Circulating anti-follicle-stimulating hormone immunoglobulin A in women: a sperm-prone reaction of mucosal tolerance?

Antibodies against follicle-stimulating hormone (anti-FSH) are present in infertile female sera. Follicle-stimulating hormone as antigen is present in female sera and introduced to the genital tract mucosa as a constituent of semen. The female immune system is activated by semen constituents during insemination to induce mucosal tolerance. We found that circulating anti-FSH IgA correlated with IgA against sperm surface antigens in female patients undergoing IVF. Our results suggest that anti-FSH and anti-sperm IgA could share antigenic origin, being induced possibly by mucosal tolerance to semen. (Fertil Steril 2007;_88_:1114–1117. ©2007 by American Society for Reproductive Medicine.)

The endometrium and cervix constitute part of the common mucosal immune system where humoral and cell-mediated immunity can be induced after contact with antigens of various origins (1). Seminal “priming” by insemination can be viewed as an immunizing event that elicits changes in the maternal immune system required to facilitate embryo implantation and successful pregnancy (2). In the process of building tolerance, the woman’s immune system responds to semen constituents and produces nonprecipitating or blocking antibodies to some antigens of sperm, along with some conserved antigens that are present both in semen and in maternal tissues (3). Because of the presence of similar antigens in semen and the mother, activated T-cell clones are eliminated to prevent pregnancy-induced autoimmunity (3). To our knowledge, the repertoire of shared antigens involved in tolerance induction has yet to be determined, and the antigenic source cannot be distinguished easily by measuring antibodies in female sera. In the context of pregnancy-favorable antibodies, only a few antigens have been targeted in the literature to date, including antibodies specific for spermatozoa (4), major histocompatibility complex (MHC) class I antigens (1), and heat shock proteins (3).

We have previously demonstrated that naturally occurring antibodies against FSH are predominantly present in patients with endometriosis and polycystic ovary syndrome (PCOS) (5) and those undergoing IVF (6). Follicle-stimulating hormone is present in female sera and also introduced to the genital tract mucosa as a constituent of semen (7). Consequently, we hypothesize that anti-FSH IgA detected in the sera of infertile women could represent alloantibodies that were developed in response to seminal FSH. Accordingly, levels of anti-FSH IgA would correlate with IgA antibodies produced against sperm surface antigens. To test this hypothesis, we measured antisperm antibodies in the sera of patients undergoing IVF and compared them with the levels of serum anti-FSH antibodies detected in the same patients (6).

MATERIALS AND METHODS
The Ethics Committee of the University of Tartu approved the study, and informed consent was obtained from 129 infertile women (mean age ± SD: 33.8 ± 4.6 years) before undergoing IVF. For analysis, patients were grouped according to their expected similarities in immunotolerating conditions in the genital tract: [1] the tubal factor infertility group—women with tubal factor infertility and normal semen quality observed in their partners; [2] the male factor infertility group—healthy women and impaired sperm quality observed in their partners; and [3] a combined group of patients—women with endometriosis, PCOS, or unexplained infertility and normal semen quality observed in their partners.

Blood samples were taken during the 3 to 5 days of the patients’ spontaneous menstrual cycle. Antisperm IgG, IgA, and IgM antibodies were detected by flow cytometry as previously reported (9) with some modifications. Antisperm antibody–negative donor motile spermatozoa were used as antigens and fluorescein isothiocyanate (FITC)–labeled rabbit F(ab')2 fragmented anti-human IgG, IgA, and IgM (DAKO, Glostrup, Denmark) were used as secondary antibodies. Living spermatozoa were distinguished with 7-aminoactinomycin D (7AAD; Invitrogen, Carlsbad, CA). Samples were analyzed with use of an...
FACScalibur flow cytometer (Becton Dickinson Immuno-cytometry Systems, Mountain View, CA). The percentage of antibody-positive sperms was defined as the ratio of the FITC-positive and 7AAD-negative sperm population to the total 7AAD-negative living sperms. The levels of antisperm antibodies were expressed as corrected values (percentage) of antibody-positive spermatozoa, calculated by using the following formula: (IgG, IgA, or IgM sample mean %) – (Median of IgG, IgA, and IgM negative controls %). Indirect ELISA with purified FSH (Postimon 75; IBSA, Lugano, Switzerland) as antigen was used to detect anti-FSH antibodies of IgG, IgA, and IgM isotypes, with a protocol completely reported in our previous study (6). Anti-FSH antibody levels were expressed as corrected optical density (OD) values and, similar to the antisperm antibodies, were calculated as follows: (IgG, IgA, or IgM sample mean OD) – (Median of IgG, IgA, and IgM blank OD). The OD signal of the blank reaction was measured from control wells where all but serum sample was incubated. The results of anti-FSH and antisperm antibody tests were analyzed as continuous numeric values.

The R2.3.1 A Language and Environment (Free Software Foundation, Boston, MA) was used for t-test and Pearson’s correlation test. A P value of <.05 was considered statistically significant.

