Concentrations of follicle regulatory protein, steroids and gonadotrophins in antral fluids from women stimulated with metrodin and hCG*

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Summary. Concentrations of immunoreactive follicle regulatory protein (FRP) were determined in 184 follicular fluid samples recovered from 30 patients in whom ovarian stimulation before oocyte recovery and in-vitro fertilization was induced with FSH (150 i.u./day). Ovum recovery was scheduled when the diameters of ≥ 2 follicles reached 15–17 mm and serum oestradiol values were 740 pM/follicle. The mean level of FRP in fluid from follicles yielding oocytes which fertilized and cleaved within 48 h of recovery (24.4 ± 3.08 immunoreactive units [IRU]/ml; 1 IRU = ~ 1 ng pig FRP) was higher than that in fluid from follicles yielding oocytes which did not fertilize (10.5 ± 1.67 IRU/ml, $P < 0.05$). FRP in fluid from follicles yielding oocytes which fertilized but did not cleave within 48 h of recovery was 17.2 ± 2.89 IRU/ml. Overall, concentrations of FRP did not correlate with follicular fluid volume or with FSH or LH concentrations, but were positively related to prolactin, oestradiol and total protein levels ($P < 0.04$). The results indicate that the FRP content of follicular fluid may be predictive of follicle/oocyte maturity. A dose-dependent increase in release of FRP by pig granulosa cells cultured in medium supplemented with 10–100 ng prolactin/ml was demonstrated. Prolactin may, therefore, be an important determinant of FRP production by granulosa cells.

Keywords: in-vitro fertilization; ovarian proteins; stimulated cycles; women

Introduction

Follicle regulatory protein (FRP) has been isolated and partly purified from pig follicular fluid (Holmberg *et al.*, 1986; Ono *et al.*, 1986). This protein of M_r 15 000 can inhibit granulosa cell aromatase and alter 3β -hydroxysteroid dehydrogenase activity *in vitro*, and can suppress the ability of follicle-stimulating hormone (FSH) to induce cyclic adenosine 3',5'-monophosphate (cAMP) and luteinizing hormone (LH) receptors on granulosa cells (diZerega *et al.*, 1982; Montz *et al.*, 1984; Battin & diZerega, 1985a, b; Ujita *et al.*, 1987). FRP is secreted by pig granulosa, but not theca, cells (Tonetta *et al.*, 1988), and its occurrence in serum and in urine indicates that it might affect responses beyond the ovarian follicles. In the present study we have examined the inter-relationships between concentrations of steroids, gonadotrophins, prolactin and FRP in follicular fluid of women, and whether the content of FRP in follicular fluid is associated with conventional indices of maturity of human follicles.

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Materials and Methods

Experiment 1: concentrations of FRP and other hormones in human follicular fluid

Patients. Thirty-one healthy women, 18–37 years of age, consented to participate in this study. All subjects were menstruating normally, had two accessible ovaries, and were diagnosed as being infertile due to tubal factors. Semen characteristics of all male partners were within normal limits. Twenty-four of the subjects were presenting for their first treatment cycle of in-vitro fertilization. Patients with any medical condition requiring concurrent medication (including renal, hepatic, endocrine, cardiovascular or gastrointestinal disorders) were excluded from the study. Similarly, subjects who hyperstimulated in response to FSH or clomiphene citrate therapy during previous cycles of ovarian stimulation were excluded.

Ovarian stimulation. All patients began treatment with Metrodin (purified human urinary FSH [hFSH]; Serono Laboratories, Inc., Randolph, MA, USA) on Day 2 of the menstrual cycle. A standardized dose of 150 i.u. hFSH was injected (intramuscularly) daily to induce multiple follicle development. From the 5th day of the fixed regimen of FSH treatment, dosages of exogenous gonadotrophin were adjusted in accord with daily changes in serum concentrations of oestradiol and expansion of follicular diameters, determined by vaginal ultrasonography (Ultramark 4, Advanced Technology Laboratories, Bothell, WA, USA). While on Metrodin treatment, all patients were monitored as follows:

- (a) ultrasound and daily determination of serum oestradiol, commencing on Day 5 of the menstrual cycle and continuing until ≥ 2 follicles exceeded 1.5 cm in diameter;
- (b) clinical examination daily from Day 5 of the menstrual cycle;
- (c) laparoscopic oocyte recovery was scheduled after the leading follicle(s) exceeded 1.8 cm in diameter and serum values of oestradiol approached 740 pm per follicle of > 1.5 cm in diameter;
- (d) final oocyte maturation was induced by intramuscular injection of 10 000 USP units of human chorionic gonadotrophin (hCG; Profasi, Serono).

