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Original Article

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

Ying Li [a], Yipeng Wang [b], Yanmin Ma [a], Yonglian Lan [a], Chanwei Jia [a], Yu Liang [a], Shuyu Wang [a,*]

[a] Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China
[b] Beijing Youan Hospital, Capital Medical University, Beijing, China

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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.

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.

* Corresponding author. 251 Yaojiayuan Rd, Chaoyang District, Beijing 100026, China.
E-mail address: yushu572000@126.com (S. Wang).

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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

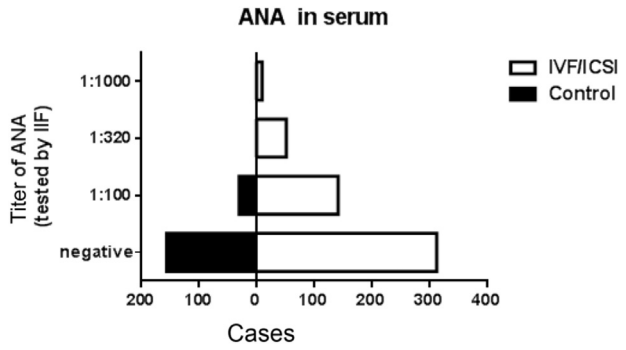

Figure 1. ANA expression in the IVF/ICSI and control groups. ANA = antinuclear antibody; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization.

Table 1
Serum ANA expression in the IVF-ET/ICSI and control groups.

		IVF/ICSI	Control	*p*
Cases		517	186	
Age (y)		30.78 ± 3.04	31.03 ± 2.83	0.327
ANA positive		204 (39.45)	30 (16.13)	<0.001
Titer of ANAs	1:100	142 (27.46)	30 (16.13)	0.002
	≥1:320	62 (11.99)	0	<0.001

Data are presented as *n*, *n* (%), or mean ± standard deviation.
ANA = antinuclear antibody; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization; IVF-ET = *in vitro* fertilization-embryo transfer.

and anti-Ro-52, and one tested positive for anti-PM-scl and anti-scl-70. Immune blot tests, by contrast, failed to identify the anti-ENAs in the serum of the 96 patients, and this might have been due to the lower sensitivity of immune blot compared to that of IFA. Furthermore, there were some antibodies against unusual ENAs in the samples, which could not be discovered by current methods and have therefore been placed in the unclassified subgroup. Detailed information about the anti-ENA screening in the IVF/ICSI and control groups has been provided in Table 2.

Impact of ANAs on IVF/ICSI treatment

Comparing the clinical information of the ANA-positive and -negative patients from the IVF/ICSI group, we found that the total gonadotropin dose was higher in the ANA-positive patients. We also discovered that the number of oocytes retrieved from the ANA-positive patients was fewer than that from the ANA-negative patients after cycles of treatment. As far as the outcome of the treatment is concerned, we found that implantation rate and clinical pregnancy rate were lower and early miscarriage rate was higher in the ANA-positive patients than in the ANA-negative patients (Figure 2). Detailed clinical information of the ANA-positive and ANA-negative patients from the IVF/ICSI group has been provided in Table 3.

We further analyzed the effect of the level of ANAs on the outcome of IVF/ICSI treatment. Our data showed that the E2 level was higher on the HCG day, and the numbers of retrieved oocytes and available embryos were more in patients with a low ANA titer (1:100) than in those with a high ANA titer (≥1:320). Furthermore, there was a lower early miscarriage rate in low-ANA-titer (1:100) patients (Figure 3). Detailed clinical information of patients with high and low ANA titers from the IVF/ICSI group has been given in Table 4.

Table 2
Anti-ENA expression in the IVF/ICSI and control groups.

