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REVIEW

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Antinuclear antibodies and recurrent miscarriage: Systematic review and meta-analysis

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Abstract

Studies have investigated the relationship between antinuclear antibodies (ANA) and recurrent miscarriage (RM). The objective of this paper is to evaluate the presence of ANA as a risk factor for spontaneous abortion in patients with RM. By considering the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analysis, the authors performed systematic review and meta-analysis by searching the databases of PubMed/Medline and SCOPUS. Review Manager, Version 5.3 performed the statistical analysis. Binary variables were analyzed by odds ratio (ORs) and 95% confidence interval (CI). The subgroup analysis compared the effect of different ANA titers. The authors analyzed the ANA patterns of immunofluorescence staining. Seven case-control studies were selected. The frequency of positive ANA was statistically higher in the RM group (20.6%, 288/1400) as compared to the control group (6.7%, 72/1080). The meta-analysis of the positive ANA showed a statistical difference between the two groups (OR 3.30, 95% CI 1.41-7.73; I^2 = 87%, P = .006). Studies have revealed different frequencies of ANA patterns of immunofluorescence. This meta-analysis suggested that positive ANA might increase the risk of RM. However, it was not possible to conclude which ANA pattern of immunofluorescence staining is more frequent in the RM group.

KEYWORDS

antinuclear antibody, meta-analysis, pregnancy loss, recurrent miscarriage

1 | INTRODUCTION

According to the guidelines of World Health Organization, recurrent miscarriage (RM) is defined as the occurrence of three or more spontaneous and consecutive gestational losses in pregnancies of <20 weeks length.[1] Presently, the widely accepted definition for this obstetric condition is the occurrence of two or more consecutive pregnancy losses. It is classified as primary, when the couple has had no pregnancy that advanced further than 20 weeks, or secondary, when the couple had at least one pregnancy beyond 20 weeks.[2]

Recurrent miscarriage affects about 2%-5% of couples on their reproductive age. Its presence has seen an increase over the last decade.[3] Based on the current recommendations from the American Society for Reproductive Medicine and European Society of Human Reproduction and Embryology, it is possible to discover a cause (genetic, anatomical, hormonal, and antiphospholipid syndrome) for pregnancy loss in about half of the couples with RM, whereas it would be difficult to determine the etiological factors in the other half.[4,5]

Epidemiologic studies have indicated that the number of children among the patients with autoimmune diseases is lower than

the normal population.[6] This fact can be attributed to several factors such as psychological factors, disease-related symptoms, sexual dysfunction, anti-rheumatic drugs, and the impact of these autoimmune disorders on an individual's reproductive capacity.[7] However, based on current protocols on infertility and pregnancy losses, the antiphospholipid syndrome, an acquired thrombophilia of autoimmune origin, is the only immune disorder which is recommended to be investigated and treated.[4,5]

A breakdown of the immune self-tolerance mechanism leads to the formation of autoantibodies. The relationship between autoantibodies, with or without a systemic disease, with infertility and RM is not well established in the literature. Antithyroid antibodies (anti-thyroglobulin and anti-thyroperoxidase), antiphospholipid antibodies (anti-cardiolipin, anti-β2-glycoprotein-I, and lupus anticoagulant), antispermatozoa antibodies, anti-endomysium, anti-DNA, and antinuclear antibodies (ANA) are the most studied autoimmune markers, which are related to reproductive problems.[8,9]

Antinuclear antibodies are the autoantibodies that bind to both nuclear and cellular antigens present in the cell (DNA, RNA, proteins, and/or their complexes). In 2019, European League Against Rheumatism/American College of Rheumatology suggested that ANA assessment may be performed by immunofluorescence on HEp-2 cells or solid-phase ANA screening immunoassay with at least equivalent performance.[10] Indirect immunofluorescence is the widely used laboratory test for the detection of ANA. The result is expressed in titers, which are used to describe the antibody concentration in blood. Positive ANA expressed in low titer is commonly found in healthy women, whereas the presence of high titers (>1:160) is strongly associated with autoimmune diseases such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and Sjögren's syndrome (SjS).[11-13]

