Regulatory Peptides, 48 (1993) 157–163

REGPEP 01566

Insulin-like growth factor binding protein-2, 28 kDa and 24 kDa insulin-like growth factor binding protein levels are decreased in fluid of dominant follicles, obtained from normal and polycystic ovaries

A.G.P. Schuller[a], D.J. Lindenbergh-Kortleve[a], T.D. Pache[b], E.C. Zwarthoff[c], B.C.J.M. Fauser[b] and S.L.S. Drop[a]

[a] *Department of Pediatrics, Subdivision of Pediatric Endocrinology,* [b] *Department of Obstetrics and Gynecology, Section of Reproductive Endocrinology and Fertility, and* [c] *Department of Pathology, Erasmus University, Dijkzigt University Hospital, Sophia Children's Hospital, Rotterdam (The Netherlands)*

(Received 21 April 1993; accepted 25 May 1993)

Key words: Insulin-like growth factor binding protein; Insulin-like growth factor; Ovary; Follicle; Human

Summary

In order to investigate potential changes in insulin-like growth factor binding proteins (IGFBPs) during human follicle maturation, we examined the IGFBP profiles in follicular fluid from follicles in different stages of maturation. Samples were obtained from ovaries of women with regular menstrual cycles and of subjects with cycle abnormalities and polycystic ovaries (diagnosed as polycystic ovary syndrome (PCOS)) and analyzed by Western ligand blotting. IGFBPs of 43 kDa, 37 kDa, 31 kDa, a doublet around 28 kDa and a minor band of 24 kDa were detected in follicle fluid of normal non-dominant (size < 10 mm) and atretic (androstenedione/estradiol ratio > 4) follicles of both regularly menstruating women and PCOS patients. The 43 and 37 kDa IGFBPs could be identified as IGFBP-3 and the 31 kDa IGFBP as IGFBP-2, whereas the 28 kDa IGFBP could not be identified as IGFBP-1, all by immunoblotting techniques. A dramatic decrease in IGFBP-2, the 28 kDa and 24 kDa IGFBPs was observed in follicular fluid of dominant follicles (size > 10 mm) of both regular menstruating individuals and one PCOS patient as compared with follicular fluid of normal non-dominant or atretic follicles. These observations indicate that the PCOS follicle may not be different from normal with respect to IGFBP profiles. Furthermore, these results suggest that at least one of these IGFBPs might be involved in human folliculogenesis.

Correspondence to: A.G.P. Schuller, Laboratory for Molecular Endocrinology, Erasmus University Rotterdam, Room 1022, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Introduction

The insulin-like growth factors (IGFs) are low molecular weight peptides with both metabolic and mitogenic properties believed to play a role in ovarian function [1,2]. In the human preovulatory granulosa cell, expression of IGF-II mRNA but not IGF-I mRNA has been detected [3]. Human granulosa cells contain transcripts for both the type I and type II IGF receptor [4] and contain the type I IGF receptor on their surface [5]. In human granulosa cells, isolated from polycystic ovaries, IGF-I synergizes with follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG) in increasing 17β-estradiol (E_2) production, suggesting a role for IGF-I in enhancing gonadotropin action in human folliculogenesis [6].

In serum and extracellular fluids, the IGFs are present bound to high affinity binding proteins (IGFBPs). These IGFBPs comprise a family of six structurally related proteins [7], which modulate IGF action [8]. In human follicular fluid several of these IGFBPs have been found and one such species, IGFBP-1, was localized in luteinized granulosa cells [9]. Human granulosa cells express mRNA for IGFBP-1 [10]. In addition, IGFBP-1 was reported to inhibit IGF induced proliferation of human granulosa cells, suggesting that IGFBP-1 is one of the endogenous factors regulating growth and differentiation of human granulosa cells [11]. IGFBP-2, -4 and -5 mRNA expression was found mainly in granulosa of atretic follicles and IGFBP-4 and -5 gene expression was located primarily in theca and stromal cells. Gene expression of IGFBP-3 was detected in theca of all stages tested and was selectively expressed in granulosa cells of dominant follicles [12]. IGFBP-3 has been shown to inhibit IGF-I and FSH induced estrogen production by cultured human granulosa cells [13], suggesting that this binding protein may modify FSH actions.

