UiT THE ARCTIC UNIVERSITY OF NORWAY

Faculty of Health Sciences

Department of Clinical Medicine

A novel risk classification system for preconception health and sero-epidemiological map of Toxoplasma, Rubella and Cytomegalovirus infections among couples planning a pregnancy in rural China: A nationwide study

Qiongjie Zhou

A dissertation for the degree of Philosophiae Doctor – August 2019

Cover photo

My son found this grass and told me it was a lovely butterfly. I do hope myself having a heart of appreciation beauty.

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Qiongjie Zhou

LIST OF ABBREVIATIONS

BMI	Body mass index
BPA	Bisphenol A
CLP	Classification, labelling and packaging
CMV	Cytomegalovirus
CRS	Congenital Rubella syndrome
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
DOHAD	Developmental origins of health and disease
ELISA	Enzyme-linked immunosorbent assay
EQA	External quality assessment
HeLTI	Healthy Life Trajectories Initiative
HSV	Herpes simplex virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MMR	Measles, Mumps and Rubella vaccine
MMRV	Measles, Mumps, Rubella and Varicella vaccine
NICU	Neonatal intensive care unit
NIH	National Institutes of Health
NTD	Neural tube defect
NPHCP	National Preconception Health Care Project
NNDRS	National Notifiable Diseases Reporting System
PCC	Preconception care
PCR	Polymerase chain reaction
PE	Pre-eclampsia
PPROM	Premature rupture of membranes
QFP	Quality Family Planning Services
RPR	Rapid plasma reagin
SD	Standard deviation
SNHL	Sensory-neural hearing loss
STI	Sexually transmitted infection
ToC	Theory of Change
TORC	Toxoplasma, Rubella and Cytomegalovirus

ABSTRACT

Introduction

Preconception care (PCC) is benificial for optimizing women's health before pregnancy for a better pregnancy outcome. In China, a vast majority of pregnancies among married couples are planned, providing a unique opportunity for promoting preconception health. However, more efficient systems are required to classify prepregnancy risk factors and then manage them appropriately. Furthermore, it is important to evaluate the prevalence of preconception risk factors and their geographic distribution for health policy planning and appropriate allocation of resources. Preconception screening of couples for vertically transmittable infections, such as Toxoplasma, Rubella and Cytomegalovirus (TORC), might potentially allow implementation of preventive strategies or treatment before conception. However, nation-wide prevalence of TORC infection in preconception period and their geographical distribution in China is not known.

Aim and objectives

The overall aim of this thesis was to evaluate the novel risk classification system in preconception care project in China. We have established a sero-epidemiological map of TORC infections among married women in China before pregnancy.

The main objectives were:

1. To evaluate the preconception health status of married couples by a novel risk classification system developed by NPHCP.
2. To investigate the sero-epidemiology of Toxoplasma gondii infection, regional difference and related risk factors.
3. To investigate the sero-epidemiology of Rubella virus infection, geographic characteristic and associated socio-demographic factors.
4. To study sero-epidemiology of Cytomegalovirus infection (CMV), and also its geographic and socio-economic factors.

Methods

This study utilized data extracted from NPHCP during 2010–12. This study was a population-based, cross-sectional and nation-wide. Its implementation covered 220 rural counties in mainland China. All married couples intending to conceive a pregnancy within six months were provided free preconception care, and those couples who signed a consent were eligible

for inclusion to the study. We excluded those couples who did not sign a consent or failed to complete the preconception health examination, and those women did not have their TORC infection status.

Local community staff interviewed married couples of reproductive age regarding their conception plans. Those with intention to conceive within six months were enrolled into the program. Detailed information on demographic and socioeconomic status was obtained, a medical history was taken, and the community health workers performed physical examination.

All the information that was recorded and uploaded in a web-based electronic data collection system. Venous blood samples were taken for TORC infection testing. According to the amenability to prevention and treatment, preconception risk factors were categorized as followed: **A**-avoidable before conception, **B**-benefiting from targeted intervention before conception, **C**-controllable but requiring close monitoring during pregnancy, **D**-diagnosable prenatally but not modifiable before conception, and **X**-pregnancy not advisable.

Results of TORC serology and collected socio-demographic and clinical data of the participating couples were extracted and further analyzed to estimate sero-prevalence in the sample population, explore regional differences, and identify associated risk factors.

Results

Our study enrolled a total of 2142849 couples. Most couples (92.36%) were from rural areas in China. 89.2% women and 88.3% men had education below university level. 68.29% couples had risk factors before conception, and the risks were mainly of category A, B or C. 38.13% men were of category A (i.e. avoidable) prepregnancy risk factors, and their female countparts were of 11.24%.

Of 2008561 women who had Toxoplasma gondii serology results, 45405 (2.3%) women were IgG positive and 6884 (0.3%) were IgM positive. Advanced maternal age, occupation of a farmer, vegetarian diet and exposure to cats were significantly related with Toxoplasma gondii IgG and IgM sero-positivity (P=0.000).

There were a total of 1974188 women with Rubella virus IgG serology results and vaccination history. 1161129 (58.4%) were Rubella virus IgG positive and 91604 (4.6%) reported history of Rubella virus vaccination. Their self-reported vaccination status did not significantly correlate with Rubella virus IgG sero-positivity (P>0.05).

CMV serology results from a total of 2019555 samples were available, of which 42.1% (n=850592) were CMV IgG positive with 0.4% (n=9290) IgM positive. CMV IgG negative women were of younger age, whereas women of advanced age were at higher risk of IgM positive ($P<0.0001$). Provincial CMV IgG sero-positivity was significantly associated with resident consumption level ($r=0.437$; $P=0.014$) but not with gross domestic product ($r=0.167$; $P=0.369$).

Significant regional variations were observed in TORC sero-positivity among married women during preconception period.

Conclusions

This project provided some new insights into preconception care. Preconception health screening and use of risk novel risk classification system could effectively identify important risk factors and stratify couples into different risk categories. Evaluation of preconception health status and stratification of risk showed that avoidable risk factors are most common among men, demonstrating the importance of including male partners in preconception care. Sero-epidemiological map of TORC infection in China showed that a significant proportion of women are susceptible in preconception period. Therefore, targeted screening of these infections followed by referral, diagnosis, treatment, counseling, health education and vaccination before pregnancy should be considered as appropriate to reduce the risk of vertical transmission.

LIST OF ORIGINAL PAPERS

Paper I

Zhou QJ, Zhang SK, Wang QM, Shen HP, Tian WD, Chen JQ, Acharya G, Li XT. China's community-based strategy of universal preconception care in rural areas at a population level using a novel risk classification system for stratifying couples´ preconception health status. *BMC Health Serv Res. 2016 Dec 28;16(1):689.*

Paper II

Zhou Q, Wang Q, Shen H, Zhang Y, Zhang S, Li X, Acharya G. Seroepidemiological map of Toxoplasma gondii infection and associated risk factors in preconception period in China: A nation-wide cross-sectional study. *J. Obstet. Gynaecol. Res. 2018 Jun;44(6):1134-1139.*

Paper III

Zhou Q, Wang Q, Shen H, Zhang Y, Zhang S, Li X, Acharya G. Rubella virus immunization status in preconception period among Chinese women of reproductive age: A nation-wide, cross-sectional study. *Vaccine. 2017 May 25;35(23):3076-3081.*

Paper IV

Zhou Q, Wang Q, Shen H, Zhang Y, Zhang S, Li X, Acharya G. Sero-epidemiology of Cytomegalovirus infection and its geographic and socio-economic determinants in preconception period among Chinese women planning a pregnancy within six months: A nationwide study. (*submitted manuscript)*

1 INTRODUCTION

Preconception care (PCC) is the provision of health care for women and men planning to have a child before conception by World Health Organization (WHO) [1]. Despite the advances in prenatal, intrapartum and postpartum care, adverse birth outcomes remain prevalent worldwide, even in affluent countries. PCC provides an opportunity to make a difference. PCC aims to identify risk factors, promote preventive strategies and provide interventions when appropriate. PCC potentially benefits not only the safety of pregnancy and childbirth for both mothers and babies, but also long-term consequences on child health and wellbeing [2-4]. PCC is shown to prevent unintended pregnancies, congenital birth defects, fetal and neonatal infections, and reduce pregnancy complications, as well as maternal and child mortality. Therefore, the Centers for Disease Control and Prevention (CDC) recognized that preconception health be a critical component of healthcare for women of reproductive age in "Providing Quality Family Planning Services" [5-6].

Ideally, preconception health should lead to satisfactory improvement in maternal and infant outcomes, however, there are still gaps between available evidence and implementation. On one hand, as approximately one third of births are intended [7], in some countries PCC is recommended for all women of childbearing age [8]. On the other hand, not all couples acknowledge that PCC is important and necessary before conceiving and take pregnancy as a biological process without recognizing associated risks. Take folic acid supplement and maternal obesity for example. Despite solid evidence that folic acid supplementation is significantly benefical for reducing the risk of neural tube defects in the offspring, only approximately 30% women take the supplement [9]. Maternal weight has become a public concern as overweight and obesity are prevalent worldwide, and maternal obesity has adverse effects not only on pregnancy but also on offspring's health later in life as well [10]. Therefore, it is still a challenge to provide effective PCC in the real world, although it is well recognized that preconception health matters. PCC is not universally provided even in high-income countries. Professional guidelines are often lacking and official policies are not always in place. Furthermore, the content varies substantially even among European countries [11].

2 HISTORY OF PRECONCEPTION CARE

Since the twenty-first century, the focus of maternal health care has shifted from intrapartum, antenatal, to preconception care. During the period from the 1960s to the 1980s, maternal health care during labor and delivery greatly improved with safer hospital deliveries, improved hygiene, availability of antibiotics, safe blood transfusion and surgical techniques, and introduction of electronic fetal heart rate monitoring for those high risk pregnancies [12-15]. This largely contributed to the huge decline of not only maternal mortality caused by infection and bleeding, but also of stillbirth and neonatal deaths. The emphasis slowly shifted towards antenatal care in the 1980s with the intention of reducing pregnancy complications such as anemia, preeclampsia, congenital birth defects and preterm birth. In the past few decades, with the realization of the fact that conventional antenatal care is not enough for primary prevention, and the growing evidence demonstrating that PCC is effective in improving maternal and fetal outcomes, efforts have been made to integrate preconception care into traditional antenatal care, interpregnancy care, family planning services and primary care.