HEp-2 cells, the current substrate for the indirect immunofluorescence assay, are permeabilized and incubated with the patient's serum. After incubation with anti-human antibodies conjugated with fluorescent molecules, antibodies bound to the intranuclear antigens are envisaged. Thereafter, different staining patterns can be observed: homogeneous, speckled, nucleolar, nuclear membranous, centromeric, and nuclear. These patterns bear relation with the ANA subtypes and specific autoimmune diseases.[11,13]

The pathophysiological process responsible for pregnancy loss in women with a history of RM and positive ANA is still unknown. However, some studies have proposed that the poor quality of oocytes, changes in embryonic development, and changes in the pattern of uterine blood flow are some of the possible mechanisms.[14-16] Positive ANA has also been associated with a worse prognosis in couples with RM, who were treated with immunotherapy.[17,18] Inflammation at the embryonic implantation site is a possible mechanism of pregnancy loss in ANA-positive RM patients. Complement activation is one inflammatory pathway involved in this situation. Veglia et al[19] observed higher C3 activation with increased C3 deposition and immune complexes in placental tissue in a study of pregnant mice receiving immunoglobulin G (IgG) obtained from RM patients compared with a group treated with ANA IgG from women with at least two previous uncomplicated pregnancies.

Therefore, by taking the possible association between autoantibodies and RM, along with large number of couples with RM of undetermined etiology, into account, it is important to evaluate whether ANA can be considered as a biomarker for a miscarriage of immunological origin. The objective of this paper (which is both systematic review and meta-analysis) is to assess whether ANA is a risk factor for spontaneous abortion among women with a history of RM.

2 | METHODS

By complying with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis[20] and, later, searching for relevant studies published in the medical literature up to the time of this research, the authors of this paper (both systematic review and meta-analysis) evaluated the frequency of ANA in couples with a history of RM.

2.1 | Search strategy

The population selected for this review was of non-pregnant women with a history of RM. The control group was non-pregnant women with no history of pregnancy loss. Up to September 10, 2019, the authors searched the following keywords in the databases of PubMed/Medline and Scopus: “recurrent miscarriage,” “antinuclear antibodies,” “ANA,” and “meta-analysis.” Data related to the ANA titers and microscopic patterns were also analyzed. After this initial searching, it was also performed a new search in the EMBASE and Cochrane Library, but there were no additional studies to those already selected for review and meta-analysis.

2.2 | Selection (inclusion and exclusion) criteria

Two independent reviewers assessed the titles and abstracts of the identified studies (MBC and CTMBC). Thereafter, selected studies were thoroughly and completely read in order to decide about their inclusion or exclusion from this review. The randomized controlled trials and cohort or case-control studies that reported the relationship between ANA and RM were included in this review. Other studies such as reviews, case reports, experimental animal studies, letters to the editor, editorials, and book chapters were excluded. Studies involving patients with a history of two or more unexplained gestational losses were also included. However, cases that evaluated the presence of ANA in women during a pregnancy were excluded.

2.3 | Quality assessment

All the manuscripts conforming to the selection criteria were assessed for their methodological quality. The quality of case-control studies included in this meta-analysis was assessed by the Newcastle-Ottawa scale (NOS). According to NOS, each study can be awarded a maximum of one star for each numbered item within the selection (maximum of four stars) and exposure categories (maximum of three stars), and a maximum of two stars can be given for comparability. The maximum score was nine stars, and adequately qualifying studies were rated with five stars.[21]

2.4 | Statistical analysis

The Review Manager (RevMan) software, Version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration) performed the statistical analysis in this study.[22] Binary variables were analyzed by odds ratio (OR) and 95% confidence interval (CI). Heterogeneity was quantified by the I^2 statistic, with values >50% considered to represent a substantial heterogeneity. A random-effects model was used when I^2 was >50%; otherwise, the fixed effects model was employed. Subgroup analyses compared the effect of different ANA titers (≥1:80 and ≥1:160). $P < .05$ was considered to be statistically significant.

3 | RESULTS

The electronic search resulted in 109 and 67 records in PubMed/Medline and Scopus, respectively. In total, 176 studies were downloaded to the EndNote Library Program, and duplicate records were excluded (19 duplicate records), thereby obtaining a total of 157 studies. After the initial evaluation of titles and abstracts, 21 studies were excluded for language and 121 for other reasons (review articles, case reports, letters, animal research), and 15 publications were selected for a full-text assessment. Finally, seven case-control studies were selected for meta-analysis[23-29] (Figure 1).

