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Intrauterine Infections and Birth Defects[1]

XIAO-YING ZHENG[※,+], TING ZHANG[*], YI-FEI WANG[#], CHEN XU[§], GONG CHEN[※], RUO-LEI XIN[*], JIA-PENG CHEN[※], XU-MEI HU[#], QING YANG[§], XIN-MING SONG[※], LI-HUA PANG[※], YING JI[※], HONG-MEI SUN*, LEI ZHANG[※], JU-FEN LIU[※], YAN-LING GUO[※], AND YAN ZHANG[※]

[※]Institute of Population Research/WHO Collaborating Center on Reproductive Health and Population Science, Beijing 100871, China; [*]Department of Infection and Immunity, Capital Institnte of Pediatrics, Beijing 100020, China; [#]Medical School of Shanghai Jiatong University, No. 1954, Huashan Road, Shanghai 200030, China; [§]Department of Embryology, the Second Medical University, No. 280, Chongqing Nanlu, Shanghai 200025, China

Intrauterine infection is an important cause of some birth defects worldwide. The most common pathogens include rubella virus, cytomegaloviurs, ureaplasma urealyticum, toxoplasma, etc. General information about these pathogens in epidemiology, consequence of birth defects, and the possible mechanisms in the progress of birth defects, and the interventions to prevent or treat these pathogens' infections are described. The infections caused by rubella virus, cytomegaloviurs, ureaplasma urealyticum, toxoplasma, etc. are common, yet they are proved to be fatal during the pregnant period, especially during the first trimester. These infections may cause sterility, abortion, stillbirth, low birth weight, and affect multiple organs that may induce loss of hearing and vision, even fetal deformity and the long-term effects. These pathogens' infections may influence the microenvironment of placenta, including levels of enzymes and cytokines, and affect chondriosome that may induce the progress of birth defect. Early diagnosis of infections during pregnancy should be strengthened. There are still many things to be settled, such as the molecular mechanisms of birth defects, the effective vaccines to certain pathogens. Birth defect researches in terms of etiology and the development of applicable and sensitive pathogen detection technology and methods are imperative.

Key words: Intrauterine infections; Birth defects; Rubella virus; Ureaplasma urealyticum; Toxoplasma

INTRODUCTION

Intrauterine infection refers to the pathogen-induced fetus infections through vertical transmission during pregnancy. Birth defects are the structural and/or functional abnormalities

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[+]Correspondence should be addressed to Dr. Xiao-Ying ZHENG, xzheng@pku.edu.cn

Biographical note of the first authors: Dr. Xiao-Ying ZHENG, professor in population health at Institute of Population Research/ WHO Collaborating Center on Reproductive Health and Population Science, Beijing 100871, China. E-mail: xzheng@pku.edu.cn; Dr. Ting ZHANG, professor in pediatrician at Capital Children Hospital, Beijing 100020, China. E-mail: Zhangting@263.net.cn

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of the newborns before birth. It is, nowadays, one of the predominant causes of neonatal death and disabilities of children and adults. It is estimated that over 0.8-1.0 million actual birth defects take place in China annually, viz. one birth-defect newborn every 30-40 seconds. As such a problem develops, the population quality of Chinese people will suffer severe consequences. Birth defects may be the result of various factors, including environmental factors, hereditary factors and the interaction of the two types of factors. The action of environmental factors on birth defect was clarified early in the 1940s. The environmental factors that affect birth defects include physical factors (radiation, physical pressure, etc.), chemical factors (teratogenic chemicals, drugs, etc.), biological factors (infections of viruses, toxoplasma gondii, ureaplasma urealyticum, etc.), heavy drinking, smoking, hypoxia, severe malnutrition, etc.

Such pathogens as virus, mycoplasma, chlamydia, parasite, bacteria, etc. cause intrauterine infections and seriously damage the fetuses. In this article, we introduce the developments of researches on the main viruses, mycoplasmas, and toxoplasmas that induce birth defects.

VIRAL INFECTION AND BIRTH DEFECTS

Intrauterine viral infection may result in birth defects such as fetal death, abortion, deafness, cataract, mental retardation, etc.[1-5]. Rubella virus (RV), cytomegalovirus (CMV), herpes simplex virus (HSV) are commonly involved in the infection. In this paper, we make a summary of birth defects caused by intrauterine infection of RV and CMV, their infection routes, and interventions.

Prevalence of Viral Infection

Human groups are generally susceptible to RV, and human body is the only host of the virus. The virus does not do severe harms to the general public or the non-pregnant women, which makes it easily ignored as an infectious disease with slight clinical symptoms[6]. However, an infection of RV during pregnancy may have immediate impacts upon fetus. Known as one of the major pathogens to cause serious deformity and other birth defects to fetus, RV should be properly noted. The infection rates of the virus are different in reports, approximately 25%-50%. A survey in India showed that the infection rate in women during their child-bearing age ranged 10%-25%.

