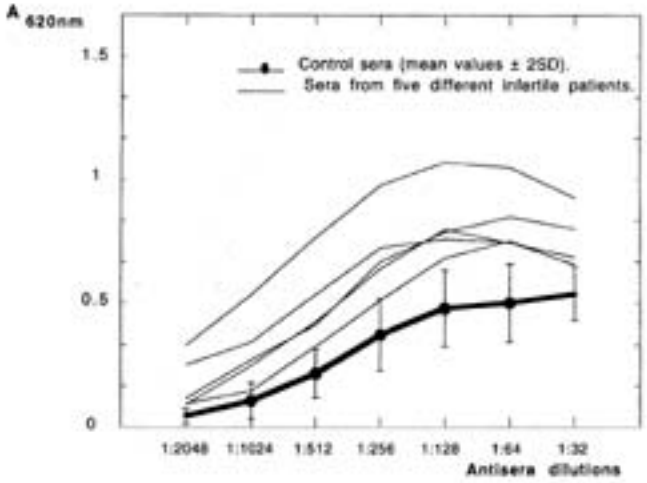

Fig. 2. Anti-endometrial antibodies (AEA) detected by ELISA.

the diagnosis of the patients (Table I). Good correlation was observed between the presence of these antibodies and the tubal obstruction (77.8 and 66.7%, respectively) or hormonal ovulatory dysfunction (54.5 and 45.5%, respectively). In the analysis of unexplained infertility in the couple, 40% of the women showed antibodies. In the couples with male factor diagnosis, 25 and 31.3%, respectively, women were positive for AEA.

DISCUSSION

This study shows the presence of AEA in a group of women with tubal obstruction and/or ovulatory dysfunction by a reproducible ELISA technique, using a stable cancer cell line as the source of the antigen.

Antibodies to endometrial tissue bind to the glandular component of normal endometrial preparations in tissue culture by indirect immunofluorescence,[15] and some endometrial antigens are detected from endometrial extracts.[16] Some authors, by using tissue from an uterine and from an ectopic endometrium for the detection of antibodies by immunohistochemistry, conclude that the antigens from the ectopic endometrium are also immunogenic.[2] Endometrial cell membrane extracts from an eutopic and from an ectopic endometrium were used for the coating of ELISA microplates by Odukoya et al.[5] to investigate the frequency of antibodies in the serum of patients with endometriosis. This determination is of interest because of the association of AEA with infertility. The effect of these autoantibodies was to interfere with implantation.

Some problems arise working with human endometrial cells from biopsies: the small number of cells, and the slow growth rate that we have after tissue treatment. Under these conditions a small number of samples can be tested with human endometrial cells as a source of ELISA assay in each experiment. Alternatively, a human endometrium cancer cell line has been used by growing the cells in monolayers to determine by indirect immunofluorescence the presence of AEA in patients with endometriosis.[12] An endometrial cell line was used for the analysis of AEA in patients with endometriosis by Hatayama et al.[9] Although these authors do not compare the results obtained with the results that could derive from the analysis with endometrial cells from biopsies, good reproducibility of the experiments was shown. Hatayama et al.[9] discuss the advantages of using cell lines in an ELISA for the detection of AEA. We proved the validity of this technique by the use of both cell lines 1671 and 113 and by the good correlation of the results that we obtained compared with endometrial cells from biopsies.

It is possible that endometrial autoimmunity may be due to a gamete predisposition to organ autoimmunity. It is described that patients with endometriosis with antibodies to the endometrium also show elevated titers to the ovary. Having in mind that the immune system is linked to implantion and that abnormal autoimmunity can affect the reproductive process at various stages, we investigated the presence of AEA in infertile patients without endometriosis. In the literature there are no data on the presence of AEA related to pathologies associated with the Fallopian tubes or ovary, although the presence of anti-ovarian antibodies has been reported.[11] We have found a high correlation between the presence of AEA and tubal obstruction (77.8 and 66.7%, respectively). The immune response to endometrial cells is triggered by the local tubal inflammatory response. The ovulatory dysfunction group of infertile patients showed a high percentage of the presence of endometrial antibodies (54.5 and 45.5%, respectively). The hormonal dysfunctions in these patients could be due to the inhibitory or blocking effect of ovarian autoantibodies on hormonal receptors or to down-regulation of the receptor. The inflammatory mechanism may be critical for the establishment of autoimmune oophoritis and may