Since there is mounting evidence that the IGF system, complete with ligands, IGF-receptors and IGFBPs, is present in the human ovary, we decided to investigate potential changes in IGFBPs during follicle maturation. Therefore, we examined the IGFBP profiles in fluid from follicles in different stages of maturation obtained from ovaries of women with regular menstrual cycles and of PCOS patients presenting with cycle abnormalities.

Materials and Methods

Follicular fluid samples

16 women volunteered to participate in this study, which was approved by the local Ethics Review Committee. Their mean age was 33 years (range 27–44) and they all were regularly menstruating with a mean cycle length of 28 ± 2 days (mean $\pm$ S.D.). Follicular fluid samples were collected through puncture of individual follicles as described previously [14]. In addition, follicle fluid was obtained from five infertile PCOS women. Four patients presented with amenorrhea and one with severe oligomenorrhea (cycle length 72–84 days). All five fullfilled our criteria for transvaginal sonographic diagnosis of polycystic ovaries; (1) ovarian volume above 8.0 ml, (2) > 11 follicles between 2 and 10 mm in size in each ovary, and (3) increased ovarian stroma echogenecity [15]. The mean age of these patients was 28 years, two women were obese (BMI > 25 kg/m^2) and hirsute, one woman was hirsute only. Serum hormone concentrations were 10.6 (range 7.9 to 12.1) IU/l for luteinizing hormone (LH), and 6.9 (range 3.9 to 8.3) IU/l for follicle stimulating hormone (FSH) estimated by immunoradiometric assays. Radioimmunoassay serum determinations for serum testosterone were 2.9 (range 1.6 to 7.2) nmol/l. All FF samples were divided into three groups (depending on their androstenedione/estradiol (AD/E_2) ratio as described by McNatty et al. [16]) and pooled per individual. Group A (normal non-dominant follicles): AD/E_2 ratio < 4 and a diameter < 10 mm, group B (atretic follicles): AD/E_2 ratio > 4 and group D (dominant follicles): AD/E_2 ratio < 4 and a size > 10 mm.

Western ligand blotting

Western ligand blots were prepared essentially as described by Hossenlopp et al. [17]. FF samples (3 μl) were applied to a 9% sodium dodecylsulfate (SDS)-polyacrylamide gel, run under non-reducing conditions, and the separated proteins were transferred onto nitrocellulose filters by electroblotting. The filters were washed with 3% NP-40 in 100 mM Tris-HCl, pH 7.5/0.9% NaCl (TBS), 3% BSA in TBS, and then incubated with 500,000 cpm [^{125}I]IGF-II (kindly provided by Dr. S. van Buul-Offers, Wilhelmina Childrens Hospital, Utrecht The Netherlands) for 14 h at 4°C. Subsequently, filters were washed and exposed to Kodak X-Omat AR film with intensifying screen at −70°C. for up to 14 days.

Immunoblotting

After autoradiography, filters were immunostained using either a monoclonal antibody against IGFBP-1 [18], a polyclonal antibody against IGFBP-2 (kindly provided by Dr. J. Schwander, Kantonspital Basel, Switzerland) or a polyclonal antibody against IGFBP-3 (kindly provided by Dr. R. Rosenfeld, Stanford University Stanford, USA) using standard procedures. Briefly, filters were incubated with 3% BSA in TBS followed by incubation with an IGFBP specific antibody. Filters were washed in 0.1% NP-40 in TBS and incubated with an alkaline-phosphatase coupled second antibody. Finally, filters were washed and stained with 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate (Boehringer, Mannheim).

