

Article

Anti-FSH antibodies associate with poor outcome of ovarian stimulation in IVF



Dr Kadri Haller received her MD (2000), medical internship (2001), and PhD (2007) from the University of Tartu, Estonia. Since 2006 she has been a Research Fellow in the Department of Obstetrics and Gynaecology and in the Department of Immunology, University of Tartu. Her scientific research is currently focused on immunological and genetic factors of female and male infertility, and previously also on the genetic markers of autoimmune diabetes. Dr Haller is a member of the Estonian Society of Immunology and Allergology (a member of the European Federation of Immunological Societies).

Dr Kadri Haller

Kadri Haller^{1,2,3,5}, Andres Salumets^{2,3,4}, Raivo Uibo¹

¹Department of Immunology, Institute of General and Molecular Pathology, Centre of Molecular and Clinical Medicine, University of Tartu, Ravila Str. 19, Tartu 50411, Estonia; ²Department of Obstetrics and Gynecology, University of Tartu, Estonia; ³Department of Biotechnology, Institute of Molecular and Cell Biology, University of Tartu, Estonia; ⁴Nova Vita Clinic, Centre for Infertility Treatment and Medical Genetics, Estonia

⁵Correspondence: Department of Immunology, University of Tartu; e-mail: kadri.haller@ut.ee

Abstract

FSH is required for spontaneous folliculogenesis and is widely used in ovarian stimulation in IVF. Previously, increased concentrations of antibodies against FSH (anti-FSH) have been demonstrated in infertile women. This study aimed to: (i) assess the possible association of anti-FSH with an adverse outcome of IVF with regard to clinical parameters characterizing the ovarian reserve; and (ii) compare serum and follicular fluid (FF) anti-FSH concentrations in relation to follicle size and endocrine markers. IVF patients ($n = 182$) subjected to gonadotrophin-releasing hormone-antagonist protocol were assessed for anti-FSH using enzyme-linked immunosorbent assay. Increased concentrations of serum anti-FSH immunoglobulin (Ig)G and IgA were associated with impaired ovarian stimulation outcome, with cut-off values >1.0 arbitrary units predicting poor ovarian response (≤ 3 oocytes) (adjusted odds ratio for IgG = 6.95, $P = 0.005$ and IgA = 3.60, $P = 0.039$). FF anti-FSH IgG and IgA were positively associated with serum anti-FSH concentrations and FSH concentration in FF. Additionally, FF anti-FSH IgG increased with follicle growth (linear regression coefficient = 0.02, $P = 0.022$). Collectively, these data suggest that serum anti-FSH antibodies are associated with poor ovarian response to FSH stimulation in IVF, with anti-FSH IgA and IgG potentially exerting a local FSH antagonizing effect in maturing follicles.

Keywords: anti-FSH antibodies, follicular fluid, IVF, ovarian stimulation

Introduction

FSH is one of the two pituitary gonadotrophins involved in regulating ovarian function. It has some influence on the development of preantral follicles (Thomas and Vanderhyden, 2006), but the growth of antral follicles becomes critically dependent on FSH support (Knight and Glister, 2006). Currently, FSH is widely used in ovarian stimulation in IVF. During IVF, exogenous FSH stimulates numerous follicles to mature. The effectiveness of ovarian stimulation can be measured by the quality and quantity of oocytes obtained. However, the outcome of ovarian stimulation may be impaired by increased age and/or low ovarian reserve (Kligman and Rosenwaks, 2001; Levi Setti, 2006), the patient's genetic background (Perez Mayorga *et al.*,

2000; Altnae *et al.*, 2007), and ovarian autoimmunity (Meyer *et al.*, 1990; Luborsky *et al.*, 2002).

