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Incidence of active maternal infections and autoantibodies in pregnant women with recurrent foetal loss

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Abstract
Recurrent foetal loss (RFL) is defined as the loss of three or more pregnancies and its causes include maternal infections and immunological status. Present study was conducted in Nair Hospital, Mumbai to check the incidence of active maternal infections and autoantibodies in pregnant women with RFL. Study comprised of 143 women in study group having history of RFL and 140 women in control group. Anticardiolipin antibodies (ACA) and Antinuclear Antibodies (ANA) and Lupus Anticoagulant (LA) giving prolonged "Activated Partial Thromboplastin Time" tests were carried out by the kits. Bacterial, viral and fungal infections were detected by standard procedures. In study group, 46.85% and 41.26% of women had active maternal infections and autoantibody respectively. While in control group, 13.57% women had active maternal infections but absence of autoantibodies. Highest infection rate was found to be of CMV, followed by *T. gondii* and budding yeast. While lowest was of *N. gonorrhea.* Highest incidence of ACA (31.47%) was found in the study group followed by LA (19.35%) and ANA (15.22%). In study group, 45.98% of women had a history of spontaneous abortion. Among them maternal infections were found more as compared to autoantibodies, but not statistically significant. Since the risk of developing a placental or fetal infection depends on the immune reactivity of pregnant woman, immunological investigations should include immunoglobulin levels and detection of autoantibodies.

Keywords: Maternal infections, autoantibodies, pregnant women, recurrent foetal loss

1. Introduction
Foetal loss is found to be the unavoidable complication of pregnancy. Episodes of foetal loss become more distressing when it occurs recurrently. The terminology recurrent foetal loss (RFL) is defined as the loss of three or more pregnancies. These losses can manifest as spontaneous abortion, history of intrauterine foetal death, intrauterine growth retardation, stillbirth, early neonatal death and or congenital anomalies. The various causes reported to be responsible for RFL include endocrine defects (25-50%), miscellaneous factors 15%, reproductive tract anomalies (10-12%), genetic factors (10%), immunological status (5-10%), and maternal infections (2-3%) [1].

The last few decades have witnessed a growing awareness and concern regarding maternal infections and their effect on perinatal morbidity and mortality [2]. Foetal death after the 20th week of pregnancy accounts for one half of all perinatal mortality. The role of maternal infections ranges from trivial even in apparent to profoundly damaging. These infections may be transplacentally transmitted from mother to the foetus at any gestational age especially first and second trimester. Nearly 2% foetuses are infected inutero and up to 10% of neonates during childbirth [3]. Similarly a significant percentage of perinatal death due to inutero infections has also been reported in India [4].Infections acquired inutero may result in resorption of the embryo abortion, stillbirth, malformation, intrauterine growth retardation, prematurity and the untoward sequelae of chronic postnatal infection. The immediate as well as long-term effects are a major problem throughout the world [3].

Immunological responses could be the cause in many cases of infertility and miscarriage. Some immunological reasons that contribute to infertility are reproductive autoimmune failure syndrome, the presence of anti-phospholipid antibodies, and antinuclear antibodies [5].

Many researchers have been conducted to identify the underlying mechanisms of RFL and accumulating evidence reveals that an immunologic mechanism is involved in some miscarriages. However, several studies of the immune interactions at the feto-maternal interface and genetic-epidemiologic studies document an immunological background for many RFLcases [6]. Several autoantibodies, synthesized by the immune system directed against one or more of the individual's own proteins have been investigated as possible influences on reproductive success and failure. Autoantibodies may persist for many years in the circulation as a marker of a prior autoimmune attack, but their presence does not necessarily indicate a current disease process. The Anti-Phospholipid antibodies such as Lupus Anticoagulant, Anti-Cardiolipin and anti- β2 glycoprotein are associated with RFL or as possible factors involved in infertility [7]. RFL throughout gestation has also been reported in presence of abnormal autoantibodies [8]. An interest in autoimmune causes of recurrent abortion has greatly increased with the discovery of an association between the presence of Anti-Phospholipid antibodies and Anti-Nuclear antibodies [9].

Of the various causes reported to be responsible for recurrent pregnancy wastage, an attempt was made to diagnose maternal infections and immunological status of the mother and cause reduction in pregnancy wastage. Hence, the present study aimed to check the incidence of active maternal infections and autoantibodies in pregnant women with RFL.