Human beings are commonly vulnerable to CMV. The infection rate varies with areas and economic status. Most of the infections are latent, which can be activated by pregnancy. The virus is one of the most common sources of perinatal infections. It was found in studies that the newborns of the primi gravidae were infected at a higher rate than those of the multiparae[7]. The newborns in countryside and economy-constrained areas suffered from more serious infection[8]. The infection of CMV is classified as the primary infection and the recurrent infection. The congenital CMV infection rate in newborns is 0.4%-2.3% in Europe and United States, and 0.9%-3.5% in China. Pregnant woman infection may result in bad outcomes in fetuses. The mother-to-child transmission rate is 24%-75%. It is estimated that out of 24 million newborns in China every year, 0.216-0.72 million are born infected with CMV. The chance of a newborn to bear symptoms is very low (<1%), but it happens at a higher rate after birth.

Relation Between Viruses Inducing Intrauterine Infections and Fetus Development

After the infection of a pregnant woman, RV and CMV are transmitted across placenta

and infect the fetus vertically[2,9]. This is the main transmission route of intrauterine infections, which often take place in the first trimester of pregnancy. During delivery, the babies may also be infected with CMV and HSV when they pass through the birth canal, and the infections may as well occur during perinatal lactation. In some cases, the infection may be resulted from hematogenous infection[9], contact infection (HSV transmitted between mother and baby or father and baby on the skin) and iatrogenic infection (infections that are brought about during health care like neonatal blood transfusion and body contacts), etc.

The infection of RV in the first trimester of pregnancy often results in severe congenital fetal abnormalities[1,2], namely congenital rubella syndrome (CRS). During an outbreak and prevalence of rubella, the CRS rate among live births was as high as about 1‰-2‰.

If RV is infected in the first 3 months of pregnancy, the virus passes across the placenta and infects the unborn baby. The cell division in systems is affected, which further involves organs such as ears (severe hearing loss), heart (patent ductus arteriosus, pulmonary valve stenosis, interatrial septum deficiency, interventricular septum deficiency), eyes (cataract, glaucoma, retina diseases), etc.[1,2,10]. Subacute rubella viral panecephalitis is a complication that occurs later, often at the age of 10-20 years. The disease is characterized by mental retardation, ataxia, epilepsy and convulsion, and abnormal T-cells. Diabetes, thymus aplasia, ecthyreosis, or growth hormone insufficiency may also take place.

The severity of RV influence on the organ development of an unborn baby is related with the stage of pregnancy. It is most dangerous if the infection occurs between the 3rd and the 6th weeks, the mother's asymptomatic RV infection can induce serious diseases in the fetus. If a pregnant woman is infected with RV in the 1st month of pregnancy, about 50% of the babies will suffer birth defects, if infected in the 2nd month, about 20% will, and if the 3rd, about 10%. In about 25% of the babies, the RV infection in the first trimester of pregnancy causes one or more birth defects, but it rarely ends up with any defects if the infection happens after 20 weeks of pregnancy, when RV infection leads to abortion or stillbirth with a high rate of birth defects. Some of infected unborn babies appear normal on birth or even during babyhood, but they must be well monitored because some abnormalities of vision, hearing, learning and behavior are not notable until childhood.

RV infection causes eye lesions such as congenital cataract (the incidence is up to 54.6%-66% at its peak), hearing loss (70% are bilateral), etc.[11]. It also induces diseases of digestive system, skeletal system, blood system, and central nervous system. The diseases above may occur alone or in combination, among which the incidence of hearing loss is 57%-76%, eye lesions 50%-90%, heart damage 37%-86%, blood system damage 32%-50%, and low birth weight 50%, and mortality rate in the first year after birth is 10%-14%.

A 60-year follow-up research of 50 congenital RV infection cases that was carried out by Gregg indicated that (ten patients died of malignant diseases before 23 years old), 68% of the patients had slight sclerosis of artery valves, and the incidences of diabetes, thyroid disorder, osteoporosis, etc. exceeded those of the normal people[12].

Studies in recent years have shown that intrauterine RV infection is closely associated with adult non-affective psychosis and schizophrenia spectrum psychosis (SSP). But as was indicated by previous studies, before or after schizophrenes' acute onset or aggravation, the specific antibody of the virus did not significantly increase[13]. The relation involved demands further studies.

CMV infection during pregnancy can induce abnormal fetal development, leading to abortion, premature delivery, fetal development retardation, birth defects, long-term effects after birth and other serious outcomes[7,14,15]. About 5%-10% of the babies with congenital CMV infection manifest cytomegalic inclusion disease, which occurs almost in mothers who are primarily infected during the first trimester of pregnancy. Congenital CMV

infection damages the nervous system, cardiovascular system, lungs, spleen, and other organs of fetus, causing stillbirth or abortion[16]. The newborns who survive suffer hepatosplenomegaly, jaundice, hepatitis, thrombocytopenic purpura, hemolytic anemia as well as tardive CNS disorder. Among the babies infected, only 10% are significantly symptomatic with a mortality rate of 10%-20%, while over 90% demonstrate no symptoms or diseases at birth. About 5%-15% of the babies will have long-term effects in childhood, including sensorineural hearing loss (SNHL), intelligence disorder, development retardation, vision loss and so on[4,14,15].