Cancellation of cycles. Treatment cycles were cancelled if only one dominant follicle developed, if concentrations of oestradiol in serum declined for 2 consecutive days during treatment with Metrodin, or if serum oestradiol values decreased $\geq 10\%$ during the 12 h after injection of hCG.

Oocyte retrieval. Laparoscopy was performed 35–37 h after treatment with hCG. All visible follicles were aspirated by laparoscopic needle puncture. To minimize the likelihood of contamination of aspirates with aspirates of preceding follicles, the aspiration needle and lines were flushed with culture medium between consecutive follicle punctures. We could not, therefore, discount a possible dilution of follicle aspirates with residual culture medium in the lines. However, this volume would have been small in comparison to the relatively large follicular volumes (average near 4 ml), and would have contributed similarly to all follicular aspirates. Morphological characteristics of the oocytes recovered from the follicular aspirates were recorded, and standard laboratory protocols for egg maturation and insemination were observed (Marrs, 1986).

Recovery and analysis of follicular fluids. Immediately following recovery of oocytes from follicular aspirates, the volume of fluid collected from each follicle was determined via syringe, aspirates were centrifuged to precipitate blood cells and debris (2000 g, 20 min, 4°C), and supernatants were aspirated, divided into 10 aliquants and stored at -80°C until analysed.

Oestradiol assay. After dilution ($\times 441$) with charcoal-cleared human serum, oestradiol concentrations in follicular fluids were determined using a double-antibody radioimmunoassay (RIA; Pantex, Santa Monica, CA, USA), sensitive to 6 fmol oestradiol/tube. The average intra-assay coefficient of variation was $< 6\%$, and the interassay coefficients of variation (calculated from levels of oestradiol in control samples determined in each assay; Lyphochek immunoassay control, BioRad ECS Division, Anaheim, CA, USA) were 9.8% at a mean oestradiol value of 369 pm, 6.3% at 823 pm, and 10.9% at 3634 pm (average values from 16 consecutive assays).

Follicular fluids were diluted with steroid-free human serum to minimize differences between matrices of samples and standards, standards being a solution of authentic oestradiol in charcoal-treated human serum. To confirm parallelism, determination of oestradiol in diluted samples, a sample of human serum was spiked with authentic oestradiol-17 β (Sterealoids, Wilton, NH, USA) to provide 1.0 μM -oestradiol, then assayed after dilution ($\times 80, 160, 320, 640, 1280$ and 2560) with steroid-free human serum. Following correction for dilution, concentrations of oestradiol through the dilution series were calculated to be 1.01, 1.16, 1.02, 1.27, 1.06 and 0.76 nM, respectively. Acceptable parallelism at a dilution of $\times 441$ was, therefore, presumed. In any event, follicular fluid comprised $< 0.23\%$ of the total volume of aliquants of the assayed samples, and this proportion was identical for all samples.

Progesterone assay. After dilution ($\times 441$) with charcoal-treated human serum, progesterone concentrations in follicular fluids were measured by double-antibody RIA (Pantex), sensitive to 32 fmol progesterone/tube. The average intra-assay coefficient of variation was $< 10\%$, and the interassay coefficients of variation (calculated from levels of progesterone in control samples determined in each assay; Lyphochek immunoassay control, BioRad) were: 10.5% at a mean value of progesterone of 3.4 nM, 10.0% at 40.4 nM and 9.7% at 88.4 nM (average values from 16 consecutive assays).

In accord with the rationale outlined above, a sample of human serum was spiked with authentic progesterone (Sterealoids) to provide 6.37 μM -progesterone, then assayed after dilution ($\times 40, 80, 160, 320$ and 640) with steroid-free

human serum. Calculated concentrations of progesterone through the dilution series were 6.10, 6.00, 5.90, 5.63 and 5.54 μM , indicating a slight (11–13%) underestimation of the progesterone content of follicular fluids after dilution ($\times 441$) in the present study. It was presumed that dilution would affect all determinations similarly, and further adjustments for dilution were not made.