Infertile women with anti-ovarian antibodies often display antibodies against FSH (anti-FSH) (Gobert *et al.*, 2001; Shatavi *et al.*, 2006). It has been previously demonstrated that anti-FSH antibodies are elevated in infertile women (Haller *et al.*, 2005) and more recent data suggest that these antibodies are associated with dysregulation of immune reactions and repeatedly performed IVF procedures (Haller *et al.*, 2007). Moreover, the majority of anti-FSH antibodies are directed against the 78–93 amino acid epitope of the FSH hormone β -chain (Gobert *et al.*, 2001; Haller

et al., 2005), which plays a key role in the specificity of hormone-receptor interactions (Fox *et al.*, 2001). These data have prompted this investigation into the effects of anti-FSH on IVF outcome. Specifically, this study aimed to: (i) assess the associations between serum anti-FSH immunoglobulin G (IgG), IgA, and IgM concentrations and ovarian stimulation outcome with regard to clinical parameters influencing folliculogenesis; and (ii) compare serum and follicular fluid (FF) anti-FSH concentrations in relation to follicle size and endocrine markers.

Materials and methods

Study population and ovarian stimulation

A total of 182 women (mean age \pm SD: 34.4 ± 5.0 years) undergoing IVF, who entered Nova Vita Clinic between July 2004 and December 2005, were enrolled in the study. The study was approved by the Ethics Committee of the University of Tartu and informed consent was obtained from all participants. The infertility diagnoses included: tubal factor infertility (48.4%, 88/182), male factor infertility (27.5%, 50/182), endometriosis (9.9%, 18/182), unexplained infertility (9.3%, 17/182) and infertility due to the other reasons (4.9%, 9/182). All patients had been suffering from infertility for at least a year before entering the study. Patients with cancelled current ovarian stimulation or with polycystic ovary syndrome were excluded. Patients undergoing IVF (49.5%, 90/182) or intracytoplasmic sperm injection (ICSI, 50.5%, 92/182) were included in the study, with an overall clinical pregnancy rate after day-2 embryo transfer of 25.8% (47/182) per ovarian stimulation. Ovarian hormonal stimulation was conducted according to the gonadotrophin-releasing hormone (GnRH) antagonist protocol (Cetrotide®, Serono, Rome, Italy or Orgalutran®, N.V. Organon, Oss, The Netherlands) with administration of recombinant FSH (Gonal-f®, Serono, Rome, Italy) as described previously (Altmäe *et al.*, 2007).

Transvaginal ultrasonography of ovaries was performed prior to IVF during the first 5 days of each patient's spontaneous menstrual cycle. Ovarian volume was estimated according to the following formula: $0.5(A \times B \times C)$, where A was the longitudinal diameter, B the anteroposterior diameter, and C the transverse diameter of the ovary (Sample *et al.*, 1977). The number of small antral follicles was determined by ultrasound scanning of each ovary in a longitudinal cross-section. Mean follicle number and mean ovarian volume were calculated as the sum of left and right ovaries divided by two. Serum ($n = 182$) and FF ($n = 170$) samples for anti-FSH determination were obtained on the day of oocyte retrieval. FF was retrieved from the single largest follicle and the diameter of the follicle was measured by ultrasound scanning. Serum FSH as a fertility parameter was measured on days 3–5 of the patient's spontaneous menstrual cycle. Both serum and FF FSH concentrations were measured using chemiluminescence immunoassay (Immulite 2000® station, DPC, Los Angeles, CA, USA). Albumin concentration in FF was measured by bromocresol green dye-binding assay (Cobas Integra® 800, Roche Diagnostics, F. Hoffmann-La Roche Ltd, Basel, Switzerland).

Multiple parameters were used to evaluate the efficacy of ovarian stimulation, including the numbers of follicles punctured (follicles) and cumulus–oocyte complexes obtained (oocytes). The number of mature oocytes was calculated for both IVF and ICSI. IVF oocytes were assessed for maturity 1 day after oocyte

retrieval and insemination by counting fertilized and meiosis II (MII) unfertilized oocytes. ICSI oocytes were considered mature if they reached MII stage 4–6 h after oocyte retrieval. The total number of embryos was calculated by counting embryos with two pronuclei (embryos). The embryos with at least four blastomeres and <20% fragmentation on day 2 after insemination/ICSI were classified as good-quality embryos. The following parameters were calculated from the total dose of FSH used for ovarian stimulation, FSH (IU) per: follicle, oocyte, mature oocyte, embryo and good quality embryo.

