The Prevalence of Anti-Phospholipid Antibodies and Effects on the Outcomes of in Vitro Fertilization

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Abstract

Background

The problem of infertility is common worldwide. In vitro fertilization remains the most widely used technique of assisted reproductive technology. Embryo implantation failure is a common cause of unexplained infertility and recurrent miscarriages, thus the relationship between antiphospholipid antibodies and embryo implantation failure after in vitro fertilization and embryo transfer is an important problem in fertility medicine that must be studied.

Patients and Methods

The study included 1000 cases, of women that underwent IVF between 2017–2021, through a Cohort study.

Results

The average age of the women was 31.91, the success rate of assisted fertilization in inducing pregnancy in the last trial was 52.8%, the prevalence was 4.4% of anticardiolipin IgG, 2.6% of anticardiolipin IgM, and 4% of Lupus anticoagulant. No relation was found between the antiphospholipid value and the age of the women, the number of times the experiment was conducted, the rate of success and failure, the type of infertility, and the number of oocytes induction, while the duration of sterility was affected by positive values of IgG and lupus coagulant antibodies.

Conclusion

There is no need for a routine antiphospholipid assay for female candidates for IVF, and when it is confirmed, treatment is required by Anticoagulants.

Introduction

Antiphospholipid antibodies, such as anticardiolipin antibodies, lupus anticoagulant antibodies, and/or anti-\(\beta_2\) glycoprotein I, are antibodies directed against the phospholipid membrane and proteins associated with phospholipids such as IgM, IgG, and IgA. These antibodies are the serological markers of antiphospholipid syndrome, a systemic autoimmune disease that affects any age, and have a higher association with women(1). Its etiology is still unclear, genetics and activation of the system of coagulation may play a role. APS is also associated with thrombotic events (arterial or venous) and fetal loss, miscarriage, preeclampsia, stillbirth, and preterm delivery (2–4).
Infertility is the inability of a couple to conceive for over 12 months of being sexually active, and without using contraceptives. Female and male infertility are equally prevalent, therefore both couples should be investigated. Men should undergo two semen analyses, while the assessment of female fertility should initially include serum progesterone measurement, screening for chlamydia antigen, and a hysterosalpingogram to evaluate tubal patency, then a diagnostic laparoscopy with dye transit should be performed. Despite a full diagnostic evaluation, approximately 20% of infertility cases remain unexplained (5–7).

Many physicians routinely screen for aPL and treat infertile women with aPL positivity whilst they are undergoing assisted reproduction technologies (ART).

aPL, especially IgG, provides the most frequent acquired risk factor for pregnancy complications like fetal loss and growth restriction. Anticardiolipin antibodies may be responsible for the occurrence of repeated miscarriages and implantation failure (4, 8).

Assisted reproduction technologies (ART) include ovulation induction, in vitro fertilization (IVF) and subsequent embryo transfer to the uterus, and treatment with exogenous human chorionic gonadotropin or progesterone (9).

The primary objective of the research is to study the prevalence of antiphospholipid antibodies in in vitro fertilization patients and their relationship with clinical pregnancy rate, while, the secondary aims are their relationship with the number of ovals and the age of the patients.

Patients & Methods

Study design and sample size

A retrospective and prospective cohort study were conducted at the Laboratory department of the Damascus University Faculty of Medicine, and Al-Sharq Hospital from June 2017 to June 2021. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Damascus University. The sample size was 1000 participants, 900 of them were retrospective (2017–2020), and 100 were prospective (2020–2021). Informed consent was obtained from each participant. The ethical approval was conducted according to the principles of the Declaration of Helsinki.

Patients

Inclusion criteria

-Women undergoing in vitro fertilization, and in the retrospective study, patients who undergo in vitro fertilization and who have antibody data.

-Positivity of phospholipids (IgM, IgG) and/or anticardiolipin (IgM, IgG), and/or B2 glycoprotein I.

-Age less than 40 years old.
The presence of the gestational sac on ultrasound as a criterion for pregnancy.

**Exclusion criteria**

- Patients with incomplete data.
- Women who have an independent risk factor for pregnancy complications (uterine anomalies).
- Women who have Diabetes Mellitus, Systemic Lupus Erythematosus, thyroid diseases, and thrombotic disorders.
- Women who refused to sign the informed consent.