2. Material and methods

2.1 Place of work

Study was carried out over a period of three years, from February 1996 to March 1999after taking the permission from Institutional Ethics committee of T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai, in Department of Microbiology in association with Department of Obstetrics and Gynecology. Part of the major study is presented in this paper.

2.2 Participants, Sample Collection, microbiological and serological analysis

A special proforma was designed for the present study and accordingly the obstetric history of each woman was recorded. All the women in this study were between the reproductive age group and were pregnant at the time of screening. Those women who had history of RFL due to any known genetic or endocrine defects or haematological disorders were excluded. A total of 283 subjects were studied, comprising of 143 in the study group and 140 in the control group. Study group comprised of all those women who had a history of RFL and Control group comprised of clinically normal women with at least one previous full term normal delivery and no history of RFL

Blood was collected from the subjects and serum was separated. Serum samples were stored at -20^{o}C and thawed when the serological tests were conducted.

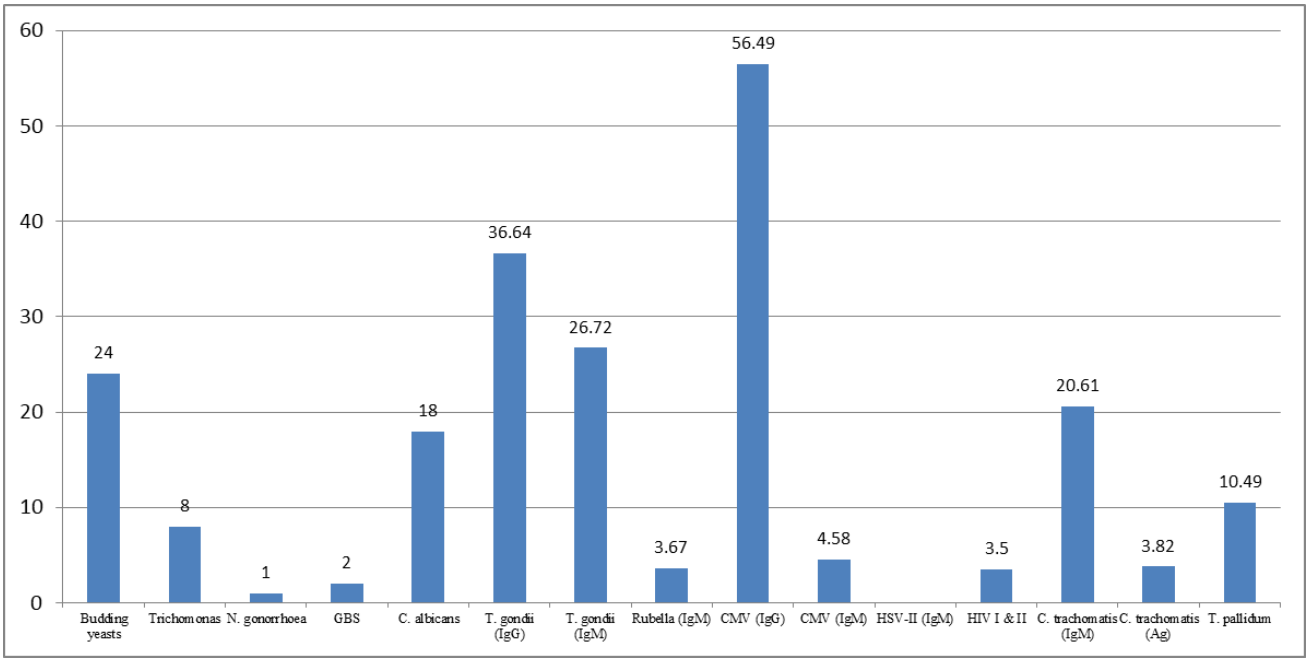
Toxoplasma gondii (IgG and IgM), *Rubella* virus (IgM), *Chlamydia trachomatis* (IgM), *Cytomegalovirus* (CMV), *Herpes Simplex* virus (HSV-II), Syphilis and Human Immunodeficiency Virus (HIV) I II were detected by standard serological methods using kits by Labo biomedical products by, Netherlands. [10]

Anti Cardiolipin antibodies (ACA) (IgG and IgM) BINDAZYME, Birmingham) [11] and total Anti-Nuclear Antibodies (ANA) (IgG and IgM combined) (DIASTAT ELISA) [12] and Lupus Anticoagulant giving prolonged "Activated Partial Thromboplastic Time" (APTT) (CEPHOTEST) [13] tests were carried out by the kits as per the manufactures instructions.

