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ΤΙΤΛΟΣ ΔΙΠΛΩΜΑΤΙΚΗΣ ΕΡΓΑΣΙΑΣ

Επίδραση αντιφωσφολιπιδικών αντισωμάτων στην έκβαση των τεχνικών υποβοηθούμενης αναπαραγωγής. Συστηματική ανασκόπηση και μετα-ανάλυση.

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Presence of antiphospholipid antibodies is associated with increased failure rates of assisted reproductive techniques:

A systematic review and Meta-analysis

A thesis submitted in fulfillment of the requirements

for the degree of

Master of Science in ‘Research in Female Reproduction’

by

Eirini Papadimitriou

A MSc thesis submitted in fulfillment of the requirements
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Abbreviations

ART: assisted reproductive technology

IVF: in vitro fertilization

ET: embryo transfer

APS: antiphospholipid syndrome

anti-PL: antiphospholipid antibodies

anti-CL: anticardiolipin

anti-b2GPI: anti-beta2 glycoprotein I

LA: lupus anticoagulant

anti-PS: anti-phosphatidylserine

anti-PC: anti-phosphatidylcholine

anti-PE: anti-phosphatidylethanolamin

anti-PI: anti-phosphatidylinositol

anti-PG: anti-phosphatidyl-glycerol

anti-PA: anti-phosphatidic acid

CI: confidence intervals

RR: relative risk

n: number of participants studied

ABSTRACT

Fifteen observational studies, which included healthy women of reproductive age without a diagnosis of antiphospholipid syndrome, were selected in this study to evaluate the association between the presence or absence of antiphospholipid antibodies and implantation outcome after in vitro fertilization and embryo transfer (IVF-ET). This meta-analysis compared the presence of antiphospholipid antibodies in women of reproductive age experiencing at least two implantation failures in IVF-ET and in controls (control 1: women who had a successful implantation after IVF-ET or control 2: women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET).

Main outcomes were the presence of any type of antiphospholipid antibodies; anticardiolipin; anti-beta2 glycoprotein I; lupus anticoagulant; anti-phosphatidylserine; anti-phosphatidylcholine; anti-phosphatidylethanolamin or anti-phosphatidylinositol or anti-phosphatidyl-glycerol or anti-phosphatidic acid antibodies in association with implantation outcome after IVF-ET. Effects were reported as relative risks and their 95% confidence intervals (CIs).

The presence of any type of aPL antibodies and of only anticardiolipin antibodies is associated with a significant 3.44 and 5.11 relative risk for implantation failure after IVF-ET, respectively, as compared to women experiencing one successful IVF-ET. The presence of either anticardiolipin or lupus anticoagulant or anti-β-2GPI or anti-phosphatidylserine antibodies is associated with a significant 14.91, 4.57, 55.6 and 226.89 relative risk for implantation failure, respectively, as compared to women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET.

The presence of antiphospholipid antibodies without the diagnosis of antiphospholipid syndrome was significantly higher among women with implantation failure after IVF-ET compared with either women with implantation success after IVF-ET or unselected healthy women with no history of IVF-ET.

ΠΕΡΙΛΗΨΗ

Σε αυτή τη μελέτη επιλέχθησαν δεκαπέντε μελέτες παρατήρησης, οι οποίες περιελάμβαναν υγιείς γυναίκες αναπαραγωγικής ηλικίας χωρίς διάγνωση αντιφωσφολιπιδικού συνδρόμου, με σκοπό να αξιολογηθεί η συσχέτιση μεταξύ της παρουσίας ή της απουσίας αντιφωσφολιπιδικών αντισωμάτων και της εμφύτευσης μετά από εξωσωματική γονιμοποίηση και εμβρυομεταφορά (IVF-ET). Αυτή η μετα-ανάλυση συνέκρινε την παρουσία αντιφωσφολιπιδικών αντισωμάτων σε γυναίκες αναπαραγωγικής ηλικίας που εμφάνισαν τουλάχιστον δύο αποτυχίες εμφύτευσης στην IVF-ET και στις ομάδες ελέγχου (γυναίκες που είχαν επιτυχημένη εμφύτευση μετά από IVF-ET ($1^{η}$ ομάδα ελέγχου) ή γυναίκες με τουλάχιστον μία επιτυχή κύηση μετά από φυσιολογική σύλληψη ή μη επιλεγμένες υγιείς γυναίκες χωρίς ιστορικό IVF-ET ($2^{η}$ ομάδα ελέγχου).

Κύρια αποτελέσματα ήταν η παρουσία οποιουδήποτε τύπου αντιφωσφολιπιδικών αντισωμάτων, αλλά και μεμονομένα αντισωμάτων έναντι της αντικαρδιολιπίνης, της β-2 γλυκοπρωτεΐνης I, του αντιπηκτικού του λύκου, της φωσφατιδυλοσερίνης, της φωσφατιδυλοχολίνης, της φωσφατιδυλοαιθανολαμίνης, της φωσφατιδυλοϊνοσιτόλης, της φωσφατιδυλογλυκερόλης, του φωσφατιδικού οξέος σε συνδυασμό με την αποτυχία εμφύτευσης μετά από IVF-ET. Τα αποτελέσματα εκφράστηκαν ως σχετικοί κίνδυνοι και τα διαστήματα εμπιστοσύνης με 95% .

Η παρουσία οποιουδήποτε τύπου αντιφωφωλιπιδικών αντισωμάτων και μεμονωμένων των αντισωμάτων έναντι καρδιολιπίνης σχετίζεται με σημαντικό σχετικό κίνδυνο 3.44 και 5.11 αντιστοίχως για αποτυχία εμφύτευσης μετά από IVF-ET σε σύγκριση με γυναίκες που είχαν μία επιτυχή IVF-ET. Η παρουσία αντισωμάτων έναντι καρδιολιπίνης, αντιπηκτικού του λύκου, αντι-β-2GPI , αντι-φωσφατιδυλοσερίνης συσχετίζεται με σημαντικό σχετικό κίνδυνο 14.91, 4.57, 55.6 και 226.89 αντιστοίχως για αποτυχία εμφύτευσης σε σύγκριση με γυναίκες με τουλάχιστον μία επιτυχή κύηση μετά από φυσιολογική σύλληψη ή μη επιλεγμένες υγιείς γυναίκες χωρίς ιστορικό IVF-ET.

Η παρουσία αντιφωσφολιπιδικών αντισωμάτων χωρίς τη διάγνωση του αντιφωσφολιπιδικού συνδρόμου ήταν σημαντικά υψηλότερη στις γυναίκες με αποτυχία εμφύτευσης μετά από IVF-ET σε σύγκριση είτε με γυναίκες με επιτυχία εμφύτευσης μετά από IVF-ET είτε με μη επιλεγμένες υγιείς γυναίκες χωρίς ιστορικό IVF-ET.

Introduction

Infertility according to World Health Organization is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. It has an estimated global prevalence of 186 million people, which has not decreased over the last two decades[i-ii]. Infertility is a global public health problem posing significant burden to women and couples by affecting 1:10 women of reproductive age[i-ii]. Infertility is a heterogeneous condition that may be related to mechanical agents, infectious inflammation, luteal phase disorders and immunological mechanisms. Polycystic ovary syndrome, hypothalamic dysfunction, premature ovarian insufficiency, tubal infertility, endometriosis and/or uterine and cervical causes (cervical stenosis, polyps, tumours) are some of the pathological conditions underlying female infertility[iii].

Assisted reproductive technology (ART) is increasingly used to assist subfertile couples to achieve pregnancy. This expanding technology has led to a significant rise in live birth following the introduction of *in vitro* fertilization (IVF) attributed to improvement of clinical and laboratory protocols[iv]. Over 1,250,000 ART cycles, resulting in the birth of over 225,000 babies, were reported by 2,419 clinics globally in 2007. This represented a 19.2% increase from 2006 and was followed by significant annual increase in the number of ART cycles thereafter. The availability of ART varies by country, from 12 to 4,140 treatments per million population[Error! Bookmark not defined.]. In Greece, the estimated or reported total number of cycles was 10,788 (1,008/million) for the year 2007 whereas the number of total babies reported from participating clinics is 769 and estimated number from all clinics is 3.247[v].

Awareness is rising regarding issues related to reproduction in chronic diseases. Rheumatic diseases can affect quality of life and reproduction. Improvement in reproductive medicine has made possible for most patients with autoimmune diseases to have a successful pregnancy and childbirth, implantation failure after IVF and embryo transfer (ET) is an obstacle to overcome. Pregnancy complications are increased in patients with systemic lupus erythematosus and antiphospholipid syndrome (APS) and these patients are seeking assistance in reproduction more often than general population[v].

APS is an autoimmune acquired thrombophilia, which occurs either alone or in combination with other autoimmune diseases; mainly with systemic lupus erythematosus[vi]. Clinical manifestations of APS include thrombotic events, affecting the arterial as well as the venous circulation, and/or pregnancy complications, in the persistent positivity for circulating antiphospholipid antibodies (aPL). The updated classification criteria for APS for obstetric morbidity comprise otherwise unexplained pregnancy loss and/or premature birth due to eclampsia, preeclampsia, or placental insufficiency[vi] (Table 1). Obstetric APS is a complex entity that can affect both mother and the fetus throughout the pregnancy with repeated miscarriages, preeclampsia/eclampsia, intrauterine growth retardation and stillbirth. Evaluation of circulating antiphospholipid antibodies (aPL) are part of the biochemical work-up which follows miscarriage or preterm labor. When found positive on two occasions six weeks to six months apart the diagnosis of the APS is evoked.

Persistent antiphospholipid antibodies (aPL) are documented by a solid phase serum assay (anticardiolipin [aCL] or anti b2-glycoprotein-I [b2GPI] enzyme-linked immunosorbent assay) or by an inhibitor of phospholipid-dependent clotting (lupus anticoagulant [LA] test) or by both[vii]. It is widely accepted that aPL represent a heterogeneous group of antibodies and recognize various phospholipids, phospholipid-binding proteins, and phospholipis protein complexes. The relationship between the presence of aPL and reproductive failure has been queried. Several studies have suggested the association of aPL not only with early miscarriage but also with implantation failure. Studies support that aPL alone and not within antiphospholipid syndrome impair female fertility by interfering from conception to endometrial decidualization and implantation.

