THE ROLE OF ANTI-NUCLEAR ANTIBODIES IN RECURRENT ABORTION OF IMMUNOLOGICAL ORIGIN

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ABSTRACT

Recurrent miscarriage (RM) is a condition that occurs when three otherwise more independent & consecutive miscarriages happen throughout the first twenty weeks of pregnancy. Although the pathogenesis of recurrent pregnancy loss (RPL) differs in reliance on the gestational age & maternal age, multiple mechanisms may ultimately converge on a common pathway that initiates the loss of pregnancy. Frequent mechanisms encompass chromosomal errors occurring in the fetus-maternal interface &collapse of the aforementioned junction, both of which lead to hemorrhaging, cramping, and miscarriage. Several risk factors, including structural uterine abnormalities & autoimmune disorders, have been linked to recurrent pregnancy loss; however, the reasons why they affect only a subset of pregnancies and not all remain unknown. In more than fifty percent of women, no risk factors for pregnancy loss are identified. Antinuclear antibodies (ANA) are autoantibodies that target substances contained within the cellular nucleus. ANAs are generally divided into two categories: those that target nuclear material, and those that target DNA and histones. Antibodies specific to DNA and histones consist of anti-dsDNA & anti-histone antibodies, respectively. The remaining category comprises an additional nuclear antigen that is targeted. The anti-Smith antibody was the initial one to be identified within this particular category. In laboratories, indirect immunofluorescence is the most prevalent method for ANA detection. The outcome is quantified in titers, a unit intended to denote the concentration of the antibody in the blood. A low titer of positive ANA is frequently detected in women who are in good health; conversely, the presence of high titers (>1:160) is significantly correlated with autoimmune disorders.

Key words: RPL, ANA, Polycystic ovarian syndrome

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INTRODUCTION

Self-reporting (diuretic symptoms & home pregnancy testing) & clinical testing—including transvaginal ultrasonography, histopathology, & declining serum human chorionic gonadotrophin (hCG) levels—are utilized to diagnose pregnancy loss. Given that the fundamental cause of recurrent pregnancy loss can vary with gestational age, it is advisable to differentiate among embryonic, fetal, & biochemical losses. Nevertheless, the prognostic significance of categorizing first-trimester pregnancy losses by gestational age remains ambiguous, leading to its frequent omission from research reports. Pregnancy loss during the first and second trimesters is frequently differentiated in clinical practice. [1].

Recurrent miscarriage impacts an estimated between two and five percent of couples during their fruitful years. Its prevalence has experienced a surge in the past decade. [2].

In accordance with the prevailing guidelines set forth by the European Society of Human Reproduction & Embryology & the American Society for Reproductive Medicine, it is feasible to ascertain the underlying factors contributing to pregnancy loss in approximately half of the couples afflicted with RM (genetic, anatomical, hormonal, & antiphospholipid syndrome). However, in the remaining half, such identification would prove challenging. [1,3].

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The purpose of this investigation was to evaluate the role of ANA in repeated miscarriages of immunologic origin in Egyptian pregnant females.

Recurrent pregnancy loss

Lost pregnancy refers to any miscarriage that occurs naturally prior to the embryo attaining viability. It includes all miscarriages that occur during the 20th to 24th week of the gestation period. In contrast, the phrase "miscarriage" refers to the confirmed loss of an intrauterine pregnancy prior to twenty to twenty-four weeks of gestation via ultrasonography or histology [3]. Recurrent pregnancy loss is defined by the American Society for Reproductive Medicine (ASRM) & the European Society of Human Reproduction & Embryology (ESHRE) as the unsuccessful outcome of two or more clinically recognized pregnancies. [4].

Epidemiology

On the basis of data from large-scale research projects conducted in Europe & the United States, it is estimated that one percent to four percent of all women who become pregnant experience recurrent pregnancy loss on average. [5].

Importantly, subsequent live birth rates are high for the majority of women who experience recurrent pregnancy loss, regardless of medical intervention. A prospective cohort investigation revealed that 66.7 percent of women who were referred to a specialist clinic for recurrent pregnancy loss achieve a live birth within five years. It is worth noting that this estimate incorporates couples who subsequently forsake any subsequent attempts at conceiving, potentially leading to an underestimation. Additionally, the probability of a live birth is influenced by the existence of fertility-related risk factors, including uterine abnormalities and ovulation errors. [6].

Etiology

Genetic Etiologies

Repeated abortions is often accompanied by embryonic aneuploidy as the underlying reason for the pregnancy loss. [7]. According to traditional genetic analyses, as karyotyping, women who experience recurrent pregnancy loss exhibit a comparatively lower incidence of abnormalities of chromosomes in comparison to those who experience sporadic pregnancy loss. [8,9].

Meiotic non-disjunction, which refers to the inability of chromosomes to separate & results in such conditions as trisomy & monosomy, & structural chromosomal abnormalities like balancing translocations otherwise inversions, are the prevailing causes of embryonic chromosomal defects. [10].

Comparable numbers of distinct types of cytogenetic abnormalities are observed in cases of recurrent & sporadic miscarriage. Aneuploid embryos that were miscarried are less prevalent in women with six or more previous miscarriages compared to those with two or three prior miscarriages (60.9% vs. 24.4%). This finding indicates that the likelihood of experiencing a euploid loss escalates as the number of previous miscarriages elevates. In a large retrospective cohort research, the adjusted proportion of miscarriages involving chromosome abnormalities was greatest among nine & fourteen weeks of gestation. [11,12] A smaller investigation which exclusively focused on women who experienced recurrent pregnancy loss reached the same conclusion, indicating that the occurrence of aneuploidy in losses occurring after thirteen weeks of gestation is less frequent. [12].

