Insulin and insulin-like growth factor I in follicular fluid after induction of ovulation in women undergoing *in vitro* fertilization

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Summary. This study was undertaken to evaluate the relationship between concentrations of insulin and insulin-like growth factor I (IGF-I) in follicular fluid and fertilization and cleavage of human oocytes fertilized *in vitro*. The concentration of oestradiol, progesterone, luteinizing hormone, follicle-stimulating hormone, testosterone, insulin and IGF-I was determined in 36 follicular fluids, free of visible blood contamination and containing mature oocyte–corona–cumulus complexes, obtained from 12 women undergoing *in vitro* fertilization. Follicular development was induced by clomiphene citrate and human menopausal gonadotrophin, and follicular aspiration was performed 35 h after an ovulatory dose of human chorionic gonadotrophin. Concentrations of IGF-I were significantly higher in follicular fluids associated with mature oocytes that fertilized and cleaved, than in follicular fluid associated with mature oocytes that did not fertilize ($P < 0.001$). There was no difference in the concentration of insulin between follicular fluids from which fertilized oocytes were obtained and those with oocytes that remained unfertilized. No significant correlations were found between rates of embryo cleavage, concentrations of insulin and IGF-I. Multiple linear regression analysis demonstrated that the concentrations of IGF-I in follicular fluid were predicted statistically by a negative regression coefficient for the concentration of testosterone, and by a positive regression coefficient for the concentration of progesterone in follicular fluid. No candidate variable was included in the model to predict concentrations of insulin. These data suggest an important role for IGF-I in the mature follicle.

Keywords: follicular fluid; insulin; insulin-like growth factor I; *in vitro* fertilization; human

Introduction

Several reports have indicated that growth factors are involved in paracrine and autocrine regulation in the ovary (Adashi *et al.*, 1985; Dornington *et al.*, 1987; Tonetta & diZerega, 1989). Insulin and insulin-like growth factor I are present in follicular fluid, and their role in ovarian steroid synthesis and metabolism has been studied in detail in recent years (Erickson *et al.*, 1989; Holtorf *et al.*, 1989; Reed & James, 1989; Geisthovel *et al.*, 1990). Although much is known of the action of these substances on theca and granulosa cells, very little is known of the relationship between the concentration of insulin and IGF-I in human follicular fluid and parameters of oocyte quality such as fertilizability and cleavage.

The concentration of insulin has been reported to be higher in the follicular fluid of unfertilized oocytes than in the fluid of fertilized oocytes (Diamond *et al.*, 1985), although Ben-Rafael *et al.* (1987) were unable to confirm this. These authors showed that insulin concentrations in follicular

fluid were not related to the outcome of *in vitro* fertilization (IVF) of mature oocytes. Conflicting results have also been reported for the relationship between human follicular fluid IGF-I and oocyte maturity, and between the former and IVF outcome (Eden *et al.*, 1988, 1990; Geisthovel *et al.*, 1989; Roussie *et al.*, 1989; Barreca *et al.*, 1990; Rabinovici *et al.*, 1990; Giudice *et al.*, 1990; Hamori *et al.*, 1991; Suchanek *et al.*, 1991).

In an attempt to broaden our understanding of intraovarian regulation, we studied the relationship between the concentrations of insulin and IGF-I in follicular fluid, the concentrations of steroid and gonadotrophin in follicular fluid, and the fertilization and cleavage of human oocytes fertilized *in vitro*.