Swabs from cervix and vagina of subjects were collected by standard procedures [14, 15] and sent in appropriate transport media to laboratory. Further suitable staining methods [14, 15], culture media [14, 15] and confirmatory tests [14, 15] were used for identification of *Candida albicans*, *Neisseria gonorrhoeae*, *Streptococcus agalactiae* and *Chlamydia trachomatis.*

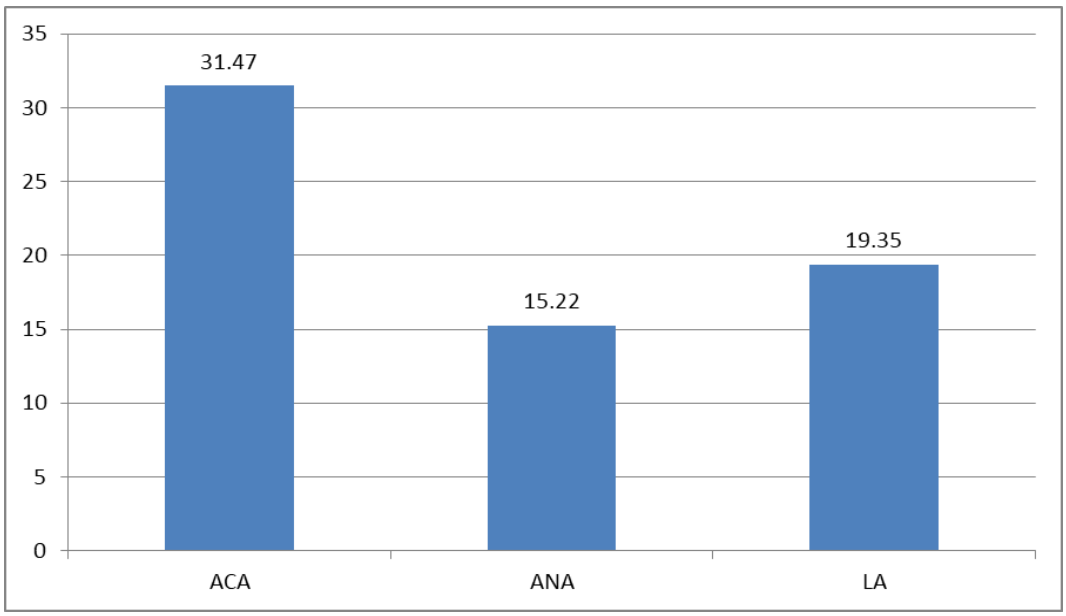
Observation and results were noted and appropriate statistical analysis was carried out wherever necessary.

3. Results



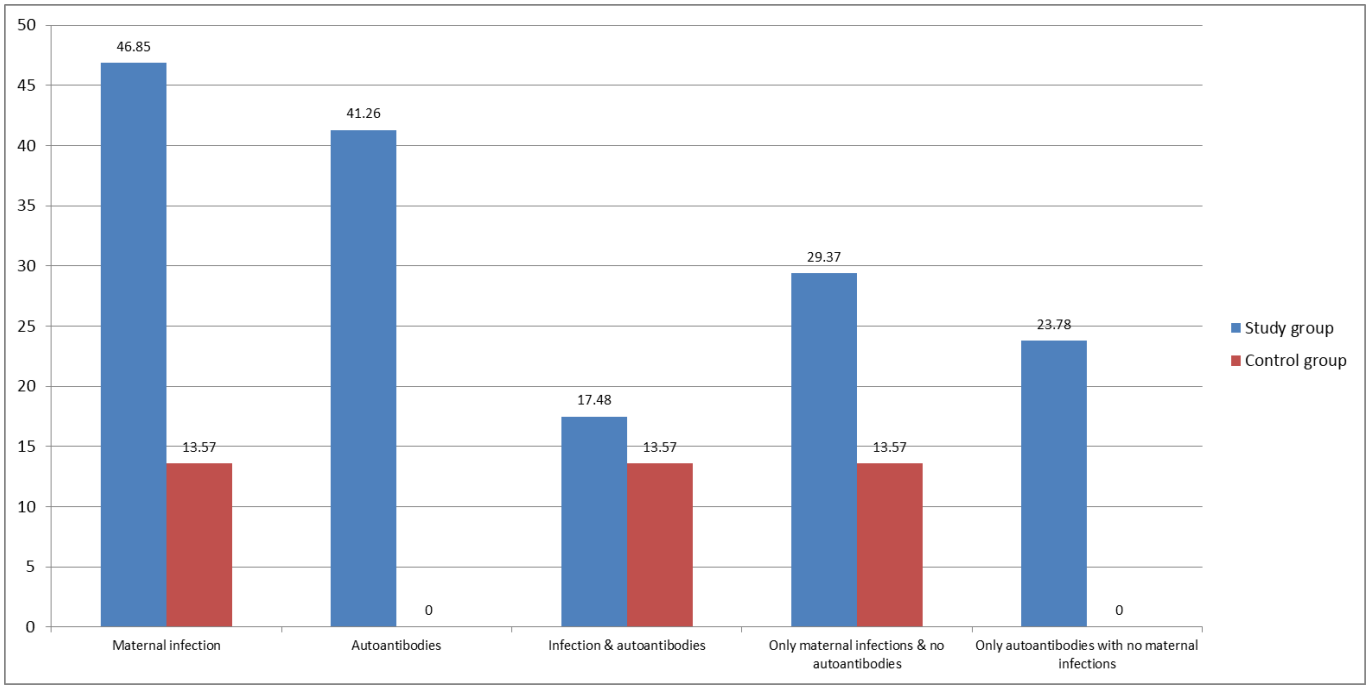
Graph 1: Active maternal infections in study group. n= 143