CMV infection during the first trimester of pregnancy tends to cause abortion and stillbirth, while that in the third trimester mainly results in microcephaly, intelligence development disorder, SNHL, and the occurrences of congenital heart disease, umbilical hernia, foot deformity and hepatosplenomegaly have also been reported. The risk of the primary infection for fetus during the last three months before pregnancy and the four weeks after the last menstrual period is not yet certain. Revello *et al*. found that only 1 of the babies that were born of 12 mothers with infections before pregnancy manifested sub-clinical infection. Among 20 pregnant women, who were infected during the early period of pregnancy (four weeks), 8 terminated pregnancy while the rest 12 gave birth to 3 babies that were diagnosed as congenital CMV infection[17]. The study suggested that, compared with that of the primary infection before pregnancy, the primary CMV infection during the first trimester of pregnancy was more risky to induce adverse pregnancy outcomes and congenital CMV infection.

Harrison *et al*. found in their study that if the mother had CMV infection during the first trimester of pregnancy, her viremia lasted longer than that during the third trimester, her immunological reaction to CMV infection was delayed and her fetus tended to have intrauterine growth retardation (IUGR) and diffusing fetal CMV infection[16].

Mechanism of Virus' Induction of Birth Defects

When a pregnant woman is infected with RV in the first 20 weeks during pregnancy, the fetal deformity rate is very high. The virus maintains the longest in the lens, internal ears and brain, resulting in cataract, visual and hearing disorder, heart deformity, etc.[1,10]. After infection, RV grows in cells. Rather than killing them the virus drives the infected cells to divide at a low rate. During the period of fetal organ development, such an affection influences cell growth, the normal differentiation of tissues and organs, and the development of the fetus. Recent research findings demonstrate that spherical change takes place in the frame structure of chondriosomes in RV infected cells, which may affect the function of chondriosomes. RV infection changes the frame structure of infected cells, and the myofilamental protein is depolymerized[18]. And it induces the apoptosis of infected cells through the signal transmission route p53[18,19]. RV has a LPCAE motif to bind the cellular growth regulatory protein, Rb specifically. The binding changes the regulation properties of the cell growth regulator protein Rb[20]. Prenatal RV infection affects the maturation of important structural and functional tissues in the brain through its infection and onset mechanism and the pathophysiologic lesions induced. The affection may be associated with mental disorders such as adolescent and adult schizophreniae[21]. The factors stated above are likely to be related with birth defects such as RV-infection-induced deformity.

The cytokine levels in amniotic fluid and the blood of newborns that experience intrauterine viral infections, for example the levels of IL-6 and IL-10, are significantly raised. The cytokines that are related with fetal inflammatory reaction may be the cause of brain lesions and nerve development disorder.

In pregnant CMV infection, viremia is more likely to develop in the primary infection than in the recurrent infection. A small amount of viruses can pass across placenta in blood and replicate in some fetal tissues. Spontaneous abortions indicate that CMV initially infects the placenta rather than the embryo or the fetus. In hemochorial placenta, the maternal blood goes to the syncytiotrophoblast directly. The virus infects the endothelial cells of microvascular system in the uterus, followed by the infection of trophoblastic cells, which affects the differentiation and infiltration of trophoblasts. The increased level of cytokines/chemotactic factors in the local area induces inflammatory cell infiltration, which ends up with immune pathological lesions. This is probably the transmission route and nosogenesis of the virus. In the case reported by Gabrielli, *et al*., the infection in the right fetus of twins in pregnancy might be transmitted to the left fetus across placenta in blood. In addition, the right fetus' auditory brainstem response at 80dB disappeared[9], which indicates a severe hearing loss caused by the infection. CMV membrane protein identifies TLR2 receptor and CD4 molecule of sensitive cells, and triggers the production of inflammatory cytokines by activating TLR2 dependent NF-κB to cause immunopathological lesions[22]. CMV infects sensitive cells and proteins encoded by the virus interact with Rb protein, which enhances DNA synthesis and further damages chromosomes to terminate mitosis[20]. It was found in studies that CMV gB genotype I was related with viremia[23], and gB genotype I and III were associated with the adverse outcomes of congenital CMV infection[24,25]. Histological researches were carried out for those fetuses that were diagnosed to be infected prenatally. The findings showed that the virus was detected in the tissues of brain, eyes, hypothyroid, heart, lungs, spleen, kidneys, pancreas, etc., which proved that intrauterine CMV infection caused a general infection of the virus. The onset time of the mother's viremia, the stage of the fetus' development, and the time length of the fetus' exposure to CMV, found in research, all can vary the incidence of congenital CMV infection as well as the degree of baby's deformity[16].

Interventions for Birth Defects Caused by Virus Infection

Prolonged immunity is obtained after RV infections. Clinical positive reinfections are rare. But the question of the risk of reinfections for pregnant women remains arguable. After RV vaccination, the antibodies that are induced by vaccines last for at least 16 years, even whole lifetime in about 95% of the infected people. The vaccination intervention is effective and has changed the prevalence pattern of RV infection. For example, in developed countries, before vaccination was available, the rubella high-risk group was the pre-schooling children (5-9 years), but after the application of the vaccine, it turned to be adolescent and young people[18].