Materials and Methods

Patients

Thirty-six samples of follicular fluid from 12 infertile women with irreparable tubal damage were aspirated at laparoscopic oocyte collection for IVF. The patients ranged in age from 26 to 37 years, with a mean of 30.4 years. Follicular growth was stimulated by administration of 100 mg of clomiphene citrate (CC Omifin: Merrell Dow, Madrid) for five consecutive days beginning on the third day of the menstrual cycle and human menopausal gonadotrophin (hMG; Pergonal: Serono, Madrid) from the seventh day of the menstrual cycle in decreasing doses (from 225 to 75 iu per day) (Molina *et al.*, 1989). Follicular growth was monitored by measuring daily serum concentrations of oestradiol and by the development of follicles measured ultrasonically from the seventh day. When oestradiol output reached 300 to 400 pg ml⁻¹ per growing follicle (18 mm in diameter), 5000 iu human chorionic gonadotrophin (hCG; Profasi: Serono, Madrid) was given, and laparoscopy under general anaesthesia for oocyte retrieval was performed 35 h later, after an overnight fast. Ten millilitres of blood were obtained from an antecubital vein before anaesthesia for determination of insulin and IGF-I plasma concentrations.

The laboratory procedures following oocyte recovery have been described previously and were not varied throughout the duration of this study (Molina *et al.*, 1989). Follicular fluid free from visible blood contamination and containing only mature oocyte-corona-cumulus complexes was used in further analyses. The follicular fluid specimens were centrifuged at 1500 g for 10 min immediately, and aliquots of the cell-free supernatant were frozen and stored until assayed for hormone and IGF-I content.

The oocytes were inseminated with spermatozoa at a concentration of 5–10 × 10 ml⁻¹. All semen samples satisfied the World Health Organization criteria of normality (sperm counts, motility and morphology) (WHO, 1987), and it was confirmed that all semen samples used had fertilized at least one oocyte. Normal fertilization was confirmed microscopically by the observation of two polar bodies and two pronuclei 14–16 h after insemination. The stage of embryo development was assessed by the rate of cleavage 36–40 h after insemination. The follicular fluid samples were divided into three groups according to oocyte development after IVF as follows: all follicles containing unfertilized oocytes (*n* = 12), all follicles yielding ova that were fertilized and cleaved to less than four cells (*n* = 12), and follicles yielding ova that were fertilized and cleaved to four or more cells (*n* = 12).

Hormones and IGF-I assays

Concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured with commercial enzyme-immunoassay kits (Terumo Medical Corporation, Elkton, MD). Respective intra- and inter-assay coefficients of variation were 7.2 and 8.3%, for LH, and 6.1 and 8.9% for FSH. Oestradiol, progesterone and testosterone determinations were performed with radioimmunoassay (RIA) kits (Diagnostic Products Corporation, Los Angeles, CA). Respective intra- and interassay coefficients of variation were 6.8 and 9.9% for oestradiol, 8.0 and 8.7% for progesterone, and 6.3 and 9.1% for testosterone. Insulin was measured in serum and follicular fluid with a highly specific RIA, using a kit for Sorin Biomedica (Verelli, Italy). IGF-I was measured with a commercially available RIA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA) on diluted unextracted samples as described by Roussie *et al.* (1989). Sensitivities and intra- and interassay coefficients of variation were 2.2 µU ml⁻¹, 6.8 and 7.0% for insulin and 0.1 iu ml⁻¹, 6.0 and 9.6% for IGF-I. Crossreactivity of the insulin antiserum with human C-peptide was <0.01%, and of the IGF-I antiserum with hCG, LH and prolactin were <0.01, <0.01 and <0.02%, respectively.

Statistical analyses

The results are presented as means ± standard error of the mean (SEM). After multiple analysis of variance, comparisons were done with Scheffe's and Tukey's test. Significance was defined as *P* < 0.05. To predict insulin and IGF-I concentrations in follicular fluid, stepwise multiple regression was used. The variable 'oestradiol' was strongly correlated with the other explanatory variables; therefore, to avoid problems of multicollinearity, oestradiol was

omitted (Armitage & Berry, 1987). In the stepwise multiple regression analysis, a variable was included if its partial regression coefficient was significant at the 0.05 level and was eliminated if its partial regression coefficient failed to reach significance at the 0.10 level. Statistical analyses were performed using the BMDP statistical package (BMDP Statistical Software, Los Angeles, CA).