	IVF/ICSI[a]	Control
ANA positive	204	30
Scl-70	7	0
PM-Scl	23	0
nRNP/Sm	9	1
SSA	18	3
Ro-52	18	1
SSB	4	0
PCNA	1	0
Jo-1	3	1
CENP-B	7	0
dsDNA	9	1
Histones	13	2
Rib-p	8	0
Unclassified ENA	96	21

Anti-ENA = antiextractable nuclear antigen; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization; Rib-p = ribosomal P-proteins.
[a] Nine anti-dsDNA and antihistone positive, two anti-SSA and anti-Ro-52 positive, and one anti-PM-scl and anti-scl-70 positive cases were included.

Impact of anti-ENAs on the outcome of the IVF/ICSI group

Out of the 15 anti-ENAs screened in this study, anti-PM-scl and anti-Scl-70 are most likely to lead to the failure of pregnancy after IVF/ICSI treatment. Unclassified anti-ENAs also influence the pregnancy rate after IVF/ICSI treatment. However, common anti-ENAs, such as anti-SSA, anti-Ro-52, and antihistone, did not influence the pregnancy rate in our cohort (Figure 4). Detailed information of the impact of anti-ENAs on the outcome of the IVF/ICSI treatment group has been provided in Table 5.

Out of the 15 anti-ENAs tested during our study, six did not hinder pregnancy after IVF/ICSI treatment. Interestingly, during the analysis of pregnancy rate after IVF/ICSI treatment, we found that the two pregnant patients tested positive for Rib-p miscarried in the first 3 months of pregnancy. Three of the patients who tested positive for Jo-1 got pregnant successfully; however, two of them miscarried early. The same trend was also found in dsDNA-positive patients. Moreover, unclassified anti-ENAs positivity was also found to impact the abortion rate, further underlining the possible impact of these unclassified ENAs on the success rate of the treatment (Figure 5). Detailed information about the impact of anti-ENAs on the outcome of pregnancy has been given in Table 6.

Discussion

In the early stages after fertilization, stability of the nucleus is the key factor in determining whether the fertilized egg would successfully develop into an embryo. In the procedure of mitosis,

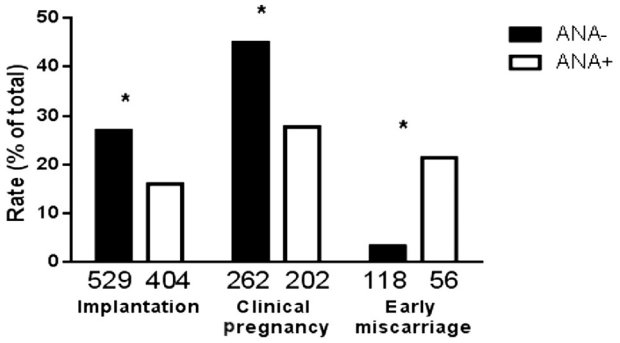

Figure 2. Pregnancy outcome in IVF/ICSI cycles. In the ANA+ group, the implantation rate (16.09% vs. 27.03%) and clinical pregnancy rate (27.72% vs. 45.03%) decreased significantly, while the early miscarriage rate (21.43% vs. 3.39%) increased in the ANA- group. *$p < 0.001$. ANA = antinuclear antibody; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization.

Table 3
Clinical information of patients in the IVF/ICSI group.