The concept of preconception care is not new. However, the ideas and models of preconception care have evolved and have been adapted as formalized health policies in several countries around the world. Preconception care is focused on the factors prior to pregnancy, which need appropriate medical education, prevention and management [16]. Each country may have its own model of preconception care with content and purpose suitable for the target population. However, the optimal strategy of delivering PCC is yet to be determined. The Preconception Service in Hungary [17-18] is mainly targeted at prevention of congenital abnormalities and preterm birth from three months with an intended pregnancy until the 12th week of pregnancy, including preconception check-up, a 3-month preparation for conception and achievement of optimal pregnancy. In this Hungarian preconception service, it was found that: (i) the rate of preterm births was reduced from 9.2% to 5.0%, mainly due to sexually transmitted infections, (ii) the rate of congenital abnormalities was reduced to 2.9%, which mainly linked with periconception multivitamin supplementation, (iii) the rate maternal smoking was reduced, (iv) male partners were involved, (v) couples at high risk were identified and further transferred to secondary specialist care. The Canadian government also launched a national preconception health care project, named the Healthy Life Trajectories Initiative (HeLTI) in 2018 [19]. According to the Public Health Outcomes Framework in the United Kingdom [20], PCC is thought to be relevant to provide across the whole reproductive life span of couples, in all relevant health and social care pathways. There is a general consensus that PCC is beneficial for achieving a better pregnancy outcome and a healthier life of the offspring. In the framework of PCC, both women’s as well as men’s health before conception equally contribute to the health of the offspring.

In China, PCC has become a priority concern for maternal and child health since 1990s. A series of laws have been passed and policies have been published, including the "Maternal and Child Health Care Act” in 1994 which legalized the prevention of birth defects, such as by folic acid supplement and avoiding exposure to teratogens for primary prevention, screening, diagnosis and termination of pregnancy when a serious defect is found for secondary prevention, and diagnosis and treatment after birth. In the decade of 1998-2008, relevant guidelines and technology standards were issued as listed in Figure 1, improving the preventative strategies of birth defects. In the process of health reform, more financial support has been provided, including a national free folic acid supplement program, neonatal health insurance for babies born with congenital heart disease, hemophilia, and cleft lip/palate, and thalassemia screening in Guangxi, Hainan and Yunnan province. A systemic network of birth defects monitoring has been built up since 1986, covering nearly 800 hospitals at county and city level. According to the requirement of “National Medium-and Long-Term Science and Technology Development Plan (2006-2020)” on the prevention of birth defects, abundant national grants and funding have been offered for the related basic and clinical research.

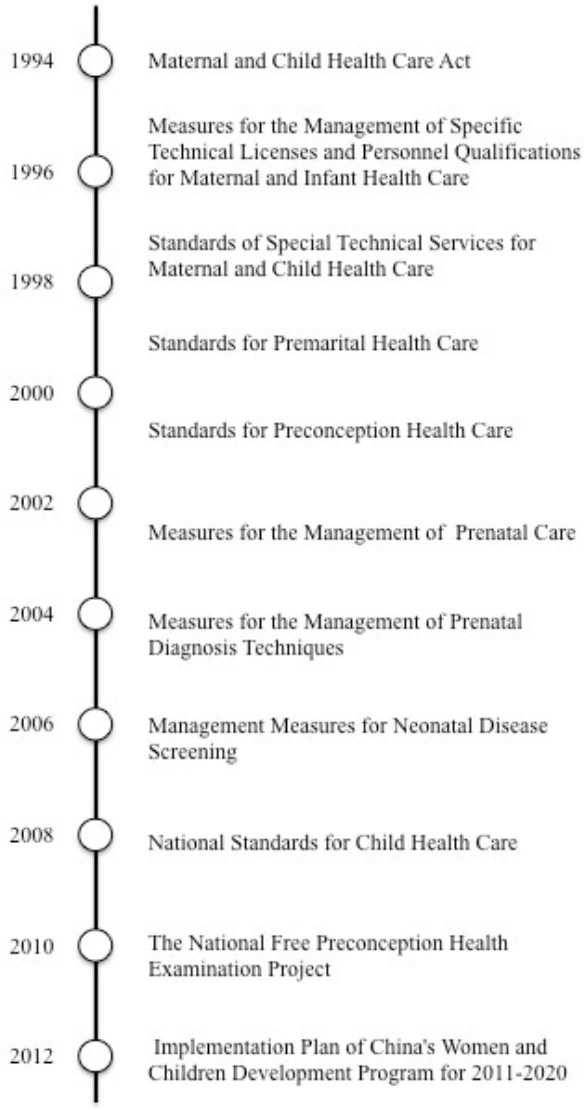

Figure 1 Timeline for governmental policy and measures for improving maternal and child health during 1994-2012 in China.

3 THEORIES OF PRECONCEPTION CARE

The importance of improving maternal and child health is well recognized and some components of PCC are shown to have a positive effect, whether the concept of universal PCC is necessary, feasible and effective still remains controversial. Hence, a careful analysis theory is needed.

3.1 Precautionary principle for the necessity of preconception care

Initially, the concept of PCC originated from the precautionary principle. According to this theory, uncertain issues should be acted on, implying that we're responsible to protect the public from harmful exposure. Further scientific evidence is required to relax the protection. The essence of this principle is captured in PCC (Figure 2).

The use of bisphenol A (BPA) is a good example of the application of the precautionary principle. Exposure to BPA is common in daily life, as it is used in many things including computers, cooking appliances, bottles, food containers, etc. Considering the potential risk to human health and environment, in the European Union, use of BPA is within a comprehensive legislative framework. The classification, labeling and packaging of BPA are required to comply with the CLP (classification, labelling and packaging) Regulation (EC No 1272/2008) to ensure that workers and consumers are clearly communicated for potential hazards. In Canada, consumer exposure to BPA is reconfirmed as very low, but the use of BPA in baby feeding bottles has continued to be restricted in Canada since 2010.

Similarly, according to epigenetic principles, intrauterine stress may impact offspring's' health without alteration of the gene sequence, which could possibly be related with DNA methylation and histone modifications [21-22]. Evidence from animal models showed epigenetic modifications in offspring's health, and epidemiological evidence suggests that this could also be the case in humans [23-24], such as impaired deoxyribonucleic acid (DNA) methylation of insulin-like growth factor-2 (IGF-2) among offspring of women prenatally exposed to starvation during the Dutch famine [25-27]. Therefore, it is logical to think that adequate and balanced nutrition before and during pregnancy is likely to be beneficial for the health of woman as well as long-term wellbeing of the child.

In the "Barker hypothesis" [28-30], the concept of developmental origins of health and disease (DOHAD) proposes that one's intrauterine environment and early childhood health affects one's health later in life. Therefore, eliminating or reducing maternal exposure to harmful substances (e.g. teratogens, smoking, drugs etc.), unfavorable environments (e.g. hypoxia), malnutrition (such as overweight, underweight), endocrine disbalance (e.g. hyperglycemia, hypothyroidism, etc.), could potentially improve perinatal outcomes and help achieve a healthier status in later life.

Thus, health policy makers and healthcare professionals are getting interested in making PCC a high priority.

Figure 2. Precautionary principle for the necessity of preconception care.

3.2 Theory of change for the feasibility of preconception care

The implementation of PCC is more than a combination of healthcare and social reform. In a public health perspective, Theory of Change (ToC) is applied to the practice of PCC. In ToC, long-term goals are defined first, and then the necessary preconditions are identified backwards. The practice of PCC in China is based on the ToC, considering the factors of society, economics, health service, knowledge of couples and social behaviors. Its framework consists of four aspects, i.e. background, activities, changes and results, considering political environment, service system and targeted population (Figure 3).

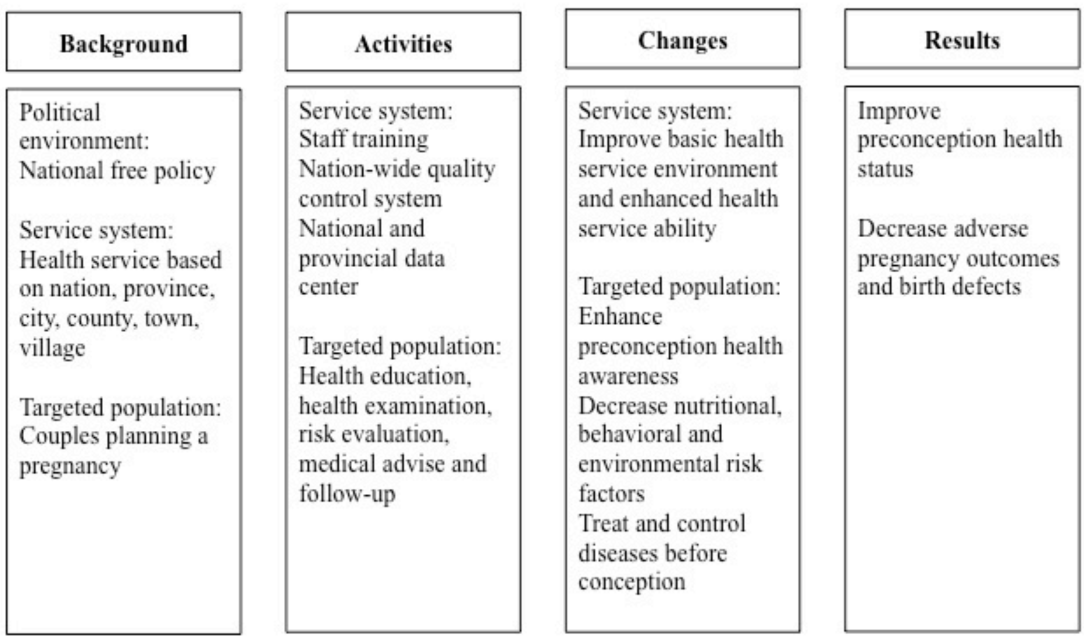

Figure 3. Theory of Change as applied to preconception care in China.