Rubella vaccine should not be administered in pregnant women, particularly not in the first trimester of pregnancy. Clinical RV reinfection may happen in pregnant women who have received the vaccine previously[1,2,10]. In the report of Ushida *et al*., a 34-year-old Japanese woman who had rubella vaccination had RV specific hemagglutination inhibition (HI) antibodies before pregnancy (titre 1:16), but during her third pregnancy, her second child was clinically diagnosed as RV infection. In the 9th week of the third pregnancy, the titre of her rubella specific HI antibody was 1:512. The third pregnancy turned out to be a preterm baby (38 w 2 d), who was clinically diagnosed to have congenital pneumonia, patent ductus arteriosus, sever neuropathic deafness, etc. and manifested mental and physical development retardation. RV gene was found in its lens by RT-PCR test[1]. The questions of vaccine protective duration and reinfection's affection on the fetus were thus aroused. In a RV infection outbreak on ships of German navy in spring 1996, the susceptible

group contained 35 persons (12%), 20 of them suffered, indicating that RV infection may be transmitted by close contact, and the immunity of adolescent and young group to RV decreases, which may prompt the chance of the mother-to-child transmission. RV reinfections increase the antibodies in normal individuals and enhance immunity. However, as corticosteroids increase and cell-mediated immunity decreases during pregnancy, the reinfections that are harmless in the normal may cause the virus to spread in fetus and result in CRS. In order to get a sufficient antibody level to prevent CRS, double-vaccination is crucial (one vaccine during babyhood, the other during youth stage).

The safety of RV vaccine has become the focus of studies in recent years. It has been known that Measles, Mumps, Rubella (MMR) vaccination may cause thrombocytopenia, but what has attracted most attention is the relation between MMR vaccine and infant autism[26-28], which is not supported by epidemiological data at present. The RV vaccination coverage is very high in European countries, but reports of pregnant RV infections are also in existence[29]. Because rubella vaccine's protective effects and the fact that the relation between rubella vaccine and autism is still under theoretical discussion, RV vaccination should be advocated at present. In addition, RV vaccine should be further improved or new vaccines should be developed, the vaccinated group should be adjusted and the amount of vaccines administered should be changed.

CMV infections can be treated with medicines (ganciclovir, GCV or foscarnet). Although teratogenesis was observed in animal experiments, GCV that was used in the first trimester of some pregnant women with transplanted livers demonstrated no adverse pregnant outcomes[30]. There are presently no such well-recognized therapies for prenatal or postnatal congenital CMV infections. The prenatal diagnosis technology is applicable and accurate to detect intrauterine CMV infections. The amniotic fluid cells and the umbilical cord blood sample of newborns are ideal materials for prenatal congenital CMV infection tests. To discover intrauterine CMV infections during pregnancy may be an effective measure to prevent or reduce congenital CMV infection. As indicated in studies, the CMV-IgM antibody level is low (about 4%) while the intrauterine CMV infection rate is high in pregnant women. Therefore, the potential harms of CMV recurrent infections are not ignorable in the tests and prevention for intrauterine CMV infection. The pregnant women's immunity before pregnancy and the pregnant age above 25 years are the two factors to influence the occurrence of congenital CMV infections in the pregnant. The immunity that is naturally acquired before pregnancy can reduce 69% of the risk of congenital CMV infection. The CMV immunity before pregnancy has but partial protection against the virus' intrauterine spread. When a CMV-seropositive pregnant woman is infected by another CMV strain, she may have intrauterine viral spread and symptomatic congenital infection[31]. It is not yet certain whether mothers can acquire protective immunity through an infection before pregnancy, which restraints the development of CMV vaccines[32]. Recently, CMV gB DNA vaccine was experimented on guinea pigs by intramascular injection and induced good humoral immunoreaction to produce gB protein specific antibody and neutralization antibodies[33].

The effective measures to prevent congenital CMV infection include preventing the infections caused by medical care operations, notifying the high-risk group to avoid virus infection, providing newborns or pregnant women with safe and effective diagnostic and therapeutic measures, and developing CMV vaccines, etc.[34].

Other viruses that cause intrauterine infections include herpes simplex virus, hepatitis B virus, HIV, parvovirus, etc. Their nosogeneses and mechanisms to induce intrauterine infections and to harm the fetuses have not yet been systematically reported, which demands further studies.

UREAPLASMA UREALYTICUM AND BIRTH DEFECTS

Epidemiology of ureaplasma urealyticum infections

Ureaplasma urealyticum (Uu) belongs to the ureaplasma of the mycoplasmal family, which is the smallest prokaryote and can be cultured on an artificial medium. It is a common opportunistic pathologic organism in human genitourinary tract, which is transmitted by sexual intercourse, close contact and the vertical mother-to-child route. The Uu detection rate in the genitourinary tracts of different groups varies considerably: the Uu detection rate in patients with sexually transmitted diseases was as high as 67.8%, while 10%-22.8% in normal people. The detection rate in pregnant women was high up to 80%, which might be related with pregnant women's increase in estrogen and decrease in immunity. Among the babies born of the women with Uu positive in the genital tracts, the mature newborns had a Uu detection rate of 18%-55%, preterms 29%-58%, and very low birth weight newborns up to 95%. The Un detection rate in female neonates' genital tracts was higher than that in male, but the rates are similar in other parts of the body. By the increase in the babies' day-age, the detection rate of Uu drops. The rate is lower than 10% during childhood but rises rapidly during adulthood as sexual activities mount[35-38].