	ANA expression		*p*
	Positive	Negative	
OPU cycles	204	313	
ET cycles	202	262	
Age (y)	30.93 ± 3.09	30.69 ± 3.01	0.382
Duration of infertility (y)	4.61 ± 4.76	4.23 ± 3.87	0.315
BMI (kg/m^2)	22.82 ± 3.48	22.60 ± 3.83	0.493
bFSH (IU/L)	6.48 ± 1.77	6.40 ± 1.78	0.633
bE2 (ng/mL)	38.46 ± 19.14	41.12 ± 20.74	0.144
bLH (IU/L)	4.86 ± 2.60	4.94 ± 3.28	0.767
Antral follicles	11.05 ± 5.37	11.18 ± 4.64	0.770
Total Gn dose (U)	2487.5 ± 547.03	2303.12 ± 641.60	0.001
E2 on HCG day (ng/mL)	3586.21.75 ± 1953.25	3768.88 ± 1987.89	0.304
Retrieved oocytes	11.33 ± 5.49	12.76 ± 6.97	0.010
ICSI proportion (%)	16.67 (34/204)	17.25 (54/313)	0.862
MII oocyte proportion (%) (ICSI cycle)	85.26 (295/346)	84.72 (671/792)	0.816
2PN proportion (%)			
ICSI cycle	79.66 (235/295)	77.65 (521/671)	0.484
IVF cycle	72.43 (1424/1966)	71.76 (2298/3202)	0.606
Cleavage rate (%)	96.93 (1608/1659)	96.99 (2734/2819)	0.912
Available embryos	5.94 ± 2.88	6.16 ± 3.66	0.478
Transferred embryos/cycle	2.00 ± 0.37	2.02 ± 0.58	0.674
High-quality embryo rate overall transferred (%)	97.03 (392/404)	97.73 (517/529)	0.502
Implantation rate (%)	16.09 (65/404)	27.03 (143/529)	<0.001
Pregnancy rate (%)	37.62 (76/202)	54.97 (144/262)	<0.001
Clinical pregnancy rate (%)	27.72 (56/202)	45.03 (118/262)	<0.001
Early miscarriage rate (%)	21.43 (12/56)	3.39 (4/118)	<0.001

ANA = antinuclear antibody; bE2 = basal estradiol; bFSH = basal follicle-stimulating hormone; bLH = basal luteinizing hormone; BMI = body mass index; ET = embryo transfer; E2 = estradiol; Gn = gonadotropin; HCG = human chorionic gonadotropin; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization; OPU = oocytes were picked up; 2PN = two-pronuclear embryos.

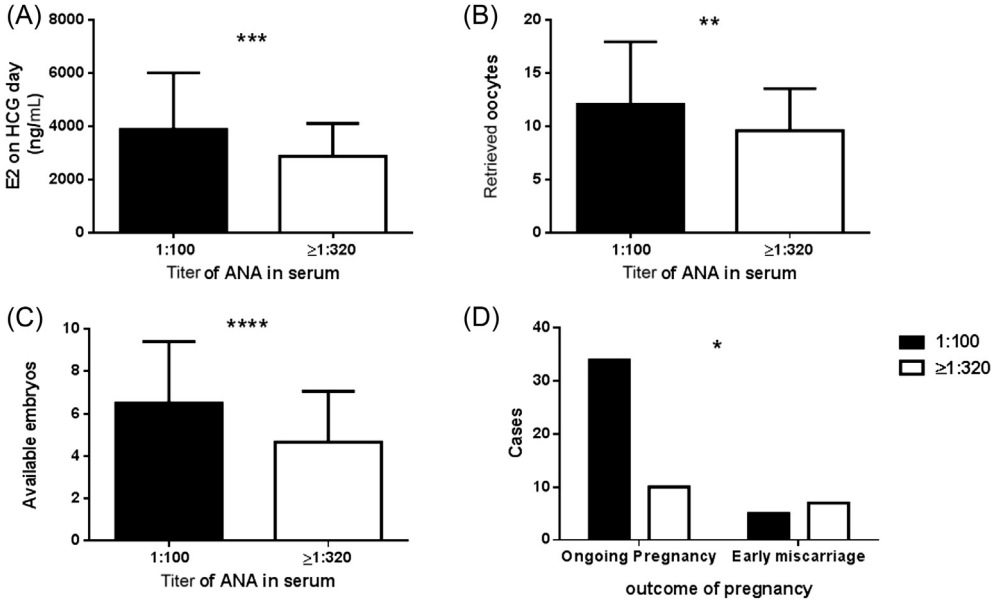

Figure 3. Impact of a high ANA titer on IVF/ICSI treatment. High titer of ANA in serum (≥1: 320) lead to lower level of E2 on HCG Day (A); lower number of retrieved level of oocytes (B); lower number of available embryos (C) and worse outcome of pregnancy (D). ANA = antinuclear antibody; E2 = estradiol; HCG = human chorionic gonadotropin; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization.