Anatomic Etiologies

Between ten fifteen percent of instances of RPL are attributed to anatomic abnormalities, which are commonly believed to induce miscarriage by disrupting the endometrial vascular system, thereby causing aberrant & insufficient placentation. Therefore, abnormalities that have the potential to disrupt the endometrium's vascular supply are considered potential etiological factors for RPL. Congenital uterine anomalies, intrauterine adhesions, & uterine fibroids or polyps are examples of such conditions. While RPL is more commonly linked to complications during the second trimester otherwise premature labor, congenital uterine anomalies do also contribute to this condition. The uterine septum is the congenital

uterine anomaly most strongly associated with RPL, with affected individuals carrying a sevevty six percent risk of spontaneous pregnancy loss. [13].

Endocrine Etiologies

Approximately seventeen percent to twenty percent of RPL cases are associated with endocrinologic illnesses, including luteal phase defect , polycystic ovarian syndrome (PCOS), DM, thyroid illness, &hyperprolactinemia. [14].

The measurement of thyroid-stimulating hormone (TSH) levels should be incorporated into the assessment of endocrine illnesses. In rare instances, additional diagnostic evaluations such as luteal phase endometrial biopsies may be warranted in response to the the individual's presentation; these involve antithyroid antibody testing, insulin resistance testing, ovarian reserve testing, & serum prolactin testing because of the existence of irregular menstrual cycles. Recently, there has been an increase in the use of insulin-sensitizing agents to treat RPL present in conjunction with PCOS. [15].

Infectious Etiologies

A number of infections have been identified as potential contributors to sporadic spontaneous pregnancy loss, as rubella, Listeria monocytogenes, Toxoplasma gondii, herpes simplex virus (HSV), measles, cytomegalovirus, and coxsackieviruses. A proposed incidence of 0.5 percent to five percent indicates that the function of infectious substances in repeated loss is less clear. [14].

Infectious reasons for pregnancy loss may be attributed to the following mechanisms: direct infection of the uterus, fetus, or placenta; chronic endometritis or endocervicitis; amnionitis; otherwise an intrauterine device that is infected. Due to the fact that the majority of these incidents are isolated occurrences, the function of infections as a causal factor in RPL appears to be limited. Specific infections, including ureaplasma, mycoplasma, L monocytogenes, Chlamydia trachomatis, & HSV, are hypothesized to be involved in RPL. In patients with compromised immune systems, chronic infection poses the greatest risk for RPL. [15].

Immunologic Etiologies

Given the lack of genetic similarity between the fetus and its mother, it is logical to deduce that certain immunological processes must take place for the mother to sustain pregnancy without experiencing fetal rejecting. In fact, at least ten such mechanisms have been suggested. [16].

Particular consideration must be given to antiphospholipid syndrome (APS), a distinct autoimmune disease that has been unequivocally associated with numerous adverse obstetric results, involving RPL. A prevalence of between three and five percent in the general population makes APS the most commonly acquired risk factor for thrombophilias; therefore, it could also be discussed in the context of thrombophilias. The APS diagnostic criteria consist of a minimum of one clinical criterion & one laboratory criterion. [17].

Thrombotic Etiologies

Thromophilias, whether inherited otherwise acquired in combination, are prevalent. It is estimated that over fifteen percent of the white population possesses an inherited thrombophilic mutation. The prevailing mutations include those in the promoter region of the prothrombin gene, the gene encoding methylene tetrahydrofolate reductase (MTHFR), & factor V Leiden. Mild thrombotic hazards are associated with these prevalent mutations, & the extent to which homozygous MTHFR mutations contribute to vascular disease remains debatable. Conversely, antithrombin & protein S deficiencies, which are more severe thrombophilic deficiencies, are considerably less prevalent among the general populace. [18].

As soon as hereditary or acquired thrombophilia is identified, the appropriate treatment regimen should be initiated. Supplemental folic acid is prescribed for individuals with hyperhomocysteinemia; prophylactic anticoagulation is utilized to treat isolated errors when there is no personal otherwise familial history of thrombotic complications; & therapeutic anticoagulation is employed to treat combined thrombophilic defects. After initial treatment, homocysteine levels should be reassessed, & prophylactic anticoagulation should be considered if hyperhomocysteinemia does not respond to dietary intervention. [19].

Environmental Etiologies

Individuals are frequently especially anxious regarding the potential causation of their miscarriages of pregnancy by environmental exposures, due to the tendency for such thoughts to elicit emotions of responsibility & remorse. There have been suggestions of associations among sporadic & /otherwise RPL & occupational & environmental exposures to toxins, pharmaceuticals, ionizing radiation, & organic solvents. However, it is challenging to establish definitive conclusions from the studies that have been conducted due to their retrospective nature & potential confounding effects from alternative otherwise additional environmental exposures. [14,18].

3 specific exposures—namely, caffeine, alcohol, & smoking—have garnered considerable attention & warrant special scrutiny due to their pervasive utilization & modifiable characteristics. While there is consistent evidence linking maternal alcoholism (otherwise frequent intoxicating alcohol consumption) to increased rates of spontaneous pregnancy loss, the relationship between moderate alcohol consumption & such outcomes continues uncertain. [15].