Relation Between Uu Infection And Fetal Development

Uu contributes not only to general genital tract infections that result in sterility and infertility, but also to intrauterine fetal infection leading to abortion, intrauterine development retardation, low birth weight and other adverse pregnant outcomes. In recent years, due to the advance in the theories and technologies of reproductive immunology, microbiology and molecular biology, Uu's influence on fetal development and pregnant outcomes has aroused experts' attention at home and abroad.

The paths, through which Uu causes intrauterine embryo or fetal infections, include: (1) ascending infection: Uu that resides in pregnant women's lower genital tracts may go upwards and infect the amnia and amniotic fluid, which results in chorioamnionitis and umbilical cord blood IgM positive and raised newborn Uu detection rate. This is the main route of Uu vertical mother-to-child transmission[39]; (2) blood-borne infection: Uu is found in placenta, umbilical cord blood, and blood of parturients and newborns, which evidences that Uu can be transmitted to fetus across placenta in maternal blood circulation[40]; (3) birth canal infection: it was demonstrated in studies that the neonatal Uu positivity of babies born through cesarean section was significantly lower than that of the ones through virginal deliverly ($P<0.05$), indicating that birth canal infection is an important route[41]. Moreover, after infection, Uu can also be transmitted to fetus through fertilization.

The birth defects caused by Uu infection include: abortion, premature delivery, fetal death, stillbirth, low birth weight, intrauterine fetal development retardation, fetal deformity, mental development disorder, neonatal diseases, etc.

Epidemiological surveillance data have shown that patients suffering unexplained spontaneous abortions have a higher Uu positive rate in cervical secretion, placenta, or aborted tissues than those in the control group. The rate was positively correlated with the number of abortions[42]. In addition, the premature delivery rate was significantly higher in women with Uu positive amniotic fluid than in those with Uu negative. Clinical survey showed that about 50% of the preterm deliveries were because of the amniotic fluid and placenta infected by pathogens, Uu the most common one. As the pregnant age rose, the Uu isolation rate in placenta turned lower[43-45].

Severe Uu infection produces intrauterine fetal death or stillbirth. China reported a

woman who had 6 pregnancies, one of which died of pharynx infection 3 weeks after birth, 1 was spontaneously aborted 8 weeks after pregnancy, and the other 4 turned out fetal deaths about 5 months after pregnancy. All the results of the woman's venous blood tests were negative for antibodies to toxoplasma, brucella, campylobacter, legionella, hemophilus influenzae and salmonella, cervical smear test results negative for fungus, Gonococcus, Trichomonas, and amniotic fluid culture negative for Chlamydia but positive for Uu and Mh, indicating that mycoplasma infection is the major cause of the abortion, fetal deaths and neonatal death. Uu infection is a most important cause of fetal or neonatal perinatal deaths[46].

Low birth weight and IUGR are two important reasons for high morbidity and mortality rate of perinatal babies and infants. As was shown in studies, compared with the neonatal group, from whose placentae no Uu was isolated, the average birth weight was significantly lower in the group with Uu isolated, and most of the babies whose birth weight was less than 2500 g were in the Uu positive group[47]. Yoon *et al*. found that it was more likely for the babies with amniotic fluid culture positive for Uu to suffer low birth weight and perinatal death[48]. IUGR affects the babies' physical and mental development after birth and is associated with even some diseases in adulthood. Intrauterine fetal infection is one of the causes of IUGR. When Uu infection is mixed with other pathogens, IUGR occurs at an even higher chance. The Uu detection rate in the umbilical cord blood and placenta was higher in the newborns with IUGR than those in the control group[49].

Uu infection's induction of fetal deformity and mental development retardation has not yet been reported. But in *in vitro* tests, it was observed that, when the amniotic fluid cells were contaminated by mycoplasma, chromosomes were broken, lost or translocated[50]. It requires further evidence to decide whether intrauterine Uu infection can lead to chromosome aberration to trigger deformity or not.

Uu infection in the genital tracts of pregnant women still contributes to many neonatal diseases, especially diseases of respiratory system and nervous system. Valencia *et al*. reported that, the newborns, whose Uu culture of throat swab or trachea secretion was positive, had a significantly higher morbidity than those whose culture was negative[51]. The severity of the clinical manifestations of neonatal pneumonia varies. Some severe cases may result in respiratory failure followed by death. The course of disease in preterms, especially in those with a very low birth weight, defers and tends to result in chronic lung disease (CLD) or bronchopulmonary dysplasia (BPD), which are manifested as prolonged oxygen-demanding time and chronic pulmonary malfunction often complicated by leukopenia and thrombocytopenia. Uu-induced nervous system infections are most likely to occur in preterms and low-birth-weight newborns and cause neonatal meningitis. Moreover, in rare cases, Uu also leads to neonatal urinary system infection, pericarditis, pulmonary hypertension or osteomyelitis.

Generally speaking, the influence of Uu infection in pregnant women's lower genital tracts on fetal development concerns many aspects. The specific outcomes are related with factors like infection time, severity, pregnant woman's immunity etc.