new compositions of the cells are synthesized. Some of these compositions, including proteins, polysaccharides, and glycoproteins, may get exposed at the surface of the cells. In normal situations, these compositions would not be recognized by the immune system. However, in an imbalanced immune system, these compositions may trigger the activation of autoimmunity. As a group of autoantibodies mainly associated with the compositions of nucleus, ANAs have been suspected as an important immune reason of the failure of implantation [15].

As commonly seen autoantibodies, ANAs are linked to several kinds of autoimmune diseases such as systemic lupus erythematosus and systemic sclerosis. Clinically, there are various methods for testing serum ANAs; however, IFA has generally been accepted as the most sensitive method for ANA screening [16]. In our study, we screened the participants for the presence of ANAs by IFA and further classified the targeted antigens to investigate which autoantibody was responsible for the failure of IVF/ICSI treatment.

Table 4
Clinical information in the 1:100 and ≥ 1:320 ANA titer subgroups.

	Titer of ANAs in serum		*p*
	1:100	≥1:320	
OPU cycles	142	62	
ET cycles	140	62	
Age (y)	31.19 ± 3.22	30.32 ± 2.68	0.065
Duration of infertility (y)	4.85 ± 4.36	4.10 ± 5.56	0.303
BMI (kg/m^2)	22.86 ± 3.63	22.74 ± 3.12	0.821
bFSH (IU/L)	6.61 ± 1.96	6.20 ± 1.83	0.118
bE_2 (ng/mL)	36.02 ± 14.57	41.87 ± 23.76	0.128
bLH (IU/L)	5.02 ± 2.60	4.51 ± 2.56	0.202
Antral follicles	11.23 ± 5.62	10.65 ± 4.77	0.479
Total Gn dose (U)	2495.54.60 ± 638.12	2435.71 ± 459.62	0.086
E_2 on HCG day (ng/mL)	3892.08 ± 2128.13	2885.68 ± 1229.33	<0.001
Retrieved oocytes	12.08 ± 5.89	9.63 ± 3.94	0.001
ICSI proportion (%)	17.61 (25/142)	14.52 (9/62)	0.586
MII oocyte proportion (%) (ICSI cycle)	85.45 (235/275)	84.51 (60/71)	0.841
2PN proportion (%)			
ICSI cycle	77.45 (182/235)	88.33 (53/60)	0.062
IVF cycle	72.92 (1050/1440)	71.10 (374/526)	0.426
Cleavage rate (%)	96.83 (1193/1232)	97.18 (415/427)	0.714
Available embryos	6.50 ± 2.91	4.66 ± 2.40	<0.001
Transferred embryos/cycle	2.01 ± 0.40	1.97 ± 0.31	0.414
High-quality embryo rate overall transferred (%)	97.16 (274/282)	96.72 (118/122)	0.759
Implantation rate (%)	16.67 (47/282)	14.75 (18/122)	0.631
Pregnancy rate (%)	37.14 (52/140)	38.71 (24/62)	0.832
Clinical pregnancy rate (%)	27.86 (39/140)	27.42 (17/62)	0.949
Early miscarriage rate (%)	12.82 (5/39)	36.84 (7/17)	0.043

ANA = antinuclear antibody; bE2 = basal estradiol; bFSH = basal follicle-stimulating hormone; bLH = basal luteinizing hormone; BMI = body mass index; ET = embryo transfer; E2 = estradiol; Gn = gonadotropin; HCG = human chorionic gonadotropin; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization; OPU = oocytes were picked up; 2PN = two-pronuclear embryos.