Quantification of IGFBP levels was performed by densitometric scanning of autoradiograms using an image analyzer (IBAS 2000 Zeiss Kontron, Oberkochen, Germany) and the Kontron IBAS1 version 4.4 software program supplied. Mean intensities were calculated from measurements of autoradiograms of at least four samples and statistical confidence of differences calculated using Student's *t*-test.

Results

Follicle fluid (FF) samples obtained from individuals with regular cycles were subjected to SDS-PAGE and Western ligand blotting using iodinated IGF-II. As shown in Fig. 1, two major bands of 43 and 37 kDa, corresponding to the glycosylated forms of IGFBP-3, were detected. In addition a band of 31 kDa, a doublet around 28 kDa and a minor band of approx. 24 kDa (corresponding to the molecular mass of IGFBP-2, IGFBP-1 and IGFBP-4, respectively) were seen (Fig. 1). Using a polyclonal antibody specific for IGFBP-3, we were able to show that the 43 and 37 kDa bands were indeed isoforms of IGFBP-3 (Fig. 2a). The 31 kDa band was identified as IGFBP-2 by immunoblotting (Fig. 2b). However, the 28 kDa IGFBP doublet did not react with a specific antibody against IGFBP-1, whereas the antibody clearly detected the positive control (IGFBP-1 in amniotic fluid), indicating that this 28 kDa IGFBP was not IGFBP-1 (Fig. 2c).

Comparison of the Western ligand blot profile of the IGFBPs in FF of follicles in different stages of development, revealed that in all dominant follicles, IGFBP-2, the 28 kDa and the 24 kDa IGFBP were strongly decreased, as compared to normal follicles

Fig. 1. Autoradiograph of a representative Western ligand blot of equal volumes (3 μl) of follicular fluid from normal non-dominant follicles (left lane) and atretic follicles (right lane) obtained from one individual. Molecular mass markers (in kDa) are indicated by arrows.

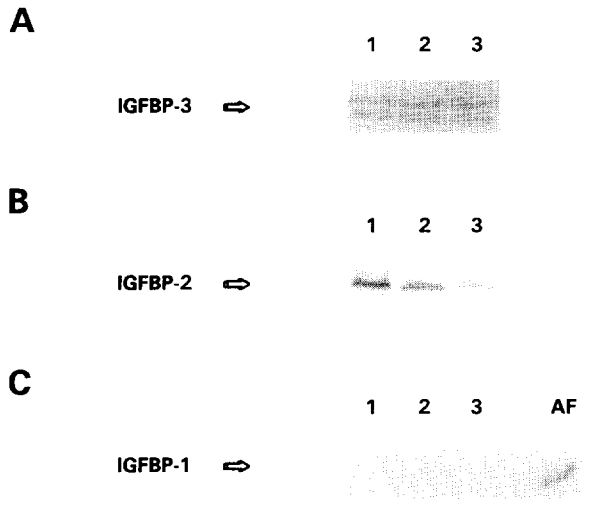

Fig. 2. Immunostaining of a representative Western blot of equal volumes (3 μl) of follicular fluid from normal non-dominant (lanes 1), atretic (lanes 2) and dominant (lanes 3) follicles obtained from one regularly menstruating individual. Filters are stained using a polyclonal antibody against IGFBP-3 (A), IGFBP-2 (B) and a monoclonal antibody against IGFBP-1 (C). Amniotic fluid (lane AF) was used as a positive control to detect IGFBP-1.

(Fig. 3). Most atretic follicles, diagnosed based on Ad/E_2 ratio >4, showed no difference in IGFBP profiles as compared to normal follicles (Fig. 3). Only in two samples classified as FF of atretic follicles, a decrease in IGFBP-2 and the 28 kDa and 24 kDa IGFBPs was observed (results not shown).

In FF samples obtained from five PCOS patients IGFBP profiles were also determined. When compared to FF of normal and atretic follicles of regular cycling women, no dramatic difference in IGFBP profiles could be observed (Fig. 4). One of the PCOS patients developed a dominant follicle. In FF of this follicle the same decrease in IGFBP-2, 28 kDa IGFBP and 24 kDa IGFBP was observed as seen in the dominant follicles of regular cycling individuals (Fig. 4), showing no difference in IGFBP profile between FF of follicles obtained from PCOS patients or regularly menstruating women.