**Methods**

Data was collected, including patients’ demographics, type and duration of fertility, and anti-phospholipid antibody values.

We performed a PTT LA for the control group (20 healthy women), then a PTT LA test was conducted for each sample, and if it exceeded 40 sec. (37.4 sec. ± 2) (10). The blending test was performed, and the values were considered positive if the correction was not made. The PTT LA test is a screening test that neither confirms nor negates lupus coagulant antibodies.

A volume of the control group's plasma was mixed with a volume of the patients' samples, and LA was tested using STA Compact (code: 607450589863 – SNCC39108033).

For IgG/IgM anticardiolipin, we performed a titer of anticardiolipin antibodies with immunofluorescence using Aeskulisa kits, and we considered the values to be positive if they were greater than 20 units/ml) (11).

Immunoassays for beta-2 GP1 antibodies can be performed using beta-2 GP1 substrate without phospholipid (so-called direct assays) and are referred to simply as “beta-2 GP1 antibodies”. Results that showed a high concentration of beta 2 glycoprotein I (beta-2 GP1) antibodies (> 40 U/mL for IgG and/or IgM) were considered positive, due to a lack of specificity, as it may also be found in the plasma of healthy individuals in low concentrations.

**Statistical Analysis**

We used the SPSS version 24 (IBM, Armonk, NY, USA). P < 0.05 is considered statistically significant.

Descriptive statistics were conducted for the women's age variable, as a result of assisted fertilization, IgG/IgM anticardiolipin antibodies, lupus coagulant antibodies, and the relationship of these variables with each other.
A Spearman test, independent T-test, Fisher Exact test, Mann-Whitney test, Chi-Square test, and Pearson test were used for the differences between the tests used to evaluate the effect of the presence of antibodies on pregnancy rates, the number of eggs, the number of previous fertilization failures, and the success of the current experience.

Results

1- Description of the sample search:

Age

The age of the entire sample ranged between 18 and 40 years, with a mean of 31.91 ± 4.863 years, while the average age of the retrospective sample was 31.88 ± 4.933 years and the prospective sample was 32.19 ± 4.187 years.

The sample was divided into three age groups. The first was patients younger or equal to 25 years old (115 women out of 1000, with a percentage of 11.5%, the second between 26 and 32 years old (372 women out of 1000, or 37.2%), and the third group between 33 and 40 years old (513 women out of 1000, or 51.3%).

Distribution of the auxiliary fertilization variable

The sample was divided into two groups according to the final results of IVF, the first group is the group in which assisted reproduction succeeded in inducing pregnancy which included 528 patients (52.8%), and the second group is where assisted reproduction failed which included 472 patients (47.2%).

Distribution of the anti-cardiolipin ACL (IgG) antibody variant in the studied sample:

The value of the IgG anticardiolipin antibody titer in the studied sample of women ranged between 3 and 48 with an average of 5.460 ± 7.0048, as shown in Table-1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrospective</td>
<td>5.358</td>
<td>900</td>
<td>6.7556</td>
<td>3.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Prospective</td>
<td>6.382</td>
<td>100</td>
<td>8.9303</td>
<td>3.9</td>
<td>48.0</td>
</tr>
<tr>
<td>Total</td>
<td>5.460</td>
<td>1000</td>
<td>7.0048</td>
<td>3.0</td>
<td>48.0</td>
</tr>
</tbody>
</table>

When studying the values of these opposites and the extent of their height, they were divided into two groups, the first group is pf values greater than 20, and therefore the probability of a positive
antiphospholipid syndrome in 44 patients out of 1000 increases by 4.4%, and the second group with antibody values less than 20, according to (Figure-1).

**Distribution of ACL anticardiolipin (IgM) variable in the studied sample**

Its value ranged between 0.4 and 52, with a mean of 3.441 ± 4.7232, as shown in Table-2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>N</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrospective</td>
<td>3.497</td>
<td>900</td>
<td>4.8890</td>
<td>.4</td>
<td>52.0</td>
</tr>
<tr>
<td>Prospective</td>
<td>2.936</td>
<td>100</td>
<td>2.7878</td>
<td>1.2</td>
<td>26.0</td>
</tr>
<tr>
<td>Total</td>
<td>3.441</td>
<td>1000</td>
<td>4.7232</td>
<td>.4</td>
<td>52.0</td>
</tr>
</tbody>
</table>

When studying the values of these opposites and the extent of their height, they were divided into two groups, the first group has values greater than 20, and therefore the probability of positivity for antiphospholipid syndrome in 26 out of 1000 patients increases by 2.6%, and the second group with antibody values less than 20, according to (Figure-2).