3.3 Translational medicine for the effectiveness of preconception care

The translation from research populations to general population is the requisite for an effective PCC. In the practice and policy decision making of PCC, translational medicine works based on the following four aspects: (1) risk factors before pregnancy are associated with adverse pregnancy outcomes in epidemiological study and basic experimental laboratory-based research; (2) intervention before conception is feasible; (3) the preconception risk is an actual problem in the real world; (4) intervention before conception is effective in real world population (Figure 4).

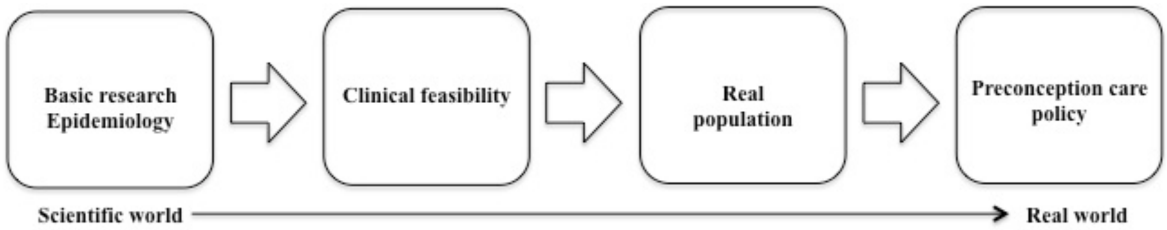

Figure 4. Translational medicine for the effectiveness of preconception care.

There are several examples of translational research influencing health policies regarding preconception care, such as national recommendations on folate intake for women of reproductive age and folic acid supplement for reducing the risk of congenital birth defects [31].

4 RISK FACTORS DURING PRECONCEPTION PERIOD

There is abundant evidence showing that PCC is beneficial to women and children’s health as it has the potential to eliminate or modify pre-pregnancy risk factors. Interventions including avoiding exposure to alcohol, smoking, and substance abuse, are expected to have a positive effect on promoting healthier lifestyle [32], besides that folic acid supplementation [33], immunizations, and treatment of sexually transmitted infections are well recognized to improve pregnancy outcomes. Since PCC is defined as *biomedical, behavioural and social health interventions* by WHO [1], the above three aspects of risk factors during preconception period are addressed here. Therefore, the prevalence, amenability to modification, and feasibility of intervention of prepregancy risk factors need to be evaluated in well-defined populations taking into account their ethnic, social, cultural and economical backgrounds.

4.1 Biomedical risk factors

Immunization is practical and feasible before pregnancy and it is known to improve pregnancy outcomes from vaccine preventable diseases. Rubella infection in pregnancy has greatly declined since the introduction of MMR immunization, and therefore, the risk of fetal loss and congenital rubella syndrome (CRS) has significantly decreased [34].

Taking folic acid supplement is widely accepted as it substantially reduces the risk of neural tube defects (NTDs) [35] and cardiovascular malformations.

Effective treatment of chronic diseases including diabetes and hypertension, during the preconception period seems to be benefical for reducing adverse maternal and neonatal outcomes [36-38].

4.2 Behavioral risk factors

Smoking during pregnancy is closely related with premature births, miscarriages, stillbirth and low birthweight [39]. Passive (second-hand) smoking is significantly prevalent among Chinese women as maternal smoking rate before conception was reported to be only 0.45% with a paternal smoking rate of 29.07% [40]. Concern regarding the role of paternal smoking on birth defects is growing. Animal studies have found potential toxic effect of paternal exposure to smoking on sperm development [41], and epidemiological evidence indicates an association between fathers' smoking and conotruncal heart defects, limb reduction defects and amniotic band syndrome [42].

Alcohol is a 'teratogen' affecting fetal development and causing birth defects [43]. Alcohol consumption is associated with a higher risk of not only fetal growth restriction, but also preterm birth and perinatal mortality as well. Prevalence of maternal alcohol consumption in preconception period was approximately 3.40% and, therefore, it is important to stop or reduce alcohol consumption before pregnancy in order to eliminate potential risks [40].

A comprehensive family planning service as a component of PCC is instrumental for avoiding unintended pregnancies, which could avert up to 44% maternal mortality [44]. It can also reduce the risk of sexually transmitted diseases, Hepatitis B virus and HIV infections.

4.3 Social risk factors

Maternal age is an important social factor related with adverse pregnancy outcomes. Teenage pregnancy is at increased risk of preeclampsia, stillbirth, preterm birth, perinatal infection and neonatal intensive care unit (NICU); a similar association has been observed among women of advanced age, with the threshold age at 35 or 40 years old [41-49]. In China, the two-child policy has been implemented since 2016, replacing the previous one-child policy. Related social and psychological risks have grown along with the aging population and cultural change [50].

5 NATIONAL FREE PRECONCEPTION HEALTH EXAMINATION PORJECT IN CHINA

5.1 Background of the National Preconception Health Examination Project

A well-organized delivery system of PCC, awareness among future parents about the benefits and awareness among healthcare professionals about the effectiveness are requiste to achieve a good PCC service [51-52].

In China, PCC has become more important than ever. Population health has greatly imporoved with a rapid economic development in recent decades. Healthcare facility-based delivery strategy including birth care in the community by a skilled provider significantly reduced neonatal mortality for the Millennium Development Goal 4 [53]. According to national health surveillance, maternal mortality has decreased by 75.6%, from 88.8/100,000 in 1990 to 21.7/100,000 in 2014, and the neonatal mortality was 0.89%. However, there are some concerns. Firstly, according to the Birth Defects Report in China in 2012, the prevalence of birth defects was increased to 5.6%, which has been attributed to decreased rate of voluntary premarital medical examination [54]. Secondly, China has shifted to a two-child policy for countermeasuring the aging population and shrinking labor supply [55], which has led to an increasing portion of women of advanced age and with previous cesarean section being pregnant. Thirdly, geographic diversity is prominent; the maternal mortality rate in western areas is 2.6 times higher and child mortality before the age of 5 is 3.1 times higher compared to that in eastern areas. Additionally, there are gaps in accessibility and affordability of healthcare between urban and rural areas.

Although the benefits of PCC have been well recognized, the integration of PCC into women's healthcare still remains challenging. PCC in rural China used to be insufficient and inadequate as there are limited medical resources and there is a lack of adequate healthcare facilities and coverage by national health insurance. Women in rural China are more likely to be under-nourished, anemic, and have infectious diseases. They are likely to be less educated, have low socio-economic level and work as farmers or as urbanized immigrant workers. Thus, the Chinese government introduced a series of health policies to establish a new health insurance system, including the provision of maternity leave and economic support after delivery, and the free National Preconception Health Care Project (NPHCP).

5.2 Framework of the National Preconception Health Examination Project

This project is a nationwide, community-based, welfare program sponsored by the government of People`s Republic of China. The project is based on the concept that preconception care is a beneficial option for the prevention of birth defects, stopping smoking, controlling alcohol consumption, promoting balanced nutrition, screening population for genetic risk factors, chronic and infectious diseases, providing education, counselling and medical advice, and facilitate referral to specialist healthcare when needed.

5.3 Implementation of the National Preconception Health Examination Project

Preconception health evaluation is free to all married couples living in the 220 pilot counties. The target population consists of both wife and husband with an intention to conceive within six months [40, 55]. The healthcare is provided during the preconception period, and extended from early pregnancy to postpartum period, with the goal of achieving a healthier prepregnancy status and improving pregnancy outcomes [56]. Practical implementation of the project is carried out in village/town-county bases or in direct county bases as illustrated in the flow charts (Figure 5, 6). Community staff and local hospital staff who are trained, qualified and are familiar with the published project contents and standards, are responsible for the recruitment and follow-up of participating couples.