To start from pre-conception and conception process APS women and aPL positive women seem to have impaired ovarian reserve and to present more frequently with premature ovarian failure. Among women with APS a lower sonographic count of antral follicles was reported as compared to healthy controls while anti-corpus luteum antibodies were detected in 11% of women with APS and none in healthy controls. AMH, FSH, LH and estradiol levels did not differ between the two groups studied[viii]. Furthermore, a significant association was found between aPL positivity and decreased levels of AMH among 351 infertile women, supporting the negative impact of these antibodies on ovarian reserve[ix]. Vascular endothelial growth factor (VEGF) is decreased in APS. When a VEGF trap was administered in marmoset, decreased

AMH expression was observed in their early and post-antral follicles compared to controls[x]. Decreased AMH expression in the ovaries makes them more sensitive to FSH contributing thus, to ovulation atresia and impaired ovarian reserve.

Antiphospholipid antibodies (aPL) in pregnancy act directly on trophoblasts by activating pro-apoptotic and pro-inflammatory mechanisms. At the same time, thrombosis of the placental chorea arteries and the activation of the complement intravascularly implicate the cell death of the trophoblast [xi]. Treatment usually comprises antithrombotic therapy using antiplatelet and anticoagulant agents. However, even with apparently adequate anticoagulation, failure to achieve pregnancy remains high.

Aim of study

The aim of this systematic review and meta-analysis is to determine whether implantation failure after IVF-ET is associated with the presence of several types of aPL without diagnosis of APS.

Methods

Protocol

Search strategy and selection of studies

This systematic review and meta-analysis was based on a protocol registered prospectively in PROSPERO database for systematic review protocols (ID: CRD42018081458)[xii] and follows Preferred reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[xiii]. The electronic databases of Medline (Pubmed) and Cochrane library were reviewed systematically from inception to December 2017, using appropriate controlled vocabulary and free search terms to identify studies evaluating fertility in women in association with presence of any type of aPL antibodies (detailed search strategy is available in appendix). Titles, abstracts and full text (when appropriate) of all identified studies were screened for eligibility by one author (E.P.). The same author extracted in a pre-specified standardized MS Excel form relevant data from the studies including: full reference; study identifiers; study design; eligibility; predefined outcomes; number of participants (population index and controls); characteristics of participants; details on the outcomes of interest. The extracted characteristics of participants were: age; cause of subfertility wherever applicable; number of years of subfertility wherever applicable; numbers of IVF/ET attempts wherever applicable; number of retrieved and fertilized oocytes; quality of embryos (defined as regular blastomeres, or according to the presence of even cleavage; even cell sizes; less than 20% fragmented blastomeres); number of transferred embryos wherever applicable; previous medical history; time period in which participants were enrolled; provenance of participants; laboratory technique for measurement of any type of aPL antibodies. All these steps were validated by a second reviewer (A.G.M.). Disagreement was resolved by discussion or adjudication by a third investigator if necessary (G.M.)

Criteria for studies inclusion in the meta-analysis

Studies fulfilling all of the following criteria were included in this meta-analysis:

(i) Studies published in English with prospective or retrospective observational design

(ii) All study populations should be healthy women of reproductive age not suffering any known autoimmune, endocrine or infectious diseases.

(iii) Studies comparing the prevalence of any type of aPL between women experiencing at least two failures in IVF-ET (population index) *vs.* either women experiencing one successful IVF-ET (controls 1) or women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET (controls 2). Studies with controls 1 were included in *subgroup A of selected studies*, while studies with controls 2 were included in *subgroup B of selected studies*.

Study outcomes extracted for the meta-analysis

The primary outcome extracted from the selected studies was the presence or not of any type of aPL antibodies. Secondary outcomes extracted from the selected studies were the presence or not of: anticadiolipin (anti-aCL) antibodies; anti-phosphatidylserine (anti-PS) antibodies; anti-phosphatidylcholine (anti-PC) antibodies; anti-phosphatidylethanolamin (anti-PE) antibodies; anti-phosphatidylinositol (anti-PI) antibodies; anti-beta2 glycoprotein I (anti-β2-GPI) antibodies; lupus anticoagulant (LA) antibodies; any other specific type of aPL antibodies assessed in the included studies (i.e. anti-phosphatidyl-glycerol (anti-PG); anti-phosphatidic acid (anti-PA)).

Risk of Bias Assessment

Risk of bias was assessed by two authors independently (E.P. and A.G.M.) by employing the Newcastle-Ottawa Scale[xiv]. The Newcastle–Ottawa scale is a tool used for assessing the quality of non-randomized studies included in a systematic review and/or meta-analyses. Using the tool, each study is judged on eight items, categorized into three groups: the selection of the study groups; the comparability of the groups; and the ascertainment of either the exposure or outcome of interest for case-control or cohort studies respectively (*see also* appendix). Disagreement was resolved by discussion or adjudication by a third investigator if necessary (G.M.). It was also

planned to evaluate publication *bias* by means of a funnel plot in case more than 25 studies were included in the meta-analysis.

Statistical analyses

A systematic review of studies addressing a common question will inevitably bring together material with an element of diversity. Studies will differ in design and conduct as well as in participants, interventions, exposures or outcomes studied. Such diversity is commonly referred to as methodological or clinical heterogeneity, and may be responsible for observed discrepancies in the results of the studies. Statistical heterogeneity exists when the outcomes being evaluated differ between studies, and may be detectable if the variation between the results of the studies is above that expected by chance. Addressing statistical heterogeneity is one of the most troublesome aspects of many systematic reviews. The interpretative problems depend on how substantial the heterogeneity is, since this determines the extent to which it might influence the conclusions of the meta-analysis. Heterogeneity among the selected studies was evaluated in each analysis, using I^2 statistic[xv-xvi].

According to Cochrane handbook: $I^2 \geq 75\%$, I^2 between 75% and 50% or $I^2 \leq 50\%$ were considered to reflect substantial, significant or non-significant heterogeneity among the selected studies, respectively[xiv]. When I^2 was found $\geq 75\%$, possible causes were investigated by performing pre-specified subgroup analyses. Meta-analysis was not performed in case of substantial heterogeneity which could not be resolved by subgroup analysis. In these cases, findings were reported narratively.

All outcomes were dichotomous and were analyzed by calculating relative risks (RR) and 95% confidence intervals (CI). For data synthesis the fixed or random effect models were employed in the presence of non-significant or significant heterogeneity, respectively. In addition, *a priori* specified sensitivity and subgroup analyses were conducted in the meta-analysis protocol to explore significant or substantial heterogeneity and to further evaluate the soundness of results.

In pre-specified subgroup analyses, studies were grouped according to their controls (1 or 2). In addition, in pre-specified sensitivity analyses, meta-analyses performed with *the fixed effects model* were repeated using *the random effects model* and *vice versa*. All analyses were performed using Review Manager 5.3 Software[xvii].

Results

Search strategy for the systematic review

The search strategy identified 434 and 73 references in Medline and Cochrane Library, respectively (Figure 1). After removal of duplicate records between the two databases, a total of 442 studies were screened by title and abstract. Of these references, 33 were deemed potentially eligible because they reported studies regarding healthy women of reproductive age with implantation failure after IVF-ET and were assessed by full-text. Eighteen of these 33 studies were excluded either because the outcome was not precisely reported, or because the study design did not fulfill the inclusion criteria set for this systematic review. In the remaining 15 studies included in the systematic review and meta-analysis, 3,633 healthy women of reproductive age were evaluated. The selection process is described in Figure 1, while the characteristics of each of the included studies are reported precisely below.

Characteristics of the included studies

1. Coulam 1997

Reference: Carolyn B. Coulam, Brian D. Kaider, Azadeth S. Kaider, Patrick Janowicx, and Roumen G. Roussev. Antiphospholipid Antibodies Associated with Implantation Failure After IVF/ET. Journal of Assisted Reproduction and Genetics, Vol. 14, No. 10, 1997

Methods	Group of 312 women with implantation failure was compared with group of 100 fertile control women.
Participants	All women had unexplained IVF/ET failure without obvious ovulatory, male, or uterine factors. To be included in the implantation failure group, each woman had to have had at least 12 embryos transferred without subsequent positive pregnancy test. The number of years of infertility, the cause of infertility and the number of previous clinical, ectopic, and biochemical pregnancies were recorded and did not differ. The numbers of oocytes retrieved and fertilized and number of embryos transferred during previous IVF/ET attempts were also noted by the authors.
Interventions	Elisa assay was used to measure IgG, IgM, and IgA anticardiolipin, anti-phosphatidylethanolamine, anti-phosphatidylinositol, anti-phospatidic acid, anti-phosphatidylglycerol, anti-phosphatidylcholine and anti-phosphatidylserine.
Outcomes	Positive antiphospholipid antibodies were detected in 69 (22%) of the 312 women with implantation failure compared with 5 (5%) of the 100 control women ($P < 0.0001$). Anticardiolipin antibodies were found positive in 13 (4%) of the 312 women with implantation failure and none of the controls. Fifty-six (18%) of the 312 with implantation failure were negative for anticardiolipin antibodies but had positive values of other anti-PL.