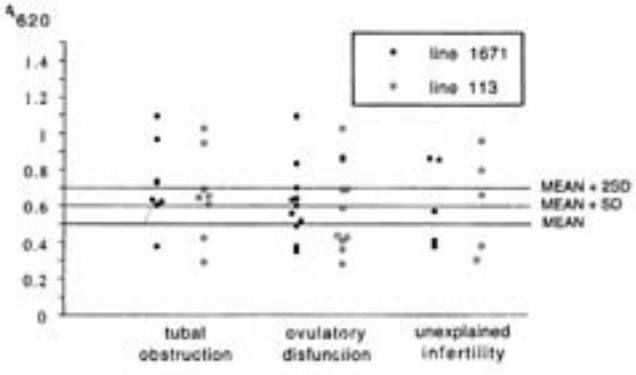

Fig. 3. Scatter plot of sera from women with tubal obstruction, ovulatory dysfunction, and unexplained infertility. a) cell line 1671 and b) cell line 113. The threshold for positivity was 0.6.

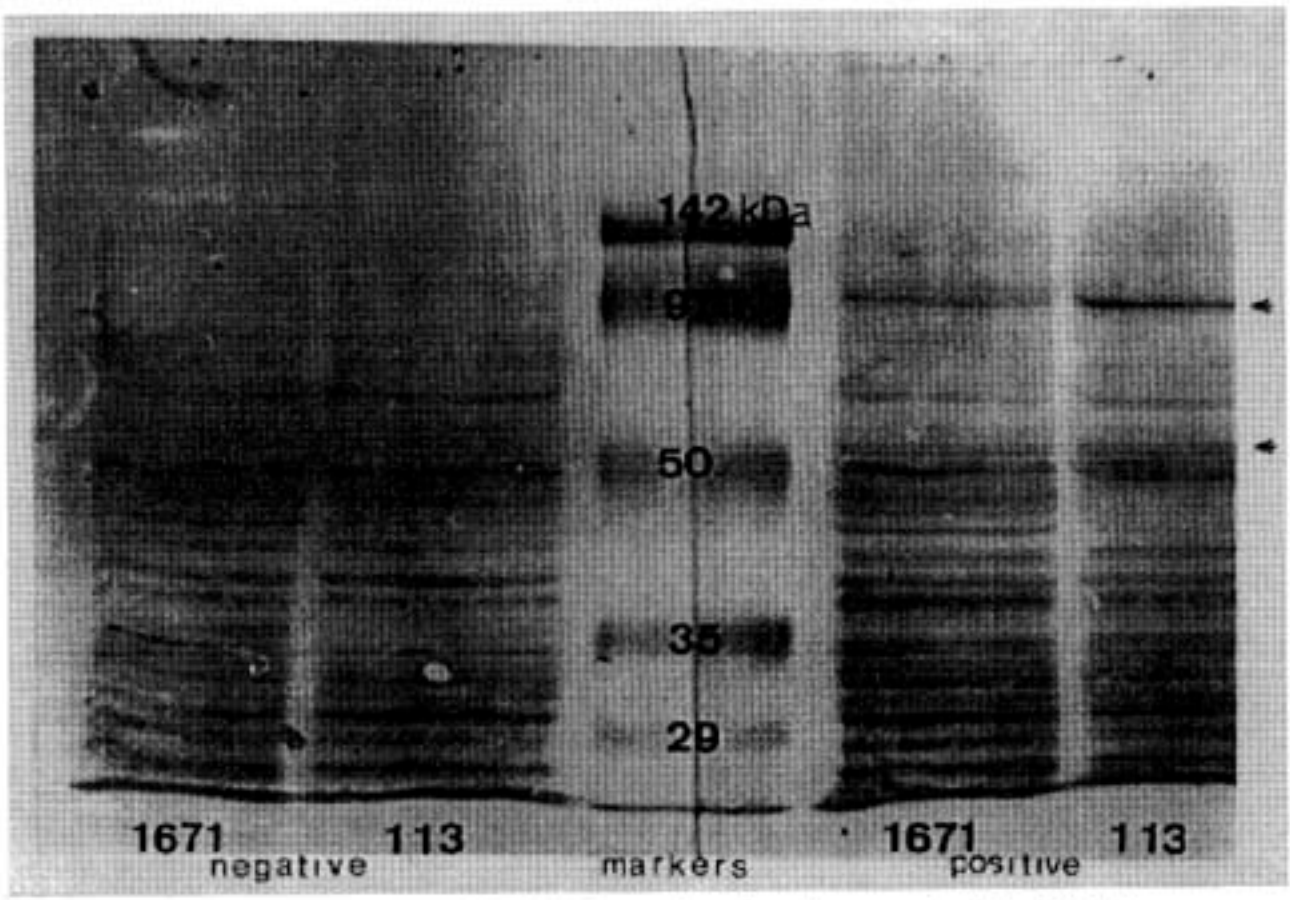

Fig. 4. Western blot analysis of antibody binding to endometrial membrane extracts (cell lines 1671 and 113) in a women with high reactivity by ELISA.

favour the induction of antibodies with other specifities. The ELISA analysis for antibodies was performed with human endometrial cells and two endometrial cancer cell lines, 113 and 1671, that seem to exhibit a similar panel of antigens, because the results are quite coincident.

Finally, some women with unexplained infertility antibodies revealed the presence of anti-endometrial antibodies (40 and 60%, respectively); this group is difficult to define and is of questionable diagnosis. Gleicher[17] describes unexplained infertility in a group of autoimmune diseases, with some characteristic features of autoimmunity, such as familiar ocurrence, polyclonal B lymphocyte activation, tissue damage, autoantibody abnormalities, and increased association of other autoimmune conditions.

In the detection of endometrial antigens, SDS-endometrial extracts were prepared from the two cell lines used in ELISA. A high positive serum revealed some antigenic bands (50 and 97 kDa) in the two cell lines. In the serum and peritoneal fluid, some authors such as Mathur et al.[16] found antibodies against antigens with molecular weights of 34, 42, 82, 94, 110, 120, and 140 kDa. We have also detected a band at around 97 kDa. Odukoya et al.