Detection of anti-FSH antibodies

Indirect enzyme-linked immunosorbent assay (ELISA), with purified FSH (Fostimon® 75, IBSA, Lugano, Switzerland) as the antigen, was used to detect serum and FF anti-FSH antibodies of IgG, IgA and IgM isotypes (Haller *et al.*, 2007). Serum samples were diluted to 1:100, corresponding to albumin concentrations of 0.35–0.50 g/l, while FF samples were diluted to albumin concentration of 0.40 g/l. A calibrator (pool of sera from 200 healthy fertile women) was used in each assay in order to allow inter-assay comparisons of antibody concentrations. Antibody concentrations were expressed as optical density (OD) ratios to the internal calibrator (arbitrary units, AU), calculated as follows: (IgG, IgA or IgM sample mean OD – IgG, IgA or IgM blank OD) / (IgG, IgA and IgM calibrator median OD – IgG, IgA or IgM blank OD). Control wells contained all the assay components except serum or FF samples.

Statistical analyses

The R2.3.1 A Language and Environment (Free Software Foundation, Boston, MA, USA) was used for chi-squared tests, Pearson's linear correlations, and all linear and logistic regression analyses. $P < 0.05$ was considered statistically significant in all cases.

Results

Association between fertility parameters and ovarian stimulation outcome

Fertility parameters were measured on days 3–5 of each patient's spontaneous menstrual cycle; results (mean \pm SD) indicated that ovarian volume was 5.0 ± 2.1 cm³, the number of small antral follicles was 4.6 ± 1.8 , and serum FSH concentration was 9.0 ± 4.9 IU/l. An average of 1918.0 ± 503.6 IU of FSH was administered during 9.6 ± 0.7 days of ovarian stimulation. The resulting mean numbers of follicles, oocytes, mature oocytes, embryos and good quality embryos obtained were 13.2 ± 6.7 , 11.3 ± 6.7 , 9.4 ± 5.5 , 6.4 ± 4.1 and 3.0 ± 2.7 , respectively. The mean amount of FSH administered was 228.8 ± 263.4 IU per follicle, 290.0 ± 309.2 IU per oocyte, 345.0 ± 359.3 IU per mature oocyte, 443.8 ± 401.2 IU per embryo and 905.7 ± 732.5 IU per good quality embryo.

Clinical fertility parameters were studied in order to consider them in models describing the relationships between anti-FSH antibodies and ovarian stimulation outcome. Bivariate linear regression analysis revealed that better ovarian stimulation outcome was linearly associated with younger age, larger ovarian volume, and higher numbers of early antral follicles, while

concentrations of serum FSH at the early follicular phase of the menstrual cycle did not accurately predict ovarian stimulation efficacy (**Table 1**).

Association between serum anti-FSH antibodies and ovarian stimulation outcome

The association between anti-FSH antibodies and ovarian stimulation outcome parameters was assessed using multivariate linear regression models adjusted for patient age and early antral follicle count. Adjusted regression models helped to investigate the role of anti-FSH antibodies in ovarian stimulation outcome independent of the influence of fertility (adjusted) parameters.

The AU ranges (means) for serum anti-FSH IgG, IgA and IgM concentrations were 0.17–3.92 (0.64), 0.14–4.10 (0.69), and 0.97–7.16 (2.62), respectively. Higher concentrations of serum anti-FSH IgG were associated with a greater amount of FSH used to obtain one oocyte [regression coefficient (r) = 92.4 IU per AU of anti-FSH IgG, $P = 0.046$] and one embryo ($r = 220.6$ IU per AU of anti-FSH IgG, $P = 0.002$). There were also positive linear correlations between serum anti-FSH IgA concentrations and the overall FSH dose used for ovarian stimulation ($r = 158.7$ IU per AU of anti-FSH IgA, $P = 0.014$), and augmented concentrations of FSH needed to mature one follicle ($r = 131.7$ IU per AU of anti-FSH IgA, $P < 0.001$), obtain one oocyte ($r = 107.2$ IU per AU of anti-FSH IgA, $P = 0.014$) and one mature oocyte ($r = 113.7$ IU per AU of anti-FSH IgA, $P = 0.029$). Furthermore, elevated anti-FSH IgA concentrations tended to be associated (although not significantly) with fewer follicles matured after ovarian stimulation ($r = -1.9$ follicles per AU of

anti-FSH IgA). Serum anti-FSH IgM was not associated with any of the ovarian stimulation parameters.

