Immune Consequences of Chlamydia Infections in Pregnancy and In Vitro Fertilization Outcome

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ABSTRACT
This review addresses the immune consequences of Chlamydial infections in pregnancy and in vitro fertilization (IVF) outcome. In pregnancy, many works have shown the risk for preterm labor, preterm birth, or miscarriage with current infection, and stress the need for screening and treatment early in pregnancy. IVF outcome needs to be studied in relation to specific markers like inflammatory cytokines and secretory antibodies to the 60 kD heat shock protein (hsp60) and to Chlamydia, to determine their ability to predict and influence pregnancy outcome. © 1996 Wiley-Liss, Inc.

KEY WORDS
preterm birth, ectopic pregnancy, spontaneous abortion, hsp60 antibodies, inflammatory cytokines, embryo implantation.

Chlamydial infection elicits an immune response involving humoral and cellular immunity. Mild or recent infection can be controlled by neutralizing antibodies like IgG and IgA including secretory IgA. Untreated or unsuspected infections can become chronic, and cause damage related to inflammation through cellular immune activation. Inflammatory cytokines are released as a consequence of prolonged antigen presentation and may not be sufficient to resolve long ongoing infections but are sufficient enough to maintain an inflammatory state. In pregnancy and IVF outcome, sequelae differ according to the specific immune responses that are induced.

PREGNANCY
Chlamydial cervicitis of recent origin, in the absence of tubal damage, does not impair fertilization or egg implantation. Detection prevalence in pregnancy varies from 6%-40% according to the studies, settings and populations. Seroprevalence varies from 15%-35% according to the populations. The risk for preterm labor and preterm birth is significantly higher in pregnant women with a positive chlamydial detection and increases further when bacterial vaginosis is also present. Intra-amniotic chlamydial infection can persist with intact membranes and without clinical symptoms. Antichlamydial antibodies have also been associated with perinatal complications. If exposure occurs during pregnancy, it may be permitted by immunosuppression and/or cervical ectopy. Chorioamnionitis can occur with cytokine emission in the amniotic fluid, promoting prostaglandin PGE2 secretion and ocytocic sequelae. Hsp60 expression have been found identical in normal pregnancies and preterm birth by immunocytochemistry, showing that hsp60 is abundantly expressed in placenta; however, antibodies to hsp60 have not been studied in that work.

In untreated mothers completing their pregnancy, the contamination risk factor for the foetus, which is mildly protected by the maternal antibodies, is 20%-50% for conjunctivitis and 10%-20% for pneumonia and respiratory disease syndrome with the likely sensitization of the the infants to further chlamydial infections. These findings stress the need for chlamydia detection at 24-26 weeks of gestation.

Pregnancy can be interrupted by chlamydial infection. Most of the studies on current infection received September 15, 1996
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correlate a late miscarriage with a positive detection. Early spontaneous abortions have been associated with chlamydial antibodies but some authors do not find significant differences with control populations. A recent screening found that chlamydial infection was the second most frequent cause for recurrent foetal losses. Another recent work finds a positive correlation between hsp60 antibodies to the chlamydial 60kD heat shock protein (chsp 60) and spontaneous abortions. More studies are needed to implicate precisely the immunopathogenesis of past or current chlamydial infection in spontaneous abortion.

ECTOPIC PREGNANCY (EP)

In contrast to infection during pregnancy, numerous authors have associated the risk for ectopic pregnancy with a past chlamydial infection. The mechanism is tubal function impairment or tubal alteration. In fact chlamydial antibody prevalence and chlamydial tubal impairment are the most frequent causes of EP, often revealing an inapparent past infection. Recent studies have demonstrated viable chlamydiae in the tubes by mRNA transcripts. Hsp60 antibodies have been associated with tubal scarring in the pathogenesis of EP through repeated exposures or sensitization to Chlamydia.

After ectopic pregnancy many women undergo IVF because of tubal removal or plasty. But it appears now that chlamydial antigens remain inside the tissues and endometrium possibly affecting IVF outcome.