Results

The mean concentrations of insulin and IGF-I in peripheral blood at the time of oocyte retrieval were significantly higher than the concentrations in follicular fluid (Table 1). Concentrations of IGF-I were significantly higher in follicular fluids associated with mature oocytes that fertilized and cleaved (0.84 ± 0.03 iu ml⁻¹), than in fluids associated with mature oocytes that did not fertilize (0.43 ± 0.06 iu ml⁻¹; $P < 0.001$). There was no difference in the concentration of insulin in follicular fluids from which fertilized oocytes were obtained and those with oocytes that remained unfertilized (6.12 ± 0.23 μ U ml⁻¹ versus 5.93 ± 0.37 μ U ml⁻¹). No significant correlation was found between rates of embryo cleavage and concentrations of insulin and IGF-I in follicular fluid.

Table 1. Concentrations of insulin and IGF-I in human peripheral blood and follicular fluid

	<i>n</i>	Insulin (μ U ml ⁻¹)	IGF-I (iu ml ⁻¹)
Peripheral blood	12	9.32 ± 0.50	1.60 ± 0.09
Follicular fluid	36	6.02 ± 0.20	0.70 ± 0.04^a
Unfertilized	12	5.93 ± 0.37	0.43 ± 0.06
Fertilized	24	6.12 ± 0.23	0.84 ± 0.03^a
Slow cleavage rate	12	6.19 ± 0.32	0.84 ± 0.05
Fast cleavage rate	12	6.05 ± 0.35	0.85 ± 0.04

^aSignificantly different ($P < 0.001$).

Concentrations of oestradiol, progesterone, LH and testosterone in follicular fluid associated with oocytes that fertilized were higher than in those associated with oocytes that did not fertilize. No significant differences in concentrations of FSH in follicular fluid were found between the group of fertilized and unfertilized oocytes. The testosterone content in follicular fluid from fertilized oocytes was significantly lower than in follicular fluid of unfertilized oocytes (Table 2). No significant correlation was found between rates of embryo cleavage and concentrations of steroid hormones and gonadotrophins.

Multiple linear regression analysis demonstrated that the concentration of IGF-I in follicular fluid was predicted statistically by a negative regression coefficient for the concentration of

Table 2. Comparisons between concentrations of steroid and gonadotrophic hormones in human follicular fluid associated with mature oocytes that were fertilized ($n = 24$) and that were not fertilized ($n = 12$)

Group	Oestradiol (ng ml ⁻¹)	Progesterone (ng ml ⁻¹)	Luteinizing hormone (mU ml ⁻¹)	Follicle-stimulating hormone (mU ml ⁻¹)	Testosterone (ng ml ⁻¹)
Unfertilized	611 ± 45	1247 ± 35	2.9 ± 0.5	11.3 ± 2.3	2.3 ± 0.2
Fertilized	944 ± 42^a	1519 ± 43^b	9.5 ± 0.7^b	13.8 ± 0.8	1.2 ± 0.1^a

^aSignificantly different ($P < 0.005$).

^bSignificantly different ($P < 0.001$).

testosterone and by a positive regression coefficient for the concentration of progesterone in follicular fluid (Table 3). No candidate variable was included in the model to predict concentrations of insulin.

Discussion

The concentration of insulin was significantly lower in preovulatory human follicular fluid than in peripheral blood. That low basal concentrations of insulin may be necessary for normal folliculogenesis is suggested by the fact that with hyperinsulinaemia, follicular development is arrested in the early or middle stage of development (Sadler & Maller, 1987). The lower concentration in follicular fluid may be due to a regulatory effect of insulin (Zelevnik & Roth, 1978) or to an increase in local insulin degradation by way of other (non-receptor) pathways (Berman *et al.*, 1980).

This study shows that concentrations of insulin are similar in preovulatory follicles regardless of the outcome of IVF of mature oocytes and are not related to steroid hormone and gonadotrophin concentrations in follicular fluid. These results are in agreement with the findings of Ben-Rafael *et al.* (1987) but not with those of Diamond *et al.* (1985). These differences may be due to the inclusion, in the latter study, of oocytes of different maturities. Insulin appears to play an important role in initial oocyte maturation, as assessed by germinal vesicle breakdown and polar body extrusion (Tsafiriri & Channing, 1975; Lessman & Schuetz, 1981).