**Distribution of the Lupus LA anticoagulant variable in the studied sample:**

Lupus anticoagulant was studied in 50 women from the prospective sample group, and its value ranged between 7 and 58, with a mean of 23.40 ± 9.549, as shown in Table-3.

<table>
<thead>
<tr>
<th>The status of LAC</th>
<th>Standard Deviation</th>
<th>Mean</th>
<th>Maximum</th>
<th>Minimum</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid N (listwise)</td>
<td></td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

When studying the values of these opposites and the extent of their height, they were divided into two groups, the first group of values of these opposites being greater.

Thus, the probability of positive antiphospholipid syndrome in two patients out of 50 increases by 4%, and the group of antibody values in it is less than 40, 46 women out of 50, with a percentage of 96%.

**The distribution of digital research variables:**
The Shapiro-Wilk test was conducted to study the distribution of numerical variables data in the studied sample. The results of this test showed that the age variable is the only variable with a normal distribution, thus, we will use it for analysis.

Standard tests, on the other hand, did not follow the normal distribution of other numerical variables so they will be subject to non-standard analytical tests (Table-4).

**Table-4** The distribution of digital research variables in the studied sample

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tests</th>
<th>Result</th>
<th>Sig</th>
<th>Df</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Parametric tests</td>
<td>.058</td>
<td>86</td>
<td>.972</td>
<td></td>
</tr>
<tr>
<td>Number of IVF</td>
<td>Non-parametric tests</td>
<td>.000</td>
<td>86</td>
<td>.772</td>
<td></td>
</tr>
<tr>
<td>Number of failed IVF</td>
<td>Non-parametric tests</td>
<td>.000</td>
<td>86</td>
<td>.795</td>
<td></td>
</tr>
<tr>
<td>Duration of Infertility</td>
<td>Non-parametric tests</td>
<td>.000</td>
<td>86</td>
<td>.866</td>
<td></td>
</tr>
<tr>
<td>ACL_G value</td>
<td>Non-parametric tests</td>
<td>.000</td>
<td>86</td>
<td>.321</td>
<td></td>
</tr>
<tr>
<td>ACL_M value</td>
<td>Non-parametric tests</td>
<td>.000</td>
<td>86</td>
<td>.454</td>
<td></td>
</tr>
<tr>
<td>LAC value</td>
<td>Non-parametric tests</td>
<td>.000</td>
<td>86</td>
<td>.575</td>
<td></td>
</tr>
<tr>
<td>Number of Oocytes</td>
<td>Non-parametric tests</td>
<td>.000</td>
<td>86</td>
<td>.838</td>
<td></td>
</tr>
</tbody>
</table>

2- Statistical Analysis

- The association of a variable ACL anticardiolipin (IgG) value with the patient's age:

Using Spearman's Correlation Test, no correlation was observed, where the value of the age variable was $P=0.144$, which is greater than the 5% specified in our study (Table-5).

**Table-5**: The association of a variable ACL anti-cardiolipin (IgG) antibody value with the patient's age.
<table>
<thead>
<tr>
<th>Spearman's rho</th>
<th>Age</th>
<th>ACL_G value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Correlation Coefficient</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.</td>
<td>.144</td>
</tr>
<tr>
<td>N</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>ACL_G value</td>
<td>Correlation Coefficient</td>
<td>.046</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.144</td>
<td>.</td>
</tr>
<tr>
<td>N</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

For the ACL (IgG) anticardiolipin antibody variant groups, the average age of women in the positive group was 31.503 ± 5.046 years old, while the negative group's average age was 31.933 ± 4.858 years old. By conducting the Independent Samples T-test, the value was determined to be P = 0.564 which is greater than the 5% approved in our study.

- The correlation of the variable anti-cardiolipin (MACL) antibody (Ig) value with the patient's age:

By performing a Spearman's Correlation Test, a weak positive correlation was observed between the value of the age variable and the value of anti-cardiolipin ACL (IgACL) IgACL (IgM) antibodies in the studied sample. The significance value was P < 0.001, which is less than the 5% determined in our study, while the value of the correlation coefficient was rho = 0.135.