In order to quantify differences in IGFBP levels, autoradiograms were analyzed by densitometric scanning. The mean relative IGFBP-2 levels in fluid of normal non-dominant ($100 \pm 52\%$) and atretic follicles ($50 \pm 23\%$, $n = 6$) was not significantly different ($P > 0.05$). Also no significant difference between the relative 24 kDa IGFBP levels could be observed ($100 \pm 39\%$, $n = 6$ for normal non-dominant and $69 \pm 21\%$, $n = 6$ for atretic follicles). Valid quantifi-

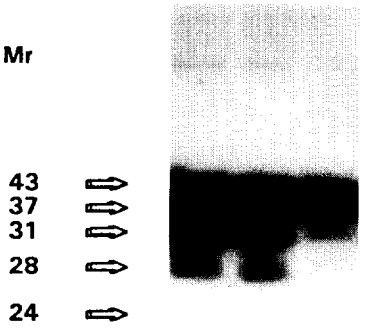

Fig. 3. Autoradiograph of a representative Western ligand blot of equal volumes (3 μl) of follicular fluid from normal non-dominant (left lane), atretic (middle lane) and dominant (right lane) follicles obtained from a single regularly menstruating individual. Molecular mass markers (in kDa) are indicated by arrows.

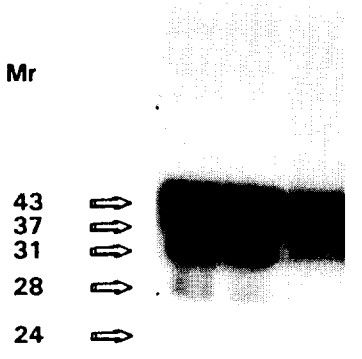

Fig. 4. Autoradiograph of a representative Western ligand blot of equal volumes (3 μl) of follicular fluid from normal non-dominant (left lane), atretic (middle lane) and dominant (right lane) follicles obtained from a single PCOS patient. Molecular mass markers (in kDa) are indicated by arrows.

cation of the differences in IGFBP-2, the 28 kDa IGFBP and the 24 kDa IGFBP levels between follicle fluid of normal non-dominant and dominant follicles could not be made due to the dramatic decrease in the intensities of these IGFBPs in fluid of the dominant follicles.

Discussion

Results presented show that follicle fluid of healthy follicles obtained from regularly menstruating women contain IGFBPs with a molecular mass of 43, 37, 31, 28 and 24 kDa. Using standard immunoblotting techniques, we were able to identify the 43 and 37 kDa bands as IGFBP-3 and the 31 kDa protein as IGFBP-2. The 28 kDa doublet, however, could not be identified as IGFBP-1. Recently, Cataldo and Giudice also reported the presence of a 28 and 24 kDa band in follicular fluid. The 28 kDa IGFBP was shown to be glycosylated, and upon deglycosylation this protein most likely comigrated with 24 kDa IGFBP, because no additional band was found. Since indeed a glycosylated variant of IGFBP-4 was reported [19,20], the authors suggested that the 28 kDa and 24 kDa IGFBPs found in follicular fluid were the glycosylated and non-glycosylated forms of IGFBP-4 [21]. Alternatively, the 28 kDa glycosylated IGFBP could be IGFBP-6. This IGFBP has been shown to contain a potential Asn-linked glycosylation site, but is up to now only detected in porcine follicular fluid [22]. Furthermore, no IGFBP-6 mRNA expression could be observed in any cell type of the human ovary [12]. Finally, since the 28 kDa IGFBP is a doublet, the possibility exists that this doublet represents both IGFBP-4 and IGFBP-6.