2. Vaquero 2006

Reference: Elena Vaquero, Natalia Lazzarin, Donatella Caserta,Herbert Valensise, Marina Baldi, Massimo Moscarini, Domenico Arduini. Diagnostic evaluation of women experiencing repeated in vitro fertilization failure. European Journal of Obstetrics & Gynecology and Reproductive Biology 125 (2006) 79–84

Methods	Group of 59 patients with at least two unsuccessful IVF attempts were included in the study and compared with 20 healthy controls.
Participants	Fifty nine (59) non pregnant patients with at least two unsuccessful IVF attempts, in the period of January 2003 to December 2003, were included in the study. These past IVF attempts were characterized by good quality embryos (i.e.regular blastomeres and no minor fragments). This group compared with 20 non pregnant healthy fertile women, who had at least two previous uncomplicated pregnancies. No differences were observed between groups in term of age and BMI.
Interventions	Elisa assays for anticardiolipin antibodies and β-2-glycoprotein-IgM and IgG class were performed.
Outcomes	The presence of anti-PL was detected in 19% (11 out of 59) of women experiencing IVF failure, whereas none of the healthy patients were aPL positive. Among the 11 positive patients, 6 were positive for aPL, 3 showed the presence of LA and in 2 we found positive for both.

3. Alves 2005

J Delgado Alves, EL Radway-Bright, S Lee, B Grima, J Hothersall, CT Ravirajan, DA Isenberg. Antiphospholipid antibodies are induced by in vitro fertilization and correlate with paraoxonase activity and total antioxidant capacity of plasma in infertile women. Lupus, 2005;14(5):373-80

Methods	Group of 70 infertile women (18 before IVF, 13 submitted to one IVF cycle and 39 after three cycles) compared with 28 healthy controls.
Participants	Group of 70 consecutive infertile females undergoing routine IVF treatment were selected. None of the patients had any other disease. IVF indications were tubal factor, male partner, unexplained infertility and endometriosis. A group of infertile women before the first IVF treatment (n=18) was included as a second control group. *(This group was not be included in the meta-analysis)*. Of those who had three IVF cycles, 11 had a successful outcome after the third cycle and 28 were unsuccessful (18 miscarriages and 10 implantation failures). 28 age-matched healthy fertile women, as evidenced by at least one healthy pregnancy selected as control group.
Interventions	ELISA assays for anticardiolipin and antiphosphatidylserine IgG and IgM. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS). Nonparametric tests were employed to compare differences between groups (Kruskall–Wallis test), and to evaluate associations between variables (Spearman's rank).
Outcomes	Of the 52 patients with unsuccessful IVF: positive for IgG anti-CL was 56%, for IgM anti-CL 96%, for anti-PS IgG 92 % and for anti-PS IgM 27%. None of the two control groups were positive for anti-CL or anti-PS antibodies. There was a significant difference between antiphospholipid titers, when the IVF patients were compared with infertile patients and normal controls ($P < 0.001$ for both isotypes of anti-CL and anti-PS). There were no significant differences in anti-CL and anti-PS titers among different types of infertility.

4. Ulcova-Gallova 2005

Zdenka Ulcova-Gallova, Vladimir Krauz, Pavla Novakova, Lucie Milichovska, Zdenka Micanova, Katerina Bibkova, Renata Sucha, Jiri Turek, Miroslav Balvin, Zdenek Rokyta. Anti-Phospholipid Antibodies against Phosphatidylinositol, and phosphatidylserine are More Significant in Reproductive Failure than Antibodies against Cardiolipin only. American Journal of Reproductive Immunology 54 (2005) 112–117

Methods	Group of 2965 women with reproductive failure compared with 391 healthy fertile women.
Participants	A total of 2965 patients aged 22–44 years (average 27.5 years) attended the Special Division for Infertility and Reproductive Immunology at the Department of Obstetrics and Gynecology, Charles University, and Faculty Hospital, Pilsen, Czech Republic during 1998–2003. Group 1=1073 women after one in vitro fertilization (IVF), group 2=853 women after two and more IVF, group 3= 627 women after three and more repeated spontaneous miscarriages or missed abortions, group 4= 412 women after diagnostic laparoscopy. *Groups 3 and 4 were not included in the meta-analysis.*
Interventions	ELISA assay was used for detection of aPL against phosphatidic acid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol.
Outcomes	Positive anti-PL: 928/1926, positive anti-CL: 421/1926, anti-PS: 778/1926, anti-β-2GLP1: 209/1926. The control group of 391 fertile healthy women had very slight positivity against anti-PE IgG (0.5%) 2/391, against anti-PG IgM (1.0%) 4/391, against anti-CL IgG (1.3%) 5/391, anti-CL IgM (0.77%) 3/391.

5. Coulam 2002

Coulam CB, Roussev R. Chemical pregnancies: immunologic and ultrasonographic studies. AJRI 2002; 48:323–328

Methods	Group of 122 women with implantation failure after IV-ET versus 107 normal control women.
Participants	The study population consisted of 20 women who underwent IVF-ET and experienced a chemical pregnancy. The results of this group were compared with those from three groups of women: (1) 122 women with implantation failure after IVF-ET associated with a negative pregnancy test, (2) 302 women experiencing two or more recurrent spontaneous abortions (positive controls) and (3) 107 normal control women (negative controls). A chemical pregnancy was defined as at least two rising values of serum human chorionic gonadotropin (hCG) concentrations without demonstration of a gestational sac after 3 weeks from ET. IVF implantation failure was defined as failure of implantation after transfer of cumulatively at least eight 'good' cleaving embryos or four blastocysts.
Interventions	ELISA assay was used to detect antiphospholipid antibodies.
Outcomes	Women experiencing chemical pregnancies had a higher frequency of anti-PL than fertile control women (16/20=80% versus 7/105=6%, $p < 0.0001$) and women with implantation failure associated with a negative pregnancy test (16/20=80% versus 34/122=28%, $p < 0.0001$) and women experiencing recurrent spontaneous abortion (16/20=80% versus 68/302=22%, ($p < 0.0001$).

6. Sanmarco 2007

M. SANMARCO, N. BARDIN, L. CAMOIN, A. BEZIANE,F. DIGNAT-GEORGE,M. GAMERRE, G. PORCU. Antigenic Profile, Prevalence, and Clinical Significance of Antiphospholipid Antibodies in Women Referred for in Vitro Fertilization. Ann. N.Y. Acad. Sci. 1108: 457–465 (2007)

Methods	Group of 101 infertile women with at least three unsuccessful IVF attempts were compared with age-matched healthy fertile women (n = 160) were included as controls.
Participants	101 non-pregnant women were recruited to this study between January 2003 and January 2000, referred for IVF treatment to three Centers for assisted Medical Procreations and Medical Institutes of Reproductive Medicine in the South of France. The inclusion criteria were age from 18 to 38 years (mean±SD, years=32±3.8) and at least two previous unsuccessful IVF-ETs. Women with a history of prior pregnancy, one or more prior miscarriages, uterine malformation; a myoma; a history of clinical thrombosis or autoimmune disease; diabetes mellitus; a history of acute or chronic infectious disease; epilepsy or neuroleptic treatment were excluded. The control population consisted of 160 non-pregnant, age-matched healthy fertile women without any history of either reproductive problems or thrombosis or any autoimmune disorder or infectious disease.
Interventions	ELISA assay was used to detect anti-PL (anti-CL, b-2-GPI, anti-PE antibodies) and LA. Patients with an initial positive result for any anti-PL underwent a second test (12 weeks apart) just prior to the next ovulation induction treatment. The chi-square test with Fisher's exact test was used to assess the relationship between antibodies and implantation.
Outcomes	Positive for at least one anti-PL were 40/101 (39.6%) infertile women whereas 8 controls were positive (8/160; $p < 0.0001$), for anti-CL 6/101, for anti-PE 24/101, for anti-β2GPI 16/101 were positive. No results for anti-CL, anti-PE, anti-β2GPI for control group were reported.

7. Steinvil 2010

Arie Steinvil, Raanan Raz, Shlomo Berliner, David M. Steinberg, David Zeltser, David Levran4, Orit Shimron; Tal Sella, Gabriel Chodick, Varda Shalev, Ophira Salomon. Association of common thrombophilias and antiphospholipid antibodies with success rate of in vitro fertilization. Thromb Haemost 2012; 108: 1192–1197

Methods	Group of 509 women with unexplained IVF failure were compared with 637 healthy controls.
Participants	Women with unexplained infertility initiating IVF treatments not older than 38 years from registry database of Maccabi Healthcare Services (MHS), undergoing IVF treatments from January 2000 through December 2010. Women with previous venous thromboembolism and/or treated with low-molecular-heparin, as well as identified reason for infertility i.e. ovarian insufficiency, male factor, mechanical factors etc were excluded.
Interventions	ELISA assay was used to detect IgG anticardiolipin and beta 2 glycoprotein I antibodies using Org 515 and Org 521 kits respectively. Lupus anticoagulant was determined by using a dilute Russell's viper venom time-based assay.
Outcomes	17/509 of the study population were positive for at least one anti-PL whereas 30/637 were positive of the control group.

8. Paulmyer-Lacroix 2014

Odile Paulmyer-Lacroix,Laura Despierres, Blandine Courbiere, Nathalie Bardin, Antiphospholipid Antibodies in Women Undergoing In Vitro Fertilization Treatment: Clinical Value of IgA Anti-β2glycoprotein I Antibodies Determination. Hindawi Publishing Corporation BioMed Research International Volume 2014, Article ID 314704

Methods	Group of 40 women after at least 2 IVF failures compared with 100 healthy controls
Participants	Women undergoing IVF from 2005 to 2011 and no pregnancy occurred after at least two IVF attempts with good quality embryos available for the transfer (i.e. even cleavage, even cell sizes and <20% fragmentation). IVF treatment was performed at the Reproductive Department of University Hospital La Conception and was proposed to couples in female (ovulation disorders, tubal obstruction, and endometriosis), male, mixed, or unexplained infertility. Mean women's age was 35 ± 4.15 years at the time of aPL detection. IVF indications were distributed as follows: female infertility (8 patients), male infertility (21 patients), mixed infertility (10 patients), and unexplained infertility (1 patient).
Interventions	ELISA were used to detect aCL (IgM, IgG, and IgA) and aβ2GPI IgA antibodies.
Outcomes	Total prevalence of 20% (8/40) of aPL was found in study population significantly different from that of the control population ($p < 0.0005$) and anti-β2GPI IgA antibodies significantly higher in IVF patients (12.5%, 5/40) than in controls (1%, 1/100) ($p= 0.01$).

