Ovarian antibodies, FSH and inhibin B: independent markers associated with unexplained infertility*

J.Luborsky1,2,4, B.Llanes1, R.Roussev3 and C.Coulam3

1Department of Obstetrics & Gynecology, Rush Medical College, Chicago IL, and 2Department of Physiology & Biophysics, University of Illinois, Chicago, IL, and the 3Center for Human Reproduction, Chicago, IL, USA

4To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Rush Medical College, 1653 W Congress Parkway, Chicago, IL 60612, USA

Premature menopause and unexplained infertility are associated with ovarian antibodies, a marker of ovarian autoimmunity. In premature menopause, FSH is also elevated while in unexplained infertility FSH concentrations are often normal. The relationship of ovarian antibodies and FSH and inhibit B, as markers of follicle function, was investigated in unexplained infertility. Ovarian antibodies were determined by immunoassay in comparison to normal controls (n = 12); 51.9% were positive at two SD (P < 0.05) and 38.5% were positive at three SD above the control mean (P < 0.01). In this study three SD above the control mean was considered positive. In unexplained infertility, three out of 10 (30%) had elevated day 3 FSH (>10 mIU/ml) and ovarian antibodies, while 17/42 (40%) had normal FSH (<10 mIU/ml) and ovarian antibodies. In women with normal FSH, two out of seven (29%) had low inhibit B concentrations (<33 pg/ml) and ovarian antibodies, and 15/35 (43%) had normal inhibit B concentrations (>33 pg/ml) and ovarian antibodies. Similarly, when women with and without ovarian antibodies were compared there was no difference in mean FSH or mean inhibit B concentrations. Thus, unlike other endocrine autoimmune disorders, hormone concentrations are not predictors of potential ovarian autoimmunity. This suggests that in unexplained infertility ovarian antibodies are an independent marker of potential ovarian failure, and may precede changes in regulatory hormones.

Key words: FSH/inhibit B/infertility/ovarian antibodies

Introduction

An altered concentration of regulatory hormone or an end-organ secretory product is usually the first indication of a potential autoimmune endocrine disease (Drexhage and Wulffraat, 1994; Baker, 1997). Ovarian autoimmunity is associated with a significant proportion of premature menopause (Irvine et al., 1968; Rebar et al., 1982; Coulam, 1983; LaBarbera et al., 1986; Moncayo et al., 1989; Luborsky et al., 1990; Cohen et al., 1991; Moncayo and Moncayo, 1992; Hoek et al., 1997). Premature menopause, like natural menopause is associated with elevated FSH and reduced production of oestrogen and inhibit B. In addition, ovarian antibodies are often detected in the peripheral circulation of women with premature ovarian failure (Coulam and Ryan, 1979; Elder et al., 1981; Damewood et al., 1986; Pekonen et al., 1986; Ahonen et al., 1987; Miyake et al., 1987; Moncayo et al., 1989; Moncayo-Naveda et al., 1989; Bannatyne et al., 1990; Luborsky et al., 1990; Muechler et al., 1991; Fenichel et al., 1997).

Unexplained infertility is also associated with ovarian autoimmunity (Dunbar, 1989; Moncayo et al., 1989; Luborsky et al., 1990; Meyer et al., 1990; Barbarino-Monnier et al., 1991; Gobert et al., 1992; Hovav et al., 1994; Narayanan et al., 1995; Geva et al., 1996b; Luborsky et al., 1999a). Since ovarian antibodies are associated with elevated FSH in premature menopause, the expectation was that in unexplained infertility, ovarian antibodies (evidence of autoimmunity) would be associated with elevated FSH. In previous studies it was noted that women with unexplained infertility and ovarian antibodies had normal day 3 FSH concentrations (Wheatcroft et al., 1997; Luborsky et al., 1999a). In contrast, Ahmed Ebbiary reported that regularly cycling infertile women with elevated FSH had more autoantibodies than those with low FSH and only women with elevated FSH concentrations had ovarian antibodies (Ahmed Ebbiary et al., 1994).

