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Original Article

Investigation of the impact of antinuclear antibody on the outcome of *in vitro* fertilization/intracytoplasmic sperm injection treatment

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ABSTRACT

Objective: The aim of this study is to investigate the influence of antinuclear antibodies (ANAs) on the pregnancy and early miscarriage rates, thereby evaluating the outcome of *in vitro* fertilization and intracytoplasmic sperm injection (IVF/ICSI) treatment.
Materials and methods: A total of 517 infertile female patients undergoing IVF/ICSI treatment (experimental group) were chosen for this study, and 186 women with normal reproductive history (control group) were designated as the control. Serum ANAs from the participants were tested using indirect immunofluorescence assay, while antiextractable nuclear antigens were tested by immune blot assay.
Results: The ANA expression in the infertile patients (39.45%) was higher than that in the control group (16.13%). A high ANA titer (≥1:320) was found only in infertile patients. ANA positivity significantly decreased the pregnancy rate and increased the early miscarriage rate after IVF/ICSI treatment. The rate of early miscarriage was higher in the high-ANA-titer individuals after IVF/ICSI treatment. Clinical pregnancy rate in anti-scl-70- and anti-PM-scl-positive individuals after IVF/ICSI treatment was lower than that in the ANA-negative individuals. Anti-Rib-p, anti-Jo-1, and anti-dsDNA were found to cause high risk of early miscarriage in pregnant women.
Conclusion: ANA positivity may not only be the cause of bad outcome during IVF/ICSI treatment, but also pose as a risk factor for IVF/ICSI treatment.
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Introduction

In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) is a technology that has brought hope and possibility of fertilization to infertile couples. At present, the global incidence of infertility rate in couples of childbearing age is about 15% and seems to be increasing day by day. *In vitro* fertilization-embryo transfer is a very effective technique that has been evolving over the past 30 years, but yet, the overall clinical pregnancy rate after IVF and ICSI treatment still remains 30–40%, implying that multiple factors could be involved in the mechanism of infertility [1–5].

Over the past few years, there has been a lot of research work that has clearly demonstrated that the immunological status of the body is a key factor for a successful pregnancy. The balance of immunologic tolerance between the mother and the embryo has been given increasing importance during the process of embryo implantation and fetal development [6–8]. It is increasingly clear that the immunological background and environment during fertilization are very crucial and decisive factors for impregnation.

While investigating the mechanisms underlying the failure of IVF/ICSI treatment, several researchers have laid focus on autoimmune factors such as the antisperm antibody, antiovarian antibody, antiendometrial antibody, and antiphospholipid antibody. Hence, over the years, some of these antibodies have become routine tests for infertility patients in many centers. However, literature has shown that only a few infertile patients were tested positive for any of the aforementioned autoantibodies, implying that there must be other factors and mechanisms underlying the implantation failure.

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Nevertheless, the correlation between implantation failure and immune factors remains unproven [9,10].

Antinuclear antibodies (ANAs) are a large group of autoantibodies targeting the entire cell including DNAs, RNAs, proteins, and/or their complexes. ANAs are commonly seen autoantibodies in autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. However, it has previously been reported that while ANA is related to a decline of oocyte quality and an impairment of embryo development, it is also relevant to recurrent spontaneous abortion, endometriosis, infertility, IVF failure, and ovarian dysfunction [11–15].

In short, in order to determine the role of ANA in IVF/ICSI treatment, in this study, we compared the ANA expressions in infertile women with a fertile control model to see if infertility and IVF/ICSI treatment failure were related to ANA.

Materials and methods

Patients and grouping

A total of 517 infertile women who were undergoing the first cycle of IVF/ICSI treatment in our faculty were enrolled in the IVF/ICSI group. By contrast, 186 patients who had given birth to healthy children without any history of spontaneous abortion over the recent 2 years were recruited as normal controls. All participants enrolled in the IVF/ICSI and control groups were duly briefed about the study, and they signed informed consent forms at the beginning of the study. They were all recruited at the Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China, between January 2012 and June 2014. This study was approved by the local ethics committee.

All patients enrolled in the IVF/ICSI group had to satisfy the following criteria: (1) age $\leq$ 38 years; (2) undergoing IVF/ICSI for the first time, satisfying all indications for IVF/ICSI treatment with basal follicle stimulating hormone < 10 mIU/L; and (3) no prior history of ovarian surgery, chemotherapy, or autoimmune diseases. By contrast, individuals enrolled for the control group had, in turn, to satisfy the following criteria: (1) age $\leq$ 38years; (2) given birth to healthy child over the past 2 years without any prior history of spontaneous abortion; and (3) no existing pregnancy complication or autoimmune disease condition.