In addition to the linear associations between anti-FSH antibodies and ovarian stimulation outcome, the best cut-off values for anti-FSH IgG and IgA for poor ovarian response (≤ 3 oocytes after ovarian stimulation) were calculated (**Table 2**). Patients with anti-FSH IgG and IgA concentrations of >1.0 AU revealed a substantially increased risk for poor ovarian response compared with patients with lower concentrations of the respective antibodies. The association of anti-FSH IgG and IgA with a risk for poor ovarian response was not confounded by the patient's age, mean ovarian volume and early follicle count, as measured by adjusted logistic regression models (**Table 2**). The cumulative association of anti-FSH with poor ovarian response from multivariate logistic regression models is illustrated in **Figure 1**.

Follicular fluid anti-FSH

The AU ranges (means) for FF anti-FSH IgG, IgA and IgM concentrations were 0.14–1.78 (0.48), 0.07–2.83 (0.40), and 0–1.90 (0.15), respectively. The mean (\pm SD) diameter of punctured follicles was 20.5 ± 3.1 mm and FSH concentration in FF was 2.2 ± 1.2 IU/l. Multivariate linear regression models suggested FF anti-FSH IgG concentrations increased with follicle diameter ($r = 0.02$ AU of FF anti-FSH IgG per 1 mm of follicular diameter, $P = 0.022$), and were also associated with serum anti-FSH IgG concentrations ($r = 0.34$ AU of FF anti-FSH IgG per AU serum anti-FSH IgG, $P < 0.001$). Anti-FSH IgA concentrations in FF were not associated with follicular diameter, but were associated with serum anti-FSH IgA concentrations (r for diameter = -0.01 , not significant; and r for antibodies in sera = 0.23 , $P = 0.005$). IgM type anti-FSH in

Table 1. Basic fertility parameters predicting the outcome of ovarian stimulation.

| Ovarian stimulation parameter | Regression coefficient ^a (P-value) | | | |
|----------------------------------|---|--|---|------------------------|
| | Age (per year) | Mean ovarian volume (per cm ³) | Day 3–5 mean follicle number (per follicle) | Day 3–5 FSH (per IU/l) |
| Duration of stimulation (days) | 0.03 (0.007) | −0.1 (0.004) | −0.1 (<0.001) | 0.02 (NS) |
| Total FSH used (IU) | 51.9 (<0.001) | −57.4 (0.001) | −134.4 (<0.001) | 10.4 (NS) |
| No. of follicles | −0.5 (<0.001) | 1.1 (<0.001) | 1.6 (<0.001) | −0.1 (NS) |
| No. of oocytes | −0.4 (<0.001) | 1.1 (<0.001) | 1.2 (<0.001) | −0.2 (0.032) |
| No. of mature oocytes | −0.3 (<0.001) | 0.9 (<0.001) | 1.0 (<0.001) | −0.1 (NS) |
| No. of embryos | −0.2 (0.007) | 0.6 (<0.001) | 0.6 (<0.001) | −0.1 (NS) |
| No. of good quality embryos | −0.1 (0.028) | 0.2 (0.014) | 0.2 (0.036) | −0.02 (NS) |
| FSH per follicle (IU) | 21.3 (<0.001) | −19.8 (0.006) | −57.7 (<0.001) | 4.3 (NS) |
| FSH per oocyte (IU) | 25.7 (<0.001) | −32.6 (<0.001) | −70.0 (<0.001) | 8.1 (NS) |
| FSH per mature oocyte (IU) | 27.4 (<0.001) | −38.5 (<0.001) | −75.7 (<0.001) | 7.6 (NS) |
| FSH per embryo (IU) | 23.4 (<0.001) | −57.2 (<0.001) | −67.8 (<0.001) | 5.6 (NS) |
| FSH per good quality embryo (IU) | 48.9 (<0.001) | −123.3 (<0.001) | −145.4 (<0.001) | 15.1 (NS) |

The associations between clinical parameters and ovarian stimulation outcome were analysed utilizing bivariate linear regression models.

NS = not statistically significant.