Testosterone assay. After dilution (1:1) with phosphate-buffered saline (PBS, 0.1 M, pH 7.0), concentrations of testosterone in follicular fluids were determined using an antibody-coated tube RIA (Pantex), sensitive to 17.3 fmol testosterone/tube. The average intra-assay coefficient of variation was <10%, and the interassay coefficient of variation (calculated from testosterone values in control samples determined in each assay; Lymphochek immunoassay control; BioRad) were: 12.4% at a mean testosterone concentration of 4.3 nM, 8.4% at 16.6 nM, and 9.4% at 56.2 nM (average values from 13 consecutive assays). Testosterone standards (0.35–88.8 nM) were prepared in PBS.

To determine effects of dilution of samples on the radioimmunoassayable content of testosterone, a pooled source of follicular fluid was assayed neat, and after dilution with PBS ($\times 2$ and $\times 4$, v/v). The testosterone concentrations were measured as 75.3, 66.78 and 75.5 nM, respectively, confirming linearity in samples diluted 1:1.

FSH assay. FSH concentrations in undiluted follicular fluids were determined using an immunoradiometric assay (MAIAclone, Sero Diagnostics, Norwell, MA, USA), sensitive to 0.013 mIU. FSH (second IRP 78/549) per tube. The average intra-assay coefficient of variation was <10%, and the interassay coefficients of variation (calculated from FSH values in control samples determined in each assay; Lymphochek immunoassay control, BioRad) were: 10.6% at a mean FSH level of 5.61 mIU./ml, 5.7% at 19.7 mIU./ml, and 14.3% at 54.7 mIU./ml (average values from 10 consecutive assays).

LH assay. An immunoradiometric sandwich assay (MAIAclone, Sero Diagnostics) was used. This assay was selected on the criterion of low cross-reactivity with hCG (<3%). The assay was sensitive to 0.025 mIU. LH (first IRP 68/40) per tube. The average intra-assay coefficient of variation was <10%, and the interassay coefficients of variation (calculated from amounts of LH in control samples determined in each assay; Lymphochek immunoassay control, BioRad) were: 11.8% at a mean LH level of 1.95 mIU./ml, 7.9% at 4.42 mIU./ml, and 9.7% at 6.86 mIU./ml (average values from 21 consecutive assays).

Prolactin assay. Prolactin concentrations were determined using an immunoradiometric sandwich assay (MAIAclone, Sero Diagnostics) sensitive to 0.05 ng human prolactin/tube. The average intra-assay coefficient of variation was <10%, and the interassay coefficients of variation (calculated from amounts of prolactin in control samples determined in each assay; Lymphochek immunoassay control, BioRad) were as follows: 12.7% at a mean prolactin level of 4.62 ng/ml, 12.9% at 30.38 ng/ml, and 9.1% at 83.23 ng/ml (average values from 24 consecutive assays).

Protein measurements. Total protein values in aliquants of follicular fluids were determined using a micromethod based on the shift in maximum absorbance of Coomassie Brilliant Blue G-250 when binding to protein occurs (Sedmak & Grossberg, 1977; BioRad Protein Assay, BioRad Chemical Division, South Richmond, CA, USA).

Measurement of follicle regulatory protein. FRP was measured by an indirect enzyme-linked immunoassay (Katt *et al.*, 1988). Polystyrene 96-well culture plates (Immulon I) were coated with 0.1 ml of a 3 $\mu\text{g}/\text{ml}$ solution of 138G3 (a murine monoclonal antibody to FRP) in veronal buffer, pH 9.6 for 16–20 h at 4°C. The plates were blocked for 2 h at room temperature with 0.2 ml PBS with 0.5% bovine serum albumin (BSA) and 0.1% Tween 80. After the blocking step, the plates were stored frozen at -20°C . Test samples or standard were diluted in PBS with PBS–0.2% BSA. An aliquant (0.1 ml) of sample or standard was added in triplicate and incubated for 3 h at room temperature. Following this incubation, the plates were washed 3 times with PBS containing 0.1% Tween 80 to remove excess antigen. The detector antibody, 0.1 ml anti-FRP rabbit antiserum diluted 1/2000 in PBS–0.2% BSA, was then added to each well and the plates were incubated overnight at 4°C then washed three times in PBS–0.1% Tween 80. Goat anti-rabbit IgG antiserum conjugated with peroxidase, diluted 1/2000 in PBS–0.5% BSA, was then added to each well and incubated an additional 2 h at room temperature. The plates were then washed three times with PBS–0.1% Tween 80, and 2,2'-azinobis 3-ethylbenzthiozoline (0.1 ml of 0.5 mg/ml in citrate phosphate buffer, pH 4) with 0.015% H_2O_2 , as a catalyst, was added to each well. After 30 min, the reaction was stopped by the addition of 0.05 ml 3.3 M-NaF. The optical density at an absorbance of 410 nm was measured using a Dynatech MR 650 microplate reader connected to an Apple IIc computer. The cross-reactivity of the FRP assay with prolactin was <0.01%, determined at a prolactin concentration of 100 ng/ml. In addition, hFSH, hLH, oestradiol, progesterone, testosterone, androstenedione, insulin, insulin-like growth factors I and II, transforming growth factor alpha and beta, epidermal growth factor, fibroblast growth factor, transferrin, and purified pig inhibin did not cross-react in this assay.