There is relatively little information on the relationship of ovarian antibodies and markers of ovarian function. Two previous studies of FSH concentrations and ovarian antibodies, do not agree (Ahmed Ebbiary, et al., 1994; Wheatcroft et al., 1997). There are no studies of inhibit B and ovarian antibodies. Therefore the objective of this study was to test the hypothesis that there is a relationship between ovarian antibodies and serum FSH and inhibit B concentrations in women with unexplained infertility.

Materials and methods

Subjects

The study groups consisted of women with unexplained infertility (n = 52) entering the clinic for evaluation or treatment in December 1997 and between March and May of 1998. Within the study group, 33 women were initiating an IVF cycle and 19 were undergoing a baseline evaluation prior to ovulation induction (no IVF). Relevant diagnostic and treatment information, including peak oestradiol (day
of HCG) concentrations during ovulation induction, was obtained from patient records. Women with polycystic ovarian syndrome (PCOS) were excluded based on a history of oligomenorrhea (menses interval of >42 days) and ultrasound evidence of cystic ovary (Franks, 1989). Women with recurrent fetal loss were excluded based on a history of more than three unexplained spontaneous abortions. Unexplained infertility patients had normal results on standard clinical evaluation. The evaluation criteria included: (i) inability to conceive for ≥1 year during unprotected intercourse; (ii) normal semen analysis, postcoital test, ovulation (luteal phase progesterone) and FSH concentrations were measured by a chemiluminescence immunoassay, and HCG (25 IU, day 28) (Dorflinger et al., 1983; Luborsky et al., 1990) with a capture antigen composed of a pool of pregnant mare serum gonadotrophin (50 IU, day 26) and HCG (25 IU, day 28) (Dorflinger et al., 1983; Luborsky et al., 1984; Meyer et al., 1990). Ovaries were removed on day 2 after HCG. The results (based on optical density; OD) with rat and human ovary were highly correlated (correlation coefficient = 0.98, P < 0.0001). All sera were tested at a dilution of 1:100, except when the titre was assessed. The captured antibodies were identified by a goat anti-human immunoglobulin (IgG)–alkaline phosphatase conjugate (FAB specific; Sigma, St Louis, MO, USA). Bound alkaline phosphatase (AP) was reacted with AP substrate (Sigma) and the product read at 405 nm (Thermomax; Molecular Devices, Sunnyvale, CA, USA).

### Ovarian autoimmunity FSH and inhibin B

FSH concentrations were measured by a chemiluminescence immunoassay (Chiron; Bayer, Tarrytown, NY, USA). The normal range for day 3 FSH was 2.5–10.2 mIU/ml (95% confidence interval). The sensitivity of the assay was 0.3 mIU/ml and the inter-assay and intra-assay coefficients of variation were 1.7 and 4.5% respectively. In this study values for normal FSH were those below 10 mIU/ml. Inhibin B was measured by enzyme immunoassay (Sero-tec; Harlan Bioproducts, Indianapolis, IN, USA). The mean range for day 3 inhibin B was 33–45 pg/ml (95% confidence interval). Values above 33 pg/ml were considered normal for inhibin B in this study.

### Statistical analysis

For measurement of ovarian antibody, samples were assessed in duplicate against the control samples and a positive control. Inter-assay and intra-assay coefficients of variation for specific OD signals were <15 and 10% respectively. The inter-assay and intra-assay coefficients of variation based on positive/negative values were <5%. In this study positive values were those >3 SD (P < 0.01) above the control mean. To compare OD values directly, OD values were normalized between plates against a positive control serum used in all assays; where normalized value = (OD test sample/OD positive control in same plate)×(expected OD from same positive control in first plate).

### Power analysis estimate for ovarian antibodies between normal FSH and increased FSH was 80%. Results were analysed by χ² test, paired Students t-test or one-way analysis of variance with individual comparisons by the least significant difference method, as appropriate. P < 0.05 was considered to be statistically significant. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) or Excel statistical software.