[5] found one band at 48 kDa with most of the sera of women with endometriosis, irrespective of the antigenic source, eutopic or ectopic. This would be coincident with the 50 kDa and that we describe. If women with autoimmune diseases secrete large amounts of antigens, AEA to some relevant antigens would be induced more easily. The two bands reported here are not reactive with sera from fertile control patients. Gorai et al.[6] reported some endometrial antigens that were reactive with serum samples from endometriosis patients, but to a lesser extent with samples from normal control women.

We have reported the presence of AEA in patients with tubal obstruction and ovulatory dysfunction. These antibodies could be induced by an inflammatory response in the female genital tract, and they could produce as a consequence the above-mentioned pathologies. More samples from each group need to be evaluated before establishing a correlation between the presence of AEA and the severity of the described diseases. We propose an ELISA technique for the detection of AEA of infertile women. This

TABLE I. ELISA Reactivity of the Serum from Infertile Women (n = 38) with Two Endometrial Cell Lines

	Cell line 1671				Cell line 113			
Diagnosis	Positive response	%	Negative response	%	Positive response	%	Negative response	%
Tubal obstruction (n = 9[a])	7	77.8	2	22.2	6	66.7	3	33.3
Ovulatory dysfunction (n = 11[a])	6	54.5	5	45.5	5	45.5	6	54.5
Unexplained infertility (n = 5)	2	40.0	3	60.0	3	60.0	2	40.0
Male factor (n = 16)	4	25.0	12	75.0	5	31.3	11	68.7

[a]Three patients showed tubal obstruction and ovulatory dysfunction. Positivity defined as ≥ 1 SD above the established cutoff.

analysis can be useful in the prediction of pregnancy if the presence of these antibodies correlates well with the failure of implantation. It remains to be elucidated what antigens are responsible for this immunological response.

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