9. Stern 1998

Catharyn Stern, Lawrence Chamley, Lyndon Hale, Michael Kloss, Andrew Speirs, H. W. Gordon Baker. Fertility and Sterility, Vol. 70, No. 5, Nov. 1998

Methods	Group of 105 patients undergoing IVF without clinical pregnancy compared with 106 fertile women.
Participants	Patients aged 24 to 47 years (mean age, 35 years) who enrolled in IVF program between January 6, 1996, and July 8, 1997, and who had undergone multiple ETs and previously had at least 10 embryos transferred without clinical pregnancy. A clinical pregnancy was defined as a pregnancy diagnosed initially by biochemical means at 17 days after ET (serum βhCG level of .100 IU) with consequent evidence of a gestational sac with or without a fetal heart observed on transvaginal ultrasound 28 days after ET). The fertile control group consisted of 106 women who had at least one child born at term without any major pregnancy complications and without any period of subfertility (i.e., .6 months of trying) before conception. These women ranged in age from 19–45 years (mean age, 33.6 years).
Interventions	ELISA was used to detect IgG and IgM isotypes of each of anti-CL antibody, anti-PS, anti-PE, anti-PI and anti-b2 GPI antibodies. Activated partial thromboplastin time (normal, 27–38 seconds), kaolin clotting time ratio (normal<1.2), kaolin clotting time mixing test ratio (normal<1.2) and dilute Russell viper venom ratio were used to evaluate lupus anticoagulant. Statistical analysis included chi-square and Fisher's exact tests for differences between groups, and multiple linear regression analysis and Spearman's nonparametric tests for relations between results.
Outcomes	30/105 of the patients were positive to at least one anti-PL test whereas 16/106 of the control group were positive. Anti-b-2 glycoprotein I IgM and antinuclear antibodies were significantly associated with both IVF implantation failure and recurrent miscarriage.

10. Bellver 2008

Jose´ Bellver, Sergio R. Soares, Claudio A lvarez, Elkin Munoz, Alberto Ramirez,Carmen Rubio, Vicente Serra, Jose Remohi, Antonio Pellicer**,** The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion. Human Reproduction Vol.23, No.2 pp. 278–284, 2008

Methods	31 women with unexplained infertility (UI), 26 implantation failure (IF), 30 with recurrent abortion compared with 32 controls.
Participants	This is a prospective study, in which women were enrolled between first March, 2004 and first January, 2007. The experimental group consists of 31 women with UI more than one year, 26 with IF at least twice with embryo transfer of at least two good quality embryos, 30 women with recurrent abortions. All were younger than 38 years old, with absence of autoimmune or endocrine disorders, with normal ovarian function, normal hysterosalpingography and transvaginal ultrasound scan of the uterus and ovaries, normal karyotype, and whose partner had normal karyotype and normozoospermia. The control group included 32 women of 18–35 years old, all Caucasian, with normal karyotype, no history of spontaneous abortions, autoimmune disorders or endocrine diseases, and previous term pregnancies without complications.
Interventions	ELISA assay was used to detect IgM and IgG anticardiolipin antibodies. Lupus anticoagulant was performed using a clotting assay with LA1 screening reagent/LA2 confirmation reagent result was expressed as positive or negative.
Outcomes	Positive for anti-CL IgG: 0 controls, 3 UI, 1 IF, 0 RSA (non-statistical significant). Positive for anti-CL IgM: 6 controls, 3 UI, 1 IF, 6 RSA (non-statistical significant). Positive for Lupus anticoagulant: 0 controls, 0 UI, 3 IF, 1 RSA (non-statistical significant -p= 0.056).
Notes	Women included in the control group were significantly younger and had more live births than in the other three groups.

11.Qublan 2006

Hussein S.Qublan, Suhair S.Eid, Hani A.Ababneh, Zouhair O.Amarin, Aiman Z.Smadi,Farakaid F.Al-Khafaji, Yousef S.Khader. Acquired and inherited thrombophilia: implication in recurrent IVF and embryo transfer failure. Human Reproduction Vol.21, No.10 pp. 2694–2698, 2006

Methods	The study group comprised of 90 consecutive women with three or more previously failed IVF–ET cycles (group A) compared with two control groups: women who have had successful pregnancy after their first IVF–embryo transfer cycle (n = 90/group B) and women who conceived spontaneously with at least one uneventful pregnancy and no previous history of miscarriage (n = 100/group C).
Participants	Women with a history of at least three previously failed IVF–ET treatments, presenting to the infertility clinic between January 2001 and August 2005, were included in this study (group A). Women's age ranged from 23 to 44 years (mean ± SD, 31 ± 4.2). Women's age of group B ranged from 22 to 40 years (mean ± SD, 30 ± 3.1) and group C ranged from 17 to 41 years (mean ± SD, 30 ± 2.8). Women with endometriosis, hydrosalpinx, abnormal uterine cavity on the hysterosalpingogram and history of thromboembolic disease and those who were receiving hormonal treatment were not included in the study group. In the IVF/ET cycles, only cycles in which grade 1 and 2 embryos were transferred were included in the study group. Indications for IVF treatment included anovulation, unexplained infertility and male and tubal factor.
Interventions	ELISA assay was used to detect IgM and IgG anti-CL antibodies. LA test was performed by the kaolin cephalin clotting time utilizing sensitive reagents and by the dilute Russell's viper venom time with a neutralization procedure using frozen–thawed platelets.
Outcomes	Positive for LA were: 8/90 of group A, 2/90 of group B and 2/100 of group C. Positive for anti-Cl antibodies were: 9/90 of group A, 2/90 of group B and 3/100 of group C.

12. E.Geva 1995

E.Geva, A.Amit, L.Lerner-Geva, F.Azem, I.Yovel and J.B.Lessing, Autoimmune disorders: another possible cause for in-vitro fertilization and embryo transfer failure. Human Reproduction vol.10 no. 10 pp.256O-2563, 1995

Methods	50 infertile patients with 3 or more IVF failures versus 40 patients who had conceived and delivered following < 3 IVF-ET.
Participants	The study group comprised 50 IVF patients with three or more previously failed cycles after embryo transfer. The study group consisted of two subgroups according to the cause of infertility: 24 patients were diagnosed as infertile from mechanical factors [tubal occlusion proven by hysterosalpingography, no evidence of endometriosis in laparoscopy and normal postcoital test (PCT) prior to treatment], and26 as unexplained infertility (unexplained infertility was defined as normal history and examination of both partners, a history of at least 2 years of infertility, regular ovulation, normal sperm analysis, normal PCT, normal uterus, patent tubes, no pelvic disease and no sperm antibodies). The control group comprised 80 computer-matched women: 40 who had conceived and delivered following IVF and embryo transfer cycles and were matched for age, duration and type of infertility cycles, and 40 who were healthy nulligravidas (*the second control group was not used in the metanalysis)*
Interventions	ELISA was used to detect IgG aCL. LA was estimated using the clotting time of citrated plasma was measured by a co agulometer MLA 1000 (Medical Laboratory Automation Inc., Pleasantville, NY, USA).
Outcomes	6.0% (3/50) of patients were positive for anti-CL antibodies and none of the control group. LA was negative in both groups.

13. Birkenfeld 1994

A. Birkenfeld, T. Mukaida, L Minichiello, M. Jackson, N.G. Kase,M. Yemeni. Incidence of Autoimmune Antibodies in Failed Embryo Transfer Cycles. Birkenfeld A, Mukaida T, Minichiello L, Jackson M, Kase NC, Yemini M. AJRI 1994; 31 :65-68

Methods	Three groups were studied: group I, 56 patients who failed to conceive following ET, group II, 14 patients who have conceived following IVF-ET and delivered or are carrying an uncomplicated ongoing pregnancy and group III, 69 patients who were new candidates for IVF-ET.
Participants	Participants included in group I, (56 consecutive patients) failed to conceive following one or more ETs (either fresh or frozen) between October 1992 to September 1993 were studied for the presence of autoimmune factors. In group II (14 patients) conceived following IVF-ET during the same period and delivered or are carrying an uncomplicated ongoing pregnancy beyond the 12^{th} gestational week, were studied as the control group. In group III 69 patients were new candidates for IVF-ET programs between February and July 1993 were prospectively studied for the presence of autoimmune factors. The indication for IVF in all patients was tubal mechanical factors. Couples with male factors were excluded from this study.
Interventions	Lupus anticoagulant was detected by modified Russell Viper Venom assay through demonstration of prolonged coagulation times. ELISA assay was used to detect the presence of IgG and IgM anticardilipin antibodies. Statistical analysis was performed using Fisher's exact test and Chi-square analysis (two-tailed).
Outcomes	18/56 (32.1 %) of patients in group I tested positive for one or more of the autoimmune antibodies. 0/14 of group II tested positive for autoimmune antibodies *($p<0.02$).* 7/69 (10%) of group III were found positive to one or more of the autoimmune factor. This rate is significantly lower than the rate of positive autoimmune antibodies detected in group I ($P<0.003$). 10/18 from group I were positive for anti-CL and 10/18 of group I were positive for LA.
Notes	All patients in the study group, who were positive for one or more of

	the autoimmune factors during their first cycle and underwent a subsequent IVF-ET cycle, were treated with 10 mg of prednisone daily and 80 mg of aspirin daily, beginning 2 week prior to the initiation of the cycle. Prednisone was then increased to 20 mg daily with the first positive beta human chorionic gonadotrophin (~hCG).