### Results

In the study group the average age, years of infertility, gravidity, parity, number of prior intrauterine inseminations (IUI) or IVF cycles were not significantly different for those with normal

### Table I. Infertility patient information. Values are given as mean ± SD with ranges shown in parentheses

<table>
<thead>
<tr>
<th></th>
<th>All (n = 52)</th>
<th>Normal FSH (n = 42)</th>
<th>Elevated FSH (n = 10)</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34.9 ± 4.9 (20–44)</td>
<td>34.6 ± 4.9 (20–42)</td>
<td>36.4 ± 5.3 (28–44)</td>
<td>NS</td>
</tr>
<tr>
<td>Years infertility</td>
<td>4.0 ± 3.9 (1–16)</td>
<td>3.9 ± 4.3 (1–16)</td>
<td>4.2 ± 2.7 (2–10)</td>
<td>NS</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.9 ± 1.2 (0–5)</td>
<td>0.95 ± 0.4 (0–5)</td>
<td>0.7 ± 1.3 (0–4)</td>
<td>NS</td>
</tr>
<tr>
<td>Parity</td>
<td>0.3 ± 0.7 (0–5)</td>
<td>0.4 ± 0.7 (0–2)</td>
<td>0.2 ± 0.5 (0–1)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of IUI cycles</td>
<td>0.9 ± 1.3 (0–4)</td>
<td>1.1 ± 1.6 (0–4)</td>
<td>0.7 ± 0.8 (0–2)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of IVF cycles</td>
<td>0.6 ± 1.0 (0–4)</td>
<td>0.7 ± 1.1 (0–4)</td>
<td>0.3 ± 0.8 (2–10)</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3 FSH (mIU/ml)</td>
<td>8.2 ± 4.6 (2.5–22.3)</td>
<td>6.3 ± 1.9 (2.5–9.4)</td>
<td>15.8 ± 4.7 (10.4–22.3)</td>
<td>0.019</td>
</tr>
<tr>
<td>Day 3 inhibin B (pg/ml)</td>
<td>54.4 ± 27.3 (nd–120)</td>
<td>60.8 ± 24.9 (22.9–120)</td>
<td>27.4 ± 22.8 (nd–73.6)</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

IUI = intrauterine insemination; NS = not significant; nd = not detected.

aComparison of normal and elevated FSH values.
Figure 1. Distribution of ovarian antibodies and hormone markers. Ovarian antibody positive (POS) sera were identified by comparison to control sera (see methods). FSH and inhibin B were negatively correlated (solid regression line, \( R^2 = 0.6; P = 0.00003 \)) as expected. The samples positive for ovarian antibody (\( \bigcirc \)) were distributed across all hormone concentrations. NEG = negative.

Figure 2. Comparison of hormone concentrations for ovarian antibody positive (POS) and negative (NEG) groups. Inhibin B concentrations (upper graph) and FSH concentrations (lower graph) are shown for women with and without ovarian antibodies. The data are displayed as a boxplot with median value (dark horizontal line), range of values (whiskers), the interquartile range (box) and expected (Figure 1). The peak oestradiol concentration minor (\( \ast \)) and extreme outliers (\( \ast \ast \)) The median and interquartile ranges were similar. There was no significant difference in inhibin B or FSH concentrations in women with and without ovarian antibodies.

Of correlation between hormone concentration and ovarian antibodies.

In the first analysis, an estimated threshold value for FSH of 10 mIU/ml was used to designate ‘elevated’ or ‘normal’ FSH groups. Likewise, the threshold value of 33 pg/ml for inhibin B was based on the lower limit of the normal range defined by the immunoassay kit. In order to determine whether these threshold values were appropriate and whether there were differences in the FSH or inhibin B concentrations by ovarian antibody result, women were grouped by ovarian antibody result (Figure 2). The mean FSH value was ~8 mIU/ml and was similar but slightly lower than the empirical value of 10 mIU/ml used to separate the study group by FSH concentrations. The mean inhibin B value was ~55 pg/ml and was higher than the empirical value used to separate the study group by inhibin B concentrations. Nonetheless, there was no difference in the mean FSH concentration (8.5 versus 8.0 mIU/ml respectively) or the mean inhibin B concentration (54.7 versus 54.5 mIU/ml) for women with or without ovarian antibodies. There was also no difference in the mean FSH or inhibin B concentration when only women with normal FSH concentrations were assessed according to the presence or absence of ovarian antibodies.
Discussion