In agreement with previous reports (Adashi *et al.*, 1985; Eden *et al.*, 1988; Geisthovel *et al.*, 1989; Rabinovici *et al.*, 1990; Giudice *et al.*, 1990; Hamori *et al.*, 1991), we found that the concentration of IGF-I was significantly lower in preovulatory follicular fluid than in peripheral blood. Increased IGF-I in peripheral blood and follicular fluid has been associated with polycystic ovaries (Eden *et al.*, 1990; Iwashita *et al.*, 1990). The source of IGF-I in human follicular fluid is unknown. Although circumstantial evidence suggests that IGF-I may be synthesized and secreted by the granulosa cells (Adashi *et al.*, 1985; Geisthovel *et al.*, 1990), no definitive studies in humans have been published. The strong positive correlation reported by different authors (Geisthovel *et al.*, 1989; Rabinovici *et al.*, 1990; Hamori *et al.*, 1991) between plasma and follicular fluid IGF-I concentrations suggests that intrafollicular IGF-I is derived mostly from the circulating pool. Our values for IGF-I in follicular fluid may have been overestimated as IGF-I binding proteins can cause falsely increased concentrations of IGF-I (Daughaday *et al.*, 1987). However, Roussie *et al.* (1989) have shown that measurements of IGF-I in diluted unextracted follicular fluid provide adequately reliable recovery rates and an acceptable degree of reproducibility.

We noted that follicles containing mature oocytes that failed to fertilize *in vitro* had lower concentrations of IGF-I than those with oocytes that fertilized. These results contrast with the findings of Rabinovici *et al.* (1990) and Suchanek *et al.* (1991), a discrepancy that may be attributed to the use of different stimulation protocols, given that the concentration of IGF-I in serum (Gadd *et al.*, 1991) and preovulatory follicular fluid is influenced by the method of ovarian stimulation (Roussie *et al.*, 1989). Our results confirm previous data regarding the role of IGF-I in oocyte maturation. Human and rat oocytes contain receptors for IGF-I (Andersson *et al.*, 1986; Balboni *et al.*, 1987) and IGF-I has been shown to induce *in vitro* oocyte maturation (Sadler & Maller, 1987). Roussie *et al.* (1989) showed that IGF-I concentrations were higher in follicular fluids yielding a mature oocyte than in those yielding an immature oocyte.

Granulosa cells contain receptors for IGF-I, suggesting that IGF-I acts on granulosa cells in an autocrine fashion (Adashi *et al.*, 1985; Gates *et al.*, 1987). The relationship we found between the concentration of IGF-I and progesterone in follicular fluid is easily explained by recent studies showing that IGF-I modulates key steps in progesterone biosynthesis. Basal progesterone secretion by granulosa cells is enhanced by IGF-I (Davoren *et al.*, 1985; Veldhuis *et al.*, 1986), while the catabolism of progesterone to 20- α -hydroxy-progesterone under IGF-I treatment is reduced (Adashi *et al.*, 1985). Other authors have also documented an association between the

Table 3. Stepwise multiple regression analysis of concentration of IGF-I in human follicular fluid

Variables in equation						Variables not in equation		
Step	Variable	Coefficient	R^a	R^2	F_{exp}	Variable	Partial correlation	F_{exp}
1	Testosterone	-0.1274	0.668	0.446	27.40 ^b	Luteinizing hormone	0.2895	2.93
2	Progesterone	0.4×10^{-3}	0.726	0.527	5.66 ^b	Follicle-stimulating hormone	0.2249	1.70
	Constant	0.2660				Insulin	-0.1361	0.60

^aR: multiple correlation coefficient.