RESULTS

The mean age of study groups was similar (Table 1). Although patients with PCOS, endometriosis, and unexplained infertility seemed to have increased levels of antisperm IgG, IgA, and IgM antibodies compared with patients with tubal or male factor infertility, these differences were not statistically significant (t-test, Table 1). Similarly, the levels of anti-FSH IgG, IgA, and IgM were comparable between the study groups. However, a positive correlation was seen between antisperm and anti-FSH IgA in the combined group of patients with endometriosis, PCOS, and unexplained infertility (Pearson’s correlation 0.34, P=.023). Patients with tubal or male factor infertility did not show any correlation between antisperm and anti-FSH IgA. The production of IgG or IgM type of anti-FSH was not correlated with the antisperm antibodies among any patients’ groups.

DISCUSSION

The levels of anti-FSH and antisperm IgG, IgA, and IgM antibodies were similar among all groups of infertile women. Out of all antibody isotypes, only anti-FSH IgA correlated with antisperm IgA, suggesting that these antibodies may share the common seminal antigenic origin. In this context, it is supportive to refer to the absence of anti-FSH antibodies in the sera of children (10).

Somewhat surprisingly, this correlation was seen only in patients undergoing IVF who had PCOS, endometriosis, and unexplained infertility, not in patients with male factor or tubal factor infertility. The common feature for the endometriosis, PCOS, and unexplained infertility is the disturbed regulation of the immune system (11–13). Disruptions of the immune system perturb the female’s immunoresponse to semen that is necessary for partner-specific

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TABLE 1

Levels of antisperm and anti-FSH antibodies in groups of infertile women.

<table>
<thead>
<tr>
<th></th>
<th>Tubal factor infertility</th>
<th>Male factor infertility</th>
<th>Endometriosis, PCOS, unexplained infertility</th>
<th>$P$ value (t-test)$^a$</th>
<th>$P$ value (t-test)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>34.0 ± 4.4</td>
<td>33.6 ± 5.1</td>
<td>33.5 ± 4.7</td>
<td>.591</td>
<td>.948</td>
</tr>
<tr>
<td>Antisperm IgG (%)</td>
<td>1.65 ± 1.22</td>
<td>1.92 ± 2.10</td>
<td>3.25 ± 6.77</td>
<td>.127</td>
<td>.227</td>
</tr>
<tr>
<td>Antisperm IgA (%)</td>
<td>1.71 ± 3.31</td>
<td>1.56 ± 1.20</td>
<td>2.42 ± 3.43</td>
<td>.297</td>
<td>.129</td>
</tr>
<tr>
<td>Antisperm IgM (%)</td>
<td>2.45 ± 2.15</td>
<td>3.16 ± 3.83</td>
<td>3.46 ± 3.95</td>
<td>.130</td>
<td>.747</td>
</tr>
<tr>
<td>Anti-FSH IgG (OD)</td>
<td>0.41 ± 0.39</td>
<td>0.56 ± 0.53</td>
<td>0.43 ± 0.28</td>
<td>.800</td>
<td>.227</td>
</tr>
<tr>
<td>Anti-FSH IgA (OD)</td>
<td>0.36 ± 0.19</td>
<td>0.42 ± 0.25</td>
<td>0.33 ± 0.12</td>
<td>.311</td>
<td>.085</td>
</tr>
<tr>
<td>Anti-FSH IgM (OD)</td>
<td>0.86 ± 0.37</td>
<td>0.97 ± 0.46</td>
<td>0.90 ± 0.37</td>
<td>.646</td>
<td>.484</td>
</tr>
</tbody>
</table>

Note: Data are presented as means ± SD.

$^a$ Antibody levels in combined group with endometriosis, PCOS, or unexplained infertility compared with patients with tubal factor infertility.

$^b$ Antibody levels in combined group with endometriosis, PCOS, and unexplained infertility compared with patients with male factor infertility.

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Haller. Serum IgA alloantibodies to FSH. Fertil Steril 2007.
tolerance and subsequent elimination of activated clones to prevent autoimmunity (3). Semen exerts its “tolerance-inducing” effect as a result of immunomodulating factors, most importantly transforming growth factor β1 (TGFβ1) (14, 15). Seminal levels of TGFβ1 correlate with sperm count in ejaculate (15), the most decisive criterion in diagnosing male factor infertility (8). However, there is some evidence that male factor infertility is not directly associated with altered TGFβ1 levels (16). Although we did not distinguish subgroups of patients with male factor infertility by sperm parameters, generally their levels of antisperm and anti-FSH antibodies, or correlations between the two, did not differ from those of patients with tubal factor infertility. Unlike other patients participating in this study, patients with infertility caused by tubal occlusions do not have disturbances in female immune system regulation or seminal environment. Thus, the diagnosis-restricted correlation of antisperm and anti-FSH IgA cannot be explained easily.

To conclude, these results lead to speculation that the production of anti-FSH IgA detected in female circulation could be subjected to regulatory mechanisms similar to that in the case of antisperm antibodies. Therefore, anti-FSH could be a part of the mucosal response involved in inducing immunotolerance to seminal constituents. However, further experiments are warranted to verify this hypothesis.

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Immunoglobulin (Ig) A against FSH (anti-FSH) in infertile female sera correlates with circulating IgA against sperm surface antigens, suggesting that anti-FSH and antisperm IgA could share antigenic origin in mucosal tolerance to semen.