IVF

Most of the studies report an unchanged fertilization rate after IVF in chlamydial infected vs. non-infected patients. This can suggest that the oocyte capacity to be fertilized is not impaired by the infection, recent or past even with high antibodies titters. In contrast, after embryo transfer a number of patients either do not reach pregnancy, or suffer early spontaneous abortions. The results are controversial because of the great variability of anti-chlamydia antibody detection methods. Some works have used detection (culture, antigen detection DNA or mRNA detection with or without amplification), others have used chlamydial structural antibodies, or anti hsp60 antibodies, in serum or genital fluids, with various degrees of specificity (genus specific antibodies, genus or bacterial specific hsp). In some studies, there was no correlation between pregnancy rate and the presence of antichlamydia antibodies. In contrast, other authors found a negative correlation between anti chlamydia antibodies and a successful IVF outcome. Because of the established relation between tubal infertility and hsp60 antibodies it seemed possible to hypothesize a link between hsp60 antibodies and a reduced pregnancy rate.

Hsp60, stress response protein for both chlamydia and host, is a potent immunogenic agent leading to antibody secretion, and inflammation induction leading to cytokine secretion through antigen presenting cells. Being homologous to the human hsp60, bacterial hsp60 can trigger autoantibody formation in the host, and development of delayed hypersensitivity reactions building tissue damage in the upper genital tract and also in remote areas like joints and liver. Maternal or embryo hsp expression is not normally harmful to the maintenance of pregnancy, unless the mother has been sensitized previously to a bacterial hsp. In that case, expression of maternal or embryo hsp60 will reactivate the lymphocytes previously sensitized to hsp60, and this will induce a proinflammatory immune response which might lead to immune rejection. After IVF, embryo implantation in a previously infected endometrium, possibly still hiding altered or impotent elementary bodies, is a challenge.

Embryo and decidua secrete proinflammatory and anti inflammatory cytokines like TNFα, IL, growth factors, and different kinds of hsp among which is hsp60. Recent studies on animal models demonstrate that implantation and embryo development are linked to endogenous and exogenous growth factors; uterine modifications triggered by steroid hormones are settled through local growth factors. To date four cytokines are known as regulators of uterine proliferation and differentiation, and embryo implantation: EGF, CSF1, LIF, and IL1. The latter is a proinflammatory, metabolic and immunologic cytokine mediating inflammation and is necessary for endometrium-embryo dialogue. Decidua and endothelial cells produce TNFα, another proinflammatory cytokine. The embryo itself produces antigens, cytokines like IFNγ and tau, TNFα, IL10. IFNγ and TNFα are predominant in placenta of aborting foetuses (Th1 immune response). IFNγ activates NK T-cells, IL10 and IFN tau are immunosuppressive and anti-
abortive (Th2 immune response). Early inflammatory reaction is necessary for implantation (IL1, IL1f) but its deregulation can totally damage implantation.

Hypersecretion of TNFα, produced by a latent infection in the endometrium, as in chronic chlamydiosis, induces other such cytokine production as IL1, IL8, IL6 and CSF. CSF inhibits LIF in infertile women and could cause embryo rejection by PGE2 production, or at high concentrations, inhibit implantation. IL1 has been demonstrated in Chlamydia infected fallopian tube tissue and could act by anti angiogenic properties, opposite it’s physiologic proimplantation (pseudo-inflammatory action).

Embryos secrete hsp in response to stress, or physiologically as protein shedding. Hsp60 and 70 have been demonstrated during the pre-and peri-implantatory phase in the embryo. It is thus evident that in the implantation phase immune modulators are already normally present. Even without inflammation, the role of immune mediators in embryo implantation is not precisely known. Following T-cell activation or suppression, an inflammatory reaction can switch from Th1 to Th2. It has been demonstrated that chlamydial antibodies and chlamydial hsp are related to induction of inflammatory cytokines, hsp strongly induces T-cell and macrophage activation and cytokine production, and that the immune response is restricted by certain regions of the MHC. Therefore, we may assume that cytokines secreted by infection-activated T-cells and macrophages send a wrong message to the developing embryo and disrupt the balance between pro and anti-inflammatory cytokines, not only impairing embryo growth, but also suppressing embryo rejection by the maternal tissue. Furthermore, in other immunoinflammatory processes, subsequent chronic inflammation, and autoimmune reaction may be due to a non-specific stimulus, of chlamydial, microbial, or other hsp origin cross reacting with not only maternal but also embryo’s hsps.