^bSignificantly different ($P < 0.01$).

concentration of IGF-I in follicular fluid and parameters of granulosa cell function (Roussie *et al.*, 1989; Eden *et al.*, 1988, 1989, 1990; Rabinovici *et al.*, 1990). The negative correlation between IGF-I and testosterone concentrations may reflect the fact that IGF-I potentiates FSH-induced aromatase activity by enhancing the reaction velocity but not the substrate affinity of this enzyme complex (Adashi *et al.*, 1985).

We observed no relationship between the concentration of insulin and IGF-I in follicular fluid. However, insulin may regulate the biological action of IGF-I by modulating the concentration of IGF-I-binding proteins (Iwashita *et al.*, 1990). At least three (Giudice *et al.*, 1990) or four (Holly *et al.*, 1990) discrete IGF-I binding proteins are present in human follicular fluid. Some studies have shown that binding proteins increase the amount of IGF-I that binds to IGF receptors (Clemmons *et al.*, 1986), thereby enhancing the response to the growth factor (Elgin *et al.*, 1987), whereas other studies have shown that the binding proteins inhibit IGF-I binding to its receptor, thus attenuating its action (Elgin *et al.*, 1987; Ritvos *et al.*, 1988).

No significant differences were observed in the concentration of insulin and IGF-I between follicular fluids that yielded fertilized oocytes that cleaved to fewer than four cells at 43 h after insemination and samples containing fertilized oocytes that cleaved to four or more cells. Follicular concentrations of insulin and IGF-I do not predict the cleavage rate of fertilized oocytes. The mechanisms involved in the early cleavage of the human embryo remain unclear but it is obvious that they are not only oocytic in nature.

In conclusion, the concentration of insulin in preovulatory follicular fluid containing mature oocytes was a poor predictor of the ability of oocytes to fertilize. However, the concentration of IGF-I was positively correlated with the ability of the oocyte to fertilize. Thus the concentration of IGF-I in preovulatory follicular fluid may be an indicator of optimal follicular development and oocyte maturation, at least in cycles stimulated by CC/hMG/hCG.

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References

- Adashi, E.Y., Resnick, C.E., D'Ercole, J., Svoboda, M.E. & Van Wyk, J.J. (1985) Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. *Endocrine Reviews* **6**, 400–420.
- Andersson, I., Billig, H., Fryklund, L., Hansson, H.A., Isaksson, O., Isgaard, J., Nilsson, A., Rozell, B., Skottner, A. & Stemme, S. (1986) Localization of IGF-I in adult rats. Immunohistochemical studies. *Acta Physiologica Scandinavica* **126**, 311–312.
- Armitage, P. & Berry, G. (1987) Multiple measurements. In *Statistical Methods in Medical Research*, pp. 296–357. Eds P. Armitage & G. Berry. Blackwell Scientific Publications, Oxford.
- Balboni, G.C., Vannelli, G.B., Barni, T., Orlando, C. & Serio, M. (1987) Transferrin and somatomedin C receptors in the human ovarian follicles. *Fertility and Sterility* **48**, 796–801.
- Barreca, A., Minuto, A., Volpe, A., Ceccheli E., Cella, F., Del Monte, P., Artini, P. & Giordano, G. (1990) Insulin-like growth factor-I (IGF-I) and IGF-I binding protein in the follicular fluids of growth hormone treated patients. *Clinical Endocrinology* **32**, 497–505.

