The effect of antigamete antibodies on the success of assisted reproduction

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Summary

The aim of the study was to investigate the presence of antigamete antibodies in unexplained infertility patients and to prove the efficiency of IUI and IVF-ET treatments for these patients.

The study includes 46 unexplained infertility patients and as controls, a group of 21 tubal infertility patients. Serum, follicular fluid and cervical mucus samples were collected from each patient and antibodies were measured with commercial ELISA kits.

Twenty-two of the 46 unexplained infertility patients produced at least one of the antibodies against sperm or ovary. Fertilization rates were lower in immunological and unexplained infertility patients than in tubal infertility patients, being statistically significant. Pregnancy rates were lower in immunological and unexplained infertility patients than in tubal infertility patients after IVF-ET, but this was not statistically significant. Pregnancy rates after IUI treatment were equal in both immunological and unexplained infertility groups.

AGA (antigamete antibodies) were found in 45% of unexplained infertility patients and therefore may be a possible cause of infertility. IUI and IVF-ET are succesful choices for treatment of these patients.

Key words: Antisperm antibodies; Antiovar antibodies; Antizona pellucida antibodies; Intrauterine insemination; In-vitro fertilization.

Introduction

About 11.5% of the causes of infertility are unexplained [1]. Immunological factors have been estimated to occur in up to 20% of couples with unexplained infertility [2]. Antigamete antibodies like antisperm antibodies (ASA), antiovar antibodies (AOA) and antizona pellucida antibodies (AZA) are possible mediators of immunologic infertility. Many different methods (e.g. condom, corticosteroid) were performed for the therapy of immunological infertility [3]. Today assisted reproduction techniques (ART) like intrauterine insemination (IUI) and in-vitro fertilization (IVF) are possible choices of treatment. This study investigates the presence of ASA, AOA and AZA in patients suffering from unexplained infertility and the efficiency of IUI and IVF treatments for these patients.

Materials and Methods

In patients with regular menstrual cycles, normal vaginal ultrasonography results and regular FSH, LH, prolactin, testosterone and DHEA-S levels at days 3-5 of the follicular phase as well as normal progesterone results in the luteal phase, pelvic endoscopy proved both tubes to be patent. All male patients were normozoospermic according to WHO criteria [4]. Preovulatory post-coital testing did show regular results. Among these patients a subgroup was differentiated which tested positive for at least one of the antigamete antibodies (ASA, AOA, AZA). We compared this immunological infertility group (n=22) to patients with unexplained infertility (n=24) and patients with bilateral tubal occlusions (n=21). The latter two groups tested

negative for antigamete antibodies. ASA testing was performed in serum, follicular fluid and cervical mucus. AOA and AZA testing were performed in serum and follicular fluid.

All samples were collected from female patients, who were undergoing IUI and/or IVF-ET at the Department of Obstetrics and Gynecology, University of Düsseldorf. The patients for IUI were stimulated with either clomiphen citrate (CC) or with hMG. Follicle maturation was monitored by ultrasound, estradiol and LH measurements. Ovulation induction was done by hCG injection (5000 IU), when follicular size was 20 ± 2 mm by ultrasound. IUI was performed 38-44h after hCG injection. All patients for IVF were stimulated with GnRH analogue/hMG/hCG using a long protocol version as published previously [5]. Semen preparation was done by swim-up method. Culture media were not supplemented with maternal serum.

Cervical mucus samples were aspirated into an 1 ml syringe immediately before IUI or IVF-follicle aspiration were performed. Equal amounts of hyaluronidase (320 units, Sigma, St. Louis, USA) were added. After 10 minutes the samples were centrifuged at 200 g at room temperature for 5 minutes. The supernatant was taken and stored at -−20°C until measurements were performed. Serum samples were also collected at the time of anticipated ovulation and stored at −20°C. Follicular fluid (FF) was obtained during ultrasound guided transvaginal aspiration of oocytes for IVF. FF was centrifugated at 800 g for 10 minutes and the supernatant was removed and stored at −20°C until testing. ASA, AOA and AZA were qualitatively detected with commercial ELISA kits (Biogen, Rostock, Germany). Differences between groups were assessed by (Chi) X^2 and Fisher's exact test, with $p<0.05$ as the level of statistical significance.