Comparison of the IGFBP profiles in follicular fluid samples of follicles in different stages of development revealed that their was no dramatic change in IGFBPs in follicular fluid of normal healthy non-dominant follicles as compared to atretic follicles. Only in two out of 12 pools of atretic follicles a decrease in IGFBP-2, the 28 kDa and 24 kDa IGFBP (presumably IGFBP-4), was observed. Furthermore, no differences in IGFBP profiles were observed between follicular fluid samples obtained from PCOS patients and regularly menstruating women. From one PCOS patient follicular fluid was obtained from a dominant follicle, showing the same decrease in IGFBP-2, the 28 kDa and 24 kDa IGFBPs as seen in regularly menstruating women, indicating that with respect to the IGFBPs, follicular fluid of PCOS patients is not different from normal. These observations are in contrast with previous findings. Cataldo and Giudice described 3-, 6- and 19-fold higher amounts of IGFBP-2, 28 kDa and 24 kDa IGFBPs, respectively, in follicular fluid of atretic compared to healthy follicles and these changes in IGFBP levels were also observed in follicular fluid obtained from three PCOS patients [21,23]. Some of this disagreement might be explained by the way atretic and healthy follicles are defined. We have classified the follicles according to AD/E_2 ratios as validated previously [16], whereas E_2/T was used by Cataldo and Giudice. Secondly, our group of PCOS patients is defined according to rigid clinical (obesity, hirsutism), endocrine (hyperandrogenemia) and sonographic (polycystic ovaries) criteria, as described previously [15]. Finally, the most striking differences between atretic and healthy non-dominant follicles noted [21] might be found only in a subgroup of these follicles (namely those with a E_2/T ratio of 136–500).

A dramatic decrease in IGFBP-2, the 28 kDa and 24 kDa IGFBPs was observed in all samples of dominant follicles tested as compared to healthy developing non-dominant follicles. This finding seems of special interest since several studies indicate that the IGFBPs may play a role in regulating the potential of IGF to enhance gonadotropin action and subsequent follicle development [24–26]. Both IGFBP-1 and IGFBP-3 have been shown to inhibit IGF-I and FSH induced estrogen production by cultured human granulosa cells. These IGFBPs were also capable of inhibiting the IGF-I, but not FSH induced progesterone response [13], demonstrating the inhibitory effect of these IGFBPs on the IGF-I-

stimulated granulosa cell steroidogenesis. Furthermore, IGFBP-1 has been shown to inhibit the DNA amplification induced by IGFs in human granulosa-luteal cells [11]. Also, a role for the IGFBPs not directly related to their IGF binding capacity has been proposed. Bicsak et al. showed that, like an IGF-I antiserum, IGFBP-2 and -3 were capable of inhibiting steroid production by granulosa cells, but that IGFBP-3 was 2–3-fold more potent then IGFBP-2. Furthermore, the IGFBPs had no effect on the IGF-I stimulated cAMP production induced by FSH, suggesting that IGFBPs may have alternative ways of action, other than IGF sequestration [27].

In summary, it may be concluded from this study that IGFBP-2, the 28 kDa and 24 kDa IGFBPs are decreased in the dominant follicles as compared to normal healthy non-dominant and atretic follicles. A decrease of the inhibitory IGFBPs may result in an increase in free IGFs resulting in a stimulation of the mitogenic response of granulosa cells and an amplified steroidogenic response to FSH stimulation. This in turn facilitates the follicle to gain dominance. Furthermore, no differences in the IGFBP follicle fluid profile between PCOS and regularly menstruating women could be observed, which may suggest that with respect to intra-ovarian IGFBPs, PCOS patients are not different from normal.

Acknowledgements

The authors would like to thank F.L. van der Panne for photography. This work was supported by grants from the Sophia Foundation for Medical Research, The Netherlands and Novo-Nordisk Insulin laboratories, Denmark.

References

1 Adashi, E.Y., Resnick, C.E., Hurwitz, A., Ricciarelli, E., Hernandez, E.R., Roberts, C., Leroith, D. and Rosenfeld, R., Insulin-like growth factors: the ovarian connection, Hum. Reprod., 6 (1991) 1213–1219.