Statistics. The 31 treatment cycles of 28 patients yielded 205 follicles. For statistical analysis, fluids aspirated from these follicles were grouped according to the outcome of in-vitro fertilization; i.e. *Group 1*: oocyte did not fertilize ($n = 34$); *Group 2*: oocyte fertilized but did not cleave within 72 h of ovum recovery ($n = 12$); *Group 3*: oocyte fertilized and cleaved between 48 and 72 h of ovum recovery ($n = 11$); and *Group 4*: oocyte fertilized and cleaved within 48 h of ovum recovery ($n = 89$). Preliminary analysis of hormone and protein levels in follicular fluids (Shapiro-Wilkes' and Levene's tests) established that the data were normally distributed, and were homogeneous with respect to group variances (Conover, 1971). Therefore, between-group differences in these data were analysed by parametric statistics (Student's *t* test; Snedecor & Cochran, 1980). Interrelationships between levels of components in follicular fluids were analysed by least squares linear regression analysis (Snedecor & Cochran, 1980).

Experiment 2: effects of prolactin on release of FRP by cultured pig granulosa cells

With respect to the close statistical association between concentrations of FRP and prolactin in human antral fluid (Table 1), and the possibility that prolactin might modulate the FRP content of antral fluid, an experiment was carried out to establish whether human prolactin could stimulate release of FRP by cultured pig granulosa cells.

Granulosa cells were aspirated from small (<3 mm), medium (3–6 mm) and large (8–10 mm) pig follicles. Cells were washed three times in Medium 199 containing penicillin-streptomycin and amphotericin B (M199). After the last wash, viable cells were identified by trypan blue exclusion. Cells were diluted to 5×10^5 viable cells/ml and apportioned into microtitre plates (1 ml/well). Granulosa cells from follicles of each size were divided between the following treatment groups: (a) control (M199 alone), (b) human prolactin (0.1, 1, 10, 100 ng/ml, NIADDK-hPRL-I-7 [AFP-9900], National Hormone & Pituitary Program, Bethesda, MD, USA). These levels of prolactin were within the normal physiological range determined for human follicular fluid (Fig. 2). Culture media for FRP analysis were removed after 48 h of static culture. The experiment was repeated twice with 4–6 wells per group (total $n = 8$ –12 wells/treatment).

Results

Experiment 1

Concentrations of steroids and proteins in follicular fluids grouped by outcome of in-vitro fertilization are summarized in Fig. 1. The means $\pm$ s.e.m. of the volumes of follicular fluid from follicles of each group are: Group 1, 3.68 ± 0.28 ml; Group 2, 3.82 ± 0.63 ml; Group 3, 4.59 ± 0.53 ml; and Group 4, 4.72 ± 0.18 ml.

Concentrations of progesterone were higher in follicular fluid samples yielding oocytes which fertilized and cleaved within 48 h of ovum recovery, compared with the other groups studied ($P < 0.05$). Concentrations of testosterone, LH, FSH, prolactin and total protein in follicular fluid did not differ significantly between outcomes of in-vitro fertilization. However, concentrations of oestradiol in samples from follicles yielding oocytes which fertilized and cleaved between 48 and 72 h of recovery were higher than in other groups of fluids ($P < 0.05$), and total contents of FRP were higher in follicular fluid samples which yielded oocytes which fertilized, compared to those which did not ($P < 0.05$). Total contents of FRP were higher in follicular fluid samples yielding oocytes which fertilized and cleaved compared with follicular fluids yielding oocytes which fertilized but did not cleave ($P < 0.05$). Lowest total contents of FRP were determined in follicular fluid samples yielding oocytes which did not fertilize.