The characteristics of included studies are presented in Table 1. They were published from 1989 to 2014 in five different countries (the United States of America, Colombia, Argentina, Italy, and Iran). Four studies considered RM as the occurrence of two or more miscarriages, and three other studies considered RM to constitute three or more consecutive pregnancy losses. The RM

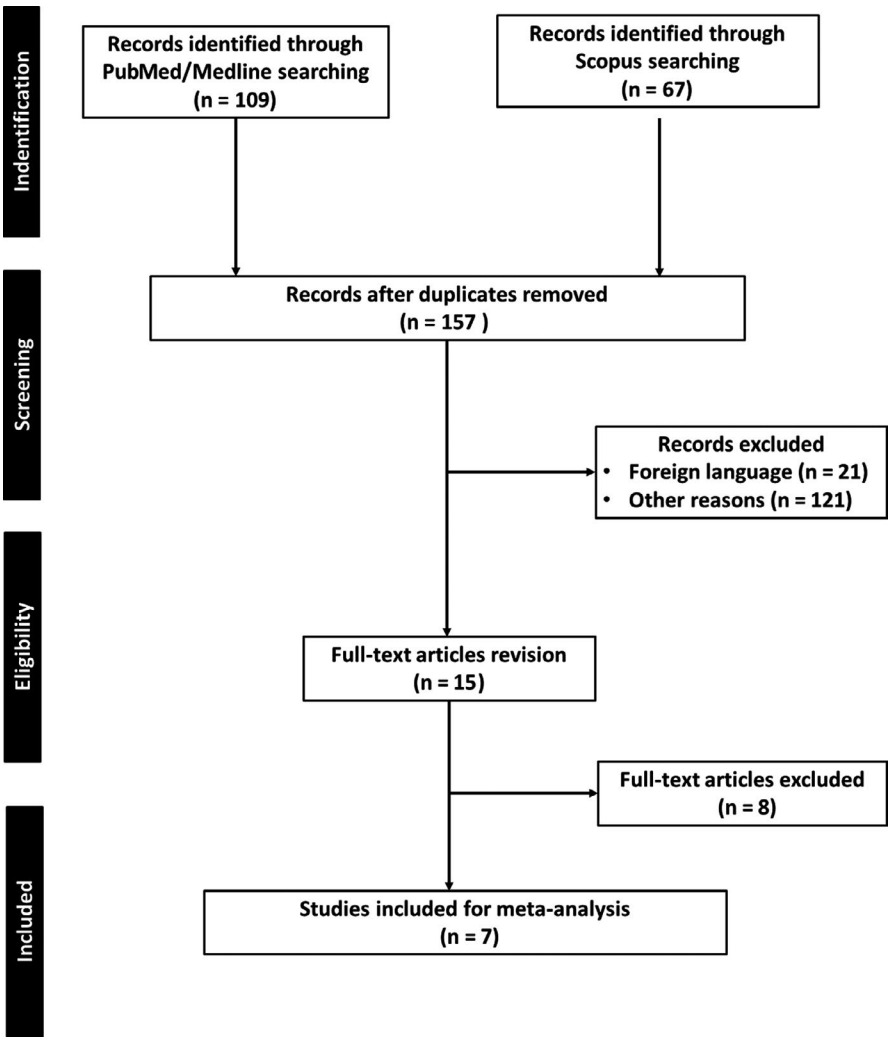

FIGURE 1 Flowchart of selection process

TABLE 1 Characteristics of the included studies

Study	Country	Design	Population	Detection method	ANA cut-off	NOS scores
Harger, 1989	United States of America	CCS	Cases: 277 non-pregnant RM patients[a] Control: 119 non-pregnant and 299 pregnant controls	Indirect immunofluorescence	≥1:40	6
Xu, 1990	United States of America	CCS	Cases: 30 explained and 30 unexplained RM patients[b] Control: 61 non-pregnant and 61 pregnant controls	Indirect immunofluorescence	≥1:40	5
Kwak, 1992	United States of America	CCS	Cases: 153 unexplained RM patients[c] Control: 90 non-pregnant controls	Indirect immunofluorescence	≥1:40	6
Ruiz, 1995	Colombia	CCS	Cases: 68 non-pregnant and 25 pregnant unexplained RM patients[d] Control: 25 non-pregnant and 31 pregnant controls	Indirect immunofluorescence	≥1:40	5
Bustos, 2006	Argentina	CCS	Cases: 118 non-pregnant unexplained RM patients[d] Control: 125 non-pregnant controls	Indirect immunofluorescence	≥1:20	6
Ticconi, 2010	Italy	CCS	Cases: 194 non-pregnant RM patients[a] Control: 100 non-pregnant controls	Indirect immunofluorescence	≥1:80	7
Molazadeh, 2014	Iran	CCS	Cases: 560 non-pregnant unexplained RM patients[e] Control: 560 non-pregnant controls	Indirect immunofluorescence	≥1:40	6