Results

There were no differences between the unexplained, immunological and tubal infertility groups regarding age of the patients (31.4, 31.5 and 32.9, respectively) and duration of infertility (4.9, 4.6 and 5.8 years, respectively).

Samples of all 24 unexplained infertility patients and 21 tubal sterility patients were negative for antigamete antibodies tested. The 22 immunological infertility patients produced at least one of the antigamete antibodies. Serum, follicular fluid and/or cervical mucus of 13 patients were positive for ASA (Ig G and/or Ig A). Positive AOA (Ig G and/or Ig A) results were detected in the serum and/or follicular fluid of 10 patients. Only 2 patients showed AZA (Ig G and/or Ig A) in the serum and/or follicular fluid (Table 1). The screening evaluated 3 patients having more than one type of antibody, two patients with ASA+AOA and one with ASA+AZA.

Fourteen of 22 patients of the immunological group were treated by IUI within 34 cycles and 3 patients became pregnant, with the pregnancy rate/cycle being 8.8%. Eighteen of 24 patients of the unexplained group were also treated by IUI within 40 cycles and 5 patients became pregnant. The pregnancy rate/cycle was 12.5%. The difference between the pregnancy rates of both groups in not statistically significant.

Eighteen patients from the immunological group were treated by IVF within 28 cycles; 61.3% of the oocytes were fertilized and 3 patients became pregnant (one ongoing and two deliveries). From the unexplained group 19 patients were treated by IVF within 35 cycles. Fertilization rate was 65.6% and 5 patients became pregnant (2 ongoing, 3 deliveries). In the group of tubal etiology 21

patients were treated within 37 cycles, 80.5% of the oocytes were fertilized and 7 patients became pregnant (2 ongoing, 5 deliveries). The fertilization rates in the three groups treated by IVF were 61.3% for immunological infertility, 65.5% for unexplained infertility and 80.5% for tubal infertility. The differences between the tubal group and the other two groups were statistically significant (p<0.01). The clinical pregnancy rate/cycle in the immunological, unexplained and tubal groups was 10.7%, 14.3% and 16.2% respectively, being not significantly different from each other (Table 2).

In patients having ASA 37 oocytes were fertilized from 48 with a fertilization rate of 77.1%. In patients having AOA 44 oocytes were retrieved out of which 21 were fertilized with a fertilization rate of 47.7%. The fertilization rate in the one patient having only antizona antibodies was 0%, no fertilization occurred in 7 oocytes. In patients with mixed antibodies 25 oocytes were retrieved and 18 were fertilized with a fertilization rate of 72%. The difference in the fertilization rate between patients with ASA and those with AOA or AZA was statistically significant (p<0.05). Clinical pregnancy rate in patients with ASA was 9.1% per cycle. In patients with AOA and with mixed antibodies the clinical pregnancy rate per cycle was 10.0% and 16.6%, respectively (Table 3). The differences in pregnancy rates between all types of antibodies were not statistically significant.

Discussion

In our study among 46 patients with unexplained infertility 22 patients (47.8%) were positive for antigamete antibodies. These results are in agreement with a previous study which reported an incidence of immunologic infertility 14-40% of patients initially suspected to have unexplained infertility [6]. The reason for our slightly higher results can be attributed to the strict selection of the unexplained infertility patients.

The female reproductive tract in general, and particularly the cervix, has the ability to synthesize and secrete immunoglobulins IgG and IgA. Circulating ASA in serum have not been observed to reach the cervical mucus in significant quantities [7]. This may explain why ASA in our study were 61.5% in cervical secretion, while in the serum only 53.8%. Monnier *et al.*, found AOA in 51% of women [8]. Our results showed AOA in 21.7% of the patients tested, which may be due to the smaller number of patients in the current study. In regard to AZA, our results showed a low prevalence of 4.3% compared to a previous study showing 13% AZA positivity in women suffering from unexplained infertility [9].