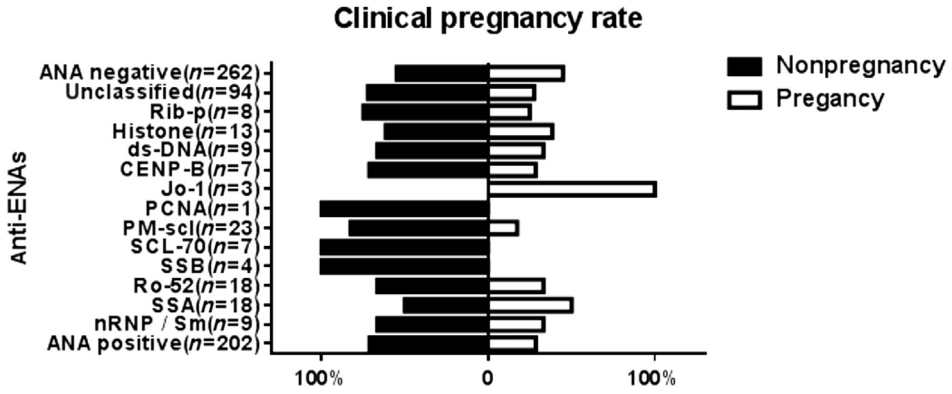

Figure 4. Impact of anti-ENAs on the pregnancy rate post IVF/ICSI treatment. ANA = antinuclear antibody; Anti-ENA = antiextractable nuclear antigen; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization; Rib-p = ribosomal P-proteins.

Few previous researches mentioned about the relationship between ANAs and infertility. It has been reported that the expression rate of ANAs in healthy controls is about 5.9–23.7% and that the expression rate varies by gender, race, area, and other factors [17–20]. In our research, the expression rate of ANAs in the control group was found to be 16.13%, which is coherent with that reported in the previous literature.

Researchers believe that the presence of ANAs acts as a risk factor for infertility and can be involved in the mechanism causing the failure of embryo implantation. Ticconi et al [14] found that about 50% of spontaneous abortion patients express ANAs, and researchers further stated that ANAs could be related to infertility, premature ovarian failure, and embryo transfer failure [11–15]. Due to the limitations of medical ethics, research work pertaining to the mechanisms underlying the association between ANAs and infertility has been difficult to pursue. Researchers can investigate only the link between different clinical phenomena and the expression of ANAs. The expression rate of ANAs in infertile women varies by researches and methods used in the research. Kikuchi et al [12] reported a 28.7% expression rate in patients who underwent IVF/ICSI treatment, as tested with IFA. In our research, a high ANA expression rate (39.45%) was found in the IVF/ICSI group, which was about 2.5 times the ANA expression rate in the control group. Interestingly, 11.99% (62/517) patients who were undergoing IVF/ICSI treatment were found to have a high ANA titer (≥1:320) in serum, while no patient with a high ANA titer in serum were found in the control group.

The presence of a high ANA titer is generally recognized as a diagnostic factor for autoimmune diseases. However, there still exist a lot of controversies about autoimmune diseases and infertility. Many researchers believe that autoimmune diseases or autoimmune phenomena have an effect on the fertility of a patient. Evidences from the previous literature showed that estrogen could also trigger autoimmune responses after binding to B lymphocytes, leading to the generation of high-affinity autoantibodies and proinflammatory cytokines [21]. Hence, the autoantibodies found

Table 5
Impact of anti-ENAs on the pregnancy rate after IVF/ICSI treatment.