Defective sperm

Three specific exposures—namely, alcohol,caffeine, Divergent opinions exist regarding as to whether sperm abnormalities are a factor in recurrent miscarriages. Recurrent miscarriages of the sperm may be influenced by epigenetic modifications of the sperm DNA [21] & enhanced DNA fragmentation in the sperm [22, 23].

Obesity

Three specific exposures—namely, alcohol,caffeine, An autonomous determinant of the likelihood of subsequent miscarriage is obesity (BMI 30 kg/m2) [24]. In women who are overweight, the substantial connection among obesity & repeated miscarriage during pregnancy (OR 1.75, 95% CI 1.24–2.47) is not observed [25]. Recurrent pregnancy loss increases the risk of euploid miscarriages in women with obesity relative to those without obesity [26].

Obesity is correlated with a number of endocrine diseases such as polycystic ovary syndrome, hypothyroidism, &DM. Nevertheless, as stated earlier, the individual associations of these comorbidities with recurrent pregnancy loss are not statistically significant [27,28].

Vitamin D deficiency

Three specific exposures—namely, caffeine,alcohol, The available clinical data is inadequate to establish a correlation among recurrent pregnancy loss & vitamin D deficiency [29]. A common treatment for vitamin D deficiency among women attempting to conceive [30] is due to its modest but significant impact on fertility & live birth rates [31].

While the precise mechanism through which vitamin D deficiency may be associated with miscarriage remains unknown, there is speculation that an increase in autoantibody concentrations, such as anti-phospholipid & anti-thyroid antibodies, may be the cause [32].

Antiphospholipid Syndrome

Long correlated with RPL, antiphospholipid syndrome (APS) is distinguished by the existence of antiphospholipid antibodies (aPL). In fact, pregnancy morbidity and vascular thrombosis are the two clinical criteria necessary to confirm the diagnosis of APS. [33].

Primary APS is observed in individuals who do not have any underlying illnesses, whereas secondary APS is associated with other conditions. Multiple hypotheses have been put forth regarding the mechanisms by which APL are correlated with additional complications of obstetrics, including preeclampsia, intrauterine growth restriction, & prematurity [33]. APL specifically targets the trophoblast, resulting in compromised trophoblastic invasion & aberrant secretion of human chorionic gonadotropin and growth factor. Additionally, it triggers syncytiotrophoblast apoptosis & an inflammatory response at the interface of the mother & fetus via complement activation [34].

Pathophysiology

RPL is a condition that can arise from a variety of factors, including immunologic, genetic, anatomical, endocrine, & antiphospholipid antibody syndrome influences. FOXD1 mutations are crucial in the development of RPL. FOXD1 has been identified as a crucial molecule that regulates endometrial & placental genes to facilitate embryo implantation in both mice & humans. FOXD1

mutations in the human species have been linked functionally to the origin of RPL. [35].

History and Physical

An exhaustive & comprehensive medical history should be obtained, encompassing every aspect of prior miscarriages. It is crucial to know the gestational age at which the prior pregnancy loss took place, as RPL typically occurs at a comparable gestational age in subsequent pregnancies. It is also crucial to be aware of the treatment method utilized for previous pregnancy losses, as curettage & dilation may heighten the likelihood of developing Asherman syndrome otherwise cervical incompetence, both of which predispose to RPL. [36].

Diagnosis of recurrent pregnancy loss

A comprehensive evaluation of couples with RPL should comprise the subsequent components:

Assessment of Medical Problems

In order to rule out thyroid issues, diabetes, & hyperprolactinemia, further research is required. [37].

Genetic Evaluation

It is necessary to conduct Karyotype assessments on the couples in order to identify potential Robertsonian translocations, mosaicism, reciprocal, balanced, otherwise reciprocal translocations that could be transferred to the embryo and result in RPL. Although these tests are costly and have a low yield, karyotype evaluation of the couples with RPL [38] is a viable option.

Assessment of the Uterine Anomalies

Numerous modalities exist for the identification of congenitally occurring & acquired uterine anomalies, with the subsequent being among the most useful [36]: MRI, saline infusion sonohysterography, pelvic ultrasound, hysterosalpingogram, & hysteroscopy are all extremely useful for detecting congenital abnormalities in the uterus.

Immunologic Work Up

Antiphospholipid antibody syndrome necessitates the implementation of studies. It is recommended that patients diagnosed with RPL undergo testing for anticardiolipin antibody, lupus anticoagulant, & anti-beta 2 glycoprotein. Recommendations exist for individuals with RPL to undergo APAS testing, as research has linked anticardiolipin antibody & lupus anticoagulant to pregnancy loss. [39].

Progesterone Level

Regular evaluation of serum progesterone levels is not advised due to its lack of predictive value for subsequent pregnancy results. [40].

Endometrial Biopsy

Multiple research studies demonstrate that this test does not accurately reflect a woman's fertility status. [41].

Testing for Infections

Routine testing for TORCH serology & vaginal & cervical cultures for chlamydia, gonorrhea, & bacterial vaginosis are not applicable to the assessment of RPL in asymptomatic healthy women. [42].