- The anticardiolipin ACL (IgM) anticardiolipin variant groups:

The average age of the women in the positive group was 32.58 ± 4.254 years old, and the negative group's average was 32.89 ± 4.87 years old, and by conducting the independent Sample T-test. P = 0.480 was not statistically significant.

- The association of lupus LA anticoagulant variant with the age of the patient:

By performing a Pearson's Correlation Test, the value of significance between the value of the variable age and the value of the lupus anticoagulant variable LA among the women of the studied sample, was observed to be: P = 0.990, which is greater than the 5% specified in our study.

For the LA lupus anticoagulant variant groups, the mean age of the women in the positive group was 33.00 ± 8.485 years old, and the negative group's was 32.42 ± 4.428 years old, by conducting a T-test, the value of P = 0.860 was not statistically significant.

**The variable number of previous assisted fertilization times with antiphospholipid antibody variables:**

- The association of variable ACL anticardiolipin (IgG) value with the number of previously assisted fertilizations:
Using Spearman's Correlation Test, no correlation was observed. In the studied sample, the value was $P = 0.057$, which is greater than the 5% specified in our study (Table-6).

**Table-6** Correlation of variable ACL anti-cardiolipin (IgG) antibody value with previous assisted fertilization times.

<table>
<thead>
<tr>
<th>Spearman's rho Number of IVF</th>
<th>The number of IVF</th>
<th>ACL_G value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation Coefficient</td>
<td>1.000</td>
<td>.060</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.</td>
<td>.057</td>
</tr>
<tr>
<td>N</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ACL_G value</th>
<th>Correlation Coefficient</th>
<th>.060</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. (2-tailed)</td>
<td>.057</td>
<td>.</td>
</tr>
<tr>
<td>N</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

For the ACL (IgG) anticardiolipin antibody variant groups, the mean number of fertilizations with previous assistance in the positive group was $2.45 \pm 2.04$ times, the negative group's was $2.13 \pm 1.398$ times, by conducting the Mann-Whitney test, the $P$ value was $0.60629$, which is greater than the 5% adopted in our study (Figure-3).

**The association of variable ACL anticardiolipin (IgM) value with the number of previous ARTs:**

By performing a Spearman's Correlation Test, a weak positive correlation was observed, where the value of $P = 0.001$, which is less than 5% specified in our study (Figure-4).

The average number of previously assisted fertilization times in the positive anti-cardiolipin ACL (IgM) group was $2.15 \pm 1.347$ times, and in the negative group it was $2.14 \pm 1.435$ times, and by performing the Mann-Whitney test, the $P$ value was $0.910$, which is greater than the 5% approved in our study.

By performing a Pearson Correlation Test and Spearman's test, no correlation was observed between the value of the variable number of previously assisted fertilizations and the value of the lupus anticoagulant variable LA in the sample women. $P = 0.592$, which is greater than the 5% specified in our study.

For the LA lupus anticoagulant variant groups, the mean number of previous ARTs in the positive group was $2.50 \pm 0.70$ times with an average rank value of 35.25, and in the negative group it was $2.15 \pm 1.571$ times with an average rank of 25.09, and by performing the Mann-Whitney test, the $P$ value was $P = 0.306$ which is statistically insignificant.

**- The variable number of previous assisted fertilization failures with antiphospholipid antibody variables:**
The association of variable ACL anti-cardiolipin (IgG) antibody value with the frequency of assisted fertilization failure:

Previous Spearman's Correlation Tests showed no correlation was observed, where the value of $P = 0.903$, which is greater than the 5% specified in our study (Table-7).

**Table-7** Correlation of a variable ACL anticardiolipin (IgG) value with the number of previous assisted fertilization failures

<table>
<thead>
<tr>
<th>Spearman's rho</th>
<th>The number of failed IVF</th>
<th>ACL_G value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation Coefficient</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1000</td>
</tr>
</tbody>
</table>

The average number of fertilization failures with former assistance in the positive ACL (IgG) anticardiolipin antibody group was $2.033 \pm 2.09$ times, while in the negative group's, it was $1.751 \pm 1.431$ times, by conducting the Mann-Whitney test, the $P$ value was 0.45, which is greater than the 5% approved in our study (Table-8).