^aRegression coefficient indicates the extent of change of an ovarian stimulation parameter per one unit of clinical parameter.

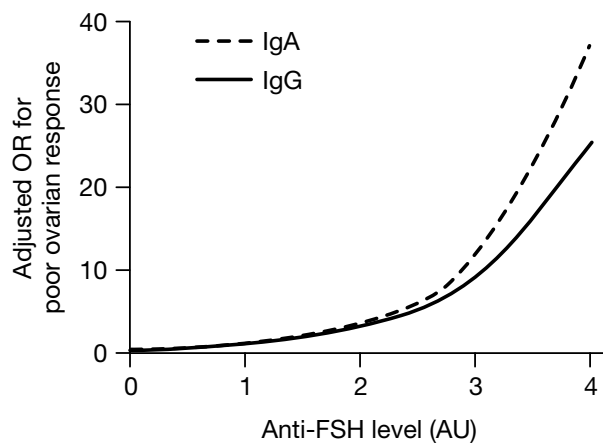
Table 2. Association between poor ovarian response (≤ 3 oocytes obtained) and elevated serum anti-FSH immunoglobulin (Ig)G and IgA concentrations.

| Anti-FSH value (AU) ^a | Poor responders (%) (n = 16) | Normal responders (%) (n = 164) | Chi-squared test (P-value) | Bivariate analysis (crude OR, 95% CI; P-value) | Multivariate analysis (adjusted OR, 95% CI; P-value) ^b |
|----------------------------------|------------------------------|---------------------------------|----------------------------|--|---|
| IgG <1.0 | 68.8 (11/16) | 90.9 (149/164) | 0.023 | 4.52, 1.38–14.74; 0.013 | 6.80, 1.77–26.16; 0.005 |
| IgG >1.0 | 31.3 (5/16) | 9.1 (15/164) | | | |
| IgA <1.0 | 62.5 (10/16) | 86.6 (142/164) | 0.030 | 3.87, 1.28–11.72; 0.017 | 3.62, 1.07–12.31; 0.039 |
| IgA >1.0 | 37.5 (6/16) | 13.4 (22/164) | | | |

^aAntibody values are expressed as sample optical density (OD) ratios to the pool OD.

^bMultivariate logistic regression analysis adjusted by the age of women, the mean ovarian volume and the mean follicle count on days 3–5 of each patient's spontaneous menstrual cycle.

AU = arbitrary units; CI = confidence interval; OR = odds ratio.

**Figure 1.** Association of serum anti-FSH immunoglobulin (Ig) G or IgA concentrations with cumulative risk for poor ovarian response (≤ 3 oocytes obtained) from multivariate logistic regression models. OR = odds ratio; AU = arbitrary units.

the FF was not associated with either follicle diameter or serum anti-FSH IgM concentrations.

Additionally, FF anti-FSH IgG and IgA were positively associated with: (i) total amount of FSH used for stimulation ($r = 276.5$ IU per AU of FF anti-FSH IgG, $P = 0.044$; $r = 335.6$ IU per AU of FF anti-FSH IgA, $P = 0.035$), if adjusted by the fertility parameters; and (ii) FSH concentrations in the FF ($r = 0.03$ IU of FSH per AU of FF anti-FSH IgG, $P = 0.064$; $r = 0.05$ IU of FSH per AU of FF anti-FSH IgA, $P = 0.022$) when anti-FSH concentrations in the peripheral blood were included in the multivariate analysis. As expected, FSH concentrations in the FF correlated positively with the amount of FSH administered during ovarian stimulation (Pearson's correlation = 0.37, $P < 0.001$). However, none of the examined parameters was associated with FF anti-FSH IgM concentrations.

Discussion

Over the past two decades, many studies have related anti-ovarian antibodies in infertile patients with poor IVF outcome

as assessed by impaired oocyte fertilization or pregnancy/birth rates (Mantzavinos *et al.*, 1993; Narayanan *et al.*, 1995; Luborsky and Pong, 2000). However, little is known regarding the role of anti-FSH antibodies in folliculogenesis. In the current study, it was shown that serum anti-FSH IgG and IgA concentrations were inversely associated with the ovarian response to FSH, with the accompanied accumulation of anti-FSH IgG in preovulatory follicles. The present study is, as far as is known, the first to investigate all three subtypes of anti-FSH antibodies in circulation and in the FF in relation to the effectiveness of IVF.