Evaluation of products of Conception (POC)

The utilization of a 24-chromosome microarray analysis substantially augments the RPL assessment suggested by the American Society of Reproductive Medicine . It should be mandatory to provide genetic analysis of miscarriage tissue obtained during the second & subsequent miscarriages to all couples who have experienced two otherwise more consecutive miscarriages. When miscarriage tissue is genetically evaluated in conjunction with an evidence-based assessment for RPL, a probable or definitive cause can be identified in over ninety percent of miscarriages. [43].

Treatment / Management

In treating RPL, attention should be paid to the underlying reason that is treatable. Each available treatment option's risks, alternatives, & success rates should be communicated to patients and their families. The efficacy of treatment can be enhanced through the provision of emotional support to these couples experiencing anxiety. In every circumstance, reproductive endocrinologists & obstetricians ought to engage in transparent communication & cooperative collaboration. [36].

Medical Conditions

As medically appropriate, women with diabetes, obesity, thyroid conditions, & other medical conditions should be treated. In addition, seeking guidance from an

endocrinologist regarding the management of uncontrolled thyroid conditions and diabetes is a viable course of action. Individuals who have elevated levels of thyroid peroxidase antibodies are at a heightened risk for RPL & require appropriate management. [44].

Chromosomal Anomalies

Priority is given to referring couples with chromosomal abnormalities to genetic counseling. Couples ought to be informed regarding the potential susceptibility to fetal chromosomal abnormalities during subsequent pregnancies. Pregnancy genetic testing, including chorionic villus sampling, preimplantation genetic diagnosis, & amniocentesis, may be selected by the individual in order to detect genetic abnormalities in the fetus & make informed decisions regarding subsequent therapeutic courses of action. [45].

Uterine Anomalies

RPL-inducing acquired & congenital uterine abnormalities are amenable to surgical treatment. Myomectomy, adhesion lysis, and hysteroscopic septum resection are a few of the procedures that are performed. [36].

Immunological

Pregnant individuals who are diagnosed with RPL & antiphospholipid antibody syndrome are typically administered aspirin & heparin, which has been shown to enhance prognoses. However, while this treatment may enhance maternal outcomes in women with thrombophilias, it does not prevent RPL. Standard medications in RPL include aspirin and low molecular weight heparin (LMWH), despite the fact that their efficacy in enhancing live birth rate has been demonstrated in only a limited number of placebo-controlled trials. Emerging evidence suggests that in some cases of RPL, new treatment options, such as TNF (tumor necrosis factor-alpha) inhibitors & granulocyte colony-stimulating factor (G-CSF), may be beneficial. However, additional comprehensive clinical trials are necessary to validate or invalidate the efficacy of these medications in the management of individuals with RPL. [46].

Unexplained RPL

A recent meta-analysis that applied stringent criteria for defining unexplained RM failed to identify any randomized controlled trials (RCTs) that utilized prednisolone. There is no evidence that intravenous immunoglobulin (IVIG) improves live birth rates for individuals with RPL, according to two recent meta-analyses. [47].

The association amongst the level of Anti-nuclear antibodies & the recurrent pregnancy loss.

Antinuclear Antibodies (ANA) Overview

Antinuclear antibody (ANA) positivity is a defining characteristic of autoimmune diseases affecting connective tissue. Antibodies of category ANAs bind to nucleic acid-protein complexes, proteins, DNA, & RNA, among other cellular components. [48].

Testing

In 1948, Hargraves & colleagues identified the "L.E. cell," which they observed in an individual with systemic lupus erythematosus (SLE), as the cell that gave rise to ANA testing. [39].

Specific subtypes of ANA are correlated with patterns of human epithelial laryngeal carcinoma type 2 (HEp-2); therefore, pattern recognition is a valuable tool in ANA testing. In general, homogeneous fluorescence indicates that the target of the antibodies is dsDNA, histones, or nucleosomes. Antibodies to membrane proteins may be detected in a membranous pattern. There is a correlation between antibodies that target distinct nuclear antigens and fluorescent patterns with pores. Additionally, the proficiency of the individual interpreting the result and the laboratory that produces the substrate cells have an impact on the initial immunofluorescence of HEp-2 cells. A unique scale must be established by each laboratory in order to delineate a positive result. Anti-Smith antibodies emit light in the form of a series of speckles. Anti-SSA/Ro & anti-SSB/La combine to create a delicate spotted array. Discrete speckles are indicative of antibodies that are specifically directed at the centromeres of interphase cells. The presence of nucleolar speckles is correlated with antibodies that target DNA topoisomerase (Scl-70). Additionally, the presence of antibodies to aminoacyl-tRNA synthetase (Jo-1) is indicated by the speckled cytoplasmic pattern [48].

Antinuclear Antibody Test Results

There are numerous techniques for quantifying ANAs. In practice, fluorescent antinuclear antibody (FANA) tests are the most prevalent. A negative or positive interpretation, ANA levels, and observed antibody patterns throughout the test are all included in the FANA test report. A negative interpretation signifies the absence of autoantibodies in the blood sample of the individual, thereby diminishing the probability of an autoimmune disease. A affirmative interpretation signifies the presence of autoantibodies in the blood sample of an individual [50].

The ANA test results might comprise a titer. The quantity of antibodies in the blood is quantified via antibody titer testing, which is frequently denoted by a ratio like 1:160. On this test, reference ranges denote the minimal titer ratio at which a positive result is deemed to have occurred [51].