**Table-8** The average number of previous ART failures in the IgG anticardiolipin group.

<table>
<thead>
<tr>
<th>Status of ACL_G</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of failed IVF</td>
<td>Negative</td>
<td>956</td>
<td>1.75</td>
<td>1.431</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>44</td>
<td>2.09</td>
<td>2.033</td>
</tr>
</tbody>
</table>

The association of a variable ACL anticardiolipin (IgM) value with the number of assisted fertilization failures

By using Spearman's Correlation Test, no correlation was observed. ACL (IgACL) antibodies in the women of the studied sample where the value of $P = 0.67$, which is greater than the 5% specified in our study (Table-9).

**Table-9** Correlation of a variable ACL anticardiolipin (IgM) value with the number of previously assisted fertilization failures.
The average number of fertilization failures with previous assistance in the positive ACL (IgM) anticardiolipin group was $1.65 \pm 1.441$ times, and the negative group's was $1.77 \pm 1.463$ times, by conducting the Mann-Whitney test, the $P$ value was 0.572.

**The association of lupus anticoagulant variant LA with the number of previous ART failures procedure:**

By using Pearson's Correlation Test, no correlation was found between the variable number of previously assisted fertilization failures and the value of the LA lupus anticoagulant variable among the sample women $P = 0.557$, which is greater than the 5% specified in our study.

In the LA lupus anticoagulant variant groups, the average number of previous ART failures in the positive group was $2.00 \pm 0.0001$ times with an average rank of 35.50, and the negative group's was $1.771 \pm 1.7161$ times with an average rank value of 25.08, and by conducting the Mann-Whitney test, the value of $P = 0.294$ was determined not statistically significant.

- Study of infertility pattern variable with antiphospholipid antibody variables.

**Study of variable ACL anticardiolipin (IgG) antibody value with infertility pattern:**

By conducting the Mann-Whitney test to compare the numerical values of the anticardiolipin variant ACL (IgG) between the group of women suffering from primary infertility $5.474 \pm 6.9989$ (mean ranks = 505.76) with a group of women with secondary infertility $5.340 \pm 7.0872$ (average ranks = 455.70), no statistically significant differences between the two groups were found, $P = 0.092$, which is greater than the 5% approved in our study (Table-10).

**Table-10**: study of the variable ACL anti-cardiolipin (IgG) antibody value with infertility pattern.
<table>
<thead>
<tr>
<th>Type of Infertility</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL_G value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>895</td>
<td>5.474</td>
<td>6.9989</td>
<td>.2339</td>
</tr>
<tr>
<td>Secondary</td>
<td>105</td>
<td>5.340</td>
<td>7.0872</td>
<td>.6916</td>
</tr>
</tbody>
</table>

For the positive ACL (IgG) anticardiolipin antibody variant, the Chi-Square test showed no statistically significant differences as the P value was 0.753 (figure-5).

**Study of variable ACL anticardiolipin (IgM) antibody value with infertility pattern:**

By conducting the Mann-Whitney test to compare the numerical values of the anti-cardiolipin ACL (Ig CL) variant (IgACL) between IgACL (IgM) in the group of women with primary infertility was $3.377 \pm 4.5157$ (average ranks = 498.41) and in the group of women with secondary infertility it was $3.985 \pm 6.2183$ (average ranks = 518.31). The statistically significant differences between the two groups was $P = 0.504$, which is greater than the 5% approved for our study (Table-11).

**Table-11** The ACL anti-cardiolipin (IgM) antibody variable with infertility pattern

<table>
<thead>
<tr>
<th>The type of Infertility</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL_M value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>895</td>
<td>3.377</td>
<td>4.5157</td>
<td>.1509</td>
</tr>
<tr>
<td>Secondary</td>
<td>105</td>
<td>3.985</td>
<td>6.2183</td>
<td>.6068</td>
</tr>
</tbody>
</table>

For the positive anticardiolipin variant ACL (IgM), the Chi-Square test showed no statistically significant differences as the value of $P = 0.141$ (Figure-6).

A study of the association of a lupus LA anticoagulant variant with the pattern of infertility:

By conducting the Mann-Whitney test to compare the numerical values of the LA lupus anticoagulant variable between the groups, the values of the group of women with primary infertility were $22.76 \pm 9.27$ (mean ranks = 24.63), and in the group of women with secondary infertility they were $30.75 \pm 10.996$ (mean ranks = 35.50). No significant differences were observed statistically between the two groups as $P = 0.1520$ which is greater than the 5% approved for our study (Table-12).

