, and colony-stimulating factor by human ovarian surface epithelium. *Biology of Reproduction* **49** 635–641.

Received 30 November 2000

Accepted 21 March 2001