- Ben-Rafael, Z., Meloni, F., Strauss, J.F., Blasco, L., Mastroianni, L. & Flickinger, G.L. (1987) Relationships between polypronuclear fertilization and follicular fluid hormones in gonadotropin-treated women. *Fertility and Sterility* **47**, 284–288.
- Berman, M., McGuire, E.A., Roth, J. & Zeleznik, A.J. (1980) Kinetic modeling of insulin fertilization to receptors and degradation in vivo in the rabbit. *Diabetes* **29**, 50–59.
- Clemmons, P.R., Elgin, R.G., Han, V.K., Lasella, S.J., D'Ercole, A.J. & Van Wyk, J.J. (1986) Cultured fibroblast monolayers secrete a protein that alters the cellular binding of somatomedin-C/insulin-like growth factor I. *Journal of Clinical Investigation* **77**, 1548–1556.
- Daughaday, W.H., Kapadia, M. & Mariz, I. (1987) Serum somatomedin binding proteins: physiologic significance and interference in radioligand assay. *Journal of Laboratory and Clinical Medicine* **109**, 355–363.
- Davoren, J.B., Hsueh, A.J. & Li, C.H. (1985) Somatomedin C augments FSH-induced differentiation of cultured rat granulosa cells. *American Journal of Physiology* **249**, E26–E33.
- Diamond, M.P., Webster, B.W., Carr, R.K., Wentz, A.C. & Osteen, K.G. (1985) Human follicular fluid insulin concentrations. *Journal of Clinical Endocrinology and Metabolism* **61**, 990–992.
- Dorrington, J.H., Bendell, J.J., Chuma, A. & Lobb D.K. (1987) Actions of growth factors in the follicle. *Journal of Steroid Biochemistry* **27**, 405–411.
- Eden, J.A., Jones, J., Carter, G.D. & Alagband-Zadeh, J. (1988) A comparison of follicular fluid levels of insulin-like growth factor-I in normal dominant and cohort follicles, polycystic and multicystic ovaries. *Clinical Endocrinology* **29**, 327–336.
- Eden, J.A., Carter, G.D., Jones, J. & Alagband-Zadeh, J. (1989) Insulin-like growth factor I as an intra-ovarian hormone. An integrated hypothesis and review. *Australian and New Zealand Journal of Obstetrics and Gynaecology* **29**, 30–37.
- Eden, J.A., Jones, J., Carter, G.D. & Alagband-Zadeh, J. (1990) Follicular fluid concentrations of insulin-like growth factor I, epidermal growth factor, transforming growth factor- α and sex-steroids in volume matched normal and polycystic human follicles. *Clinical Endocrinology* **32**, 395–405.
- Elgin, R.G., Busby, W.H. & Clemmons, D.R. (1987) An insulin-like growth factor (IGF) binding protein enhances the biologic response to IGF-I. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 3254–3259.
- Erickson, G.F., Garzo, V.G. & Magoffin, D.A. (1989) Insulin-like growth factor-I regulates aromatase activity in human granulosa and granulosa luteal cells. *Journal of Clinical Endocrinology and Metabolism* **69**, 716–724.
- Gadd, S.C., Anthony, F.W., Jenkins, J.M., Davies, D.W. & Masson, G.M. (1991) Alteration of serum IGF-I concentrations in IVF patients treated with buserelin. *Human Reproduction* **6**, Supplement 1. 74, Abstract P51.
- Gates, G.S., Bayer, S., Seibel, M., Poretsky, L., Flier, J.S. & Moses, A.C. (1987) Characterization of insulin-like growth factor binding to human granulosa cells obtained during in vitro fertilization. *Journal of Receptor Research* **7**, 885–902.
- Geisthoel, F., Moretti-Rojas, I.M., Rojas, F.J. & Asch, R.H. (1989) Immunoreactive insulin-like growth factor I in human follicular fluid. *Human Reproduction* **4**, 35–38.
- Geisthoel, F., Moretti-Rojas, I., Rojas, F.J. & Asch, R.H. (1990) Insulin-like growth factors and thecal-granulosa-cell function. *Human Reproduction* **5**, 785–799.
- Giudice, L.C., Farrell, E.M., Pham, H. & Rosenfeld, R.G. (1990) Identification of insulin-like growth factor-binding protein-3 (IGFBP-3) and IGFBP-2 in human follicular fluid. *Journal of Clinical Endocrinology and Metabolism* **71**, 1330–1338.
- Hamori, M., Blum, W.F., Török, A., Stehle, R., Waibel, E., Cledon, P. & Ranke, M.B. (1991) Insulin-like growth factors and their binding proteins in human follicular fluid. *Human Reproduction* **6**, 313–318.
- Holly, J.M., Eden, J.A., Alagband-Zadeh, J., Carter, G.D., Jemmott, R.C., Cianfarani, S., Chard, T. & Wass, J.A. (1990) Insulin-like growth factor binding proteins in follicular fluid from normal dominant and cohort follicles, polycystic and multicystic ovaries. *Clinical Endocrinology* **33**, 53–64.
- Holtorf, A.P., Furuya, K., Ivell, R. & McArdle, C.A. (1989) Oxytocin production and oxytocin messenger ribonucleic acid levels in bovine granulosa cells are regulated by insulin and insulin-like growth factor-I: dependence on developmental status of the ovarian follicle. *Endocrinology* **125**, 2612–2620.
- Iwashita, M., Mimuro, T., Watanabe, M., Setoyama, T., Matsuo, A., Adachi, T., Takeda, Y. & Sakamoto, S. (1990) Plasma levels of insulin-like growth factor-I and its binding protein in polycystic ovary syndrome. *Hormone Research* **33**, 21–26.
- Lessman, C.A. & Schuetz, A.W. (1981) Role of follicle wall in meiosis reinitiation induced by insulin in *Rana pipiens* oocytes. *American Journal of Physiology* **241**, E51–E56.
- Molina, R., Castilla, J.A., Vergara, F., Perez, M., Garrido, F. & Herruzo, A. (1989) Luteal cytoplasmic estradiol and progesterone receptors in human endometrium: in vitro fertilization and normal cycles. *Fertility and Sterility* **51**, 976–979.
- Rabinovici, J., Dandekar, P., Angle, M.J., Rosenthal, S. & Martin, M.C. (1990) Insulin-like growth factor I (IGF-I) levels in follicular fluid from human pre-ovulatory follicles: correlation with serum IGF-I levels. *Fertility and Sterility* **54**, 428–433.
- Reed, M.J. & James, H.T. (1989) Regulation of steroid synthesis and metabolism by growth factors. *Clinical Endocrinology* **31**, 511–525.
- Ritvos, O., Ranta, T., Jalkanen, J., Suikkari, A.-M., Voutilainen, R., Bohn, H. & Rutanen, E.-M. (1988) Insulin-like growth factor (IGF) binding protein from human decidua inhibits the binding and biological action of IGF-I in cultured choriocarcinoma cells. *Endocrinology* **122**, 2150–2157.
- Roussie, M., Royère, D., Guillonueau, M., Lansac, J. & Muh, J.P. (1989) Human antral fluid IGF-I and oocyte maturity: effect of stimulation therapy. *Acta Endocrinologica* **121**, 90–94.