A partner’s chlamydial infection can also play a role in the female’s rejection of the embryo: chlamydial auto antibody-coated sperm activate, after intercourse, women’s T-cells which in turn produce inflammatory cytokines. High levels of inflammatory cytokines like IL6 have been found in men affected with chlamydia-associated prostatitis, which also activate the woman’s genital tract T-cells during and after intercourse.

According to this hypothesis, to link embryo rejection to chlamydial infection, we had to demonstrate that an inapparent or subclinical inflammation could permanently activate T cells, macrophages and B-cells by presenting altered antigens and heat shock proteins to secrete IFNγ and TNFα through interleukin activation. In a recent prospective study, we have attempted to look for evidence of inflammation in the cervical mucus related to local chlamydial IgA, and we found significant correlation. Chlamydial antigenicity has been proven by local specific secretory antibodies, although circulating antibodies were not found. Those patients with a past chlamydial infection had significant titers of inflammatory cytokines. The correlation between inflammatory cytokines, chlamydial hsp, IgA, and a poor pregnancy outcome has been recently demonstrated. The possibility that a past chlamydial infection enhances Th2 inflammatory processes during early embryo implantation and thus impairs it is then assessed. But this antigen presentation is MHC restricted. Recently it has been shown that genetic susceptibility or resistance to tubal damage in CT infected macaques was correlated to MHC class I antigens. Similarly susceptibility to endometriosis has been associated with expression of MHC class I antigens, which regulate the resistance to lysis by NK T cells. Class II MHC, TNFα and HSP70 genes are very closely located on the same chromosome and play a role in up- and down-regulation of antigenicity, inflammation, and autoimmune delayed hypersensitivity. MHC class I antigens as well as polymorphism in the gene encoding TNFα has been associated with severe scarring in trachoma, confirming the genotypic susceptibility to severe infection. MHC1 antigen expression also modulates the embryo’s susceptibility or resistance to lysis by NK T-cells. If it certainly is necessary to develop research on MHC-related immune reactions towards chronic Chlamydial infection to predict IVF outcome, it seems mandatory to look for inflammation by screening for local chlamydial antibodies, and not only in Chlamydia trachomatis seropositive patients. A recent work confirmed the correlation between MHC antigens, antibodies to hsp60 and significant adverse sequelae. Although controversial results on the relation between hsp60 antibodies and pregnancy rates have been reported with significant methodological differ-
ences between studies, it seems more likely, that hsp60 antibodies are associated with adverse IVF outcome or early spontaneous abortions. In this study, the lack of correlation between pregnancy rate and serologic evidence of past chlamydial infection is consistent with the findings of Claman. Our results, like other works, correlate the secretory IgA CT- positivity to inflammation.

To see if chronic chlamydial infection could predict IVF outcome, we studied a series of infertile couples. In an ongoing retrospective study, we looked for antibodies to hsp60 and 70 from chlamydial, E. coli, and human origin in those patients. So far, the results are surprising in terms of the relation between past chlamydial infection, infertility, and IVF outcome. We tried to see in what way a past chlamydial infection could impair assisted reproduction attempts in infertile patients undergoing IVF. Some CT seropositive women did not have anti-chsp60 antibodies, some had anti-chsp antibodies and/or anti E. coli hsp60 antibodies, and some of them had only anti-human hsp60 antibodies. Those first results already suggest that all past chlamydial infections do not lead to chsp60 antibodies, and that chsp antibodies are associated with chronicity and unsuccessful treatment. Secondly, some infected patients may have only E. coli hsp60, but still have human hsp antibodies. A recent study (in press) found that women having human hsp60 antibodies were also reactive with a conserved epitope of chlamydial hsp60, showing that sensitization is sufficient for one or the other hsp60 to launch the antibody reaction, and infected women respond differently according to their genetic susceptibility. This supports the facts already reported that hsp60 antibodies are associated with severe sequelae of chronic upper genital tract chlamydial infections.

I can conclude that neither anti-structural chlamydia antibodies or anti-chsp60 antibodies are the golden marker to predict IVF outcome in Chlamydia infected couples. We need more prospective studies to define the marker, or the association of markers. After that, other studies will also be necessary to know how to improve the prognosis of IVF outcome by antibiotic, anti-inflammatory or immunomodulating treatments.

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CHLAMYDIA, PREGNANCY AND IVF

ASKIENAZY-ELBHAR

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