The results of this study show that ovarian antibodies are not correlated with FSH or inhibin B concentrations. A significant number of women with both normal FSH and inhibin B had ovarian antibodies. This is the first study that also shows that ovarian antibodies are found in individuals with normal inhibin B. Thus unlike other endocrine autoimmune diseases, concentrations of ovarian regulatory hormones are not a predictor for ovarian autoimmunity in infertility.

It has previously been shown that ovarian antibodies are associated with premature menopause and unexplained infertility (Luborsky et al., 1990; Barbarino-Monnier et al., 1991; Ahmed Ebbiary et al., 1994; Geva et al., 1994, 1996a; Hovav et al., 1994; Narayanan et al., 1995; Nip et al., 1995). There also appears to be a relationship of ovarian antibodies to poorer outcomes of infertility treatment (Meyer et al., 1990; Hovav et al., 1994; Narayanan et al., 1995; Geva et al., 1996a) and with lower pregnancy rates (Geva et al., 1996a; Luborsky and Pong, 2000).

In addition, it has been suggested that there is an association of ovarian antibodies with a subgroup of PCOS (van Gelderen et al., 1993; Fenichel et al., 1999). However there are other reports that suggest PCOS is not associated with ovarian antibodies (Rojansky et al., 1997; Luborsky et al., 1999b). The apparent lack of agreement regarding PCOS may be due to the heterogeneity of PCOS and differences in patient selection, or differences in the antibody tests and the antigens detected. Overall, there appears to be general agreement on the association of unexplained infertility with ovarian antibodies.

The question then becomes whether altered reproductive endocrine markers of ovarian function are associated with evidence of ovarian autoimmunity, or, if ovarian autoimmunity is evident in women with normal concentrations of reproductive endocrine markers. In an earlier study (Ahmed Ebbiary et al., 1994), this question was addressed and immunofluorescence was used to detect a variety of autoantibodies including ovarian autoantibodies. In this study, 27% of individuals with normal FSH and 58% of individuals with elevated FSH (>10 mIU/ml) had at least one antibody. However, ovarian antibodies (6%) were only found in the elevated FSH group. Since immunofluorescence is subjective and sections of ovary may have a variable content of specific antigens and relevant follicular structure, it is not surprising that few women with ovarian antibodies were found. In comparing immunoassay and immunohistochemical detection of ovarian antibodies, it was found that sections from different ovariessometimes produced different immunohistochemical results (Meyer et al., 1990). The results of the current study agree with those of Wheatcroft et al. who reported that ovarian, thyroid, endometrial and several non-organ specific antibodies did not differ with FSH concentration in women with unexplained infertility (Wheatcroft et al., 1997). Significantly they also found evidence of complement activation which was greatest in the elevated FSH group, and also significant in the normal FSH group but not in controls.

How can FSH and inhibin B values be normal in the face of a potential autoimmune disease? In animal models and human premature menopause, lymphocytic infiltration is seen in maturing but not primordial or primary follicles (Coulam et al., 1981; Sedmak et al., 1987; Tung et al., 1987a,b; Tung and Teuscher, 1995). Since only some follicles may be subject to an autoimmune reaction in early stages of the disorder, the endocrine function of remaining follicles may contribute to the apparently normal concentrations of serum hormones. This is consistent with the report that normal ovarian function is compatible with severe ovarian inflammation in murine autoimmune ovarian disease (Bagavant et al., 1999). Complete lack of function may not occur until there is significant disease progression or depletion of the follicle pool.