2 Giudice, L., Insulin-like growth factors and ovarian follicular development, Endocr. Rev., 13 (1992) 641–669.

3 Geisthovel, F., Moretti-Rojas, I., Asch, R.H. and Rojas, F.J., Expression of insulin-like growth factor-II (IGF-II) messenger ribonucleic acid (mRNA), but not IGF-I mRNA, in human preovulatory granulosa cells, Hum. Reprod., 4 (1989) 899–902.

4 Hernandez, E.R., Hurwitz, A., Pellicer, A. et al., Human ovarian insulin-like growth factor receptor gen expression, Thirty-seventh Annual Meeting, Society for Gynecologic Investigation, St. Louis, March 1990, abstract 414.

5 Gates, G.S., Bayer, S., Seibel, M., Poretsky, L., Flier, J.S. and Moses, A.C., Characterization of insulin-like growth factor binding to human granulosa cells obtained during in vitro fertilization, J. Recept. Res., 7 (1987) 885–902.

6 Erickson, G.F., Magoffin, D.A., Cragun, J.R. and Chang, R.J., The effects of insulin and insulin-like growth factor-I and -II on estradiol production by granulosa cells of polycystic ovaries, J. Clin. Endocrinol. Metab., 70 (1990) 894–902.

7 Drop, S.L.S., Schuller, A.G.P., Lindenbergh-Kortleve, D.J., Groffen, C., Brinkman. A. and Zwarthoff. E.C., Structural aspects of the IGFBP family, Growth Regul., 2 (1992) 69–79.

8 Clemmons, D.R., IGF binding proteins: regulation of cellular actions, Growth Regul., 2 (1992) 80–87.

9 Seppälä, M., Wahlstrom, T., Koskimies, A.I., Tenhunen, A., Rutanen, E.M., Koistinen, R., Huhtaniemi, I., Bohn, H. and Stenman, U.H., Human preovulatory follicular fluid, luteinized cells of hyperstimulated preovulatory follicles, and corpus luteum contain placental protein 12, J. Clin. Endocrinol. Metab., 58 (1984) 505–510.

10 Koistinen, R., Suikkari, A.M., Tiitinen, A., Kontula, K. and Seppälä, M., Human granulosa cells contain insulin-like growth factor-binding protein (IGF BP-1) mRNA, Clin. Endocrinol., 32 (1990) 635–640.

11 Angervo, M., Koistinen, R., Suikkari, A.M. and Seppälä, M., Insulin-like growth factor binding protein-1 inhibits the DNA amplification induced by insulin-like growth factor I in human granulosa-luteal cells, Hum. Reprod., 6 (1991) 770–773.

12 El-Roeiy, A., Roberts, V.J., Shimasaki, S., Ling, N. and Yen, S.S.C., Localization, expression of insulin-like growth factor binding proteins (IGFBPs) 1–6 in normal, polycystic (PCO) human ovaries, Program of the 48th Annual Meeting of the American Fertility Society, New Orleans, 1992, p. S9 (abstract 0–020).

13 Mason, H.D., Willis, D., Holly, J.M.P., Cwyfan-Hughes, S.C., Seppälä, M. and Franks, S., Inhibitory effects of insulin-like growth factor-binding proteins on steroidogenesis by human granulosa cells in culture, Mol. Cell. Endocrinol., 89 (1992) R1-R4.

14 Pache, T.D., Hop, W.C.J., De Jong, F.H., Leerentveld, R.A., Van Geldrop, H., Van de Kamp, T.M.M., Gooren, L.J.G. and

Fauser, B.C.J.M., 17β-Estradiol, androstenedione, and inhibin levels in fluid from individual follicles of normal and polycystic ovaries, and in ovaries from androgen treated female to male transsexuals, Clin. Endocrinol., 36 (1992) 565–571.