	Clinical pregnancy[a]			Compared with ANA negative		
	Pregnancy	Nonpregnancy	Rate (%)	*p*	RR	OR
ANA negative	118	144	45.03	—	—	—
ANA positive	56	146	27.72	0.0001	0.6393	0.4681
Unclassified anti-ENAs	24	70	25.53	0.0009	0.5167	0.4184
Scl-70	0	7	0	0.0193	0.9536	0.0813
PM-Scl	4	19	17.39	0.0141	0.9134	0.2569
nRNP/Sm	3	6	33.33	>0.05		
SSA	9	9	50	>0.05		
Ro-52	6	12	33.33	>0.05		
SSB	0	4	0	>0.05		
PCNA	0	1	0	>0.05		
Jo-1	3	0	100	>0.05		
CENP-B	2	5	28.57	>0.05		
dsDNA	3	6	33.33	>0.05		
Histones	5	8	38.46	>0.05		
Rib-p	2	6	25.00	>0.05		

ANA = antinuclear antibody; Anti-ENA = antiextractable nuclear antigen; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization; OR = odds ratio; Rib-p = ribosomal P-proteins; RR = relative risk.
[a] Nine anti-dsDNA and antihistone positive, two anti-SSA andanti-Ro-52 positive, and one anti-PM-scl and anti-scl-70 positive cases were included.

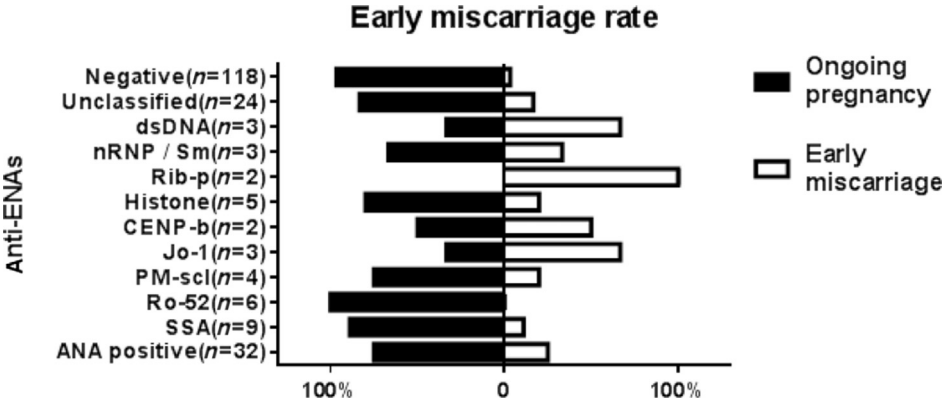

Figure 5. Impact of anti-ENAs on the outcome of pregnancy after IVF/ICSI treatment. ANA = antinuclear antibody; Anti-ENA = antiextractable nuclear antigen; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization; Rib-p = ribosomal P-proteins.

Table 6
Impact of anti-ENAs on the outcome of pregnancy after IVF/ICSI treatment.

	Outcome of pregnancy			Compared with ANA negative		
	Early miscarriage	Ongoing pregnancy	Rate (%)	*p*	RR	OR
ANA negative	4	114	3.39	—	—	—
ANA positive	12	44	21.43	0.0003	2.886	7.773
Unclassified anti-ENAs	4	20	16.67	0.0280	1.701	5.700
Jo-1	2	1	66.67	0.0061	1.487	57.00
Rib-p	2	0	100	0.0021	1.500	127.2
dsDNA	2	1	66.67	0.0061	1.487	57.00
CENP-B	1	1	50	>0.05		
Ro-52	0	6	0	>0.05		
SSA	1	8	11.11	>0.05		
PM-scl	1	3	25.00	>0.05		
histones	1	4	20	>0.05		
nRNP/Sm	1	2	33.33	>0.05		

ANA = antinuclear antibody; Anti-ENA = antiextractable nuclear antigen; ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization; OR = odds ratio; Rib-p = ribosomal P-proteins; RR = relative risk.

in the serum of infertile patients could not only indicate an imbalanced immune system, but also account for some kind of immunologic reproductive system injury. In our study, no patient was diagnosed with autoimmune diseases. However, the presence of ANA expression in patients could indicate the possibility of any sort of immune disorder in them.