Similarly, during the progression to spontaneous menopause that occurs at ~50 years of age, altered hormone concentrations precede the loss of menstrual cycles. In spontaneous menopause, the progression to ovarian failure occurs over a period of 4–5 years (World Health Organization, 1996). During this transition, hormone concentrations become erratic and irregular. Inhibin B, a direct product of the maturing follicle, is an earlier endocrine marker than FSH of reproductive decline (Prior, 1998; Welt et al., 1999). Likewise women with normal early follicular phase FSH who did not respond to stimulation with human menopausal gonadotrophin (HMG), developed elevated FSH concentrations within 1 year, indicating that FSH was not predictive of potential ovarian failure (Farhi et al., 1997).

The progression of autoimmune diseases also occurs over a prolonged time period. Autoantibodies may appear before the onset of clinical symptoms. For example, the risk of thyroiditis is higher in healthy individuals with thyroid antibodies (Mariotti et al., 1995; Massoudi et al., 1995). Similarly, autoantibodies associated with diabetes, e.g. GAD65 and IA-2 antibodies, predict future insulin dependence and may appear months or years before clinical diagnosis (Gottsater et al., 1995; Ivarsson et al., 1997; Bonifacio et al., 1999). Premature menopause eventually developed in patients with polyglanudal disease type 1 and with adrenal and steroid cell antibodies who were followed for 8 years (Coulam and Lufkin, 1981; Ahonen et al., 1987).

In addition to ovarian antibodies, the most frequently studied autoantibodies have been the antiphospholipid antibodies (APA) (Birkenfeld et al., 1994; Geva et al., 1994; Gleicher et al., 1994; Sherr et al., 1994; Dmowski et al., 1995; Birdsall et al., 1996; Roussev et al., 1996; Coulam et al., 1997; Kutteh et al., 1997; Battaglia et al., 1998). The presence of circulating APA associated with unexplained infertility is a syndrome known as reproductive autoimmune failure syndrome (RAFS) (Gleicher and El-Roeiy, 1988). The mechanisms by which APA are associated with unexplained infertility are not completely known. Targets for a direct effect of APA include endothelial cells (Roubey et al., 1999a).
and Hoffman, 1997), trophoblastic cells (Rote et al., 1998; Di Simone et al., 1999), and pre-embryos (Kaiser et al., 1999). The actions of APA on endothelial cells result in thrombosis and the mechanisms include inhibition of prostacycline generation with resultant thromboxane predom-
nance, increased platelet activation and decreased anti-
 thrombotic activation (Roubey et al., 1997). These actions on uterine vessels would result in increased resistance of blood flow through blood vessels. Increased resistance of flow through uterine arteries, measured as the pulsatility index, has been associated with unexplained infertility (Steer et al., 1992; Coulam et al., 1994). Recently, Battaglia et al. showed a relationship between unexplained infertility, APA and increased uterine artery pulsatility (Battaglia et al., 1998). Based on incidence, neither ovarian autoantibodies nor APAs alone account for all women with unexplained infertility. We showed that ovarian antibodies were not associated with anti-cardiolipin antibodies (Luborsky et al., 1999a). But, it remains to be determined whether APA and ovarian antibodies are associated with different subsets of unexplained infertility.

While the target for the detrimental effect of ovarian antibodies on reproduction has not been elucidated, the data suggest that ovarian antibodies are independent markers of potential ovarian failure. Furthermore, since ovarian antibodies are found in women with normal FSH and inhibin B concentrations, ovarian antibodies are possibly an early marker of ovarian failure. There are multiple factors involved in successful reproduction. A series of events such as follicle cell endocrinology and growth, egg maturation, fertilization, and implantation are required for a successful outcome. Since the antibodies are against ovarian antigens it is likely that follicle function rather than fertilization or implantation is the primary target. We postulate that the antibodies are involved in cellular destruction in maturing follicles. At an early stage of ovarian autoimmunity, some healthy follicles may escape destruction and continue to develop. Normal concentrations of reproductive hormones would be maintained. Eventually, cellular destruction proceeds, the pool of healthy follicles is reduced and ovarian failure occurs.

References


Received on August 23, 1999; accepted on January 19, 2000