This study demonstrated that serum concentrations of anti-FSH IgG and IgA, but not IgM, were linearly associated with poorer ovarian stimulation outcome. Ovarian stimulation outcome was assessed by the total dose of FSH used, number of follicles punctured or oocytes obtained, number of mature oocytes or embryos available, and amount of FSH required per all of these parameters. According to the findings, the role of anti-FSH antibodies was rather remarkable. For example, 1.0 AU difference in anti-FSH IgG was associated with a 220.6 IU increase in FSH needed for one embryo, while the mean amount

of FSH used per embryo was only 443.8 ± 401.2 IU. In addition, anti-FSH IgA or IgG concentrations greater than 1.0 AU were 3–6 times more likely to be associated with a poor ovarian response (≤ 3 oocytes), demonstrating the cut-off values of anti-FSH for inadequate stimulatory effect of FSH. It is generally accepted that the outcome of IVF depends more on the quality rather than on the quantity of oocytes obtained. However, based on the current study on anti-FSH concentrations, the quality of oocytes available cannot be predicted.

These results may have been due to impaired ovarian function caused by general ovarian autoimmunity, since anti-FSH antibodies are often detected in patients with anti-ovarian antibodies (Gobert *et al.*, 2001; Shatavi *et al.*, 2006). The association between serum anti-gonadotrophin (Meyer *et al.*, 1990) or anti-ovarian antibodies (Luborsky *et al.*, 2002), IgG and poor ovarian response to FSH stimulation has been shown previously. However, in addition to reflecting ovarian autoimmunity, anti-FSH antibodies may impair the function of FSH. Anti-FSH could form immune complexes with FSH and induce its clearance, as recently shown for creatine kinase auto-antibodies (Warren *et al.*, 2006). In addition, anti-FSH could interrupt binding of FSH to its receptor. This hypothesis is supported by previous data from the authors' laboratory suggesting that the majority of anti-FSH antibodies were directed against the 78–93 amino acid region of FSH β -chain (Gobert *et al.*, 2001; Haller *et al.*, 2005). This immunodominant domain determines FSH receptor-binding specificity (Fox *et al.*, 2001). On the other hand, in-vitro studies have shown that these antibodies are also present in women with good FSH response in IVF (Reznik *et al.*, 1998), suggesting that these auto-antibodies may be non-pathogenic. However, this study did not specify anti-FSH antigen epitopes. Moreover, it is interesting to note that, in the current study, 9% and 13% of normal responders also displayed high concentrations of serum anti-FSH IgG and IgA, respectively. These results indicate the need for further studies on other epitopes on FSH- β and inclusion of additional (genetic) parameters for better discrimination of the patients with anti-FSH immunity associated adverse ovarian response in IVF.

Anti-FSH antibodies may exert their pathological role locally in growing follicles. The charge- and size-selective ovarian follicular barrier is open for IgG to pass into the FF (Hess *et al.*, 1998). Similar anti-FSH IgG and IgA concentrations in FF and serum were noted, in agreement with a previous study (Clarke *et al.*, 1984). In addition, it has been shown that the concentrations of FF anti-FSH IgG increased along with follicular growth, reflecting the maturity of follicles. Furthermore, concentrations of both FF anti-FSH IgG and IgA were also dependent on the overall dose of FSH used and FSH concentrations in FF. FF concentrations of FSH increase during follicular growth (Lambert-Messerlian *et al.*, 1997; Glister *et al.*, 2006) and depend on the amount of FSH administered (Luborsky *et al.*, 2002), as this study has also shown. Thus, these results suggest that anti-FSH IgG may diffuse along with the antigen into the FF during ovarian stimulation. On the other hand, research in pigs demonstrated that the concentration of total IgG in the FF increased during follicular growth (Hussein and Bourne, 1984), suggesting that accumulation of anti-FSH IgG in the FF may not be specifically caused by FSH taxis. Although anti-FSH IgA and IgM were present in the FF, concentrations of these antibodies were not associated with follicular diameter, in agreement

with other authors (Hussein and Bourne, 1984). Moreover, FF anti-FSH IgM concentrations were very low compared with serum antibodies, in accord with the findings of Clarke and co-workers (1984), in which total IgM in the FF represented only approximately 10% of the plasma concentration (Clarke *et al.*, 1984). Exclusion of IgM from the follicular fluid could explain why the concentrations of anti-FSH IgM were not predictive for the response of ovaries to FSH stimulation in the current study.