**Table-12** Study of the association of lupus LA anticoagulant variable with infertility pattern.

<table>
<thead>
<tr>
<th>Type of Infertility</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status of LAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>46</td>
<td>22.76</td>
<td>9.274</td>
<td>1.367</td>
</tr>
<tr>
<td>Secondary</td>
<td>4</td>
<td>30.75</td>
<td>10.996</td>
<td>5.498</td>
</tr>
</tbody>
</table>
For the LA positive lupus anticoagulant variable, the Fisher test showed no statistically significant differences as the value of $P = 0.155$.

A study of the final result of IVF with antiphospholipid antibody variables:

Study of variable ACL anticardiolipin (IgG) antibody value with recent ART result:

By conducting the Mann-Whitney test to compare the numerical values of the anti-cardiolipin variant ACL (IgG) between the groups, the values in the group of women whose IVF succeeded in producing a pregnancy were $5.187 \pm 6.0911$ (Mean ranks = 505.83) while the values in the group of women whose ART did not succeed in producing a pregnancy were $5.765 \pm 7.8982$ (average ranks = 494.53), and no statistically significant differences were observed between the two groups as $P = 0.535$, which is greater than the 5% approved in our study.

For the positive ACL (IgG) anticardiolipin antibody variant, the Chi-Square test showed no statistically significant differences as the P value was 0.486 (Figure-7).

A study of variable ACL anticardiolipin (IgM) value with recent ART result:

By conducting the Mann-Whitney test to compare the numerical values of the anti-cardiolipin variant ACL (IgM) between the groups, the values with women whose assisted fertilization process succeeded in producing a pregnancy were $3.163 \pm 3.461$ (mean ranks = 503.04) and in the group of women whose ART did not succeed in inducing pregnancy they were $3.752 \pm 5.8073$ (average ranks = 497.66), so no statistically significant differences were observed between the two groups as $P = 0.7690$, which is greater than the 5% approved in our study (Table-13).

Table-13 Study of the variable ACL anticardiolipin (IgM) value with the last assisted fertilization result.

<table>
<thead>
<tr>
<th>Final result</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL_M value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Success (Pregnancy)</td>
<td>528</td>
<td>3.163</td>
<td>3.4617</td>
<td>.1507</td>
</tr>
<tr>
<td>Failure</td>
<td>472</td>
<td>3.752</td>
<td>5.8073</td>
<td>.2673</td>
</tr>
</tbody>
</table>

For the positive anti-cardiolipin ACL (IgM) anticardiolipin variant, the Chi-Square test showed no statistically significant differences as the P value was 0.278 (Figure-8).

Study of the association of lupus anticoagulant variant LA with recent ART outcome:

By conducting the Mann-Whitney test to compare the numerical values of the LA lupus anticoagulant variable between groups, values in women whose ART had succeeded in producing a pregnancy were $21.68 \pm 11.205$ (average ranks = 21.90) and in women whose ART did not succeed in producing a pregnancy they were $25.12 \pm 7.379$ (average ranks = 29.10), thus, no statistically significant
differences were observed between the two groups, $P = 0.0800$, which is greater than the 5% approved in our study (Table-14).

**Table-14** Study of the association of the LA lupus anticoagulant variable with the last ART result.

<table>
<thead>
<tr>
<th>Final result</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status of LAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Success (Pregnancy)</td>
<td>25</td>
<td>21.68</td>
<td>11.205</td>
<td>2.241</td>
</tr>
<tr>
<td>Failure</td>
<td>25</td>
<td>25.12</td>
<td>7.379</td>
<td>1.476</td>
</tr>
</tbody>
</table>

**Study of the variable duration of infertility in years with antiphospholipid antibody variables:**

Study of the association of variable ACL anticardiolipin (IgG) value with the duration of infertility in years:

By performing a Spearman's Correlation Test, a weak positive correlation was observed between the value of the variable duration of infertility in years and the value of ACL (IgG) antibodies among the sample women, where the value of $P < 0.001$ which is smaller than the 5% specified in our study. The value of the correlation coefficient reached $\rho = 0.1760$.