Mechanism of Uu Infection's Influence on Fetal Development

The nosogenesis of Uu infection's induction of abnormal fetal development remains unclear, but it is presently considered to include the following aspects: (1) the urease in Uu decomposes urea to produce an alkalescent environment with NH3, in which cytoclasis and cilium damage take place to affect the normal development of fetus[52,53]; (2) Uu forms phosphatidases, which hydrolyze the phospholipid in the host's cell membranes, to affect the

membrane biosynthesis and biofunction and to interfere with normal fetal development. Phosphatidase A2 decomposes arachidonic acid in fetal membrane to produce prostaglandins. The latter may trigger uterinerine contraction, which may result in premature delivery, premature rupture of fetal membranes and prenatal complications[54]; (3) Uu infection induces chorioamnionitis. It was also observed under electron microscope that, Uu could enter and proliferate in placenta trophocytes to degenerate plasma vesicles and enlarge chondriosomes, which affects the fetus' oxygen and nutrition intake by lowering the placenta's abilities of material exchange and transportation. This could result in intrauterine fetal development retardation, low birth weight and even fetal death[55]; (4) The endometrial tissue infected with Uu often manifests chronic lymphocyte and macrophage infiltrated inflammatory reactions. Additionally, Uu is an effective cytokine inducer to induce such cytokines as IL-1β, IL-6, IL-8, TGF, and TNF-α. The chronic inflammatory reactions and cytokines are both responsible for adverse influences on fetus development[56].

Interventions for Birth Defects Caused by Uu Infection

It is necessary to strengthen the education of sexual morality and hygiene, to advocate self-respect and self-protection, and to avoid unhealthy sexual activities. Uu examination is needed for the high-risk group and their sexual partners in order to prevent Uu infection. Once a Uu infected patient is discovered, condoms are recommended in addition to treatments in order to prevent cross infection. As Uu is communicable between husband and wife, screening and strict follow-up should be performed so as to reduce intrauterine fetal infection, and effective preventions should be applied against birth defects. Every sample collected for artificial fertilization must be examined for Uu against any adverse outcomes due to Uu in sperm.

Three major groups of medicines are available for human Uu infections: tetracycline antibiotics，including tetracycline hydrochloride, doxycycline, minocycline hydrochloride (due to its low side effects, minocycline hydrochloride is applicable for pregnant Uu infection), etc.； macrolide antibiotics, including spiramycin, erythromycin, azithromycin, clarithromycin, etc.; and quinolones antibiotics, including ciprofloxacin, levofloxacin, norfloxacin, etc.

TOXOPLASMA INFECTION AND BIRTH DEFECTS

In 1937, Wolf discovered toxoplasma in the brain of a baby who died of meningitis. The issue aroused wide interest and led to the belief that toxoplasma might go across placenta to cause congenital infection. Afterwards, many reports have come out about toxoplasma-induced fetal deformity, development retardation, abortion, premature delivery, etc.

Epidemiology of Toxoplasma

Toxoplasma gondii, an opportunistic pathological intracellular parasite that is found in human beings as well as other mammals, can cause serious zoonoses. toxoplasma infection includes, congenital and acquired infections. The former refers to the infection of toxoplasma during pregnancy. The trophonts of the parasite are transmitted across placenta to fetus vertically. Acquired toxoplasma infection is the major form of transmission. It is transmitted by ingestion of food contaminated by oocysts in cat faces or undercooked meat of domestic birds and animals containing Toxoplasma cysts. In a few cases, the infection also occurs with the parasite's transmission through injured skin, mucosa, or in blood transfusion and

organ transplantation.

It is estimated that the average infection rate is 33% globally, approximately 0.5-1 billion persons are infected. USA, Canada, Norway, and UK have an infection rate of 12%-14%, France 74%, and China around 4%-9%. By estimate, 13-15 million women of pregnant age in China are likely to give birth to 80-100 thousand toxoplasma infected babies.

Relation Between Toxoplasma Infection and Fetal Development

When a pregnant woman is infected by toxoplasma, the parasite can be transmitted transplacentally and infect fetus in the uterus, which, in addition to causing congenital defects and death of the fetus, still leads to the woman's abortion, stillbirth, premature delivery or increased coincidental complications of pregnancy. It is thus a most important perinatal infectious disease.

The fetus' infection rate differs according to pregnant stages, during which toxoplasma is infected. When it occurs primarily in the first, second and third trimesters of pregnancy, the fetal infection rates are 17%, 25%, and 65%, respectively. It is generally believed that during the first trimester of pregnancy, it is difficult for the parasite to go across the placenta and the rate of fetal infection is thus relatively low. The infection mainly causes placenta development disorder, whose outcomes can be serious like abortion, premature delivery, stillbirth and all forms of deformities. If the infection occurs in the third stage, its clinical symptoms in babies are present mostly several months, years or even longer after birth. Because toxoplasma can live in many organs and cells, it results in multi-organ lesions and various clinical manifestations.

The positive rate of early pregnant women in IFA toxoplasma test was reported to be 12.5%. The incidences of neonatal deformity in the infected and uninfected women are 4.3% and 1.7% respectively, hydrocephalus incidences 9.0% and 2.5% (P<0.01), stillbirth incidences 4.9% and 1.7%. The difference of all the incidences is close to 3 times[57], which infers that toxoplasma infection is a major reason for pregnant abnormalities. Some of the babies infected with toxoplasma before birth may express no abnormal pregnant outcomes, but without effective treatments, many will suffer clinical symptoms of congenital toxoplasma infection several months, several years or even longer after birth due to brain lesions.