Regarding ANA tests, reference ranges are contentious. The ratio that laboratories consider to be positive for ANAs may differ. Referral ranges may prove beneficial for individuals to deliberate upon with their physician. Additionally, the majority of FANA test reports detail the staining patterns generated throughout the testing process. FANA testing involves the attachment of antibodies to a fluorescent substance, the observation of which reveals patterns under a specialized microscope. Although staining patterns do not provide a definitive diagnosis of a health condition, specific patterns exhibit a tenuous association with particular illnesses. [52].

The association between the level of Anti-nuclear antibodies & the recurrent pregnancy loss.

An immune self-tolerance mechanism malfunction results in the development of autoantibodies. In the scientific literature, the relationship among autoantibodies and RM, infertility, & systemic disease, is not well established. The most researched autoimmune markers associated with reproductive issues are antithyroid antibodies (anti-thyroglobulin & anti-thyroperoxidase), antiphospholipid antibodies (anti-cardiolipin, anti-β2-Glycoprotein-I, & lupus anticoagulant), antispermatozoa antibodies, anti-endomysium, anti-DNA, & antinuclear antibodies (ANA). [53, 54].

Unknown at this time is the pathophysiological mechanism underlying pregnancy loss in women with a history of RM & positive ANA. However, some studies have hypothesized that potential mechanisms include alterations in uterine blood flow patterns, fluctuations in embryonic development, & suboptimal oocyte quality [55].

An additional association between positive ANA and a poorer prognosis has been observed in couples with RM who underwent immunotherapy. In ANA-positive RM individuals, inflammation at the site of embryonic implantation is a potential mechanism of pregnancy loss. One of the inflammatory pathways implicated in this situation is complement activation [25].

The occurrence of ANAs in expectant women may indicate the existence of an underlying autoimmune process that hinders placental development & may result in precocious pregnancy termination. Antiphospholipid antibodies & antinuclear antibodies are common autoantibodies detected in the blood of individuals who have recurrent abortions for unknown reasons [56].

ANA patterns exhibited homogeneity among individuals with positive tests in the enigmatic RM group, as determined by fluorescent microscopy analysis [57].

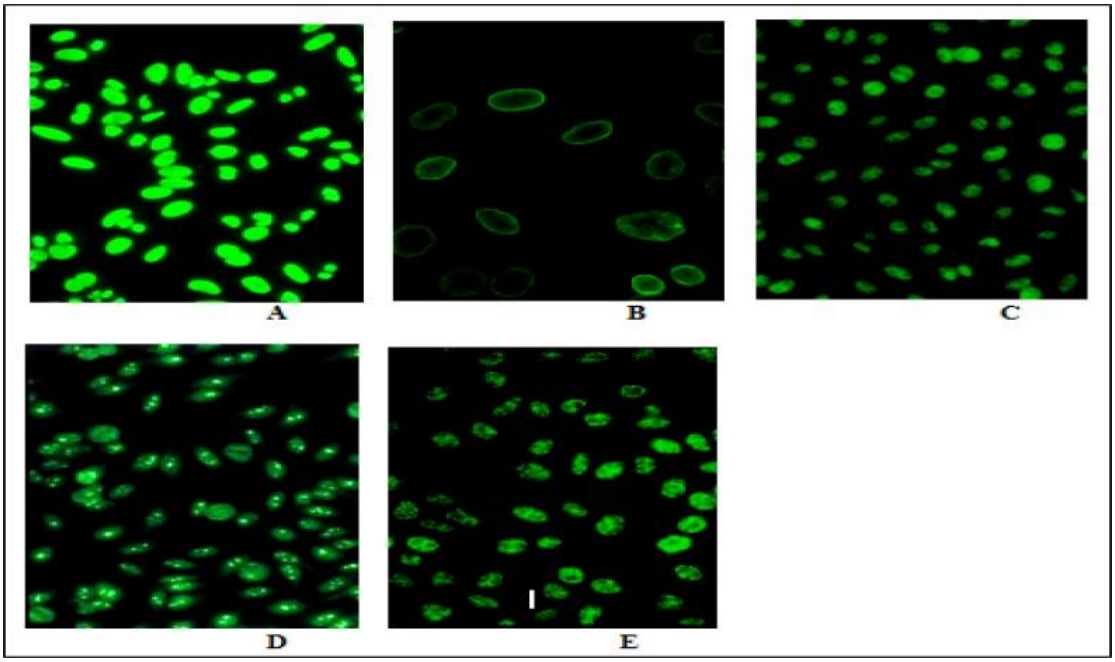

Fig (1): ANA patterns in individuals with unexplained recurrent miscarriage by immunofluorescence microscopy [57].
A: Homogenous, B: Peripheral/rim, C: Speckled, D: Nuclear and E: Centromeric

Multiple studies have documented a substantial incidence of low-titre ANA in the sera of individuals who have experienced pregnancy losses, whether they were explicable or enigmatic. Nevertheless, the implications of these discoveries remain ambiguous. Shoenfeld & his colleagues discovered a greater prevalence of antinuclear antibodies among individuals with autoimmune disease in their research. Nevertheless, no evidence suggested that they were more prevalent among individuals who had experienced recurrent pregnancy loss or infertility [58].

The occurrence of ANAs was 31.8 percent in individuals with a history of miscarriages (110 individuals), compared to 5.7 percent in thirty-five individuals with verified fertility & no history of pregnancy loss otherwise autoimmune illness [60], according to **Cubillos et al.** [58].