For the ACL (IgG) anticardiolipin antibody variant groups, the mean duration of infertility in years in the positive group was $9.07 \pm 5.60$ years (mean ranks = 592.58), and in the negative group it was $7.22 \pm 4.60$ years (average ranks = 495.73), and by conducting the Mann-Whitney test, the $P$ value was 0.029, and it is less than the 5% approved in our study indicating no statistically significant differences between the two groups.

A study of the association of variable ACL anticardiolipin (IgM) value with the duration of infertility in years:

By performing Spearman's Correlation Test, a weak positive correlation was observed between the value of the variable duration of infertility in years and the value of ACL (IgM) antibodies in the sample women, where the value of $P = 0.44$, which is less than the 5% specified in our study, the value of the correlation coefficient reached $\rho = 0.0640$.

For the ACL (IgM) anticardiolipin antibody variant groups, the mean duration of infertility in years in the positive group was $5.081 \pm 8.31$ years (mean ranks = 557.27), and in the negative group it was $7.27 \pm 4.651$ years (average ranks = 498.98), and by conducting the Mann-Whitney test, the $P$ value was $P = 0.308$, and it is greater than the 5% approved in our study, so there are no statistically significant differences between the two groups.

Study of the association of lupus LA anticoagulant variant with the duration of infertility in years:

By using Spearman's Correlation test, there is was a moderate positive correlation observed between the value of the variable duration of infertility in years and the value of the lupus anticoagulant variable LA
among the sample women.

The studied significance value was $P = 0.028$, which is less than the 5% determined in our study. As for the correlation coefficient, Spearman's showed a value of $\rho = 0.311$.

For the LA lupus anticoagulant variant groups, the mean duration of infertility in years in the positive group was $17.50 \pm 10.607$ years (average rank = 43.75), and in the negative group it was $7.33 \pm 4.795$ years (average rank = 24.74), and by conducting the Mann-Whitney test, the value $P = 0.040$ was statistically significant.

**Study of the variable number of eggs stimulated with antiphospholipid variables:**

A study of the association of variable ACL anti-cardiolipin (IgG) antibody value with the number of induced eggs:

By using Spearman's Correlation Test, no correlation was observed between the value of the variable number of eggs stimulated and the value of anticardiolipin ACL(IgG) in the sample women. The studied value was $P = 0.782$, which is greater than the 5% specified in our study.

For the ACL (IgG) anticardiolipin antibody variant groups, the mean number of induced oocytes in the positive group was $7.928 \pm 10.43$ (mean ranks = 499.74), and in the negative group it was $8.13 \pm 10.540$ (mean ranks = 500.01), and by conducting Mann-Whitney test, $P = 0.0995$ which is greater than the 5% approved in our study, so there are no statistically significant differences between the two groups.

A study of the association of variable ACL anticardiolipin (IgM) value with the number of induced eggs:

By performing the Spearman Correlation Test, a negative correlation was observed between the value of the variable number of eggs stimulated and the value of anticardiolipin ACL (IgM) antibodies in women. $P = 0.004$ which is less than the 5% specified in our study. The value of the correlation coefficient $\rho = -0.0900$.

For the ACL (IgM) anticardiolipin antibody variant groups, the mean number of induced oocytes in the positive group was $10.62 \pm 8.09$ (mean ranks = 508.06), and in the negative group it was $10.52 \pm 8.123$ (mean ranks = 500.30), and by conducting Mann-Whitney test, $P = 0.892$ which is greater than the 5% approved in our study, thus, there are no statistically significant differences between the two groups.

Study of the association of lupus anticoagulant variant LA with the number of oocytes stimulated:

By performing a Pearson Correlation test, and Spearman correlation test, no correlation was observed between the value of the variable number of eggs stimulated and the value of the variable lupus anticoagulant LA among the women of the sample studied, where the significant value was $P = 0.523$, which is greater than the 5% determined in our study.
For the LA lupus anticoagulant variant groups, the mean number of oocytes induced in the positive group was $11.0011 \pm 5.657$ times with an average rank of 31.50, and in the negative group it was $10.35 \pm 8.581$ times with an average rank value of 25.25, and by conducting a Mann-Whitney test, the $P = 0.551$, which is statistically insignificant.

Studying the correlation of the variable of age with the number of induced eggs:

By running Spearman's Correlation test, a negative correlation was observed. $P < 0.001$, and $\rho = -0.395$ (Scatter-1).