Detection of ANAs

Serum ANAs were detected by the indirect immunofluorescence assay (IFA) on a slide with human epithelial HEp-2 cell line and liver tissue (monkey) substrate (EUROIMMUN, Luebeck, Germany) in dilution ratios of 1:100, 1:320, and 1:1000. ANAs would react with the antigens in the Hep-2 cell substrate, forming antigen–antibody complexes bound to the cell nucleus. The slides were prepared following the manufacturer's recommendations and protocol, and were evaluated under the fluorescence microscope using 20× or 40× objectives. The ANA test was considered positive when the characteristic fluorescent signal was detected in the tissue or cell, with a serum dilution ratio of $\geq$1:100 (EUROIMMUN). Fluorescence intensity was interpreted semiquantitatively based on negative and positive controls.

Further assays were performed following IFA to identify the autoantibody-targeted extractable nuclear antigens (ENAs) using the immune blot method (EUROIMMUN). This test identified the 15 different anti-ENA targets including nRNP, Sm, SS-A, Ro52, SS-B, Scl-70, PM-Scl, Jo-1, CENP-B, PCNA, dsDNA, nucleosome, histones, ribosomal P-proteins (Rib-p), and AMA-M2.

All assays were performed and interpreted according to the manufacturers' protocol.

In vitro fertilization-embryo transfer/ICSI protocol

All the infertile patients recruited in the IVF/ICSI group were stimulated using the traditional gonadotropin-releasing hormone agonist flare and long luteal-phase protocols, the combinations of gonadotropin-releasing hormone agonists and gonadotropins. About 36 hours after the human chorionic gonadotropin (HCG) injection, the oocytes were picked up. The fertilization program (conventional IVF or ICSI) was selected based on the semen condition. On the 2nd day or 3rd day after the oocytes were picked up, morphologic assessment of embryos was carried out under an inverted microscope before transfer. Embryos graded 1, 2, and 3 were considered available embryos, and those graded 1 and 2 were considered good-quality or perfect embryos. Pregnancy was diagnosed by a positive blood test for β-HCG at 14 days after the embryo transfer. Clinical pregnancy was diagnosed when the gestational sac was detected by transvaginal ultrasonography.

Data collection of infertile women

The basic clinical information of infertile women treated with IVF/ICSI, including their age, body mass index, duration of infertility, and basal level of sex hormone, was collected. Serum levels of follicle-stimulating hormone, luteinizing hormone, estradiol (E2), and progesterone were measured. Other parameters that were recorded were related to the controlled ovarian hyperstimulation and IVF, including duration of gonadotropin treatment (days); total dose of gonadotropin; levels of luteinizing hormone, E2, and progesterone and endometrial thickness on the day of HCG administration; number of oocytes picked up; proportion of MII oocytes; proportion of two-pronuclear embryos; cleavage rate; number of available embryos; number of transferred embryos; implantation rate; pregnancy rate; clinical pregnancy rate; and early miscarriage rate.

Statistical analysis

Statistical analysis was performed using SPSS version 18 statistical software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA). The data were analyzed using the Chi-squared test (Fisher's exact test was used in case the sample size was small) and Student *t* test. A *p* value of <0.05 indicated that the difference was statistically significant.

Results

Higher level of expression of serum ANAs in the IVF/ICSI group than in the control group

In the IVF/ICSI group, 39.46% (204/514) patients were found to be ANA positive by IFA. Moreover, 30.39% (62/204) of the ANA-positive patients were screened by IFA to have a high ANA titer in serum. By contrast, in the control group, only 30 cases were tested to be ANA positive and no high titer of ANAs was found in the control group. The positive rate of ANA in the serum, the incidences of ANA positive or high titer of ANA between IVF/ICSI group and control group were tabulated for proper reference (Figure 1). Detailed information about the ANA expression in the IVF/ICSI and control groups is given in Table 1.

In the IVF/ICSI group, anti-ENA test results showed that out of the 204 patients tested ANA positive, 23 cases were anti-PM-scl positive, 18 anti-SSA positive, 18 anti-Ro-52 positive, and 13 anti-histone positive. Twelve patients were tested positive for multiple anti-ENAs in the serum; nine of them tested double positive for anti-dsDNA and antihistone, two tested positive for both anti-SSA

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