14. Buckingham 2006

K.L.Buckingham, P.R.Stone, J.F.Smith, L.W.Chamley. Antiphospholipid antibodies in serum and follicular fluid is there a correlation with IVF implantation failure? Human Reproduction Vol.21, No.3 pp. 728–734, 2006

Methods	Total of 99 women undergoing IVF, 28 with implantation failure and 71 with implantation success.
Participants	All women undergoing IVF treatment at Fertility Plus, National Women's Hospital, Auckland were eligible for entry. Fertilization was determined by the presence of two adjacent pronuclei 16–18 h later. Embryos were maintained in culture and transferred to the uterus transcervically under ultrasound guidance using a Sydney IVF transfer catheter, 48–72 h after oocyte retrieval. A maximum of three embryos were transferred (mean two embryos) at one time. Fresh embryo transfers occurred in 87 women, 6 women had a 'freeze all', whereby all the embryos were cryopreserved. A further 6 women did not proceed to embryo replacement. Those women who did not have an embryo replacement were all aPL negative.
Interventions	ELISA assay was used to detect IgG and IgM isotypes for anti-β2GPI, anti-CL and anti-PS antibodies.
Outcomes	5 patients were anti-PL positive of 22 unsuccessful IVF and 13 patients were anti-PL positive of 71 successfull IVF group.

15. Kaider 1996

Kaider BD, Price DE, Roussev RG, Coulam CB. Antiphospholipid antibody prevalence in patients with IVF failure. AJRI 1996; 35:388-393

Methods	Group from 42 women with IVF failure compared with 42 women who successfully conceived after IVF were tested for the presence of aPL.
Participants	Participants have had at least 12 embryos transferred during several IVF cycles without ensuing pregnancy. Successful post-IVF pregnancy was determined by obtaining two consecutive rising beta-hCG levels followed by an ultrasound to confirm a viable conceptus.
Interventions	ELISA assay was used to detect three isotypes of antibody: IgA, IgG, and IgM against seven phospholipids: cardiolipin (CL), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidyl-glycerol (PG), phosphatidylcholine (PC), and phosphatidyl-serine (PS)
Outcomes	IVF failure group 11/42 (26.2%) were positive for anti-PL, the control group, 2/42 (4.8%) were found positive only for IgA against PE *p=0,01*

Characteristics of the women in the selected studies

The women with implantation failure after IVF-ET included in all studies had at least two implantation failures (range of failures: 2-6) characterized by good quality embryos and absence of subsequent positive pregnancy tests (based on beta-human chorionic gonadotropin (β-hCG) evaluation). Two studies evaluated the presence of a gestational sac three weeks after embryo transfer[xviii-xix]. In 3 studies implantation failure was defined by the absence of signs of implantation after transfer of 12 or more embryos of good quality [xviii-xx-xxi]. All women included in these studies were not receiving any treatment.

Subgroup A of selected studies: Among the five studies of this subgroup, four included women (population index and controls 1) referred for IVF-ET with similar indications[xxii-xxiii-xxiv-xxv]. Unexplained infertility was the predominant indication and it was followed by tubal factor for the majority of these women. In one study indications for IVF-ET were not reported[xxi]. Age, duration and type of infertility did not differ between population index and controls studied in this subgroup of selected studies according to the reported data.

Subgroup B of selected studies: In some studies of this subgroup, indications for IVF-ET were not reported[xviii-xx-xxvi-xxvii-xxviii]. Therefore, it was assumed that the majority of women experiencing at least 2 failures in IVF-ET and included in these studies, were referred for IVF-ET due to unexplained infertility, tubal or male factor. Other not frequent indications for IVF-ET in studies of *subgroup B* were endometriosis, ovulation disorders or mixed infertility. In one study the controls were significantly younger than the women experiencing at least 2 failures in IVF-ET[xxix].

Characterisitcs of the included studies in the systematic review

Ovarian stimulation protocols employed: Three studies reported that a standardized protocol for ovarian stimulation was followed (a combined regimen of GnRH agonists and human menopausal gonadotrophins)[xxiv-xxv-xxviii]. The remaining studies did not report the specific ovarian stimulation protocol employed.

Assays employed for the antibodies evaluation: All selected studies employed enzyme-linked immunoabsorbent assays (ELISA) for detection of any type of aPL antibodies. Lupus anticoagulant was evaluated by the kaolin cephalin clotting time utilizing sensitive reagents and by the dilute Russell's viper venom time with a neutralization procedure using frozen–thawed platelets. Results for anti-PL and LA

antibodies were expressed as positive or negative. Three studies provided a medium-high titer cut-off to define positivity for anti-PL antibodies [xxvi-xxviii-xxix].

Subgroup A of selected studies: Five studies (n=number of women studied; n=260) evaluated the prevalence of any type of anti-PL antibodies (anti-aCL, anti-PS, anti-PC, anti-PE, anti-PI and anti-β2-GPI antibodies)[xxi-xxii-xxiii-xxiv-xxv]. Three studies (n=182) evaluated the prevalence of anti-CL antibodies only[xxi-xxiv-xxv]. One study (n=42) evaluated the presence of anti-PS, anti-PI, anti-PC, anti-PE, anti-PA and anti-PG antibodies[xxi]. Meta-analysis was performed for the prevalence of any type of anti-PL antibodies and for anti-CL antibodies only.

Subgroup B of selected studies: Eleven studies (n=3,373) evaluated the prevalence of any type of anti-PL antibodies [iv-xviii-xix-xx-xxv-xxvi-xxvii-xxviii-xxix-xxx-xxxi]. Five studies (n=2432) evaluated the prevalence of anti-CL antibodies only [xx-xxv-xxvii-xxix-xxx]. Three studies (n=2004) evaluated the presence of anti-CL-IgG as well as anti-CL-IgM antibodies[xxvii-xxix-xxx] . Three studies (n=175) evaluated the prevalence of LA antibodies only[xxv-xxvi-xxix]. The presence of anti-β-2GPI and anti-PS antibodies were evaluated by two studies each (n=1966[xxvii-xxxi] and n=1978[xxvii-xxx], respectively). One study (n=1926) evaluated the presence of anti-PI, anti-PA, anti-PE and anti-PG antibodies[xxvii]. Meta-analysis was performed for the prevalence of anti-CL-IgG, LA, anti-β-2GPI and anti-PS antibodies.

Qublan et al. evaluated women with 3 or more previous implantation failures after IVF-ET compared with both controls 1 and controls 2. Therefore, this study is included in both subgroups A and B of selected studies[xxv].

Data from one study of subgroup A[xxi] and one study of subgroup B[xxvii], which evaluated additional types of anti-PL antibodies (anti-PS, anti-PI, anti-PC, anti-PE, anti-PA and anti-PG antibodies) were not included in the meta-analysis, because they did not fulfill the inclusion criteria set for the meta-analysis. These data are described narratively further-on in the context of this systematic review (Table 2).

Risk of bias assessment

Based on the Newcastle Ottawa scale all studies of subgroup A were rated as of low risk in all assessed domains: selection bias of population index and controls 1, performance bias, detection bias and attrition bias.

Eight out of 11 studies of subgroup B (73%) were rated as having low risk and 3 (27%) as having unclear risk of bias regarding selection of population index. Regarding the controls 2 selection, 5 out of 11 studies of subgroup B (45%) were rated with unclear risk of bias. All studies of subgroup B were rated with low risk of bias regarding performance bias, detection bias and attrition bias. Detailed assessment of the risk of bias is available in figure 2.

Outcomes of Systematic Review and Meta-analysis

Initially, it was planned to include all 15 selected studies in a single analysis but this approach resulted in substantially heterogeneous results due to the different control groups the 15 selected studies. To deal with this situation, studies were analyzed according to the two different control groups (controls 1 and controls 2). Thus, the reported results derive from the distinct analyses of *subgroup A* (women experiencing at least two failures in IVF-ET *vs.* women experiencing one successful IVF-ET) and *subgroup B* (women experiencing at least two failures in IVF-ET *vs.* women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET). The remaining pre-planned sensitivity analyses did not affect the outcomes.

The majority of selected studies supported the association between positivity of any type of aPL antibodies and infertility. Studies with control women experiencing one successful IVF-ET (*subgroup A*) were fairly homogenous (I^2 range: 0-33%) and provided quite reliable results, whereas studies with control women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET (*subgroup B*) presented higher heterogeneity.

Outcomes of subgroup A of selected studies: Twenty-one percent and 7% of women experiencing at least two failures in IVF-ET and women experiencing one successful IVF-ET, respectively, had positive any type of anti-PL antibodies among anti-CL, anti-β2GPI, anti-PS, anti-PC, anti-PE, anti-PI, anti-PA and anti-PG antibodies of IgG, IgM or IgA isotypes (5 studies)[xxi-xxii-xxiii-xxiv-xxv]. Women who had at least one test positive for any type of anti-PL had increased relative risk (RR) for

implantation failure following IVF-ET 3.44, [95% CI: 1.90, 6.23, I^2 = 33%] (Figure 3, panel A).

In addition, 8.2% and 1% of women experiencing at least two failures in IVF-ET and women experiencing one successful IVF-ET, respectively, had positive anti-CL antibodies (3 studies)[xxi-xxiv-xxv]. The presence of anti-CL antibodies only was strongly associated with implantation failure (RR 5.11; 95% CI: 1.51, 17.27; I^2 =0%) among women undergoing IVF-ET (Figure 3, panel B).

Kaider et al. further evaluated anti-PI, anti-PC, anti-PE, anti-PA and anti-PG antibodies. They concluded that the presence of these antibodies is associated with implantation failure after IVF-ET procedures. Prevalences of these types of aPL antibodies are reported in table 2.

Outcomes of subgroup B of selected studies: Nineteen percent and 1% of women experiencing at least two failures in IVF-ET and women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET, respectively, had positive anti-CL-IgG antibodies (3 studies)[xxix-xxx-xxvii]. The presence of the IgG isotype of anti-CL antibodies was more strongly associated with implantation failure after IVF-ET compared to the IgM isotype. Specifically, the presence of IgG anti-aCL antibodies in women experiencing at least two failures in IVF-ET presented RR=14.91 (95%CI: 6.65, 33.45; I^2=0%) compared with women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET (figure 4, panel A).

Eight percent and 1.3% of women experiencing at least two failures in IVF-ET and women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET, respectively, had positive LA antibodies (3 studies)[xxv-xxvi-xxix]. Also the fertilization rate decreased when LA test was positive (RR=4.57; 95% CI: 1.37, 15.25; I^2=0%) compared with controls 2 (Figure 4, panel B).