Although the pathophysiological role of anti-FSH in poor ovarian response is still unclear, the association of these antibodies with poor ovarian response in the present study was striking for two reasons. Firstly, these results confirmed that a woman's age, her ovarian volume and the number of follicles counted at the early follicular phase of her spontaneous menstrual cycle are significant clinical fertility parameters predicting ovarian stimulation outcome (Kligman and Rosenwaks, 2001; Levi Setti, 2006). The association of anti-FSH antibody concentration with poor ovarian stimulation outcome was demonstrated in various associations from multivariate adjusted models. By adjusting the statistical analysis for the clinical parameters, the role of anti-FSH in ovarian response could be assessed as if the clinical parameters were constant. These data suggest that anti-FSH antibodies could represent an important addition to fertility parameters such as age, ovarian volume or follicle count to predict ovarian stimulation outcome. Secondly, if the antagonizing effect of anti-FSH on the ovarian response was apparent in IVF patients with supraphysiological doses of FSH used, the importance of these antibodies in spontaneous folliculogenesis might be even more substantial. Future research investigating IVF patients with their oocytes retrieved during spontaneous menstrual cycles or after mild FSH stimulation, rather than after ovarian stimulation, could further elucidate this issue. In addition, it would be of interest to investigate the association of anti-FSH antibodies with IVF treatment outcome in patients following the GnRH-agonist protocol. Consequently, similar to successful immunosuppressive therapy in cases of repeated IVF failure and autoantibody seropositivity (Geva *et al.*, 1999; Luborsky, 2002; Forges *et al.*, 2006), a low-dose prednisolone therapy might be beneficial in cases of increased anti-FSH production.

Acknowledgements

This work was supported by the Estonian Science Foundation (grants nos. 6498 and 6514) and the Estonian Ministry of Education and Science (core grants nos. 0182582Cs03, 0182586s03 and PBGMR07903). The authors thank Dr E Talving, Dr P Karits and K Rohtla from Nova Vita Clinic, for collecting patient data.

References

- Altmae S, Haller K, Peters M *et al.* 2007 Allelic estrogen receptor 1 (ESR1) gene variants predict the outcome of ovarian stimulation in in-vitro fertilization. *Molecular Human Reproduction* **13**, 521–526.
- Clarke GN, Hsieh C, Koh SH *et al.* 1984 Sperm antibodies, immunoglobulins, and complement in human follicular fluid. *American Journal of Reproductive Immunology* **5**, 179–181.
- Forges T, Monnier-Barbarino P, Guillet-May F *et al.* 2006 Corticosteroids in patients with antiovarian antibodies undergoing in-vitro fertilization: a prospective pilot study. *European Journal of Clinical Pharmacology* **62**, 699–705.