Abbreviations: ANA, antinuclear antibodies; CCS, Case-Control Study; NOS, Newcastle-Ottawa Scale; RM, Recurrent Miscarriage.

[a]Two or more pregnancy losses before 24 wk of gestation.

[b]Two or more pregnancy losses, gestational age was not specified.

[c]Three or more pregnancy losses before 24 wk of gestation.

[d]Three or more pregnancy losses, gestational age was not specified.

[e]Two or more consecutive unexplained RM in the first trimester.

group had a history of gestational loss <24 weeks and an unknown etiology. The detection of ANA in all seven studies was performed by indirect immunofluorescence assay. In five studies, ANA was found to be positive with titers ≥1:40, one with titer ≥1:20, and one with titer ≥1:80. It was possible to identify the ANA patterns of immunofluorescence staining in five of the seven studies (Table 1).

The NOS scores for observational studies are presented in Table 1. The studies showed an overall variable risk of bias. The median NOS score of included studies was 6 (range 5-7), and the score of each study is also presented in Table 1. One study scored a total of seven stars, four studies scored six stars, and two studies scored five stars.

The frequency of positive ANA, including all study cases, was statistically higher in the patient group (20.6%, 288/1400) as compared to the control group (6.7%, 72/1080), ranging from 13.2% to 50% between patients with a history of RM and 0.9% to 16% in the control group. The meta-analysis of the positive ANA showed a statistical difference between the two groups (OR 3.30, 95% CI 1.41-7.73; I^2 = 87%, P = .006; Figure 2).

Subgroup analysis based on different cut-off titers revealed a greater difference in the frequency of positive ANA between patient and control groups as there was a change in the highest value of titer. Among the RM group, 24.6% (116/471) had positive ANA with titer ≥80, whereas 7.8% (17/219) of the control group showed a statistically significant difference (OR 5.63, 95% CI 3.14-10.08; I^2 = 0%, P = .00001; Figure 3). Positive ANA with titer ≥1:160 was observed in 6.8% of patients, not occurring in any of the 660 people in the control group (OR 40.28, 95% CI 5.51-294.23; I^2 = 0%, P = .0003; Figure 4).

The ANA pattern of immunofluorescence staining frequency in patients with RM was different among the five included studies. The homogeneous pattern was more frequent (80% of positive ANA results) in a study of Argentine women with a history of three or more spontaneous abortions and less frequent (10.4%) in the study of Kwak et al, which evaluated American women with three or more gestational losses earlier than 24 weeks. The speckled pattern was more prevalent in the study of Kwak et al (89.6%) and less frequent in the study of Bustos et al (15%) (Table 2).