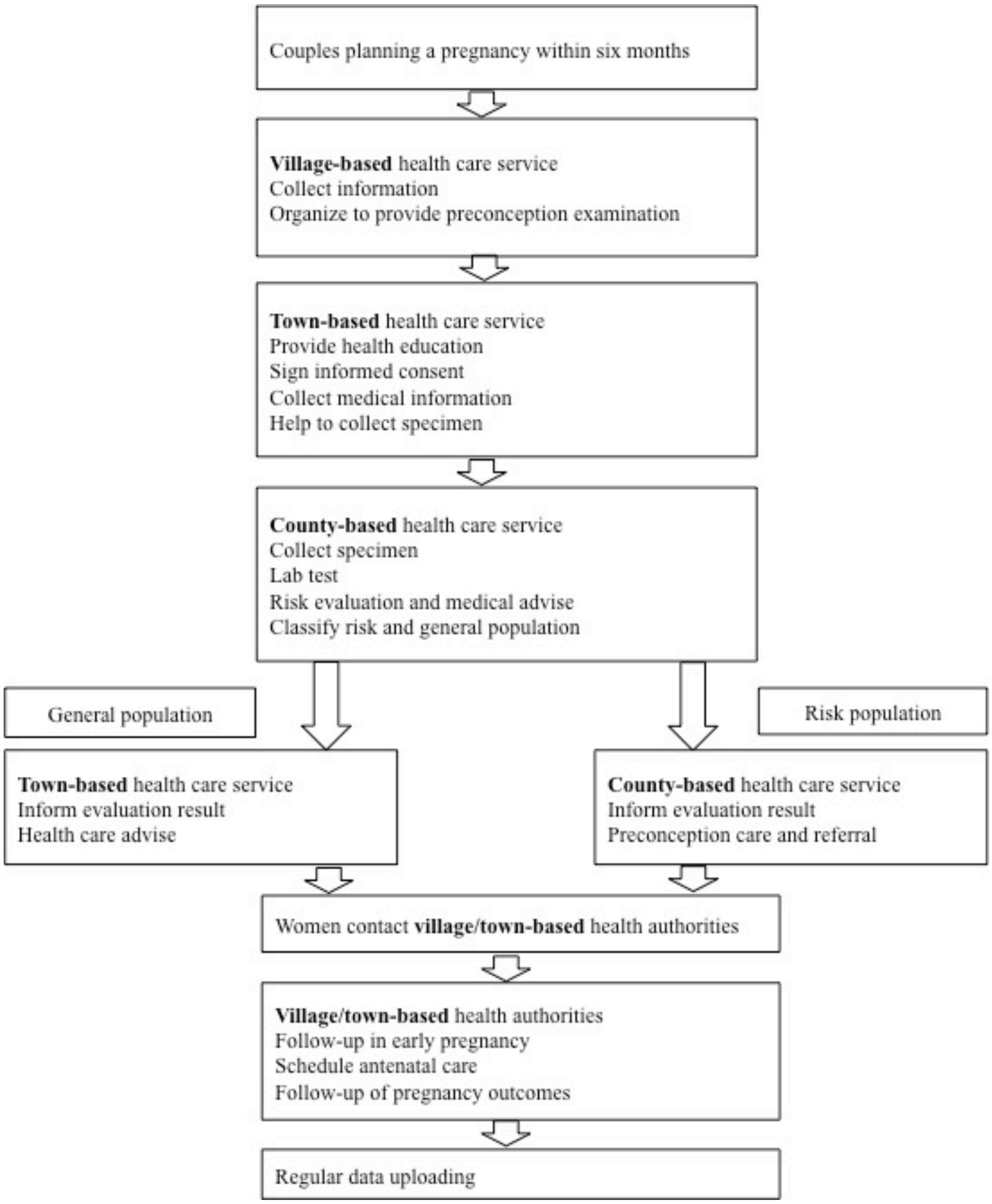

Figure 5. Flow chart demonstrating implementation of NPHCP in village/town and county base.

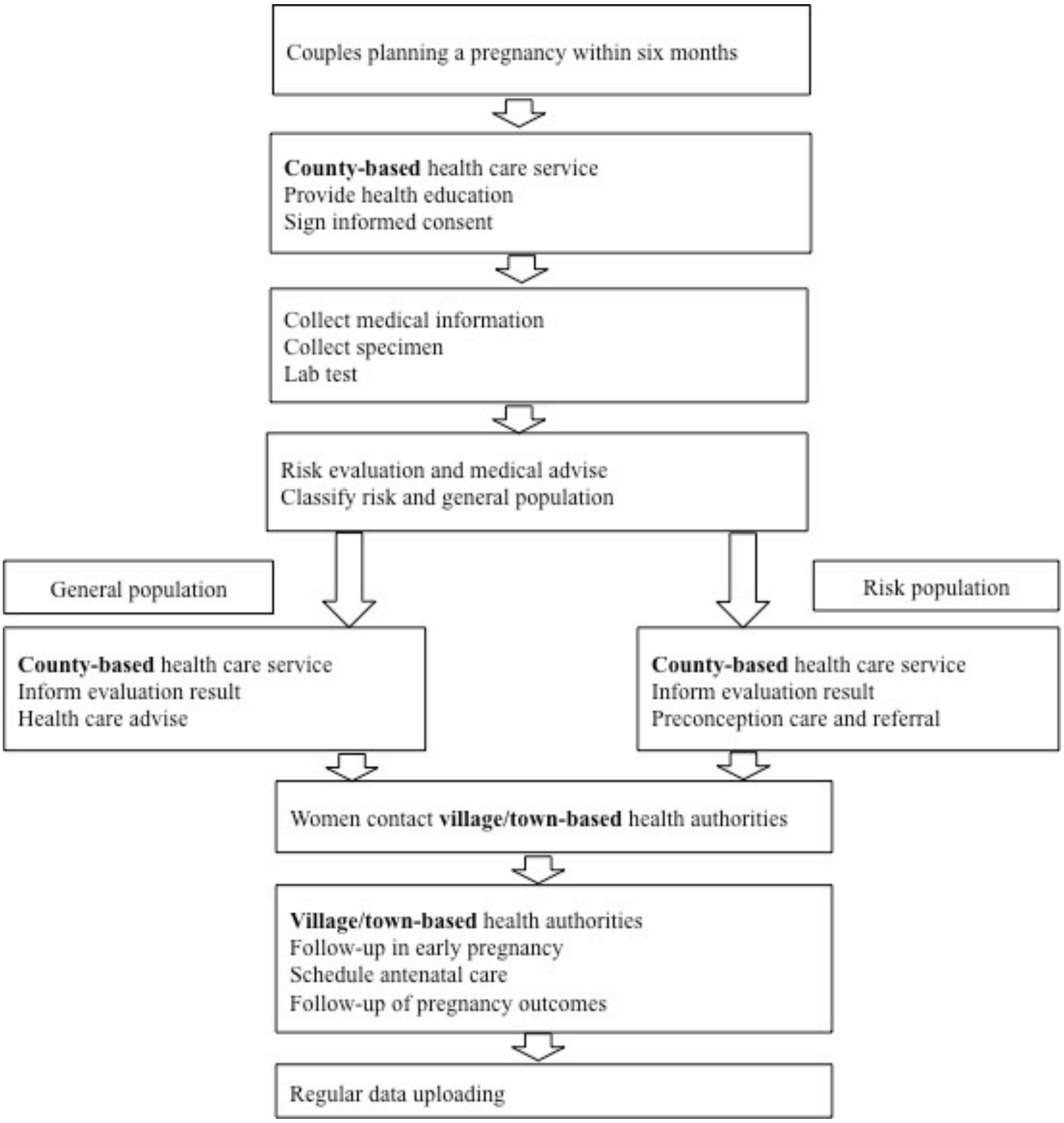

Figure 6. Flow chart demonstrating implementation of NPHCP in direct county base.

6 CURRENT PRECONCEPTION RISK CLASSIFICATION SYSTEMS AND NOVEL RISK CLASSIFICAITON IN CHINA

6.1 Current classification systems

The majority of risk factors before conception are avoidable, preventable or treatable [57]. These risks are generally classified based on the type of disease or exposure (such as genetic, reproductive, chronic disease, infectious, nutritional, behavioral, occupational, environmental and social risks). They are sometimes classified based on adverse outcomes in the mother or the fetus (maternal and fetal), or by severity of risk. Identifying and evaluating preconception risk is cumbersome and time-consuming but a comprehensive risk assessment is required. In the Netherlands, ZwangerWijzer is the most widely used self-administered online questionnaire, covering environmental, occupational, lifestyle and genetic risk factors. A personalized PCC is subsequently provided according to the identified risk factors [58-59]. Abundant prepregnancy risk factors have been reported, and thus, there is a necessity for PCC providers to manage the risk factors based on their impact, priority and feasibility of intervention to improve subsequent pregnancy outcomes.

6.2 Novel preconception risk classification model in China

In China, in order to better classify and manage the risk factors before conception, a novel risk classification system has been introduced in NPHCP that groups prepregnancy risk factors according to their amenability to prevention and treatment [56]. Preconception Health Examination and Risk Evaluation Guides were publicly published, which listed classification and treatment advices in details [40]. Preconception risk factors were categorized into the following five groups: **A**-avoidable before conception, **B**-benefiting from targeted intervention before conception, **C**-controllable but requiring close monitoring during pregnancy, **D**-diagnosable prenatally but not modifiable before conception, and **X**-pregnancy not advisable [40, 56]. Whether this novel classification system is feasible and efficient in classifying prepregnancy risk factors needs to be further evaluated. Furthermore, neither distribution of prepregnancy risk factors among Chinese couples, nor their amenability to intervention has been assessed. More research is needed regarding the impact of maternal healthcare strategy and novel risk classification model on stratifying risk factors and the preconception health status.

7 INFECTIONS AND PRECONCEPTION HEALTH

Infections are a major cause of adverse pregnancy outcomes. Approximately 30-50% preterm births (PTB) are related with maternal infections, and urogenital infections are well recognized as an important contributing factor to PTB and neonatal sepsis. Screening for group B streptococcus (GBS) is recommended in several countries. Systemic infections, such as TORCH (Toxoplasma, Rubella virus, Cytomegalovirus and Herpes Simplex), syphilis, Hepatitis B virus and HIV are a significant cause of maternal, fetal and neonatal adverse outcomes. Thus, screening to identify susceptible or infected women and offering immunization or other preventive measures as well as treating infected women before pregnancy can be expected to improve pregnancy outcomes.

Antenatal screening strategies for infections vary worldwide: in the USA, UK and most Scandinavian countries, pregnant women are screened for Rubella and syphilis at their first prenatal visit, while in some other countries, such as Germany and France, they may also be screened for Toxoplasmosis and CMV. Asymptomatic infants are generally not screened for congenital infections in most counties.

Ethnic and socio-economic disparities regarding infectious morbidity are well described. In the USA, urogenital infection is more common among Black women compared to their White ccouterparts, which is closely associated with not only preterm premature rupture of membranes but also preterm birth. Whether similar disparity exists among Chinese women of different genetic, cultural and socio-economic backgrounds has not been investigated. Furthermore, the prevalence of infections in preconception period in different geographic regions of China is not known. However, this knowledge would be important to develop policy on preconception healthcare.

8 TORCH INFECTIONS

TORCH infections have a potential risk of vertical transmission causing congenital infection. They are associated with serious risk of maternal, fetal and neonatal morbidity and mortality.

8.1 Toxoplasma gondii

Toxoplasma gondii (T. gondii) is an intracellular protozoan parasite. It infects both animals and humans, and is widely prevalent worldwide. Cats shed oocysts in their feces after ingesting any of the any stages of T. gondii, including tachyzoites, bradyzoites and sporozoites, which are contained in infected raw meat. Humans can get infected when they come in contact with oocysts shed by cats or by ingesting infected raw meat. In humans, ingested oocysts or tissue cysts transform into tachyziotes representing acute infectious stage of the disease and localize in neural and muscle tissues. Later, they develop into bradyzoites and remain dormant except in immunocompromised individuals.