Study of the correlation of the variable of age with the number of previous ART failures:

By performing a Spearman's Correlation Test, a weak positive correlation was observed. between the value of the variable number of previous ART failures and the value of the age variable in women in the studied sample, where the value of $P = 0.045$, which is less than the 5% specified in our study. The value of the coefficient of correlation $\rho = 0.056$ (Scatter-2).

We found only one patient with IgG and IgM positivity for anticardiolipin antibodies.

**Discussion**

Our sample size study is 1000 participants, which is bigger than other studies (12–15).

The average age of 31.91 years is in concordance with Steinvil A study (15).

Assisted fertilization succeeded in producing pregnancy in the last trial by 52.8%, and this is in concordance with other studies (12, 13).

The positivity of the cardiolipin (IgG) antibodies was (4.4%), while the positive values of anticardiolipin (IgM) antibodies were 2.6%, and Lupus anticoagulant antibodies were positive at 4%. This result is low compared to other cohort studies (16–18). This disparity may be due to the different ethnicity, inclusion criteria, or the numbers and types of antibodies tested.

There was no association between the age variable and the value of IgG anticardiolipin antibodies, lupus anticoagulant antibodies (17), nor the number of previous ARTs, meanwhile, a weak positive association was found between the age variable and IgM anti-cardiolipin antibody value for the entire sample, while they were not statistically significant at positive values for antibodies (19, 20).

There was no association with IgG anti-cardiolipin antibody values, Lupus anticoagulant antibodies, nor number of previous ARTs, but a weak positive correlation was observed with IgM anticardiolipin antibodies, and there was no statistical significance at the positive values of IgM antibodies, as the average number of times of the procedure in previous fertilization was 2.15, and this was in concordance with other studies (12, 13, 15), while others provided contradictory results, like Kheder, et al (21).
No correlation was observed between the numbers of previous ART failures, the primary and secondary infertility pattern, the last ART result, the values of both antibodies, the cardiolipin, and lupus coagulant antibodies. This was in concordance with the 4 studies mentioned before, and the American Reproductive Medicine Society, which recommended that aPL positivity does not reduce the success of assisted reproduction (20).

A positive correlation was observed between the duration of infertility and anti-cardiolipin (IgM and IgG) levels, but there was no statistical significance when IgM anti-cardiolipin and lupus anticoagulant antibodies were positive. The positive lupus anticoagulant in the two patients was interesting, but cannot be determined statistically significance, and this is in concordance with some studies (12.22).

There was no correlation between the oocytes number and the IgG anti-cardiolipin and lupus coagulant antibodies, while a negative correlation was found between the oocyte number and the patients’ age, besides the anti-cardiolipin antibodies IgM (22–24).

**Conclusion**

There is no need for a routine antiphospholipid assay for female candidates for IVF, and when it is confirmed, treatment is required by Anticoagulants (low molecular weight Heparin).

**Declarations**

**Acknowledgements:**

No acknowledgements given by any of the authors.

**Conflict of Interests:**

None of the authors have reported a competing interest.

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None of the authors have received any funding of any kind.

**Ethical Approval Ethics and Consent to Participate:**

Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Damascus University. Informed consent was obtained from each participant. The ethical approval was conducted according to the principles of the Declaration of Helsinki.

**Publication Consent:**

Not Applicable
Availability of Data and Materials:

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors’ Contributions:

All authors equally contributed to the collection and analysis of the data, the writing of the paper and to the proof reading.

Guarantor:

Dr. Nagham Al Halaki is the guarantor for this paper.

References


Scatter

Scatter 1 and 2 are available in the Supplementary Files section.

Figures
Figure 1

Distribution of ACL anticardiolipin (IgG) antibody classes variable in the studied sample
Figure 2

Distribution of ACL anticardiolipin (IgM) antibody classes variable
Figure 3

Mean number of previously assisted fertilizations in IgG anticardiolipin group
Figure 4

The mean number of previously assisted fertilizations in the IgM anticardiolipin group
Figure 5

Significance of positive values of anticardiolipin antibodies ACL (IgG) with the type of sterility.
Figure 6

The significance of the positive values of ACL (IgM) anticardiolipin antibodies with the type of sterility
Figure 7

Significance of positive anti-cardiolipin ACL (IgG) antibody variant with the last fertilization result.
Figure 8

Significance of positive anti-cardiolipin ACL (IgM) antibody variant with the last fertilization result

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scatter1.png
- Scatter2.png