Dinse GE et al A direct proportional relationship was also identified among age & positive ANA (in both sexes), with rates spanning from 11.2 percent among those aged between the ages of 12 & 19 to 19.2 percent among those aged seventy years & older. Non-Hispanic blacks had a greater prevalence of ANA than members of other racial and ethnic groups. Weight, ANA level, education, family income, alcohol consumption, smoking history, or serum cotinine levels have not been found to be connected with ANA. [61].

REFERENCES

1. Dimitriadis E, Menkhorst E, Saito S, Kutteh WH, Brosens JJ. Recurrent pregnancy loss. Nature reviews disease primers. 2020 Dec 10;6(1):98.
2. El Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet PE. Recurrent pregnancy loss: current perspectives. International journal of women's health. 2017 May 17:331-45.
3. Eshre Guideline Group on RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, Nelen W, Peramo B, Quenby S, Vermeulen N. ESHRE guideline: recurrent pregnancy loss. Human reproduction open. 2018;2018(2):hoy004.
4. Alves C, Rapp A. Spontaneous abortion.
5. Rasmark Roepke E, Matthiesen L, Rylance R, Christiansen OB. Is the incidence of recurrent pregnancy loss increasing? A retrospective register-based study in Sweden. Acta obstetricia et gynecologica Scandinavica. 2017 Nov;96(11):1365-72.
6. Kling C, Hedderich J, Kabelitz D. Fertility after recurrent miscarriages: results of an observational cohort study. Archives of

gynecology and obstetrics. 2018 Jan;297:205-19.
7. Wray S, Arrowsmith S. Uterine excitability and ion channels and their changes with gestation and hormonal environment. Annual review of physiology. 2021 Feb 10;83:331-57.
8. Ozawa N, Ogawa K, Sasaki A, Mitsui M, Wada S, Sago H. Maternal age, history of miscarriage, and embryonic/fetal size are associated with cytogenetic results of spontaneous early miscarriages. Journal of assisted reproduction and genetics. 2019 Apr 15;36:749-57.
9. Zhang T, Sun Y, Chen Z, Li T. Traditional and molecular chromosomal abnormality analysis of products of conception in spontaneous and recurrent miscarriage. BJOG: An International Journal of Obstetrics & Gynaecology. 2018 Mar;125(4):414-20.
10. Kaser D. The status of genetic screening in recurrent pregnancy loss. Obstetrics and Gynecology Clinics. 2018 Mar 1;45(1):143-54.
11. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, Brosens JJ, Brewin J, Ramhorst R, Lucas ES, McCoy RC. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. The Lancet. 2021 May 1;397(10285):1658-67.
12. Wang H, Yuan D, Wang S, Luo L, Zhang Y, Ye J, Zhu K. Cytogenetic and genetic investigation of miscarriage cases in Eastern China. The Journal of Maternal-Fetal & Neonatal Medicine. 2020 Oct 17;33(20):3385-90.
13. Prior M, Richardson A, Asif S, Polanski L, Parris-Larkin M, Chandler J, Fogg L, Jassal P, Thornton JG, Raine-Fenning NJ. Outcome of assisted reproduction in women with congenital uterine anomalies: a prospective observational study. Ultrasound in Obstetrics & Gynecology. 2018 Jan;51(1):110-7.
14. Berek JS. Berek & Novak's gynecology. Lippincott Williams & Wilkins; 2019 Mar 19.
15. Palla R, Peyvandi F, Shapiro AD. Rare bleeding disorders: diagnosis and treatment. Blood, The Journal of the American Society of Hematology. 2015 Mar 26;125(13):2052-61.
16. Fülöp V, Vermes G, Demeter J. The relationship between inflammatory and immunological processes during pregnancy. Practical aspects. Orvosi Hetilap. 2019 Aug 1;160(32):1247-59.
17. Esteve-Valverde E, Ferrer-Oliveras R, Alijotas-Reig J. Obstetric antiphospholipid syndrome. Revista Clínica Española (English Edition). 2016 Apr 1;216(3):135-45.
18. La Farina F, Raparelli V, Napoleone L, Guadagni F, Basili S, Ferroni P. Inflammation and thrombophilia in pregnancy complications: implications for risk assessment and clinical management. Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders). 2015 Dec 1;15(3):187-203.
19. Li X, Fang Z, Yang X, Pan H, Zhang C, Li X, Bai Y, Wang F. The effect of metformin on homocysteine levels in patients with polycystic ovary syndrome: A systematic review and meta-analysis. Journal of Obstetrics and Gynaecology Research. 2021 May;47(5):1804-16.
20. Garrido-Gimenez C, Alijotas-Reig J. Recurrent miscarriage: causes, evaluation and management. Postgraduate medical journal. 2015 Mar;91(1073):151-62.
21. Ibrahim Y, Johnstone E. The male contribution to recurrent pregnancy loss. Translational andrology and urology. 2018 Jul;7(Suppl 3):S317.
22. Zidi-Jrah I, Hajlaoui A, Mougou-Zerelli S, Kammoun M, Meniaoui I, Sallem A, Brahem S, Fekih M, Bibi M, Saad A, Ibala-Romdhane S. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss. Fertility and sterility. 2016 Jan 1;105(1):58-64.
23. Carlini T, Paoli D, Pelloni M, Faja F, Dal Lago A, Lombardo F, Lenzi A, Gandini L. Sperm DNA fragmentation in Italian couples with recurrent pregnancy loss. Reproductive biomedicine online. 2017 Jan 1;34(1):58-65.
24. Marchi J, Berg M, Dencker A, Olander EK, Begley CJ. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. Obesity Reviews. 2015 Aug;16(8):621-38.