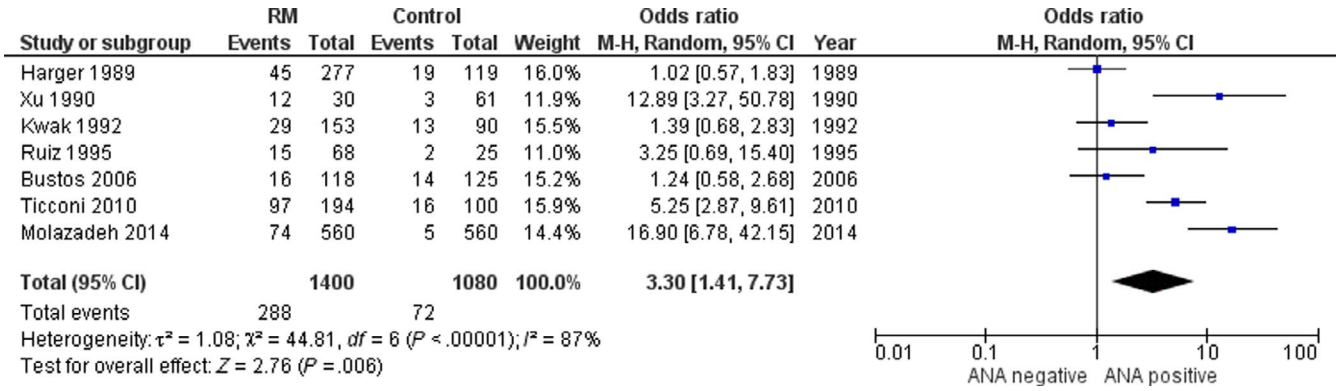

FIGURE 2 Miscarriage risk in all included studies

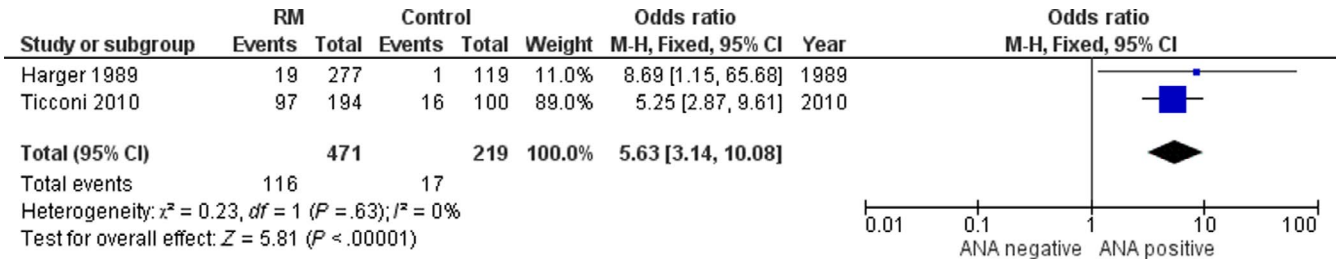

FIGURE 3 Miscarriage risk considering positive ANA ≥ 1:80

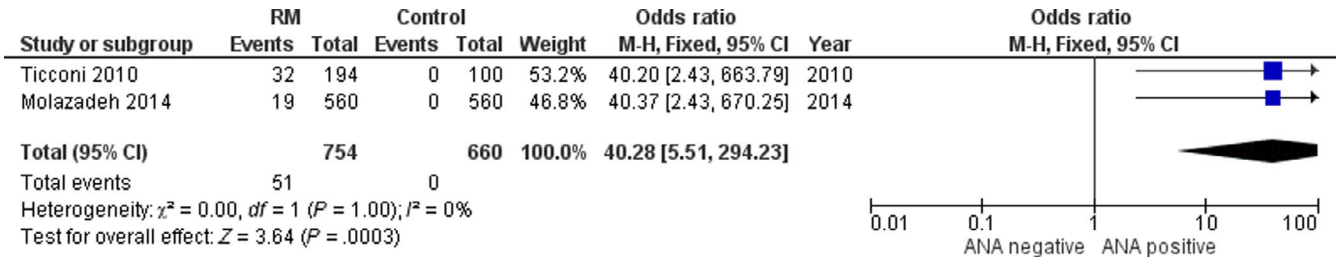

FIGURE 4 Miscarriage risk considering positive ANA ≥ 1:160

TABLE 2 Frequency of ANA pattern of immunofluorescence staining

	Recurrent miscarriages					
Study	Homogeneous (%)	Nucleolar (%)	Speckled (%)	Centromeric (%)	Peripheral (%)	Other (%)
Harger, 1989	31.1	4.4	64.5	NA	NA	NA
Kwak, 1992	10.4	0	89.6	NA	NA	NA
Bustos, 2006	80	NA	15	NA	NA	5
Ticconi, 2010	36	6	56	2	NA	NA
Molazadeh, 2014	47.3	10.8	32.4	4	5.4	NA

Note: Harger (1989): the authors described the frequency of three patterns of immunofluorescence staining in RM group, did not describe the frequency of patterns in non-pregnant control. Kwak (1992): the authors described the frequency of three patterns of immunofluorescence staining in RM group, did not describe the frequency of patterns in control group. Bustos (2006): the patterns of immunofluorescence staining were similar among patients. Ticconi (2010): no significant differences were detected between RM and control women in the available immunofluorescence staining patterns observed ($\chi^2 = 4.91$, not significant). Molazadeh (2014): the authors described the frequency of five patterns of immunofluorescence staining in RM group, did not describe the frequency of patterns in control group.