The common clinical nervous system lesions caused by congenital toxoplasma infection include meningoencephalitis, cerebral palsy, epylepsy, and decreased intelligence, with meningoencephalitis the most common. In a clinical pathological study of 120 cases of neonatal deformities, 51.9% were CNS deformities, among whom 12 suffered anencephalus, 8 hydrocephalus, 6 microcephaly, 3 encephalocele, and 2 enlarged gyrus. Calcification foci were found in 4 cases. They mostly manifested complex nervous system deformity or were complicated by other deformities of other systems[58].

Congenital toxoplasma infection is etiologically related with intelligence development disorder. Utilizing IHA, IFA, and ELISA-IgG, IgM, the infants with mental retardation detected were as high as 38.6%, which was significantly higher than that of the control group viz. 14% (P<0.01). In the toxoplasma survey for students with decreased intelligence, the positive rates of toxoplasma antigen, IgM, IgG antibodies were 16.67%, 19.70%, and 37.88%，respectively, which significantly exceeded those of normal infants which were 2.94%, 2.94%, and 11.76%. Other surveys showed that the toxoplasma positive rate was 7.50% in the group of infants with normal intelligence compared with 19.23% in those with decreased intelligence. All the studies stated above indicate the relation between toxoplasma infection and fetal intelligence disorder. Therefore, it is reasonable to provide routine

toxoplasma sero-antibody tests to women of childbearing age or in the first trimester of pregnancy to reduce the incidence of congenital deformity and protect the newborns.

About 25% of toxoplasma infected patients were found in studies to suffer ocular lesions[59]. Series toxoplasma specific tests of IHA and ELISA were given to 69 school infants coming to hospitals for ocular disorder. Eighteen had positive results (26.08%). Among them, 10 (55%) had one or both of their parents positive for toxoplasma. Besides, among 130 cases of neonatal hepatitis syndrome, 16 had toxoplasma IgM positively detected (12.3%), 12 of whose mothers were toxoplasma positive (9.23%), and anti-toxoplasma gondii therapy was effective in 6 cases with jaundice, their liver, gallbladder, and liver functions recovered after 4 weeks, and cases improved[60]. It is thus evident that congenital toxoplasma infection is associated with neonatal hepatitis syndrome.

Mechanism of Toxoplasma's Influences on Fetal Development

How seriously fetal development is affected by toxoplasma depends on the correlation between the fetus and the parasite, the mechanism of which is typically complicated with some details unknown yet.

Tachyzoites, the main form of pathogens in the acute stage of toxoplasma infection, have been reported to actively invade almost all cells. They proliferate constantly and cause severe lesions of tissues and organs, which is the major source of toxoplasma-induced abnormal fetal development and birth defects. But the mechanism of tachyzoites' entry and harms to cells at molecular level is still under research. It has been evidenced in study that, toxoplasma tachyzoites are capable of avoiding being resolved by the enzymes in host cells and damaged by the respiratory burst action, growing and multiplying in their zoitocysts to harm the host cells[61]. The penetration enhancing factor (PEF) secreted by rhoptries on the surface of toxoplasma, together with laminin, has been proved in some studies to contribute to the parasite's adhering to and entering cells, but the pH, Mg^{2+} and Ca^{2+} concentrations have immediate effects on PEF function. The same study also found that phospholipid changed the cellular fluidity to prompt epicyte's concave and the parasite's entry, phospholipase A2 (PLA2) could form micropores in the cell membranes of the host and fuse the polypide with the membrane or change the host's membrane fluidity to let in the parasites[62]. In *in vitro* experiment, both PLA2 and monoclonal antibody, which were added to the culture medium, inhibited the entry of Toxoplasma to fibrocytes[63,64]. Therefore, PLA2 is closely related with toxoplasma's invasion into host cells.

After a fetus is infected with toxoplasma, the activated T-cells, macrophages and NK cells function as predominant effector cells to eliminate and inhibit the parasite in cell-mediated immunity. The anti-infection of the host depends chiefly on the induction of T-lymphocytes and macrophages to produce cytokines with multiple biological activities in immunoloregulation[65-67]. IgM and IgG antibodies are present in serum in succession. By dectecting neonatal IgM antibody or paired IgG antibodies, supportive diagnosis is available for fetal toxoplasma infection.

Parasitemia may occur after a pregnant woman is infected with toxoplasma. The lesion is disseminated in different organs of the fetus with the parasites growing intracellularly to cause acute infection phase disease[68]. Such organs are vulnerable to toxoplasma as brain, eyes, lymph nodes, heart, lungs, liver, and muscles. By the body's formation of specific immunity, the infection tends to become still gradually, but because of the blood-brain barrier, immune substances like immunocytes, IFN-γ, antibodies, etc. cannot reach the eyes and brain, where infection also occurs. The infection, therefore, may persist or develop in the eyes and brain.

Interventions for Birth Defects Caused by Toxoplasma Infection

The early diagnosis in pregnancy for toxoplasma is crucial to reduce the incidence of congenital neonatal toxoplasma infection. Serology screening for early pregnant women followed by counterchecks every 1-3 months afterwards can timely detect cases in acute infection phase, so treatments or termination of pregnancy can be available in time. Good hygiene and the cut-off of the toxoplasma transmission routes of food, water, and pet contact are also helpful.