Two studies assessed anti-β2GPI[xxv-xxix] and anti-PS antibodies[xxviii-xxvix]. Eleven percent and 42% of women with at least two IVF-ET failures had positive anti-β2GPI and anti-PS antibodies, respectively, as compared to 0.2% and 0% of women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET, respectively. The presence of the anti-β-2GPI and anti-PS antibodies was associated with implantation failure after IVF-ET compared

with controls 2 (RR=55.60; 95% CI: 6.98, 442.84; I^2=50%, and RR=226.89; 95% CI: 27.64, 1862.61; I^2=0%, respectively) (Figures 4, panel C and panel D, respectively).

Ulcova-Gallova et al. further evaluated anti-PI, anti-PE, anti-PA and anti-PG antibodies and concluded that the presence of these antibodies is associated with implantation failure in IVF-ET procedures. Prevalence for each type of these aPL antibodies in this study are reported in table 2.

The presence of any type of anti-PL antibodies and of anti-CL antibodies only, as evaluated in 11 studies and 5 studies, respectively, in *subgroup B* were consistently associated with increased risk of implantation failure, but due to substantial heterogeneity a meta-analysis was not meaningful. The prevalence of any type of aPL antibodies and of anti-CL antibodies only are reported in table 3.

Two studies of this subgroup did not show any association of the presence of any type of anti-PL antibodies or anti-CL antibodies only with IVF-ET outcome[iii-xxvii]. However, the results of both studies should be examined with caution as *Bellver et al* were based on a very limited study population, while it is very unclear how *Steinvil et al* selected their control group (controls 2).

Discussion

Among 507 references that this systematic search yielded from Medline and Cochrane Library, 15 selected studies, involving 3,633 women of reproductive age were included in this systematic review and meta-analysis. All studies involved women with at least two IVF-ET failures *vs.* women with one successful IVF-ET or women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET. When a specific type of aPL antibodies was evaluated in heterogeneous or single studies, the reported data were not included in the meta-analysis.

We found, in this meta-analysis, that in women experiencing at least two failures in IVF-ET, the presence of any type of anti-PL antibodies and of anti-CL antibodies only is associated with a significant 3.44 and 5.11 RR for decreased implantation rate, respectively, as compared to women experiencing one successful IVF-ET. In addition, in women experiencing at least two failures in IVF-ET, the presence of either anti-CL or LA or anti-β-2GPI or anti-PS antibodies is associated with a significant 14.91, 4.57, 55.6 and 226.89 RR for decreased implantation rate, respectively, as compared to women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET. Most importantly, the presence of anti-β-2GPI and of the rarely evaluated anti-PS antibodies in 11% and 41%, respectively, of all women experiencing at least two failures in IVF-ET and in virtually none of controls 2 suggests that these may be very accurate biomarkers (more accurate than the more frequently measured anti-PL antibodies) and urges further evaluation of their potential clinical use in infertility, as well as in the APS in general. This is the first meta-analysis evaluating the presence of any type of anti-PL antibodies in women experiencing implantation failure after IVF-ET. The results from the 15 selected studies were consistent, of strong methodological quality and supported an association between recurrent IVF-ET failure and the presence of anti-PL antibodies. This meta-analysis looked into multiple anti-PL antibodies, including newer markers (i.e. anti-PS antibodies). The main limitation of this meta-analysis is the lack of relevant prospective, controlled studies and the small number of included studies.

Except the above mentioned anti-PL antibodies, which were evaluated by meta-analysis, this systematic review pointed towards other types of anti-PL antibodies, which seem to impair implantation and consequently fertility. The presence of specific types of anti-PL antibodies (i.e. anti-PS, anti-PC, anti-PI and anti-PA antibodies) was significantly increased in women with at least two implantation failures after IVF-ET and was associated with increased implantation failure rates. Of note, these antibodies were totally absent from the respective control groups (Table 2). The prevalence of these specific types of anti-PL antibodies reaches till 40.4% among women with at least two implantation failures after IVF-ET compared to controls 2 (range of prevalence among population index 2.4%-40.4%). Prevalence of anti-PE and anti-PG antibodies was also increased in women with at least two implantation failures after IVF-ET (range among population index 7.1%-19.6%) compared to their respective control groups (range among controls: 0-4.8%) (Table 2). It is noteworthy that the aforementioned types of anti-PL (anti-PS, anti-PC, anti-PI and anti-PA anti-PE and anti-PG antibodies) are rarely evaluated in clinical practice; they might be positive where other more often evaluated types of anti-PL such as anti-CL, LA or anti-β-2GPI antibodies are negative. Thus, questions emerge about their involvement in the underlying pathophysiological mechanisms not only in implantation, but also in the broader spectrum of APS.

The possible association of anti-PL antibodies with female infertility has been suggested since 1980s. However, it still remains a controversial issue particularly because no well-defined etiopathological mechanisms have been recognized as yet. Thus, *in vitro* and *in vivo* studies, have suggested that anti-PL antibodies might have a negative impact on conception, implantation, early and recurrent miscarriages, intrauterine growth restriction (IUGR), pre-term birth and stillbirth. In a 10-year follow-up of an observational, prospective study of 1000 patients (82% women) with primary or secondary APS, 127 (15.5%) women became pregnant (188 pregnancies) with 137 (72.9%) of them ending with a healthy take-home baby. The reported complications were early pregnancy loss (16.5% of all pregnancies), late pregnancy loss (4.8% of all pregnancies), pre-eclampsia/eclampsia (6.4% of all pregnancies), IUGR (26.3% of total live births) and pre-term birth (48.2% of total live births)[xxxii]. According to the authors, the frequencies of complications probably reflect the effect of therapy and medical care during that study, as well as the improvement in the management of the obstetric APS patients during the last decade with the

widespread use of antiaggregant and anticoagulant drugs and the careful monitoring of pregnancies. A retrospective study analysis reported that pregnant women with elevated anti-PL antibodies had an increased risk for preeclampsia and eclampsia, (adjusted odds ratio, aOR = 2.93, p = 0.0015) and placental insufficiency (aOR = 4.58, p = 0.0003) as compared to women without elevated anti-PL antibodies [xxxiii]. Subsequently, the authors suggested that the presence of APS and anti-PL are frequently encountered acquired risk factors for recurrent pregnancy loss and that are associated with increased risk for ischemic placental dysfunction, such as fetal growth restriction, preeclampsia, premature birth and intrauterine death.

The impact of anti-PL antibodies in pregnancy could be exerted *via* mechanisms affecting both placental and endometrial cells. It seems that these antibodies affect maternal blood vessels, decidua and trophoblasts although the physiopathologic mechanisms explaining these phenomena are not yet well elucidated. They have been suggested to target tissue plasminogen activator, plasmin, annexin A2 and thrombin[xxxvii]. β-2GPI and prothrombin mediate binding of anti-PL antibodies to target cells such as endothelial cells, monocytes, platelets and trophoblasts leading to thrombosis of placental vessels and fetal loss.

Anti-PL antibodies and particularly β-2GPI-mediated anti-PL antibodies bind to trophoblasts monolayers and can induce direct cellular injury, inhibition of proliferation and syncytia formation, apoptosis, defective invasiveness and decreased human chorionic gonadotrophin (hCG) production[xxxiv,xxxv] . *In vitro* studies indicate that anti-CL antibodies inhibit trophoblast proliferation possibly by a prostacyclin-thromboxane A2 imbalance[xxxvi,xxxvii]. *In vitro* and *in vivo* studies have shown that aPL antibodies seem to have a direct effect on maternal decidua and invading trophoblast. Anti-phospholipid antibodies can impair spontaneous as well as IVF-ET implantation as they are directed against negatively charged phopsholipids located in the blood vessels of the uterine mucous membrane, or on the surface of oocytes, or they can affect the early embryo at the initial implantation process. Implantation process and the maintenance of pregnancy in its early stages could be affected by inhibition of prostaglandin synthesis caused by anti-PL antibodies[xxxviii].

In mice β-2GPI is essential for a successful pregnancy and for optimal placental development and fetal growth[xxxix]. By experiments in rats and human trophoblast cells, anti-PS antibodies have been shown to affect negatively implantation. Furthermore, anti-PS antibodies bind to human trophoblast in a dose

dependent way affecting thus, trophoblast invasiveness and differentiation of cytotrophoblast into a syncytium. It is also shown that specifically anti-PS and not anti-CL antibodies are responsible for the decrease of hCG production by the placenta.[xl] Patients' anti-PS antibodies when co-cultured with rats embryos delay the development of rat yolk sacs[xl].

In summary, this meta-analysis has shown that the presence of any type of anti-PL antibodies is associated with decreased implantation rate among women experiencing at least two failures in IVF-ET compared to either women experiencing one successful IVF-ET or women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET. The presences of anti-β-2GPI and anti-PS antibodies suggest an excessive risk. Importantly, types of anti-PL that are not frequently measured in daily medical practice (anti-PS, anti-PC, anti-PE, anti-PI, anti-PA and anti-PG antibodies) seem to be stronger predictors of implantation failure in IVF-ET.

Additional confirmatory studies with rigorous methodology would help to confirm the findings of this meta-analysis before recommending the introduction of such biological assessments in everyday medical practice. Well-designed interventional studies might confirm the presence of anti-PL antibodies as predictive markers of implantation failure, but also target them pharmacologicaly in women suffering infertility or subfertility.