Pregnant women are at risk of acquiring infection if they eat raw meat, have contact with cat litter or contaminated soil. Infection is usually asymptomatic, but transplacental transmission can occur causing congenital infection in the fetus. The risk depends on the timing of infection (seoconversion), varying from approximately 10-15% transmission rate in the first trimester to 60-70% in the third trimester. However, congenital infections tend to be more severe earlier in gestation, potentially leading to miscarriages and stillbirths. Fetal infection is associated with increased risk of intracerebral calcifications, hydrocephalus, meningoencephalitis, and retinochoroiditis [60-62].

T. gondii is prevalent worldwide, and it infects approximately 33% of the world's population [63]. Its sero-positivity among pregnant women varies, ranging from 60% in Brazil to less than 10% in the United Kingdom [64]; geographic difference is notable for the incidence of congenital infection: varying from 0.1% live births in France, to 0.01% in the USA [65]. Prevalence of T. gondii sero-positivity in some provinces in China was relatively low [66], but nation-wide sero-epidemiology is unclear.

Some risk factors for T. gondii infection are avoidable, especially eating raw meat and contact with cats [67-68]. In addition, educational level and awareness of risk as well as hygiene play an important role in disease transmission. Considering that it is common to have domestic cats in rural areas and the possibility of lower hygienic standards compared to urban areas, the risk of Toxoplasma gondii infection was expected to be higher in rural areas. The risk factors for maternal-fetal transmission include advanced gestational age at maternal infection, high parasite load, parasite source from sporozoites in oocysts, high-virulence T. gondii strain, and maternal immunocompromise. The distribution of risk factors may vary geographically, but the information regarding regional differences in prevalence of Toxoplasmosis in China is scarcely available.

Maternal infection is generally asymptomatic, nonspecific and mild. The mother could have fever, fatigue, chills, sweating, headaches, myalgias, pharyngitis, lymphadenopathy, hepatosplenomegaly, and nonpruritic maculopapular rash. More importantly, it is critical but difficult to determine the timing of infection among asymptomatic pregnant women. Primary prevention strategy seems a safer option to reduce the risk of congenital infection, because there is no effective vaccine against Toxoplasma [69]. Also, there still lacks sufficient evidence for an effective prenatal treatment to reduce the risk of mother to child transmission [70]. It is recommended to delay pregnancy for six months after an acute infection since the parasitemia is short lived and the infected women would have developed adequate immunity by then. Studies in Europe and North America have shown that maternal treatment within three weeks of seroconversion is beneficial for reducing vertical transmission and serious neurological sequelae or death in congenitally infected offspring [71]. However, majorities of women of childbearing age are susceptible to primary infection and are at risk of congenital toxoplasmosis and its sequelae. Therefore, prepregnancy screening for T. gondii infection should be a good option for better primary prevention of congenital infection.

8.2 Rubella virus

Congenital rubella syndrome (CRS) is regarded as a public health concern. Rubella is a viral infection, which was known as German measles. Rubella virus is infectious only to humans. Rubella outbreaks have continued in some countries, and Although Rubella virus infection is generally self-limiting, the risk of transplacental transmission is high if the infection occurs during pregnancy. Rubella infection during pregnancy may cause miscarriage, preterm birth, stillbirth and intrauterine growth restriction [72]. CRS is associated with deafness, ophthalmic defects (e.g. cataracts, chorioretinitis, microphthalmia), cardiac defects, neurological abnormalities (e.g. microcephaly, meningo-encephalitis, mental retardation) and other defects, such as hepato-splenomegaly, bone defects, thrombocytopenia, purpuric skin lesions).

The risk of congenital infection varies with gestational age. Fetal infection rates are approximately 81% in the first trimester, dropping down to 25% in the second trimester, and rising to nearly 100% after 36 weeks [73]. However, severe sequalae is mostly limited to infection occurring before 16 weeks of gestation. Currently, effective treatment for in utero Rubella virus infection is still not available.

Maternal immunity is effective for protecting against intrauterine Rubella infection. It is acquired not only naturally but also by vaccine. The risk of CRS can be eliminated, by screening to identify susceptible women and offering vaccination before pregnancy. If antibodies to Rubella are negative, it provides the woman an opportunity to get vaccinated before conception, in order to avoid the risk of CRS. Immunization before conception is important in susceptible women to ensure that Rubella virus IgG are positive before or in early pregnancy because none of the CRS resulting from maternal reinfection occurred in women infected after 12 weeks of pregnancy [74]. It is recommended to avoid conception at least by one month following the administration of Rubella vaccine due to theoretical risk to the fetus with live attenuated vaccine.

In many countries screening for Rubella antibodies is performed routinely during the first trimester of pregnancy. Although it allows counseling regarding the risk, providing some advice on how to avoid infection in the current pregnancy and offer immunization postnatally, the risk of infection during pregnancy cannot be completely avoided.

Prevalence of Rubella susceptibility among women of reproductive age varies worldwide. In China, the nation-wide incidence of Rubella was 9.11/100,000 in 2008 according to the National Notifiable Diseases Reporting System (NNDRS) [75] while the annual incidence rate of Rubella was reported to be only 0.75 per 100,000 in Zhejiang province in 2013-15 [73]. The rate of CRS in live births was 0.9% in Jinan and Yantai [76]. In the Russian Federation, approximately 16.5% of pregnant women were susceptible to Rubella infection, and prevalence of CRS was 0.35% [77]. In Turkey, it was estimated that 15% of women aged 20-29 have negative titers [78], and 23% of reproductive women were reported to lack antibodies to Rubella in Nigeria [79].

Vaccination strategy is effective and recommended for protecting from Rubella virus infection. Routine vaccination with measles, mumps, and Rubella combination vaccine (MMR) in childhood, and measles, mumps, Rubella, and varicella combination vaccine (MMRV) are beneficial for reducing CRS. For example, the incidence of Rubella in the United States declined to 0.1 per 100,000 in 1999 [80-82]. However, it is not always optimal for vaccination coverage. In China, MR and MMR vaccinations during infancy and childhood were expanded in 2005 [83]. However, there lacks nation-wide prevalence of Rubella virus IgG sero-positivity among Chinese women of childbearing age before conception.

8.3 Cytomegalovirus

CMV is a ubiquitous DNA virus. It can be found in the urine or cervix of 2-28% pregnant women. Although CMV infection is common, in immunocompetent adult individuals a vast majority of infections (>90%) are subclinical. However, infection during pregnancy could possibly lead to serious fetal infection. Viral transmission occurs tranplacentally or during birth from exposure to maternal cervicovaginal secretions and blood. Maternal immunity is beneficial for the fetal protection as maternal IgG positivity significantly reduces the risk of intrauterine infection [84]. However, nearly 970,000 women of childbearing age were reported to have a primary CMV infection each year in the United States [85]. The prevalence of congenital CMV is 0.64% at birth, and it's one of the most common virus infections during pregnancy [83].

In utero CMV infection may occur after maternal primary or recurrent infection during pregnancy, but symptomatic congenital CMV are mainly observed in primary maternal infection. Hearing loss and mental impairment, including seizures and cerebral palsy, are the most common manifestations of congenital CMV infection. Prenatal antiviral treatment has been used, but it remains controversial due to associated drug toxicity. Sufficiently safe and effective treatment is still lacking. Therefore, it is suggested that women consider delaying conception at least 6 months after primary infection [86-87]. Compared with seroconversion during pregnancy, preconception seroimmunity provides a more substantial protection against maternal-fetal infection. Thus, determining CMV serology status before conception and providing proper counseling, information and support might help to reduce the risk vertical transmission.

CMV IgM and IgG are used as serological markers of infection and immunity. Women who are IgG positive are considered at low risk of infection while those who are IgM positive may have current infection. Since IgM antibodies could be detected 6-8 months after the infection, the timing of maternal infection is difficult to determine. Avidity assays may be of some help as low avidity suggests recent infection. Prenatal diagnosis by amniocentesis and polymerase chain reaction (PCR) for CMV DNA may be offered to women diagnosed as primary infection or if fetal infection is suspected on ultrasound examination.

IgG sero-positivity was reported to be 98.7% among pregnant women living in Jiangsu province in China [88]. Substantial regional variation in CMV sero-positivity has been observed in the USA with seropositivity differing by race and/or ethnicity. Compared with their non-Hispanic white Americans, non-Hispanic Black and Mexican Americans were at higher risk of infection [77].

In the United States and Europe, 40% of women of childbearing age are considered to be susceptible to CMV. However, studies on prevalence of CMV sero-positivity among women planning a pregnancy are scarce and whether identification of susceptible women before pregnancy and subsequent preventive strategies could reduce vertical transmission has not been properly explored. A recent study of women with fertility treatment showed that preconception testing and counseling is helpful for minimizing exposure to CMV by improving personal hygiene might have a positive effect [89]. CMV vaccine could be a future option for eliminating maternal-fetal transmission. Despite their potential in preventing congenital CMV, vaccines is currently not available, but they are under clinical development [90]. Therefore, preconception screening and treatment of CMV may be useful in reliefing the burden of congenital infection, at least in areas with high susceptibility rates.

8.4 Herpes simplex virus

Herpes simplex virus (HSV) is subgrouped into HSV type 1 (HSV-1) and HSV type 2 (HSV-2) [91-93]. HSV-2 primarily causes the genital herpes and HSV-1 for herpes labialis, gingivitis, stomatitis and kerato-conjunctivitis. However, genital infections due to HSV-1 are becoming common in recent years, perhaps due to changing sexual practices. HSV is transmitted through direct contact and genital herpes is a sexually transmitted infection. During primary infection, vesicles appear on the vulva which then break leaving ulcers that heal in 2-3 weeks. Reactivation of viral replication can lead to recurrent ulcerations or asymptomatic shedding of virus.

Genital HSV infection has three clinical subtypes: i) primary infection with HSV-1 and HSV-2 antibodies negative, ii) nonprimary first-episode (first genital infection with HSV-1 with pre-existing HSV-2 antibodies or genital infection with HSV-2 and pre-existing HSV-1 antibodies), and iii) recurrent infection, i.e. HSV type in the genital lesion and antibody type in the serum are same [87]. The seroprevalence of HSV differs depending on the subtype of virus: sero-positivity rate of HSV-2 decreased by 50% from 30% to 16% during 1990-2010, with a stable rate of HSV-1 at 65-69% [94-95].

