

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*® 2008;90:1253–5. ©2008 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 \pm SD: 33.8 \pm 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 (8); [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 Immunocytometry 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

Received July 5, 2007; revised August 23, 2007; accepted August 31, 2007.

Supported by the Estonian Science Foundation (grant nos. 6498 and 6514) and the Estonian Ministry of Education and Science (core grant nos. 0182582Cs03, 0182586s03, and PARNS07903).

Reprint requests: Kadri Haller, M.D., Ph.D., Department of Immunology, Institute of General and Molecular Pathology, University of Tartu, Ravila Str. 19, Tartu 50411, Estonia (FAX: 372-7-7374232; E-mail: kadri.haller@ut.ee).

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 (Fostimon 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 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 β_1 (TGF β_1) (14, 15). Seminal levels of TGF β_1 correlate with sperm count in ejaculate (15), the most decisive criterion in

TABLE 1

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

	Tubal factor infertility (n = 56)	Male factor infertility (n = 30)	Endometriosis, PCOS, unexplained infertility (n = 43)	<i>P</i> value (<i>t</i> -test) ^a	<i>P</i> value (<i>t</i> -test) ^b
Age (y)	34.0 ± 4.4	33.6 ± 5.1	33.5 ± 4.7	.591	.948
Antisperm IgG (%)	1.65 ± 1.22	1.92 ± 2.10	3.25 ± 6.77	.127	.227
Antisperm IgA (%)	1.71 ± 3.31	1.56 ± 1.20	2.42 ± 3.43	.297	.129
Antisperm IgM (%)	2.45 ± 2.15	3.16 ± 3.83	3.46 ± 3.95	.130	.747
Anti-FSH IgG (OD)	0.41 ± 0.39	0.56 ± 0.53	0.43 ± 0.28	.800	.227
Anti-FSH IgA (OD)	0.36 ± 0.19	0.42 ± 0.25	0.33 ± 0.12	.311	.085
Anti-FSH IgM (OD)	0.86 ± 0.37	0.97 ± 0.46	0.90 ± 0.37	.646	.484

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.

Haller. Correspondence. Fertil Steril 2008.

diagnosing male factor infertility (8). However, there is some evidence that male factor infertility is not directly associated with altered TGF β ₁ 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.

Kadri Haller, M.D., Ph.D.^{a,b}

Anu Sikut, M.Sc.^b

Helle Karro, M.D., Ph.D.^b

Raivo Uibo, M.D., Ph.D.^a

Andres Salumets, Ph.D.^{b,c,d}

^a Department of Immunology, Institute of General and Molecular Pathology, Centre of Molecular and Clinical Medicine, University of Tartu, Tartu;

^b Department of Obstetrics and Gynaecology,

University of Tartu, Tartu; ^c Nova Vita Clinic, Centre for Infertility Treatment and Medical Genetics, Viimsi;

and ^d Department of Biotechnology, Institute of Molecular and Cell Biology, Estonian Biocentre, University of Tartu, Tartu, Estonia

REFERENCES

- Robertson SA. Control of the immunological environment of the uterus. *Rev Reprod* 2000;5:164–74.
- Robertson SA, Bromfield JJ, Tremellen KP. Seminal ‘priming’ for protection from pre-eclampsia—a unifying hypothesis. *J Reprod Immunol* 2003;59:253–65.
- Hegde UC, Ranpura S, D’Souza S, Raghavan VP. Immunoregulatory pathways in pregnancy. *Indian J Biochem Biophys* 2001;38:207–19.
- Naz RK, Menge AC. Antisperm antibodies: origin, regulation, and sperm reactivity in human infertility. *Fertil Steril* 1994;61:1001–13.
- Haller K, Mathieu C, Rull K, Matt K, Béné MC, Uibo R. IgG, IgA and IgM antibodies against FSH: serological markers of pathogenic autoimmunity or of normal immunoregulation? *Am J Reprod Immunol* 2005;54:262–9.
- Haller K, Salumets A, Grigorova M, Talja I, Salur L, Béné MC, et al. Putative predictors of antibodies against follicle-stimulating hormone in female infertility: a study based on *in vitro* fertilization patients. *Am J Reprod Immunol* 2007;57:193–200.
- Bujan L, Miesusset R, Audran F, Lumbroso S, Sultan C. Increased oestradiol level in seminal plasma in infertile men. *Hum Reprod* 1993;8:74–7.
- World Health Organization. Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. New York: Cambridge University Press, 1999:60–2.
- Nikolaeva MA, Kulakov VI, Goukasian IA, Philippova RD, Korotkova IV, Sukhikh GT. Flow cytometry study on the effect of serum and peritoneal fluid of women on sperm-binding activity of immunoglobulin G antisperm antibodies. *Fertil Steril* 1997;67:680–6.
- Gobert B, Jolivet-Reynaud C, Dalton P, Barbarino-Monnier P, Faure CG, Jolivet M, et al. An immunoreactive peptide of the FSH involved in autoimmune infertility. *Biochem Biophys Res Commun* 2001;289:819–24.
- Fénichel P, Gobert B, Carré Y, Barbarino-Monnier P, Hiéronimus S. Polycystic ovary syndrome in autoimmune disease [letter]. *Lancet* 1999;353:2210.
- Mathur SP. Autoimmunity in endometriosis: relevance to infertility. *Am J Reprod Immunol* 2000;44:89–95.
- Luborsky J, Llanes B, Davies S, Binor Z, Radwanska E, Pong R. Ovarian autoimmunity: greater frequency of autoantibodies in premature menopause and unexplained infertility than in the general population. *Clin Immunol* 1999;90:368–74.
- Robertson SA, Ingman WV, O’Leary S, Sharkey DJ, Tremellen KP. Transforming growth factor β —a mediator of immune deviation in seminal plasma. *J Reprod Immunol* 2002;57:109–28.
- Ochsenkühn R, O’Connor AE, Hirst JJ, Gordon Baker HW, De Kretser DM, Hedger MP. The relationship between immunosuppressive activity and immunoregulatory cytokines in seminal plasma. Influence of sperm autoimmunity and seminal leukocytes. *J Reprod Immunol* 2006;71:57–74.
- Loras B, Vételé F, Malki AE, Rollet J, Soufir J-C, Benahmed M. Seminal transforming growth factor- β in normal and infertile men. *Hum Reprod* 1999;14:1534–9.