- Fox KM, Dias JA, Van Roey P 2001 Three-dimensional structure of human follicle-stimulating hormone. *Molecular Endocrinology* **15**, 378–389.
- Geva E, Fait G, Lerner-Geva L *et al.* 1999 The possible role of antiovarian antibodies in repeated in-vitro fertilization failures. *American Journal of Reproductive Immunology* **42**, 292–296.
- Glister C, Groome NP, Knight PG 2006 Bovine follicle development is associated with divergent changes in activin-A, inhibin-A and follistatin and the relative abundance of different follistatin isoforms in follicular fluid. *Journal of Endocrinology* **188**, 215–225.
- Gobert B, Jolivet-Reynaud C, Dalbon P *et al.* 2001 An immunoreactive peptide of the FSH involved in autoimmune infertility. *Biochemical and Biophysical Research Communications* **289**, 819–824.
- Haller K, Salumets A, Grigorova M *et al.* 2007 Putative predictors of antibodies against follicle-stimulating hormone in female infertility: a study based on in-vitro fertilization patients. *American Journal of Reproductive Immunology* **57**, 193–200.
- Haller K, Mathieu C, Rull K *et al.* 2005 IgG, IgA and IgM antibodies against FSH: serological markers of pathogenic autoimmunity or of normal immunoregulation? *American Journal of Reproductive Immunology* **54**, 262–269.
- Hess KA, Chen L, Larsen WJ 1998 The ovarian blood follicle barrier is both charge- and size-selective in mice. *Biology of Reproduction* **58**, 705–711.
- Hussein AM, Bourne FJ 1984 Immunoglobulin concentrations in pig follicular fluid. *International Journal of Fertility* **29**, 54–57.
- Kligman I, Rosenwaks Z 2001 Differentiating clinical profiles: predicting good responders, poor responders, and hyperresponders. *Fertility and Sterility* **76**, 1185–1190.
- Knight PG, Glister C 2006 TGF-beta superfamily members and ovarian follicle development. *Reproduction* **132**, 191–206.
- Lambert-Messerlian G, Taylor A, Leykin L *et al.* 1997 Characterization of intrafollicular steroid hormones, inhibin, and follistatin in women with and without polycystic ovarian syndrome following gonadotropin hyperstimulation. *Biology of Reproduction* **57**, 1211–1216.
- Levi Setti PE 2006 The importance of consistent FSH delivery in infertility treatment. *Reproductive BioMedicine Online* **12**, 493–499.
- Luborsky J 2002 Ovarian autoimmune disease and ovarian autoantibodies. *Journal of Women's Health and Gender-based Medicine* **11**, 585–599.
- Luborsky J, Pong R 2000 Pregnancy outcome and ovarian antibodies in infertility patients undergoing controlled ovarian hyperstimulation. *American Journal of Reproductive Immunology* **44**, 261–265.
- Luborsky JL, Rivnay B, Roussev R *et al.* 2002 Evidence for different aetiologies of low estradiol response to FSH: age-related accelerated luteinization of follicles or presence of ovarian autoantibodies. *Human Reproduction* **17**, 2641–2649.
- Mantzavinos T, Dalamanga N, Hassiakos D *et al.* 1993 Assessment of autoantibodies to the zona pellucida in serum and follicular fluid in in-vitro fertilization patients. *Clinical and Experimental Obstetrics and Gynecology* **20**, 111–115.
- Meyer WR, Lavy G, DeCherney AH *et al.* 1990 Evidence of gonadal and gonadotropin antibodies in women with a suboptimal ovarian response to exogenous gonadotropin. *Obstetrics and Gynecology* **75**, 795–799.
- Narayanan M, Murthy PS, Munaf SA *et al.* 1995 Antiovarian antibodies and their effect on the outcome of assisted reproduction. *Journal of Assisted Reproduction and Genetics* **12**, 599–605.
- Perez Mayorga M, Gromoll J, Behre HM *et al.* 2000 Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. *Journal of Clinical Endocrinology and Metabolism* **85**, 3365–3369.
- Reznik Y, Benhaim A, Morello R *et al.* 1998 High frequency of IgG antagonizing follicle-stimulating hormone-stimulated steroidogenesis in infertile women with a good response to exogenous gonadotropins. *Fertility and Sterility* **69**, 46–52.
- Sample WF, Lippe BM, Gyepes MT 1977 Gray-scale ultrasonography of the normal female pelvis. *Radiology* **125**, 477–483.
- Shatavi SV, Llanes B, Luborsky JL 2006 Association of unexplained infertility with gonadotropin and ovarian antibodies. *American Journal of Reproductive Immunology* **56**, 286–291.
- Thomas FH, Vanderhyden BC 2006 Oocyte-granulosa cell interactions during mouse follicular development: regulation of kit ligand expression and its role in oocyte growth. *Reproductive Biology and Endocrinology* **4**, 19.
- Warren GL, O'Farrell L, Rogers KR *et al.* 2006 CK-MM autoantibodies: prevalence, immune complexes, and effect on CK clearance. *Muscle and Nerve* **34**, 335–346.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 31 July 2007; refereed 12 October 2007; accepted 16 November 2007.