Maternal-fetal transmission of HSV usually results from infected genital secretions during labor and delivery. Ascending infection occurs rarely. However, primary HSV infection acquired in the first trimester is correlated with miscarriage, preterm birth and fetal intrauterine growth restriction, choriortinitis and microcephaly [94-98]. Primary infection in late pregnancy may cause localized lesions in the skin, eye, mouth and central nervous system in the neonate. Regardless of the timing of infection and virus subtype, acyclovir is recommended as suppressive therapy from 36 weeks of pregnancy until the onset of labor for reducing the risk of disease recurrence and vertical transmission [99-100], and some recommend elective cesarean section in cases with active vulvo-vaginal lesions. Whether to perform a universal antenatal serologic screening remains controversial. Serologic screening might be helpful to inform infected women regarding standard suppressive antiviral therapy, and give advice on avoiding new infection during pregnancy to women with negative HSV antibodies. However, universal screening strategy still needs further evaluation.

In NPHCP, universal TORC (Rubella IgG antibody, CMV IgM and IgG antibody, Toxoplasma IgM and IgG antibody) serological screening was provided to all participating couples. However, data on serological profile of HSV were not collected in the NPHCP project. Thus, we were not able to evaluate the seroprevalence of HSV. We focused on establishing the sero-epidemiological map of TORC infections among married women in rural China during the preconception period.

9 AIMS OF THE STUDY

Delivering PCC at a population level is complex and many questions still remain to be answered. As pregnancies among married couples in China are mostly planned, this presents the opportunity to deliver PCC services in a structured manner. However, for appropriate allocation of resources and organization healthcare systems to deliver effective PCC services, it is of importance to know the distribution of prepregnancy risk factors and their amenability to intervention. Infections, such as TORC, that can be vertically transmitted from mother to fetus are a major risk factor during pregnancy, but their nationwide prevalence, regional differences in sero-epidemiology and feasibility of intervention in preconception period to improve pregnancy outcomes has not been studied well in China.

The overall aim of this thesis was to evaluate the impact of preconception health screening strategy and a novel risk classification system of China´s "National Preconception Health Care Project (NPHCP)", and to establish a sero-epidemiological map of TORC infections among Chinese married women before conception.

The main objectives were:

1. To evaluate the preconception health status of married couples by a novel risk classification system developed by NPHCP.
2. To investigate the sero-epidemiology of Toxoplasma gondii infection, regional difference and related risk factors.
3. To investigate the sero-prevalence of Rubella virus infection, geographic characteristic and associated socio-demographic factors.
4. To study sero-epidemiology of Cytomegalovirus infection (CMV) in preconception period among Chinese women of childbearing age together with its geographic and socio-economic factors.

10 METHODS

10.1 Study design and settings

This study utilized data collected by NFHCP between 2010–12. It covered 220 predominantly rural counties located in 31 provinces and province level municipalities of China. In 2010, NFHCP was launched by the Chinese National Health and Family Planning Commission and Ministry of Finance. Local community staff interviewed married couples of reproductive age regarding their conception plans. Those with intention to conceive within six months were enrolled into the program. They were provided with a free PCC package that included 20 preconception health service items including preconception health check-ups and referral to specialized hospitals [40, 56-57]. Couples who did not complete preconception examination and women with missing TORC serology test results were excluded from analysis.

10.2 Contents of the National Preconception Health Examination Project

Basic items of NPHCP are presented in Table 1. Preconception health examination included: (1) current medical histor; (2) physical examination; (3) clinical laboratory test; (4) past medical history; (5) previous obstetric history.

Table 1. Basic items included in National Preconception Health Examination Project.

	Items			Female	Male
1	Health education			√	√
2	Medical history (pregnancy history, disease history, family history, drug use, lifestyle, nutrition, environmental factors)			√	√
3	Physical exam		Regular exam (height, weight, blood pressure, heart rate, thyroid palpation, cardiopulmonary auscultation, abdominal palpation, limb and spine exam)	√	√
			Exam of reproductive system	√	√
4	Lab test	Vaginal smear	Leucorrhea routine	√	
			Gonococcus test	√	
			Chlamydia test	√	
5		Blood routine		√	
6		Urine routine		√	√
7		Blood type		√	√
8		Fasting glucose test		√	
9		Glutamic-pyruvic transaminase		√	√
10		Hepatitis B serology		√	√
11		Creatinine		√	√
12		Thyroid stimulation hormone		√	

13	Virus test	Treponema pallidum screening	√	√
14		Rubella (IgG antibody)	√	
15		Cytomegalovirus (IgM and IgG antibody)	√	
16		Toxoplasma (IgM and IgG antibody)	√	
17	Imaging exam	Gynecological ultrasound exam	√	
18	Risk evaluation and consultation		√	√
19	Follow-up in early pregnancy			
20	Follow-up of pregnancy outcomes		√	

General information including age, residence address, education, occupation, ethnicity, medical history and reproductive history were collected using a standardized questionnaire for wife and husband respectively. A real-time central database was built up for a continuous follow-up and data collection from preconception, early pregnancy to postpartum period (Figure 7).

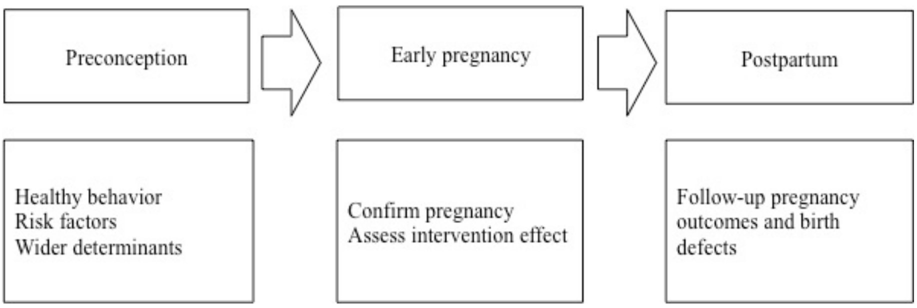

Figure 7. Diagram of the National Preconception Health Examination Project.

The detailed data collection forms included family health file, informed consent, female examination form, male examination form, examination result and evaluation notification, follow-up records in early pregnancy, follow-up records of pregnancy outcome and records of birth defects. These data were collected at the community base as demonstrated in Table 2. The details of the data collection forms translated from Chinese are included as an appendix 1.

Table 2. Data collection of the National Preconception Health Examination Project.

Data forms	Data collecting authority

Family health file	Village/town/county-based health care authority
Informed consent	Village/town/county-based health care authority
Female examination form	County-based health care authority
Male examination form	County-based health care authority
Examination result and evaluation notification	County-based health care authority
Follow-up records in early pregnancy	Village/town/county-based health care authority
Follow-up records of pregnancy outcome	Village/town/county-based health care authority
Records of birth defects	Village/town/county-based health care authority

Trained staff regularly uploaded the information into database. For the purpose of studies included in this thesis, risk factors, TORC serology and related demographic and clinical information were extracted and analyzed.

10.3 Requirements and training for the National Preconception Health Examination Project

In this project, at least 85% coverage of married couples who intended to conceive was required. The absolute numbers of couples that participated in the project were calculated based on the statistics reported by the local health administration department and compared with the estimates based on local number of births in the previous year [40, 56-57]. The requirements and training of health service authorities and staff involved in the project is demonstrated in Figure 8.

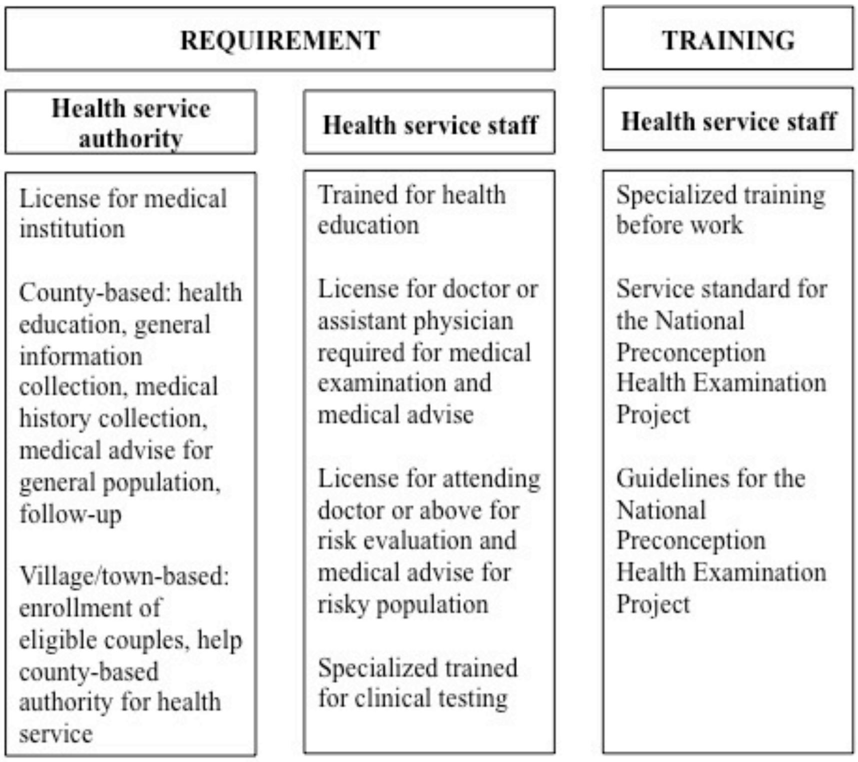

Figure 8. Requirements and training of health service authorities and staff.

10.4 Quality control of the National Preconception Health Examination Project

A comprehensive quality control system has been established for this national project. Firstly, a national center for examination of family planning services was established, which conducted sampling and recheck of lab tests. Secondly, a national database of preconception care was built and data entry was checked regularly and monthly quality reports were published. In addition, a series of policies were introduced, including quality management of health care services, clinical laboratory testing, and databases.