Abbreviation: NA, not available.

4 | DISCUSSION

Population studies have shown a decrease in the reproductive capacity in women after the diagnosis of autoimmune diseases, with a reduction of 12%-16% in the birth rate as compared to healthy women.[6] The reduced fertility of these women is due to side effects of medications, psychological factors, complications of these diseases, and the direct effect of autoimmune disorders on reproductive organs.[7]

Several immunological markers have been proposed to identify the patients with or without autoimmune diseases and their respective risks of both reproductive failures in in vitro fertilization (IVF) cycles and gestational loss. Traditionally, ANA has been used as a biomarker for autoimmune disease screening such as SLE, SSc, polymyositis, dermatomyositis, and SjS. Positive ANA is also associated with other conditions such as cancer, neurological diseases, cardiovascular diseases, medication use, endometriosis, and infertility.[30] In cases of SLE, ANA has a sensitivity of 93%-100% and a specificity of 57%. In patients with SLE, the determination of a specific antibody increases this specificity and may reach to about 98% in cases of positive anti-Sm.[30]

In 1972, Abrahams et al[31] first described the relationship between ANA and RM. Over the past decades, ANA has been studied as a biomarker for the autoimmune causes of RM. Antithyroid and antiphospholipids antibodies are other autoantibodies strongly related to RM.[3,32] The relationship between positive ANA and female reproductive performance has been described by several authors.[14,33-35] In a recent meta-analysis, Simopoulou et al observed that patients undergoing IVF with positive ANA showed lower rates of clinical pregnancy and birth along with higher rates of abortion. In the same study, the presence of antithyroid antibodies or antiphospholipid was not associated with a worse treatment outcome.[8]

The major difficulty in defining ANA as an important biomarker for these conditions is the high prevalence of this antibody in healthy populations; therefore, it is a very sensitive but unspecific marker. The presence of ANA in healthy individuals is affected by ethnicity, gender, and age. Satoh et al observed that the prevalence of positive ANA in a sample of the healthy American population was 13.8% (considering a cut-off ≥1:80), which was twice more common among women (women: 17.8% and men: 9.6%, $P < .001$; OR 2.02, 95% CI 1.57-2.60). This female-to-male positive ANA ratio can vary up to 4:1. This higher prevalence among women is believed to be due to elevated levels of estrogen and progesterone.[36]

Satoh et al also observed a directly proportional relationship between positive ANA and age (in both genders), ranging from 11.2% in the age group of 12-19 years to 19.2% in individuals aged 70 years and over. The prevalence of ANA was higher among non-Hispanic black people than those of other race/ethnic groups. Education, family income, alcohol use, smoking history, serum levels of cotinine, weight, or C-reactive protein has been not associated with ANA levels.[36]

Other population studies that evaluated ANA levels in healthy individuals corroborated the results presented by Satoh et al Differences were noticed in the positivity of ANA levels in healthy populations, which ranged from 5.92% in Chinese to 30.8% among African Americans. However, these studies used different reference values. Regardless of ethnicity, the number of positive ANA cases in healthy individuals was four times higher in women as compared to men.[36]

Racoubian et al observed a progressive annual increase in the prevalence of positive ANA in healthy individuals between 2008 and 2015. The authors attributed this elevation to a greater exposure to allergens and pollutants, as well as a greater sensitivity toward diagnostic tests. Racoubian et al and other authors also observed that the presence of positive ANA results in healthy populations occurs at low titrations. Racoubian et al[37] also described a directly proportional relationship between the age of healthy individuals and the number of cases with positive ANA, ranging from 31.6% positive ANA in women under 20 to 43.5% in those individuals over 70 years.