25. Cavalcante MB, Sarno M, Niag M, Pimentel K, Luz I, Figueiredo B, Michelon T, Lima S, Machado IN, Araujo Júnior E, Barini R. Lymphocyte immunotherapy for recurrent miscarriages: predictors of therapeutic success. American Journal of Reproductive Immunology. 2018 Jun;79(6):e12833.
26. Sugiura-Ogasawara M. Recurrent pregnancy loss and obesity. Best Practice & Research Clinical Obstetrics & Gynaecology. 2015 May 1;29(4):489-97.
27. Mayrhofer D, Hager M, Walch K, Ghobrial S, Rogenhofer N, Marculescu R, Seemann R, Ott J. The prevalence and impact of polycystic ovary syndrome in recurrent miscarriage: a retrospective cohort study and meta-analysis. Journal of Clinical Medicine. 2020 Aug 21;9(9):2700.
28. Dong AC, Morgan J, Kane M, Stagnaro-Green A, Stephenson MD. Subclinical hypothyroidism and thyroid autoimmunity in recurrent pregnancy loss: a systematic review and meta-analysis. Fertility and Sterility. 2020 Mar 1;113(3):587-600.
29. Gonçalves DR, Braga A, Braga J, Marinho A. Recurrent pregnancy loss and vitamin D: A review of the literature. American Journal of Reproductive Immunology. 2018 Nov;80(5):e13022.
30. Chu J, Gallos I, Tobias A, Robinson L, Kirkman-Brown J, Dhillon-Smith R, Harb H, Eapen A, Rajkhowa M, Coomarasamy A. Vitamin D and assisted reproductive treatment outcome: a prospective cohort study. Reproductive health. 2019 Dec;16(1):1-0.
31. Mumford SL, Garbose RA, Kim K, Kissell K, Kuhr DL, Omosigho UR, Perkins NJ, Galai N, Silver RM, Sjaarda LA, Plowden TC. Association of preconception serum 25-hydroxyvitamin D concentrations with livebirth and pregnancy loss: a prospective cohort study. The lancet Diabetes & endocrinology. 2018 Sep 1;6(9):725-32.
32. Kwak-Kim J, Skariah A, Wu L, Salazar D, Sung N, Ota K. Humoral and cellular autoimmunity in women with recurrent pregnancy losses and repeated implantation failures: a possible role of vitamin D. Autoimmunity reviews. 2016 Oct 1;15(10):943-7.
33. Devreese KM, Ortel TL, Pengo V, De Laat B. Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH. Journal of thrombosis and haemostasis. 2018 Apr 1;16(4):809-13.
34. Chighizola CB, Ubiali T, Meroni PL. Treatment of thrombotic antiphospholipid syndrome: the rationale of current management—an insight into future approaches. Journal of Immunology Research. 2015 May 5;2015.
35. Barišić A, Pereza N, Hodžić A, Krpina MG, Ostojić S, Peterlin B. Genetic variation in the maternal vitamin D receptor FokI gene as a risk factor for recurrent pregnancy loss. The Journal of Maternal-Fetal & Neonatal Medicine. 2021 Jul 18;34(14):2221-6.
36. Pillarisetty LS, Gupta N. Recurrent Pregnancy Loss [Internet]. Treasure Island (FL): StatPearls Publishing LLC. 2020.
37. Arbib N, Hadar E, Sneh-Arbib O, Chen R, Wiznitzer A, Gabbay-Benziv R. First trimester thyroid stimulating hormone as an independent risk factor for adverse pregnancy outcome. The Journal of Maternal-Fetal & Neonatal Medicine. 2017 Sep 17;30(18):2174-8.
38. Shahine L, Lathi R. Recurrent pregnancy loss: evaluation and treatment. Obstetrics and Gynecology Clinics. 2015 Mar 1;42(1):117-34.
39. Piccinni MP, Lombardelli L, Logiodice F, Kullolli O, Parronchi P, Romagnani S. How pregnancy can affect autoimmune diseases progression?. Clinical and Molecular Allergy. 2016 Dec;14:1-9.
40. Adda-Herzog E, Poulain M, de Ziegler D, Ayoubi JM, Fanchin R. Premature progesterone elevation in controlled ovarian stimulation: to make a long story short. Fertility and sterility. 2018 Apr 1;109(4):563-70.
41. Ellett L, Readman E, Newman M, McIlwaine K, Villegas R, Jagasia N, Maher P. Are endometrial nerve fibres unique to endometriosis? A prospective case–control study of endometrial biopsy as a diagnostic test for endometriosis in women with pelvic pain. Human Reproduction. 2015 Dec 1;30(12):2808-15.
42. Arora M, Tayade S. Recurrent Miscarriage. Donald School Textbook of Human Reproductive & Gynecological Endocrinology. 2018 Aug 16:364.
43. Idowu D, Merrion K, Wemmer N, Mash JG, Pettersen B, Kijacic D, Lathi RB. Pregnancy outcomes following 24-