Concentrations of FRP in follicular fluid were correlated with oestradiol, protein and prolactin content ($P < 0.05$; Table 1). Although the mean values show different patterns, the closest correlation with FRP was with prolactin ($P < 0.001$).

Experiment 2

Concentrations of FRP in spent media from granulosa cells cultured with various concentrations of prolactin are shown in Fig. 2. Prolactin supplementation did not significantly affect FRP secretion by granulosa cells from large follicles. However, prolactin induced a dose-dependent increase in secretion of FRP by granulosa cells of small and medium-sized follicles.

Discussion

During the past decade, several studies have examined interrelationships between concentrations of hormones in human follicular fluids, the maturity of oocytes recovered from those fluids, and the outcome of in-vitro fertilization (Fowler *et al.*, 1977; Wramsby *et al.*, 1981; Radwanska *et al.*, 1986; Kreiner *et al.*, 1987; Nayudu *et al.*, 1987; Marrs *et al.*, 1987; Stone *et al.*, 1988a, b). There is general concurrence in this literature with respect to positive correlations between oocyte maturity and amounts of oestradiol, progesterone and LH in follicular fluid (Rannevik *et al.*, 1979; Wramsby

Fig. 1. Concentrations of testosterone, oestradiol-17 β , progesterone, total protein, FSH, LH, prolactin and FRP in follicular fluid from patients with oocytes that did not fertilize or cleave (Group 1), fertilized but did not cleave (Group 2), fertilized and cleaved within 48–72 h (Group 3), or fertilized and cleaved in <48 h (Group 4). Values are mean $\pm$ s.e.m.

et al., 1981). The total volume and protein content of antral fluid also increases with follicular maturation (Fowler *et al.*, 1977; Simonetti *et al.*, 1985; Stone *et al.*, 1988a), protein content being an element of prediction of pregnancy outcome (Nayudu *et al.*, 1987). Collectively, the literature has identified qualitative changes in the intrafollicular milieu during final stages of oocyte–follicle maturation, and results of the present study (Fig. 1) indicate that late maturational changes in the follicular endocrine status of patients stimulated with only FSH are not widely different from values associated with other hyperstimulation regimens (Fowler *et al.*, 1977; Rannevik *et al.*, 1979; Nayudu *et al.*, 1987; Stone *et al.*, 1988a).

Concentrations of oestradiol in follicular fluid samples yielding oocytes which fertilized were not consistently higher than those in fluids yielding oocytes which did not fertilize (Fig. 1). In

Table 1. Analysis of interrelationship between the concentrations (immunoreactive units/ml) of FRP (dependent variable, y) and other components of human antral fluids ($n = 205$)

	Independent variable (x)						
	Progesterone (μM)	Testosterone (nM)	Oestradiol (nM)	LH (mi.u./ml)	FSH (mi.u./ml)	Protein ($\mu\text{g/ml}$)	Prolactin (ng/ml)
A*	12.0	13.2	11.5	3.70	3.42	0.10	0.62
B*	+0.16	+0.07	+0.022	+0.19	+0.25	+0.08	+0.04
Correlation coefficient (R)	0.09	0.05	0.16	0.05	0.07	0.22	0.28
F-ratio	1.33	0.34	4.45	0.34	0.72	8.48	14.12
Significance levels (P)	0.251	0.562	0.036	0.561	0.396	0.004	<0.001

*Constants in the equation: $y = A + B \cdot x$.

Fig. 2. Change in FRP secretion by pig granulosa cells from follicles of different sizes after treatment with prolactin. Media samples were removed at 48 h and concentrations of FRP determined by ELISA (as described in 'Materials and Methods'). Values are mean $\pm$ s.e.m. but the symbols are larger than the error bars.

addition, the average amount of oestradiol in all fluids was lower than the threshold ($6.6 \mu\text{M}$) reported by Carson *et al.* (1982), although our values were within ranges reported more recently by others (0.49 – $1.73 \mu\text{M}$; Fowler *et al.*, 1977; Radwanska *et al.*, 1986). We therefore suggest that follicles in all categories in the present study were 'oestrogenic', and that intrafollicular levels of oestradiol may not be reliable predictors of the outcome of insemination of oocytes received for in-vitro fertilization.