Highest infection rate was found to be of CMV, followed by *T. gondii* and budding yeast. While lowest was of *N. gonorrhea*.



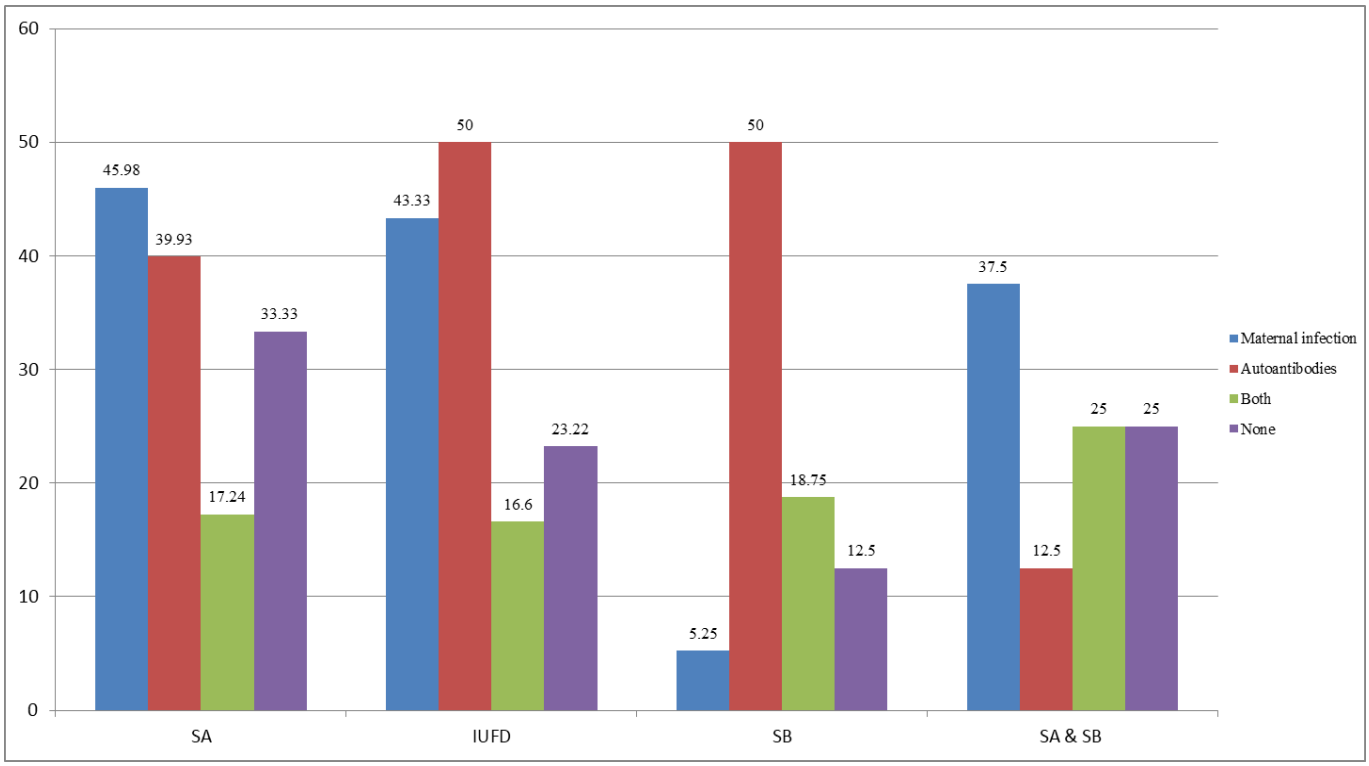
Graph 2: Incidence of autoantibodies in study group. n= 143