chromosome preimplantation genetic diagnosis in couples with balanced reciprocal or Robertsonian translocations. Fertility and sterility. 2015 Apr 1;103(4):1037-42.
44. Nazarpour S, Ramezani Tehrani F, Simbar M, Tohidi M, Alavi Majd H, Azizi F. Effects of levothyroxine treatment on pregnancy outcomes in pregnant women with autoimmune thyroid disease. European journal of endocrinology. 2017 Feb;176(2):253-65.
45. Iews M, Tan J, Taskin O, Alfaraj S, AbdelHafez FF, Abdellah AH, Bedaiwy MA. Does preimplantation genetic diagnosis improve reproductive outcome in couples with recurrent pregnancy loss owing to structural chromosomal rearrangement? A systematic review. Reproductive biomedicine online. 2018 Jun 1;36(6):677-85.
46. Toth B, Vomstein K, Togawa R, Böttcher B, Hudalla H, Strowitzki T, Daniel V, Kuon RJ. The impact of previous live births on peripheral and uterine natural killer cells in patients with recurrent miscarriage. Reproductive Biology and Endocrinology. 2019 Dec;17:1-8.
47. Rasmark Roepke E, Hellgren M, Hjertberg R, Blomqvist L, Matthiesen L, Henic E, Lalitkumar S, Strandell A. Treatment efficacy for idiopathic recurrent pregnancy loss–a systematic review and meta-analyses. Acta Obstetricia et Gynecologica Scandinavica. 2018 Aug;97(8):921-41.
48. Mejdoub S, Hachicha H, Gargouri L, Feki S, Mahfoudh A, Masmoudi H. Antinuclear antibodies in children: clinical signification and diagnosis utility. La Tunisie Medicale. 2021 Oct;99(10):982.
49. Gomariz A, Helbling PM, Isringhausen S, Suessbier U, Becker A, Boss A, Nagasawa T, Paul G, Goksel O, Székely G, Stoma S. Quantitative spatial analysis of haematopoiesis-regulating stromal cells in the bone marrow microenvironment by 3D microscopy. Nature communications. 2018 Jun 28;9(1):2532.
50. Santhosh P, Ajithkumar K. Anti-nuclear antibodies: A practical approach to testing and interpretation. Journal of Skin and Sexually Transmitted Diseases. 2021 Oct 14;3(2):175-9.
51. Bossuyt X, Claessens J, De Langhe E, Belmondo T, Westhovens R, Hue S, Poesen K, Blockmans D, Mahler M, Fritzler MJ. Antinuclear antibodies by indirect immunofluorescence and solid phase assays. Annals of the Rheumatic Diseases. 2020 Jun 1;79(6):e65-.
52. Tozzoli R, Villalta D, Bizzaro N. Challenges in the standardization of autoantibody testing: a comprehensive review. Clinical reviews in allergy & immunology. 2017 Aug;53:68-77.
53. Di Rosa R, Ferrero S, Cifani N, Ferri L, Proietta M, Diamanti AP, Del Porto F. In vitro fertilization and autoimmunity: Evidence from an observational study. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2019 Mar 1;234:137-42.
54. Simopoulou M, Sfakianoudis K, Maziotis E, Grigoriadis S, Giannelou P, Rapani A, Tsioulou P, Pantou A, Kalampokas T, Vlahos N, Pantos K. The impact of autoantibodies on IVF treatment and outcome: a systematic review. International journal of molecular sciences. 2019 Feb 19;20(4):892.
55. Fan J, Zhong Y, Chen C. Impacts of anti-dsDNA antibody on in vitro fertilization-embryo transfer and frozen-thawed embryo transfer. Journal of immunology research. 2017 Apr 28;2017.
56. Pleguezuelo DE, Cabrera-Marante O, Abad M, Rodriguez-Frias EA, Naranjo L, Vazquez A, Villar O, Gil-Etayo FJ, Serrano M, Perez-Rivilla A, de la Fuente-Bitaine L. Anti-phosphatidylserine/prothrombin antibodies in healthy women with unexplained recurrent pregnancy loss. Journal of Clinical Medicine. 2021 May 13;10(10):2094.
57. Chen S, Yang G, Wu P, Sun Y, Dai F, He Y, Qian H, Liu Y, Shi G. Antinuclear antibodies positivity is a risk factor of recurrent pregnancy loss: a meta-analysis. InSeminars in Arthritis and Rheumatism 2020 Aug 1 (Vol. 50, No. 4, pp. 534-543). WB Saunders.
58. Shoenfeld Y, Carp HJ, Molina V, Blank M, Cervera R, Balasch J, Tincani A, Faden D, Lojacono A, Doria A, Konova E. Autoantibodies and prediction of reproductive failure. American Journal of Reproductive Immunology. 2006 Nov;56(5-6):337-44.
59. Cubillos J, Lucena A, Lucena C, Mendoza JC, Ruiz H, Arango A, Quiroga G, Ferro J,

Lucena E. Incidence of autoantibodies in the infertile population. Early Pregnancy: Biology and Medicine: the Official Journal of the Society for the Investigation of Early Pregnancy. 1997 Jun 1;3(2):119-24.

60. Deroux A, Dumestre-Perard C, Dunand-Faure C, Bouillet L, Hoffmann P. Female infertility and serum auto-antibodies: a systematic review. Clinical reviews in allergy & immunology. 2017 Aug;53:78-86.
61. Dinse GE, Parks CG, Weinberg CR, Co CA, Wilkerson J, Zeldin DC, Chan EK, Miller FW. Retracted: increasing prevalence of antinuclear antibodies in the United States.