Follicles yielding less mature oocytes had higher concentrations of testosterone in their follicular fluids (Fig. 1). These follicles may be less mature than those follicles yielding oocytes that cleaved and fertilized as previous reports indicate that intrafollicular androgen content is inversely related to follicular maturity (Fowler *et al.*, 1977). The findings are also consistent with the apparent inverse relationship between average levels of testosterone and FSH in fluids in each of the 4 groups, and support evidence for a direct stimulatory effect of FSH on ovarian aromatase activity (Savard *et al.*, 1965; Armstrong & Papkoff, 1976; Baird & McNeilly, 1981; diZerega & Hodgen, 1981; McNatty *et al.*, 1985). In view of evidence that FSH can stimulate ovarian synthesis of macromolecules in a dose-dependent fashion (Mueller *et al.*, 1978; Ax & Ryan, 1979), it is of interest that intrafollicular levels of FSH were neither related to total protein content, nor to the total content of FRP. However, changes in concentrations of protein and FRP in antral fluid were closely interrelated,

and the content of either in follicular fluid appeared to be predictive of the likelihood that the nested oocyte would fertilize and cleave *in vitro*. This finding is important with respect to the potential application of intrafollicular content of FRP in predicting the outcome of in-vitro fertilization, and suggests that FRP may be a potentially important determinant of follicular maturity in women.

The close correlation between concentrations of FRP and oestradiol in follicular fluid (Table 1) supports our previous bioassay data (diZerega *et al.*, 1983). While this may seem to contradict evidence that FRP can inhibit granulosa cell aromatase activity, the ability of FRP to inhibit this enzyme activity is modulated by FSH content of the in-vitro/in-vivo medium. In the presence of high concentrations of FSH (>2 mIU/ml), the inhibitory effects of FRP can be overridden (Schreiber & diZerega, 1986). With respect to the present findings, values of FSH in the intrafollicular milieu during final follicular maturation are sufficiently high to preserve granulosa cell aromatase activity, despite rising levels of FRP in the antral fluids (Fig. 1).

A close statistical correlation was established between concentrations of prolactin and FRP in the human follicular fluids ($P < 0.001$; Table 1). With respect to the dose-dependent increase in FRP release by granulosa cells harvested from small and medium-sized follicles (Fig. 2), prolactin becomes the first hormone shown to have the ability to stimulate the release of FRP by cultured granulosa cells. On the basis of these findings, we propose that some of the effects of prolactin on granulosa cell function *in vivo* may be more directly mediated by FRP. That is, the established suppressive effects of prolactin on oestradiol production by granulosa cells (McNatty *et al.*, 1974; Veldhuis & Hammond, 1980; Wang *et al.*, 1980; Dorrington & Gore-Langton, 1981, 1982; McNeilly *et al.*, 1982; Tsai-Morris *et al.*, 1983; Cutie & Andino, 1988) may be modulated through inhibition of basal and FSH-stimulatable aromatase activity by FRP.

From a clinical perspective, disorders of follicular maturation and altered ovarian responsiveness to gonadotrophins in hyperprolactinaemic women (St Michel & diZerega, 1983) are also consistent with stimulation of release of FRP by granulosa cells, and with suppression of granulosa cell aromatase activity by high levels of FRP in the antral fluid. Low peripheral concentrations of FSH in these women will be associated with low levels of FSH in antral fluid (Stone *et al.*, 1988b). Suppressing effects of FRP on aromatase in these patients are, therefore, unlikely to be overridden by endogenous FSH.

The findings concur with evidence that the prolactin-related inhibition of aromatase activity can be overcome by increasing FSH stimulation of granulosa cells. Similarly, stimulation with FSH has been found to overcome inhibition of aromatase activity by FRP in cultured granulosa cells (Schreiber & diZerega, 1986). Prolactin has been reported to inhibit induction of granulosa cell LH receptors by FSH (Adashi & Resnik, 1987), in accord with the ability of FRP to inhibit FSH-induction of LH receptors in granulosa cells (Montz *et al.*, 1984). These provocative results raise speculation that some of the effects of prolactin on granulosa cell function *in vivo* may be directly mediated by FRP.

We thank Mae Gordon for help in the preparation of this manuscript. This study was supported in part by a grant from the National Institute of Child Health & Development (NICHD-18601).

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Received 23 June 1989