Nevertheless, many ANA-positive patients still have the ability to reproduce, which adds to the controversy regarding the relationship between autoantibodies and infertility. The pregnancy rate in IVF/ICSI patients might help us reveal the effect of ANA on fertility. Our results showed statistically significant differences in clinical pregnancy and miscarriage rates while comparing ANA-positive with ANA-negative patients in the IVF/ICSI group. In our study, we found that ANA-positive patients tend to have a lower clinical pregnancy rate and a higher early miscarriage rate. These results suggest ANAs are somehow directly or indirectly related to the failure of IVF/ICSI

treatment. We also found that ANA-positive patients were required to take a higher dosage of gonadotropin while fewer oocytes were retrieved from them. In recent studies [15,22], ANAs were found to impair the maturation of oocytes and embryo cell division. Ying et al [15] revealed that ANAs affected the rates of MII oocyte production, two-pronuclear embryo production, and division, and the number of high-quality embryos and transplantable viable embryos, causing lower pregnancy and implantation rates. Our data also underlined the impact of ANAs on the outcome of IVF/ICSI treatment.

A high ANA titer did not have a similar effect on the outcome of IVF/ICSI treatment. The E2 level, retrieved oocytes, and available embryos of high-ANA-titer patients were lower than those of low-ANA-titer patients on the HCG day. A higher early miscarriage rate was found in high-ANA-titer patients. These results indicated that the level of ANAs in serum did not affect implantation, but had an adverse effect on oocytes and embryonic development. Zhu et al [22] reported that an intake of prednisone and aspirin could help ANA-positive patients improve their IVF/ICSI treatment outcome in terms of higher numbers of two-pronuclear embryos, high-quality embryos, and transplantable embryos, and a higher rate of successful transplantation. This indicates that a proper treatment targeting the imbalance of immune system could help improve the outcome of IVF/ICSI treatment in ANA-positive patients.

Few researches have mentioned about the relationship between anti-ENAs and the outcome of IVF/ICSI treatment. ANAs mainly work against different compositions of the nucleus, such as DNAs, RNAs, proteins, or compounds of these substances. It is, therefore, necessary to find out which ENA is related to the failure of IVF/ICSI treatment. Limited by the current methods of ENA test, we could only test 15 autoantibody-targeted ENAs. Anti-dsDNA is highly suspicious of pregnancy failure [23]; although the group number is small in our research, anti-dsDNA was found to be associated with an increased possibility of early miscarriage. Interestingly, anti-scl-70 and anti-PM-scl were found to be highly related to the outcome of IVF/ICSI treatment. Anti-scl-70 is also called antitopoisomerase I, and anti-PM-scl is also called antiexosome. In an early report by Li and Wang [24], mammalian DNA topoisomerase was found to be essential in early embryogenesis. Anti-scl-70 could impair the function of topoisomerase, thereby interrupting the process of early embryogenesis. Furthermore, placenta-derived exosomes were found to be important downregulators of the immune system in the endometrium, and antiexosome could further change the immune balance in the endometrium, thus disrupting implantation [25]. Both anti-scl-70 and anti-PM-scl are linked to an autoimmune disease, scleroderma [26]. Scleroderma is a rare disease, and a research in India found a high preterm birth rate, but normal miscarriage rate, in scleroderma patients [27]. However, that research mainly included pregnant women, thus being different from our study. Anti-Rib-p and anti-Jo-1 were also found to increase the possibility of early miscarriage in pregnancy. However, there is currently no further evidence supporting the negative effect of these two autoantibodies on pregnancy.

In short, ANA positivity is a risk factor accounting for the failure of IVF/ICSI treatment. Unlike other autoantibodies commonly tested before IVF/ICSI treatment, ANAs are commonly seen in women, and the results of our study pinpoint that one cannot ignore the influence of ANAs on the overall outcome of IVF/ICSI treatment. We also discovered that anti-scl-70 and anti-PM-scl could be the new therapeutic targets in the IVF/ICSI treatment plan.

Competing interests

The authors have declared that no competing interests exist.

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