Among women, the history of at least one live child seems to increase the frequency of positive ANA among healthy women of

Control					
Homogeneous (%)	Nucleolar (%)	Speckled (%)	Centromeric (%)	Peripheral (%)	Other (%)
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
70	NA	12	NA	NA	18
7.1	7.1	85.7	0	NA	NA
NA	NA	NA	NA	NA	NA

childbearing age. However, this increase in the positive ANA cases was not related to the birth rate. High levels of estrogen, progesterone, and prolactin, as well as the presence of fetal cells in the maternal circulation (micro-chimerism) may justify this relationship between parity and positive ANA levels. On the other hand, in women with a history of RM, the increased number of gestational losses does not appear to increase the risk of having a positive ANA test.[36]

The results of our meta-analysis indicated that the frequency of positive ANA in the control population was 6.7% (72/1080) as compared to 20.6% (288/1400) in the RM group. The study with the highest number of individuals in the control group in this present meta-analysis was conducted in a population of Iranian women, with a frequency of positive ANA in the control population of 0.9%, thereby suggesting that the ethnic factor may have contributed to this low frequency.[29] Annual analysis of the included studies did not reveal a clear increase in the prevalence of positive ANA over the years in any group (RM or control).

Four of the studies included in this meta-analysis did not show a significant difference in the frequency of ANA between RM and control groups.[23,25-27] Harger et al considered a positive ANA with titer of ≥1:40 and did not find any difference between the groups (RM: 16.3% vs control: 16.6%, P = NS). However, when using a cut-off titer value of ≥1:80, they described a statistical difference between RM and control groups (6.9% vs 0.8%, $P < .0001$).[23] The other three studies that did not demonstrate a higher frequency of positive ANA in the RM group have not performed an assessment on different titers.[25-27] Three studies found a higher frequency of positive ANA in the RM group.[24,28,29] Interestingly, Xu et al[24] observed that positive ANA was more frequent in patients with RM in both conditions, known and unknown etiology. The two studies of the last decade, including one that had a greater participation in the meta-analysis, observed a higher frequency of positive ANA in the group of women with RM.[28,29] The association between ANA and recurrent miscarriage was more evident at higher serum levels (titer ≥ 1:80 [OR 5.63, 95% CI 3.14-10.08] vs titer ≥ 1:160 [OR 40.28, 95% CI 5.51-294.23]). Therefore, further studies are needed to identify the best cut-off in patients with positive ANA and a history of recurrent abortion.

The IIFA on HEp-2 cells (ANA test) is a gold standard screening test for the diagnosis of autoimmune conditions.[13] Investigation of specific autoantibodies should be performed with a positive ANA, which are strongly associated with some autoimmune diseases. Only three of the seven studies included in this review investigated the presence of specific autoantibodies.[23,25,28] Harger et al[23] observed no positive anti-dsDNA (anti-double-stranded DNA), anti-SSA (RO), and anti-SSB (LA) tests among women with a history of RM and positive ANA. Kwak et al[25] observed that anti-dsDNA and anti-ss-DNA (anti-single-stranded DNA) were more prevalent in RM group when compared to controls. Ticconi et al[28] detected the presence of other autoantibodies (antithyroid antibodies; lupus anticoagulant; anti-cardiolipin antibodies; anti-mitochondrial antibodies; anti-smooth muscle; anti-Annexin V; anti-beta2-glycoprotein-I; anti-ds-DNA) in 53.6% of patients with RM and positive ANA, with antithyroid antibodies being the most frequent. Therefore, the association of positive ANA and other autoantibodies suggests that breaking the mechanism of immune self-tolerance may play an important role in the pathophysiology of cases of unknown RM.

The ANA staining pattern is associated with specific autoantibodies as it may be helpful in the diagnosis of rheumatologic diseases.[11,13] There are few studies that evaluated the relationship between specific autoantibodies with RM and other reproductive conditions. Fan et al[16] observed that women with positive anti-dsDNA undergoing IVF treatment had fewer aspirated oocytes, fewer good quality embryos, lower fertilization, implantation and clinical pregnancy rates, and a higher miscarriage rate as compared to the women with negative anti-dsDNA controls.

Five out of seven studies included in this meta-analysis were related the frequency of ANA staining pattern in the RM group; however, there were insufficient information regarding the relationship between ANA and other specific autoantibodies.[23,25,27-29] The speckled pattern was more frequent in three out of five studies,[23,25,28]

and the homogeneous pattern was more frequent in the other two studies.[27,29]

Therefore, ANA is a vital biomarker for the screening of autoimmune factors related to systemic diseases. Its clinical application in patients with RM still shows scarce evidence. There is a need for further research into the evaluation of the best cut-off titers and determination of specific autoantibodies related to reproductive disorders.

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