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Appendix

Search Strategies

Medline and Cochrane Library

Antiphospholipid

#1 Antiphospholipid syndrome [mh]
#2 Antibodies, antiphospholipid [mh]
#3 Phospholipids/immunology[mh]
#4 Antiphospholipid [tiab]
#5 Anti-phospholipid[tiab]
#6 #1 or #2 or #3 or #4 or #5

Fertility

#7 Infertility, Female [mh]
#8 Infertility, Male [MH] NOT (Infertility, Female [MH])
#9 Infertility [mh] not #8
#10 Fertility Preservation [MH]
#11 Fertility Agents, Female [mh]
#12 Fecundity[mh]
#13 Fecundability [mh]
#14 Ovarian Reserve [mh]
#15 infertility [tiab]
#16 ovarian reserve [tiab]
#17 conception [tiab]
#18 endometrium [tiab]
#19 implantation [tiab]
#20 endometrial implantation [tiab]
#21 #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20
#22 male [mh] not (female[mh])
#23 #21 not #22
#24 #6 and #23
#25 animals[mh] not humans [mh]
#26 #24 not #25

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
a) truly representative of the average _____ (describe) in the community*****
b) somewhat representative of the average _____ in the community
c) selected group of users eg nurses, volunteers
d) no description of the derivation of the cohort
2) Selection of the non exposed cohort
a) drawn from the same community as the exposed cohort*****
b) drawn from a different source
c) no description of the derivation of the non exposed cohort
3) Ascertainment of exposure
a) secure record (e.g, surgical records)*****
b) structured interview*****
c) written self report
d) no description
4) Demonstration that outcome of interest was not present at start of study
a) yes
b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
a) study controls for _____ (select the most important factor) *****
b) study controls for any additional factor***** (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
a) independent blind assessment*****
b) record linkage*****
c) self report
d) no description
2) Was follow-up long enough for outcomes to occur
a) yes (select an adequate follow up period for outcome of interest) *****
b) no
3) Adequacy of follow up of cohorts
a) complete follow up - all subjects accounted for*****
b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) *****
c) follow up rate < ____% (select an adequate %) and no description of those lost
d) no statement

Wells, G. A, Shea, B., O'Connel, D. et al. The Newcastle-Ottawa scale (NOS) for assessing the quailty of nonrandomised studies in meta-analyses. http://www ohri ca/programs/clinical_epidemiology/oxford htm 2009 Feb 1

Legends to the Figures

Figure 1: PRISMA flow diagram of the selection process from identified studies to selected studies through Medline and Cochrane Library.

Figure 2: Risk of bias regarding selection, performance bias, detection bias and attrition bias of all selected studies based on Newcastle-Ottawa Scale.

Figure 3: Meta-analysis of subgroup A of selected studies assessing the presence or not of any type of aPL antibodies (panel A) and of aCL antibodies (panel B) between women with at least two implantation failures in IVF embryo transfer *vs.* women with one successful IVF embryo transfer (controls 1), respectively.

Figure 4: Meta-analysis of subgroup B of selected studies assessing the presence or not of aCL-IgG antibodies (panel A), LA antibodies (panel B), β-2GPI antibodies (panel C) and PS antibodies (panel D) between women with at least two implantation failures in IVF embryo transfer *vs.* women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET (controls 2), respectively.

Table 1: Revised Sapporo Classification Criteria for the Antiphospholipid Syndrome

Clinical criteria: 1. Vascular thrombosis (a) One or more clinical episodes of arterial, venous, or small vessel thrombosis in any tissue or organ. 2. Pregnancy morbidity (a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, or (b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of eclampsia, severe preeclampsia, or recognized features of placental insufficiency, or (c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.
Laboratory criteria 1. Lupus anticoagulant present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis 2. Anticardiolipin antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e., > 40 GPL or MPL, or > the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized enzyme-linked immunosorbent assay (ELISA). 3. Anti-b2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma, (in titer > the 99th percentile) present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.
Definite APS is present if at least one of the clinical criteria and one of the laboratory criteria are met. Classification of APS should be avoided if less than 12 weeks or more than 5 years separate the positive aPL test and the clinical manifestation. In studies of populations of patients who have more than one type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b, or c above.
From 'Clinical immunology Principles and Practice, fourth edition. Chapter 60

Table 2: Prevalences of anti-PI, anti-PC, anti-PE, anti-PA, anti-PG and anti-PS antibodies in women with at least two implantation failures after IVF embryo transfer *vs.* women with one successful IVF embryo transfer (controls 1) and women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET (controls 2) in references # x (Kaider et al) and y (*Ulcova-Gallova et al.)*. These data were not appropriate for inclusion in the in the meta-analysis (n/a stands for *not applicable*).

	anti-PI	**anti-PC**	**anti-PE**	**anti-PA**	**anti-PG**	**anti-PS**
Kaider et al	7.1% *vs.* 0%	21.4% *vs.* 0%	7.1% *vs.* 4.8%	7.1% *vs.* 0%	4.8% *vs.* 0%	2.4% *vs.* 0%
Ulcova-Gallova et al.	31.8% *vs.* 0%	*n/a*	19.6% *vs.* 0.5%	12.5% *vs.*0%	16.5% *vs.*1%	40.4 % vs.0%

Table 3: Prevalence of any type of anti-PL antibodies and prevalence of anti-CL antibodies in women with at least two implantation failures after IVF embryo transfer *vs.* women with at least one successful pregnancy from spontaneous conception or unselected healthy women with no history of IVF-ET (controls 2), respectively, in all relevant published studies. These data were not appropriate for inclusion in the in the meta-analysis (n/a stands for *not applicable*).

	Any type anti-PL antibodies		Anti-CL antibodies	
	Patients	Controls 2	Patients	Controls 2
Alves 2005	48/52 (92%)	0/28 (0%)	50/52 (96.2%)	0/28 (0%)
Bellver 2008	8/57 (14%)	6/32(18.8%)	8/57 (14%)	6/32 (18.8%)
Coulam 1997	69/312 (22%)	5/100(5%)	13/312 (4.2%)	0/100 (0%)
Coulam 2002	34/122 (27.9%)	7/107 (6.5%)	n/a	
Paulmyer-Lacroix 2014	8/40 (20%)	1/100 (1%)	n/a	
Qublan 2006	17/90 (18.9%)	9/100 (9%)	9/90 (10%)	3/100 (3%)
Sanmarco 2007	40/101 (39.6%)	8/160 (5%)	n/a	
Steinvil 2010	17/509 (3.3%)	30/637 (4.7%)	n/a	
Stern 1998	30/105 (28.6%)	16/106 (15.1%)	n/a	
Ulcova-Gallova 2005	928/1926 (48.2%)	5/391 (1.3%)	421/1921 (21.9%)	8/391 (2%)
Vaquero 2006	8/59 (13.6%)	0/20 (0%)	n/a	