The functions of vaccines are by inhibiting the reproduction and spread of toxoplasma, or by inhibiting formation of the cysts. It is useful to prevent congenital toxoplasmosis and reactivation of the cysts in the immunosuppressed individuals. Despite the recent molecular biological advances in recombinant vaccines, further researches are still needed.

The treatments for pregnant women with toxoplasma infection should be arranged according to conditions such as the time of infection, with or without fetal involvement, etc. Pregnancy termination is recommended if the infection happens within the first 8 weeks in pregnancy, medicines like spiramycin should be administered when the initial infection is diagnosed between the 8th to the 26th week. If the fetus is found infected between the 8th to the 26th week, pyrimethamine + sulfapyridine therapy should be carried out immediately.

In a word, it is important to conduct appropriate prevention education, timely reorganization of infection cases and proper treatments for the sake of good intelligence and health of coming generations.

PROSPECT

Intrauterine infections induced by certain pathogens are regarded as the major cause of spontaneous abortions, perinatal deaths, birth defects, etc. Therefore, it is necessary to have the pregnant women informed of the pathogens that may affect the pregnant outcomes as well as the measures to prevent such infections and the triggered adverse pregnant outcomes. The screening for infections before and during pregnancy is significant to reduce intrauterine infections and birth defects.

RV and CMV infections in pregnancy are important sources of fetal deformities and birth defects. The birth defects to be induced, especially the long-term effects, such as sensorineural hearing loss, intelligence disorder, development retardation, vision loss, schizophrenia, etc.[4,14,15], take several years to become observable. Those who are intrauterinely infected are susceptible to diseases like diabetes, thyroid function disorder, osteoporosis, etc.[12]. The immediate and potential influences of viral infection on fetuses need further studies in molecular epidemiology, and the molecular mechanism and pathophysiologic process of stillbirths, abortions, deformities and birth defects induced by intrauterine viral infection still remain unclear at present.

Vaccines are effective to prevent viral infection and protect the high-risk groups. It has been proved in practice that RV vaccines have a protective anti-virus effect. However, the protective duration and safety of vaccines have been arguable. Safe and effective vaccines as well as scientific vaccine use have been the focus in current anti-virus researches.

There are disagreements in studies on Uu infections and birth defects due to the influences of such factors as Uu virulence, human immunity, sample size, experimental methods, criteria, etc. Uu has a total of 14 serotypes. It is probable that the serotypes with a stronger virulence can infect more easily the uterus to cause abnormal fetal development. The Uu that infects pregnant women is mainly types III and VI[69,70]. Uu is a conditional pathogenic microorganism in the lower genital tracts. There are limitations in many studies which applied Uu positivity in cervical secretion alone as the criterion to decide the relation

between Uu and birth defects and adverse pregnant outcomes, so reliable results depend on appraisal experimental design, unified criteria and even larger epidemiologic surveillances.

The clinical manifestations of toxoplasma infection are complicated because of insufficient specific clinical symptoms and signs, which makes the diagnosis difficult. The pathogen-detecting method currently utilized is poor in detection rate, and the immunological diagnostic method is restricted by the extraction and processing of antigens, immunity condition, and the sensitivity and specificity of the method. The Chinese National Institute for the Control of Pharmaceutical and Biological Products spot-checked 10 toxoplasma IgG and IgM antibody diagnostic kits that were produced by 6 manufacturers and compared their quality according to International Standard Toxoplasma Serum. The result demonstrated that there were significant variations among the kits, and the routine diagnostic kits also had problems in specificity and sensitivity[68]. Thus, further standardization of toxoplasma diagnostic methods is demanded. Tachyzoites are the major form of pathogens in the acute episode of toxoplasma infection, whose molecular mechanism in its invasion of cells is still under research. In addition, the molecular mechanisms of Uu and toxoplasma to induce birth defects require in-depth studies.

Among 686 pregnant women who were diagnosed to be normal in prenatal examinations (ultrasonic test and chromosomal patterns test), 44 had viral DNA detected in the amniotic fluid (three had two types of viral DNAs)[71]. Further studies are needed to clarify the significance of virus' existence in normal amniotic fluid during pregnancy, the potential influences of viral infection on fetuses, and the long-term effects. Other viral infections in pregnancy, such as HIV, HBV, HSV, parvovirus B19 infections, etc. can trigger birth defects to some extent. Their induction mechanisms and the induced defects demand further researches.

Birth defect researches in terms of etiology and the development of applicable and sensitive pathogen detection technology and methods are imperative. At present, safe and effective interventions are still absent for congenital infections. It is meaningful to administer necessary vaccines before pregnancy to prevent viral infection. It is important to make diagnoses in the first trimester of pregnancy, especially during the first three months to distinguish intrauterine infections and protect the fetuses. Moreover, by strengthening the pregnant health care and examinations, the infections of virus, toxoplasma, Uu, and other pathogens can be prevented so as to reduce the adverse pregnant outcomes, which is significant for improving population quality and the performance of the healthy birth policy. At the same time, further researches should be conducted to delineate the mechanisms of birth defects caused by virus, Uu, toxoplasma and other pathogen infections at macroscopic and molecular levels.

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