Highest incidence of ACA (31.47%) was found in the study group followed by LA (19.35%) and ANA (15.22%).



Graph 3: Incidence of active maternal infections and autoantibodies n1 (Study group) =143; n2 (Control group) =140

In study group, 46.85% and 41.26% of women had active maternal infections and autoantibodies respectively. While in control group, 13.57% women had active maternal infections but absence of autoantibodies. Women showing both maternal infections and autoantibodies were similar in number in both the groups.



Key: SA: Spontaneous Abortion, IUFD: Intra Uterine Feotal Death, SB: Still Birth

Graph 4: Type of RFL with active maternal infections and autoantibodies in study group. n1 (SA) =87; n2 (IUFD) =30; n3 (SB) =16; n4 (SA & SB)= 8

In study group, 45.98% of women had a history of spontaneous abortion. Among them maternal infections were found more as compared to autoantibodies. Statistical analysis revealed no significant association (Chisquare X^2 (DF=9)sf = 5.6; P=0.78(NS)) between type of previous obstetric losses with active maternal infection and / or antibodies.

4. Discussion

Pregnancy loss could be in the form of recurrent consecutive SA, history of IUFD, intrauterine growth retardation (IUGR), SB, early neonatal death and or congenital anomalies [16]. The RFL problem affects 1% of all women. This incidence is higher than expected by chance alone, since 10%-15% of all clinically recognized pregnancies end in miscarriages [17]. With each pregnancy that is lost, the desire for a favorable outcome is increased. Recurrent pregnancy wastage is therefore a frustrating problem for the patient and physician alike [18].

Spontaneous abortion is reported to be the commonest complication of pregnancy. It has been documented roughly to affect one in four of all women who become pregnant [19]. Actual spontaneous abortion rate is difficult to determine as most of the women abort at home [20]. No literature has therefore documented the exact frequency of SA among recurrent pregnancy losses. In present study among RFL, majority of women (60.84%) had a history of spontaneous abortion. The various causes reported to be responsible for RFL include endocrine defects (25-50%), miscellaneous factors 15%, reproductive tract anomalies (10-12%), genetic factors (10%), immunological status (5-10%) and maternal infection (2-3%) [1]. RFL depends on numerous factors, including the pattern of pregnancy loss. A detailed obstetrical history, including gestational age at the time of fetal death, ultrasound, pathology, and cytogenetic results, has a key role in the evaluation and management of RFL. This complex reproductive disorder requires a multidisciplinary approach to evaluate and manage genetic, endocrinologic, anatomic, immunologic, infectious, thrombophilic, placental abnormalities, and iatrogenic factors [21].

Of the various diagnosable and treatable causes reported, an attempt was made to diagnose maternal infections and immunological status of the mother and cause reduction in pregnancy wastage. Present study was conducted with an aim to estimate the incidence and effect of treatment on maternal infections and immunological factors in women with a history of RFL.

Autoantibodies are found to occur in 18-43% of patients with RFL. The antibodies commonly identified include APA (14%) and ANA (7%) [17]. In present study, the incidence of autoantibodies (APA and ANA) was found in more than three recurrently aborting women was 41.25%. A statistically significant incidence of 34.96% of APA was found in study group. Thus APA was commonly found etiology in women suffering from recurrent foetal losses. Therefore the presence of these APA should be sought by both coagulations based assays and immunological assays such as those using cardiolipin in women with unexplained fetal loss [22]. Earlier literature suggested that women with APA antibodies had a significant risk of reproductive failure and adverse pregnancy outcomes [23]. In present study, we found an incidence of 15.22% of ANA in study group, while ANA were not detected in our healthy control group.