Figure 1

PRISMA Flow diagram

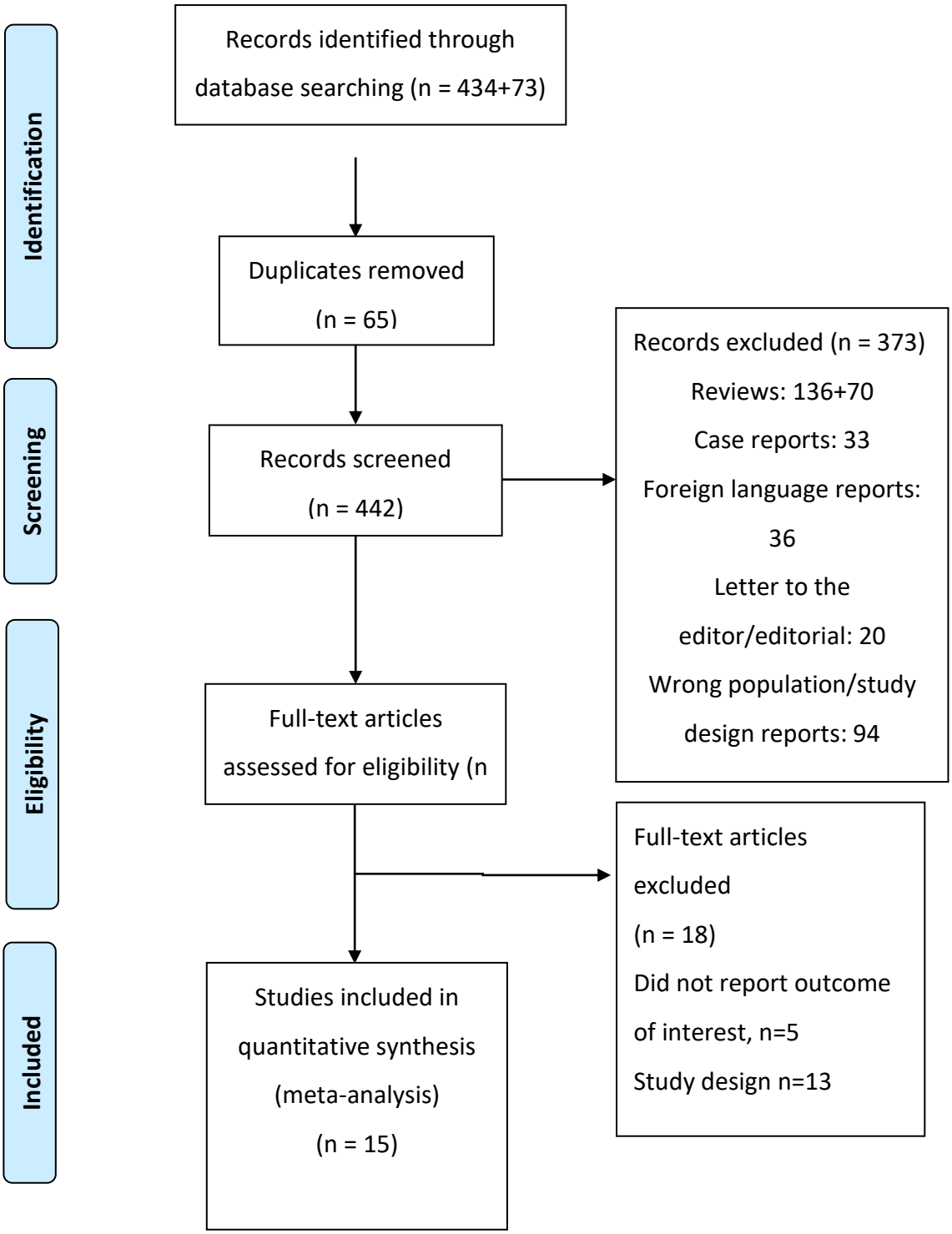


Figure 2

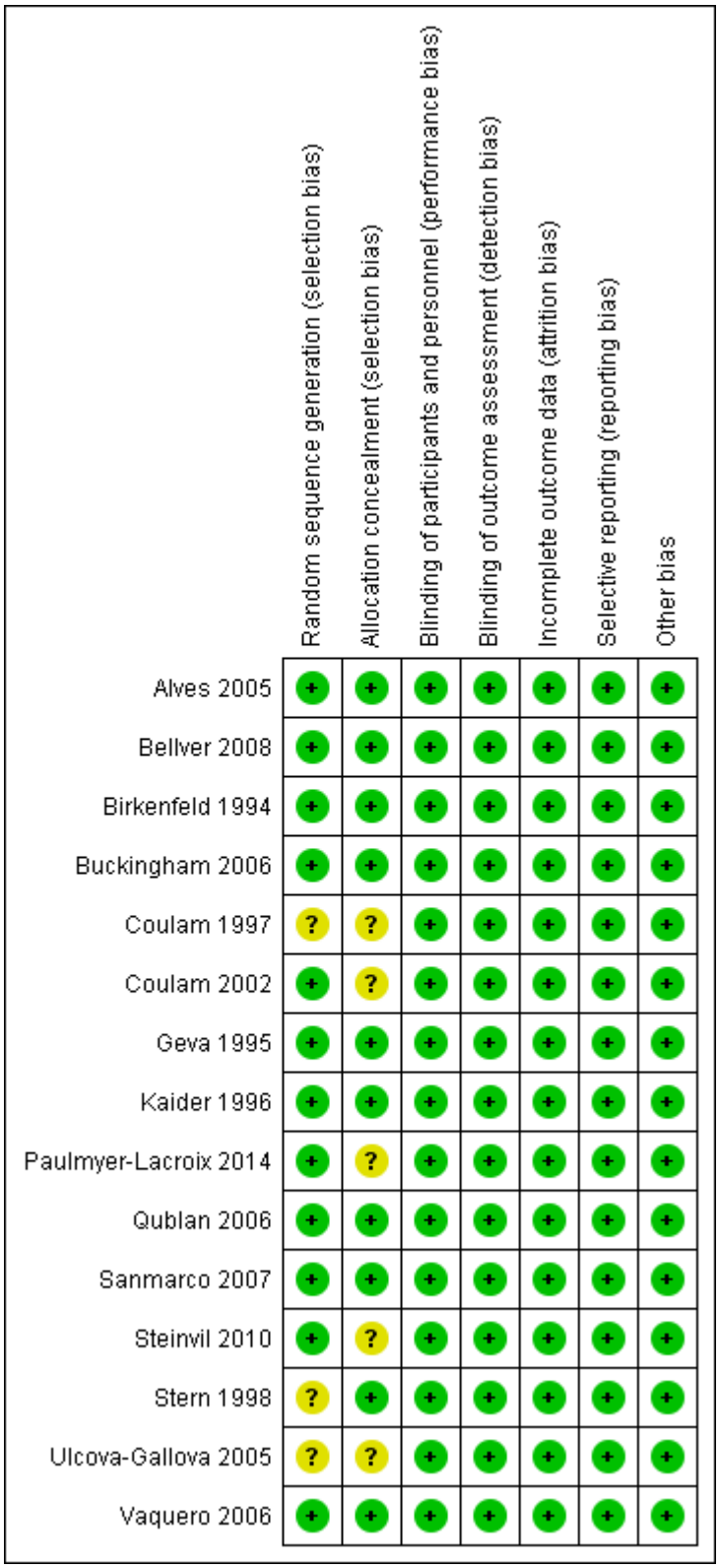

Random sequence generation (selection bias)
Allocation concealment (selection bias)
Blinding of participants and personnel (performance bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective reporting (reporting bias)
Other bias
Alves 2005
Bellver 2008
Birkenfeld 1994
Buckingham 2006
Coulam 1997
Coulam 2002
Geva 1995
Kaider 1996
Paulmyer-Lacroix 2014
Qublan 2006
Sanmarco 2007
Steinvil 2010
Stern 1998
Ulcova-Gallova 2005
Vaquero 2006

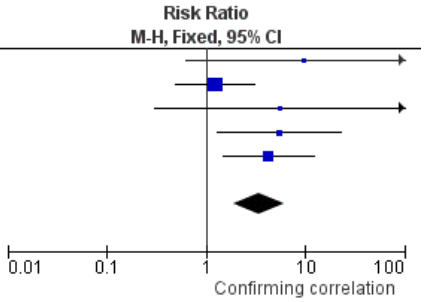
Figure 3

Panel A

Study or Subgroup	Population index Events	Total	Control 1 Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Birkenfeld 1994	18	56	0	14	5.9%	9.74 [0.62, 152.44]
Buckingham 2006	5	22	13	71	45.6%	1.24 [0.50, 3.10]
Geva 1995	3	50	0	40	4.1%	5.63 [0.30, 105.87]
Kaider 1996	11	42	2	42	14.8%	5.50 [1.30, 23.32]
Qublan 2006	17	90	4	90	29.6%	4.25 [1.49, 12.14]
Total (95% CI)		**260**		**257**	**100.0%**	**3.44 [1.90, 6.23]**
Total events	54		19			

Heterogeneity: Chi² = 6.00, df = 4 (P = 0.20); I² = 33%

Test for overall effect: Z = 4.08 (P < 0.0001)



Panel B

Study or Subgroup	Population index Events	Total	Control 1 Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Geva 1995	3	50	0	40	18.1%	5.63 [0.30, 105.87]
Kaider 1996	3	42	0	42	16.4%	7.00 [0.37, 131.47]
Qublan 2006	9	90	2	90	65.5%	4.50 [1.00, 20.25]
Total (95% CI)		**182**		**172**	**100.0%**	**5.11 [1.51, 17.27]**
Total events	15		2			

Heterogeneity: Chi² = 0.08, df = 2 (P = 0.96); I² = 0%

Test for overall effect: Z = 2.63 (P = 0.009)

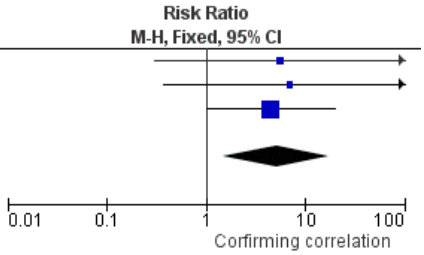
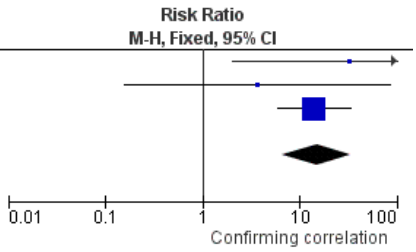


Figure 4

Panel A

Study or Subgroup	Population index Events	Total	Control 2 Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Alves 2005	29	52	0	28	6.9%	32.28 [2.05, 509.20]
Bellver 2008	1	26	0	32	4.8%	3.67 [0.16, 86.42]
Ulcova-Gallova 2005	349	1926	5	391	88.3%	14.17 [5.90, 34.03]
Total (95% CI)		**2004**		**451**	**100.0%**	**14.91 [6.65, 33.45]**
Total events	379		5			

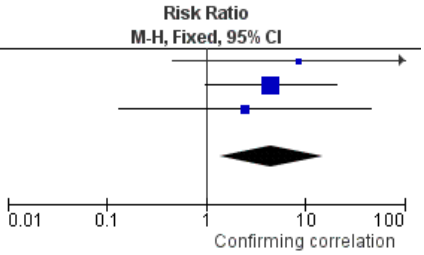
Heterogeneity: Chi² = 1.07, df = 2 (P = 0.59); I² = 0%
Test for overall effect: Z = 6.56 (P < 0.00001)



Panel B

Study or Subgroup	Population index Events	Total	Control 2 Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Bellver 2008	3	26	0	32	14.6%	8.56 [0.46, 158.51]
Qublan 2006	8	90	2	100	61.4%	4.44 [0.97, 20.38]
Vaquero 2006	3	59	0	20	24.0%	2.45 [0.13, 45.48]
Total (95% CI)		**175**		**152**	**100.0%**	**4.57 [1.37, 15.25]**
Total events	14		2			

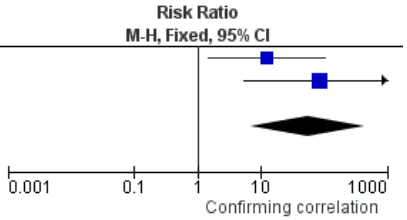
Heterogeneity: Chi² = 0.35, df = 2 (P = 0.84); I² = 0%
Test for overall effect: Z = 2.47 (P = 0.01)



Panel C

Study or Subgroup	Population index Events	Total	Control 2 Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Paulmyer-Lacroix 2014	5	40	1	100	40.7%	12.50 [1.51, 103.67]
Ulcova-Gallova 2005	209	1926	0	391	59.3%	85.24 [5.33, 1364.31]
Total (95% CI)		**1966**		**491**	**100.0%**	**55.60 [6.98, 442.84]**
Total events	214		1			

Heterogeneity: Chi² = 2.00, df = 1 (P = 0.16); I² = 50%
Test for overall effect: Z = 3.80 (P = 0.0001)



Panel D

Study or Subgroup	Population index Events	Total	Control 2 Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Alves 2005	48	52	0	46	38.9%	86.02 [5.45, 1356.70]
Ulcova-Gallova 2005	778	1926	0	391	61.1%	316.73 [19.84, 5057.50]
Total (95% CI)		**1978**		**437**	**100.0%**	**226.89 [27.64, 1862.61]**
Total events	826		0			

Heterogeneity: Chi² = 0.53, df = 1 (P = 0.47); I² = 0%
Test for overall effect: Z = 5.05 (P < 0.00001)