10.5 TORC testing and follow up

Five mL of venous blood was sampled and stored at –30°C. All serum specimens were tested for TORC antibodies using commercially enzyme immuno-assay kits in local laboratories. The cut-off value for sero-positivity was based on the manufacturer's recommendation for the enzyme-linked immunosorbent assay (ELISA) kit. As described previously [56-57], an external quality assessment (EQA) was performed by the National Center of Clinical Laboratories for Quality Inspection and Detection every six months.

The interpretation of TORC serology was as follows: positive IgM serology with negative IgG titer was considered as acute infection, and those women were referred to specialist accordingly. Negative IgM with positive IgG was considered as low risk of infection due to immunity originated from previous infection or vaccination. Negative IgG and IgM was considered to be susceptible and those women were advised on how to prevent being infected during preconception period and pregnancy, and to have vaccination before conception when appropriate. The recalled vaccination history was recorded based on the vaccination records when available, such as the vaccination cards, or otherwise self-reports.

10.6 Statistical analyses

The database of the National Data Center of Preconception Health Care has been regularly updated. According to their residential address, all participanted couples were grouped into 31 provinces and province level municipalities, and six geographical regions as well.

Socio-demographic and clinical characteristics were calculated as numbers, proportions, means and standard deviations (SD) when appropriate. The chi-squared test was applied for comparison of proportions between groups. The multivariate correlation or regression analysis were used for analyzing associations between variables. The Spearman correlation analysis was used to evaluate the association of CMV serology (IgG and IgM) status with grosss domestic product (GDP) and resident consumption level, as these variables had skewed distribution. A two-sided P-value<0.05 was regarded as statistically significant. SPSS version 22.0 (IBM Corp, Armonk, NY) and R software version 3.2.2 (https://www.r-project.org) were applied for statistical analyses. Geographic mapping was drawn using ArcGIS version 10.2.

10.7 Ethical considerations

PCC involves risk assessment, health promotion, counseling, and interventions to modify or eliminate risks. During PCC, couples' decision-making regarding pregnancy is influenced by provided information about their reproductive choices and options that may help to improve pregnancy outcomes. Through PCC the couples could be helped to optimally prepare their minds and bodies for a pregnancy. However, the implementation of universal PCC at a national level is still controversial. On the other hand, some may consider it as a moral responsibility. In China, free PCC services were introduced as a national public health program. This projected was approved by the Institutional Review Board of the Chinese Association of Maternal and Child Health Studies (IRB201001). The participating couples were provided free preconception examination and healthcare services, and their personal information was kept anonymous.

11 SUMMARY OF RESULTS

11.1 The Flow chart (Figure 9) demonstrates the number of participants included in each of the studies presented below.

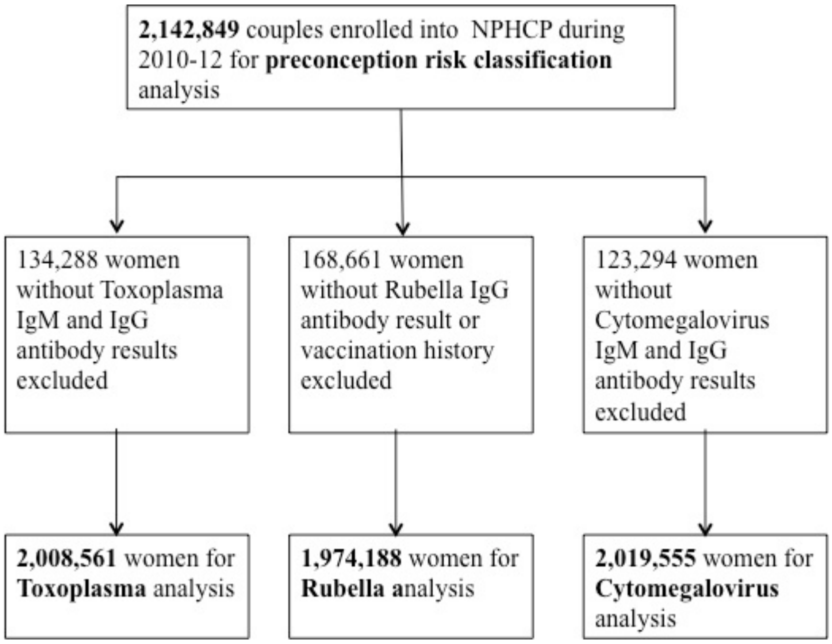

Figure 9. Number of participants included in each of the studies.

11.2 China's community-based strategy of universal preconception care in rural areas at a population level using a novel risk classification system for stratifying couples' preconception health status

During 2010-12, a total of 2142849 couples were included to the national preconception care project. 92.36% couples were from rural areas; 89.2% women and 88.3% men had education below university level. Among those couples aged 30–35 years and >35 years, Category D risk was more common, compared to those aged less than 30 years (P<0.05). More women of non-Han ethnicity had risk factors of category D and X compared to those of Han ethnicity. However, that ratio among men was not significantly different.

68.29% (1463266) couples were with at least one preconception risk factor. The main factors were of category A, B and C. The rate of category A (avoidable) risk factor was 11.24%, with 38.13% among men. 3.4% women drank and 0.45% smoked, but the rate was nearly 30% of men who reported to drink alcohol and smoke [40].

11.3 Sero-epidemiological map of *Toxoplasma gondii* infection and associated risk factors in preconception period in China: a nation-wide cross-sectional study

2008561 married women had their Toxoplasma gondii serology test, and enrolled for this analysis. Generally, participating women was of young age (28.1±4.8 years), and 1545510 (78.2%) women were occupied as a farmer. They were of lower education level: 89.6% (1634003) women had senior high school education or lower. Concerning of dietary habits, 1.3% (25850) of women did not eat meat and egg, and 1.0% (19948) did not eat fresh fruits and vegetables. 0.3% (5212) women ate raw meat or fish, and 2.9% (57385) women reported their exposing to cats.

2.3% (45405) women were Toxoplasma IgG positive and 0.3% (6884) for IgM positive. Only 0.04% (859) women were with both IgG and IgM positive. Provincial (geographical) difference in IgG sero-positivity was significant: the highest was 11.2% in Tianjing and the lowest was 0.2% in Heilongjiang. Toxoplasma IgM sero-positivity ranged from 0.9% in Anhui, to 0% in Tibet.

Regarding the risk factors of Toxoplasma gondii IgM positivity, maternal age, residence, occupation of farmer, vegetarian diet and exposure to cats were statistically significant ($P<0.05$). Moreover, those women ate raw meat or fish tended to be Toxoplasma IgG positive, whereas women with lower education level were more likely to be Toxoplasma IgM positive ($P<0.05$). After adjusting for province of residence, involvement in agricultural activitiesand exposure to cats, were statistically related with Toxoplasma gondii IgM sero-positivity ($P<0.0001$) [101].

11.4 Rubella virus immunization status

A total of 1974188 women had Rubella IgG serology and reported vaccination history. The average age of the participating women was 28 years and 76.7% (1613342) women were occupied as a farmer. 89.1% (1884504) women were of high school education or lower.

Overall, 58.4% (1161129) women were of Rubella virus IgG sero-positivity, and 4.6% (91604) women reported previous vaccination. IgG sero-positivity rates varied across the six administrative regions: the highest was in northeastern area (84.5%) while the lowest was in northwestern area (45.8%). The rates of Rubella IgG sero-positivity differed in 31 provinces: the highest rates were observed in Jilin (92.5%), Beijing (91.9%), Ningxia (82.3%) and Guangdong (81.0%), while the lowest rates were in Tibet (0.0%) and Qinghai (20.1%).

The self-reported rate of Rubella virus vaccination status was lower among women aged 40-49 years, compared with their countparts aged 20-29 and 30-39 years ($P<0.0001$). This rate appeared to be lower among women from urban areas compared to those from rural areas

(P<0.0001). In addition, there was no signifanct correlatation between Rubella IgG sero-positivity and self-reported vaccination (P=0.07) [102].

11.5 Sero-epidemiology of Cytomegalovirus infection and its geographic and socio-economic determinants in preconception period among Chinese women planning a pregnancy within six months: A nationwide study

2019555 women who had their Cytomegalovirus serology tested were included in this CMV analysis. The overall Cytomegalovirus IgG sero-positivity rate was 42.1% (850592) and 0.4% (9290) were IgM positive indicating that nearly 60% women were at risk of Cytomegalovirus infection before conception (Table 1 in Paper IV).

Provincial differences in Cytomegalovirus IgG sero-positivity ranged from 20.1% in Shaanxi to 97.5% in Zhejiang, besides the extreme low rate of 0% in Tibet and 0.2% in Heilongjiang, consistent with a substantial regional difference (Figure 1 in Paper IV). The highest IgM positive rate was observed in Liaoning of 2.3%, and the lowest rate was in Heilongjiang (0.1%) and Tibet (0%) (Table 2 in Paper IV).

CMV sero-positivity was associated with province of residence after adjusting for age, education level and occupation by multivariate correlation analysis (P<0.0001).

Analysis of correlation between CMV serology and socioeconomic factors demonstrated that provincial CMV IgG sero-positivity was statistically significantly correlated with resident consumption level (r=0.437; P=0.014), and but not with GDP (r =0.167; P=0.369). CMV IgM sero-positivity was neither significantly associated with GDP (r=0.229; P=0.216) nor with resident consumption level (r=0.049; P=0.794).

12 DISCUSSION

12.1 Main findings

This thesis provides an overview of prevalence of preconception risk factors among married couples residing mostly in rural areas of China based on a novel integrated amenability-based risk classification model. Furthermore, it presents a sero-epidemiological map of TORC infections among women with pregnancy intention. Firstly, new stratification of risk classification model demonstrated that proportional prepregnancy risk factors could be eliminated or prevented by appropriate medical intervention. Secondly, an integrated approach to PCC including male partner is justified as more than half of the husbands planning to father a child were exposed to risk factors prior to a pregnancy. Thirdly, it was demonstrated that TORC sero-positivity in preconception period in rural China had a substantial geographic variation and susceptibility to these vertically transmittable infections among women of childbearing age is substantially high.