- Sadler, S.E. & Maller, J.L. (1987) *In vivo* regulation of cyclic AMP phosphodiesterase in *Xenopus* oocytes. *Journal of Biological Chemistry* **22**, 10644–10650.
- Suchanek, E., Simunic, V., Grizelj, V., Halimi, V., Hlavati, V., Dobec, D. & Zaninovic, N. (1991) Insulin-like growth factor-I levels in human preovulatory follicular fluid. *Human Reproduction* **6**, Supplement 1, 101, Abstract P130.
- Tonetta, S.A., & diZerega, G. (1989) Intraovarian regulation of follicular maturation. *Endocrine Reviews* **10**, 205–229.
- Tsafiriri, A. & Channing, C.P. (1975) Influence of follicular maturation and culture conditions on the meiosis of pig oocytes *in vitro*. *Journal of Reproduction and Fertility* **43**, 149–152.
- Veldhuis, J.D., Rodgers, R.J. & Furlanetto, R.W. (1986) Synergistic actions of estradiol and the insulin-like growth factor somatomedin-C on swine ovarian (granulosa) cells. *Endocrinology* **119**, 530–538.
- World Health Organization (1987) *WHO Laboratory Manual for the Examination of Human Semen and Semen-cervical Mucus Interaction*. Cambridge University Press, Cambridge.
- Zeleznik, A. & Roth, J. (1978) Demonstration of the insulin receptor *in vivo* in rabbits and its possible role as a reservoir for the plasma hormone. *Journal of Clinical Investigation* **21**, 1363–1374.

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