Several authors had addressed the question of which class of APA (LA, IgG ACA or IgM ACA) was the best predictor of foetal loss. There occurred lack of agreement on the prediction values. Pattison *et al* found that ACA had higher predictive power than for foetal loss [9] and Lynch *et al* reported that an elevated IgG ACA was the only APA to be

significantly associated with foetal demise. In contrast, Out *et al* reported that whilst the presence of LA could predict foetal loss, an elevated ACA titer was a risk factor for low birth weight in live born infants. [24] In present study ACA (IgG) was 30.27% ACA (IgM) was 22.38% and LA was 19.35%. Hence we found more of elevated ACA IgG as a cause for unexplained recurrent foetal loss.

Though infectious recurrent foetal wastage is a grave problem of a developing country like India, still it has not yet attracted much desirable attention from medical fraternity and has always remained neglected [2]. Hence, early substantive diagnosis and appropriate treatment can decrease the chances of RFL to a greater extent. The severity of maternal infections is dependent on virulence, load, and tissue tropism of a pathogen. The ultimate outcome of the foetus is decided not only by the maternal infections and associated placental damage but also the stage of pregnancy at which it occurs [3]. The infectious diseases are commonly considered to cause recurrent abortion at a rate as low as 4% [25]. Indeed, all microorganisms can produce acute infection and then occasional abortion, but only a few can induce chronic maternal disease capable of causing recurrent abortion. The rate of occasional abortions due to embryo–foetal infections is believed to range from 10 to 15% but this figure is likely to be underestimated, because of subclinical abortions occurring at an early stage of gestation that remain undiagnosed.

In fact, the diagnosis of abortion from infection can only be made retrospectively, based on histological examination of feotal and placental tissues and isolation by culture or genomic detection of the suspected infectious agent. Infectious agents said to produce transplacental infection of the mammalian tissue includes viruses, bacteria, spirochetes, fungi, protozoa, and even worms. These may or may not be transmitted sexually. Usually sexually transmitted diseases, which are steadily increasing in the population, may often produce untoward deleterious effect on foetal outcome [26]. The agents causing adverse effect on the foetus are grouped together as ToRCH complex i.e. *Toxoplasma gondii* (To), *Rubella* virus (R), *Cytomegalovirus* (CMV), *Herpes simplex* Virus (HSV) – II [27]. This grouping is based on the diagnostic and therapeutic dilemma they present to the physician. Other significant agents in the list include *Chlamydia trachomatis*, *Candida albicans*, *Group B streptococcus, Neisseria gonorrhoea, Mycoplasma hominis, Ureaplasma urealyticum, Trichomonas voginalis*[28], Human Immunodeficiency Virus [29]. In our study, highest infection rate was found to be of CMV, followed by *T. gondii* and budding yeast. While lowest was of *N. gonorrhea*. Viruses appear to be the most frequently involved pathogens, since some of them can produce chronic or recurrent maternal infection. In particular, cytomegalovirus during pregnancy can reach the placenta by viremia, following both primary and recurrent infection, or by ascending route from the cervix, mostly following reactivation. Although, a definitive relationship between recurrently active infections and RFL is still lacking, mostly due to difficulties in demonstrating the pathogenic role of each individually isolated pathogen, diagnosis and therapy of RFL-related infections not been attempted. [30]

Evidence has accumulated which indicated that the components of vaginal flora may be additional risk factors for preterm delivery with various vaginal organisms being associated with preterm labor, premature rupture of membranes or the delivery of low birth weight infants [28]. The main mechanisms by which infections induce abortion include, (a) Production of toxins or cytokines (i.e. tumor necrosis factor-a), which induce uterine contractions or damage the foeto-placental unit; (b) Foetal infection, resulting in fetal death or life-threatening malformations;(c) Placental infection, with subsequent placental insufficiency and fetal death; (d) Endometrial chronic infection, interfering with embryo implantation;(e) Amnionitis, which causes abortion in the first trimester as well as preterm labor in the third trimester.

5. Conclusion

All patients with RFL should be studied, according to their own personal risk for infections, by collection of a careful history, and by physical and laboratory examinations, to exclude chronic infections. Since the risk of developing a placental or fetal infection depends on the immune reactivity of the pregnant woman, immunological investigations should include the immunoglobulin levels and detection of autoantibodiesas a first step. [48]. The diagnosis of infectious agents as a possible cause of RFL might lead to a therapeutic approach with antiviral drugs and antibiotics or using immunoglobulins, which can display both anti-infective neutralizing and immunomodulation properties.

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