IUI is a method for treatment of ASA positive patients. In the current study the pregnancy rate/patient in the immunologic infertility group was 21.4%. The results of other studies have been similar showing 23-36% [10, 11]. Our work focused on the effectiveness of IVF in the treatment of immunologically infertile patients by studying fertilization rates, as well as pregnancy rates analysing the IVF data from patients with immunological infertility,

Table 1. — *Positive samples from unexplained infertility patients (n=46)*

	S	FF	CM	S+CM	CM+FF	S+FF	Total
ASA	3	1	4	3	1	1	13
AOA	3	3	–	–	–	4	10
AZA	0	1	–	–	–	1	2

S – Serum; FF – Folicular fluid; CM – Cervical mucus

Table 2. — *Fertilization and pregnancy rate differences between groups * p<0.05*

	tubal	unexplained	immunologic
patients	21	19	18
cycles	37	35	28
fertilization %	80.5*	65.6*	61.3*
pregnancy/cyc %	16.2	14.3	10.7

Table 3. — *Fertilization and pregnancy rate differences between antigamete antibodies * p<0.05*

	ASA	AOA	AZA	Mixed
patients	7	7	1	3
cycles	11	10	1	6
fertilization %	77*	48*	0	72
pregnancy/cyc %	9.1	10	0	16.6

compared to patients with unexplained or tubal infertility as control groups. The fertilization rate in the immunological group was significantly lower than in the group with tubal infertility. This may be explained by the fact that antigamete antibodies can inhibit the fertilization by interference with the acrosome reaction or other enzyme-dependent mechanisms of sperm penetration of the oocyte or could occlude binding sites for the zona pellucida or ovum, thereby preventing sperm-oocyte attachment [12].

Regarding fertilization rates in relation to different types of antibodies there was a significant difference between the patients with ASA and those having AOA and AZA. The occurrence of AOA and AZA did effect the fertilization process more negatively than ASA. Possible reasons for this are that AOA may interfere with ovum maturation, ovulation and oocyte viability. Moreover, the antibodies directed against zona pellucida antigens could cause infertility by inhibiting sperm-egg interaction or by exerting complement mediated cytotoxic effects on the oocyte [12]. Of the two patients having AZA, the one that had AZA in combination with AOA, showed no fertilization. These results are in agreement with Papala *et al.*, who reported that the presence of AZA was positively correlated with fertilization failure [13]. No significant correlation could be demonstrated between the percentage of any specific immunoglobulin class and the percentage of fertilization (data not shown). The clinical pregnancy rate after ET did not differ significantly in patients with unexpalined, immunological or tubal infertility. A recent study reported also that pregnancy rates in immunologic infertility patients were similar to the control group [14]. The insignificant differences between the immunologic and the tubal group in regard to pregnancy rates, despite the significant difference between both groups in regard of fertilization rates, may be attributed to the fact that in case of immunoligical infertility the obstacle for pregnancy to occur is the failure of fertilization. Once fertilization is achieved and equal numbers of embryos are transfered after IVF, there seems to be no difference in pregnancy rates. In the current study we could not demonstrate an embryotoxic effect of antibodies because no difference was observed in the pregnancy rates between the three studied groups.

In conclusion there is a cosiderable prevalence of antibody titer among patients suffering from unexplained infertility. Consequently routine screening of antibodies is mandatory in such cases. Antibodies against sperm, ovarian tissues or zona pellucida may interfere with fertilization by different mechanisms possibly affecting sperm motility, sperm capacitation, acrosome reaction and sperm-egg interaction. Antiovarian and zona pellu-

cida antibodies seem to be more able to interfere with fertilization than the antisperm antibodies. IUI as well as IVF seem to be effective treatments of immunologic infertility. Nevertheless, intracytoplasmic sperm injection (ICSI) may be recommended for treating patients showing AOA or AZA. Multicenter trials may be useful to further investigate the influence of antigamete antibodies on fertilization and pregnancy rates.

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