15 Fauser, B.C.J.M., Pache, T.D., Lamberts, S.W.J., Hop, W.C.J., De Jong, F.H. and Dahl, K.D., Serum bioactive and immunoreactive luteinizing hormone and follicle-stimulating hormone levels in women with cycle abnormalities, with or without polycystic ovarian disease, J. Clin. Endocrinol. Metab., 73 (1991) 811–817.

16 McNatty, K.P., Moore Smith, D., Makris, A., Osathanondh, R. and Ryan, K.J., The microenvironment of the human follicle: inter-relationships among the steroid levels in antral fluid, the population of granulosa cells and the status of the oocyte in vivo and vitro, J. Clin. Endocrinol. Metab., 49 (1979) 851–860.

17 Hossenlopp, P., Seurin, D., Segovia-Quinson, B., Hardouin, S. and Binoux, M., Analysis of serum insulin-like growth factor binding proteins using Western blotting: use of the method for titration of the binding proteins and competitive binding studies, Anal. Biochem., 154 (1986) 138–143.

18 Schuller, A.G.P., Lindenbergh-Kortleve, D.J., De Boer, W.I., Zwarthoff, E.C. and Drop S.L.S., Localization of the epitope of a monoclonal antibody against human insulin-like growth factor binding protein-1, functionally interfering with insulin-like growth factor binding, Growth Regul., (1993) in press.

19 Fielder, P.J., Thordarson, G., Talamantes, F. and Rosenfeld, R., Characterization of insulin-like growth factor binding proteins (IGFBPs) during gestation in mice: effects of hypophysectomy and an IGFBP-specific serum protease activity, Endocrinology, 127 (1990) 2270–2280.

20 Cheung, P.T., Smith, E.P., Shimasaki, S., Ling, N. and Chernausek, S.D., Characterization of an insulin-like growth factor binding protein (IGFBP-4) produced by the B104 rat neuronal cell line: chemical and biological properties and differential synthesis by sublines, Endocrinology, 129 (1991) 1006–1015.

21 Cataldo, N.A. and Giudice, L.C., Insulin-like growth factor binding protein profiles in human ovarian follicular fluid correlate with follicular functional status, J. Clin Endocrinol. Metab., 74 (1992) 821–829.

22 Shimasaki, S., Gao, L., Shimonaka, M. and Ling, N., Isolation and molecular cloning of insulin-like growth factor binding protein-6, Mol. Endocrinol., 5 (1991) 938–948.

23 Cataldo, N.A. and Giudice, L.C., Follicular fluid insulin-like growth factor binding protein profiles in polycystic ovary syndrome, J. Clin Endocrinol. Metab., 74 (1992) 695–697.

24 Barreca, A., Minuto, F., Volpe, A., Cecchelli, E., Cella, F., Del Monte, P., Artini, P. and Giordano, G., Insulin-like growth factor-I (IGF-I) and IGF-I binding protein in the follicular fluids of growth hormone treated patients, Clin. Endocrinol., 32 (1990) 497–505.

25 Holly, J.M.P., Eden, J.A., Alaghband-Zadeh, J., Carter, G.D., Jemmott, R.C., Cianfarani, S., Chard, T. and Wass, Insulin-like growth factor binding proteins in follicular fluid from normal dominant and cohort follicles, polycystic and multicystic ovaries, Clin. Endocrinol., 33 (1990) 53–64.

26 Bicsak, T., Ling, N. and DePaolo, L.V., Ovarian intrabursal administration of insulin-like growth factor-binding protein inhibits follicle rupture in gonadotropin-treated immature female rats, Biol. Reprod., 44 (1991) 599–603.

27 Bicsak, T.A., Shimonaka, M., Malkowski, M. and Ling, N., Insulin-like growth factor-binding protein (IGF-BP) inhibition of granulosa cell function: effect on cyclic adenosine 3′,5′-monophosphate, deoxyribonucleic acid synthesis, and comparison with the effect of an IGF-I antibody, Endocrinology, 126 (1990) 2184–2189.