12.2 Methodological considerations

Sero-epdemiology is a method that uses the measurement of antigens or antibodies in the serum to identify infectious stratus as well as immunity and susceptibility to infections in the population of interest. It is an excellent tool to study the prevalence of biomarkers of infection and/or vaccination [103]. We applied this approach to study population immunity in the preconception period and identified susceptibility to TORC infections among women with pregnancy intention in the following six months. Seroprevalence data provide reliable and useful information on population immunity and susceptibility to infections especially when the infections are subclinical. However, it is not always possible to differentiate between acquired immunity due to vaccination and natural immunity due to infection, meaningful estimates of vaccination coverage and effectiveness, as well as factors associated with disease resurgences can be stipulated based on such data. However, it is important that the sampling method used provides a representative sample of the population of interest for it to be useful and that the reliable laboratory assays are used. We believe that this government-sponsored project had sufficient nationwide coverage and quality control mechanisms in place, which is able to provide reliable data although the sampling was done from the rural, low resource areas of China. This project has provided much needed experience on implementing universal preconception care and integrating it into the national healthcare system. Despite the need for providing venous blood samples, it appears to feasible and acceptable to use seroepidemiological method for detecting susceptibility and immunity to TORC infections in a country as large as China. Further studies are required to evlaute whether such a strategy is feasible cost-effective in other countries.

12.3 Limitation of the data

The effect of risk classification on the couples' health before conception did not be assessed because follow-up of pregnancy outcomes was not included in our study. It is unclear whether the counseling and advice provided lead to sustained risk modifications among couples. Secondly, there is a possibility of recall bias because dietary habits, exposure to cats, history of vaccination, adverse pregnancy outcomes and history of chronic disease, were mainly based on self-reporting, although medical records and vaccination cards were scrutinized when available. Thirdly, there could be selection bias about low sero-positivity rate in Tibet because only 53 couples were included. Finally, different laboratories used different assay kits according to the local choice and might have used different cut-off values to indicate seropositivity although all of them were approved by the China Food and Drug Administration and central inspections and quality control mechanisms were in place. Therefore, we had to use sero-positivity and negativity as categorical variables rather than using antibody levels as the quantitative variables. Standardizing and uniformly using same assay kits could help to make comparisons between different locations and regions more reliable in the future.

12.4 Novel amenability-based risk classification system adapted by NPHCP

This nation-wide free project was an integrated model of PCC. It targeted rural areas in China and included both women and men. Based on the amenability of preconception risk factors, our novel classification system stratified enrolled couples into different risk categories.

PCC is not routinely offered in most countries around the world, and where it is offered the focus has been mostly on women. Preconception risk evaluation for couples planning to conceive was expected to be meaningful: more than 60% of the recruited couples had risk factors, and moreover, 23% of maternal and 45% paternal risk factors were potentially avoidable or preventable. More importantly, the most common risk factors among male partners were smoking and alcohol consumption, which were avoidable before pregnancy. It can be speculated that the alcohol and substance abuse may be associated with social problems and domestic violence, and partner smoking usually leads to passive smoking, which is related with adverse pregnancy outcomes. This underscores the importance of an integrated approach to PCC that includes not only women but their male parterners as well. Furthermore, the effectiveness of the interventions, such as health education, recommendations about life style changes, nutrition and medical prescriptions need to be further evaluated. In addition, in order to achieve sustained amelioration, some avoidable risks including smoking, alcohol consumption and substance abuse, calls for a longer-term strategy.

In some European countries, there are PCC recommendations for women with diabetes or epilepsy, however, they are heterogeneous and inconsistent [104]. Therefore, PCC approach needs to be further innovated to achieve an optimal reproductive health status before conception and during pregnancy period [105-106]. Couples participating in this project who were identified to have a significant medical condition or disease were referred to specialists. However, we are unaware of the exact content of the care they received after referral, and it may have varied in different healthcare institutions. In addition to PCC, more uniform evidence-based maternal health care would be required for managing risk categories C and D during pregnancy period to achieve better pregnancy outcomes.

The classification system of preconception risk in this national PCC project was practical: it is helpful for stratifying the couples' health status, targeting health interventions, and further referring to specialists. The risks were classified during the preconception period, but their amenability to modification was considered during both the preconception and the prenatal periods. Because new risks factors may emerge during pregnancy, prenatal risks may be different from preconception risks. Therefore, it is important to take into account of different methods and timing of intervention. PCC provides a window of opportunity for individual health promotion for motivated couples. Regardless of abundant supportive evidence for the value of PCC [107], there lacks sufficient research focused on clinical PCC service delivery. Thus, the implementation of PCC within contemporary women's healthcare requires more consideration in details [108]. This national integrated free PCC service in rural China provides a promising model, and its effect on pregnancy outcomes needs fruther demonstrated in future.

12.5 Sero-epidemiological map of *Toxoplasma gondii* infection and associated risk factors

In this nation-wide study, we found the low rate of Toxoplasma gondii sero-positivity among Chinese women planning a pregnancy: 2.3% women were IgG positive, 0.3% were IgM positive, and 0.04% were both IgG and IgM positive. Among pregnant women in Jiangsu, Qingdao and Weihai, Toxoplasma gondii IgG sero-positivity varied from 3.8% to 15.2 %, and IgM from 1.6% to 2.9% [101, 109-110]. Our study reported a national sero-positivity rate for Toxoplasma gondii among women before pregnancy.

Secondly, we found the geographical variation in Toxoplasma gondii sero-positivity: provincial rates differed: 0.2-11.2% for IgG and 0.0-0.9% for IgM. Vertical transmission was reported to be approximately 20% if maternal infection occurred during pregnancy, and moreover, the incidence was increased from the early to the late pregnancy [102]. Thus, to achieve prevention of congenital toxoplasmosis, more targeted strategies for primary prevention for women planning pregnancy should be emphazed.

Thirdly, occupation of farmer and exposure to cats were demonstrated as risk factors for Toxoplasma gondii infection. This was reasonable as it was more likely for a farmer to be exposed to cats and contaminated soil. Li et al. reported that the main infection source of Toxoplasma gondii among the Han population would be feeding a cat [66]. Despite that fact that exposure to cats and eating raw meat were known as risk factors for Toxoplasma gondii infection [68-68], further strategy is required for strengthening current insufficient personal hygiene [111]. Moreover, it was indicated that early antibiotic therapy was benefifical for eliminating severely affected infants, but prenatal treatment during pregnancy did not reduce the risk of maternal-fetal transmission [112]. Therefore, identifying infected women before conception provides an oppounity for primary prevention before pregnancy. Our findings were useful to integrate preconception care for reducing congenital toxoplasmosis in Chinese communities.

12.6 Sero-epidemiological map of Rubella virus and immunization status

We found that more than 40% women planning a pregnancy were susceptible to Rubella in Mainland China characterized with a significant regional difference. To our knowledge, there are no other similar nationwide studies to compare our results. In a previous study of female migrant factory workers in Shenzhen, 77.6% women were immune to Rubella [113]. The relatively low rate of Rubella IgG sero-positivity could possibly due to limitation of healthcare facilities and childhood vaccination in rural China. Targeted screening and vaccination for women before conception are indicated to reduce CRS in China. Moreover, to prevent CRS in future pregnancies, postpartum Rubella vaccination is also available for those sero-negative women in Japan and Spain [114-115]. Our study opened a window for better ensuring Rubella vaccination coverage among Chinese women in rural China.

12.7 Sero-epidemiology of Cytomegalovirus infection

We found that nearly 58% women were at risk of CMV infection before conception. Nationwide prevalence of CMV sero-positivity was very different from what has been reported in some pregnant populations previously. As an example, in our study the rate of IgG sero-positivity in Jiangsu province was 47.6%, which is substantially lower than that reported among pregnant women (98.7%) from the same province [36]. Preconception serological screening might be useful in this situation as preventive strategies to minimize exposure to CMV could be implemented. It also allows postponing pregnancy when acute infection is diagnosed.

As congenital CMV infectionis mainly caused by vertical transmission, vaccines administered to adult women could reduce the burden of CMV in children by making them immune prior to pregnancy [116-117], but no approved vaccines are available yet. However, there is a growing evidence that CMV vaccination can protect adults and children from infection, and licensure paths have been defined [116-118]. Offering vaccination to susceptible women before conception could be possible in the near future.

There was significant geographic variation in CMV sero-positivity among married women planning a pregnancy. This may be related to cultural and socio-economic differences. To our knowledge, socio-economic determinants of CMV infection in China have not been reported previously. We found that CMV serology status is associated with socio-economic factors. We found a significant correlation between provincial CMV IgG sero-positivity and resident consumption level, and GDP correlated with both CMV IgG and IgM sero-positivity. Thus, economic determinant should be considered when developing and implementing preventive strategies.

12.8 Validity of the findings

The internal validity of our data was ensured by the systematic identification and inclusion of almost all eligible women in rural China. Our trained local community staff used a uniform questionnaire survey in order to collect standardized family health file, and also utilized web-based data entry to a centralized database. The government organized and conducted periodic quality controls. Some of the information collected in the project was based on self-reports, which may be prone to reporting and recall bias. Although uptake of folate, alcohol, and smoking were verified by biomarker testing, epidemiological mapping of population immunity and susceptibility to TORC infections in preconception period was based on serological screening.

Possibility of experimental bias could not be avoided as the different reagents and kits were used based on local availability. Possibility of some cases being false positive or false negative could not be excluded, as the results of repeated testing were not included in the database.

Regarding external validity, participating couples were all married and were of relatively young age, less educated, most were residing in rural areas and had a fairly homogeneous ethnic and socioeconomic background. In other words, they were representative of rural young couples in preparing for a pregnancy. Therefore, our findings are likely be valid and applicable to several other low-middle income countries around the world, but may not be generalizable and directly applicable to urban populations living in economically affluent high-income countries. Furthermore, in this national project, PCC was free, but offered only to couples who were married and had clear plans regarding pregnancy. In China, having babies without being married is rare and most pregnancies are planned. This may not be the situation in many industrialized countries. Therefore, this model of PCC may not be directly transferrable to other countries and societies with different cultural backgrounds, social structures, and healthcare delivery systems.

13 CONCLUSIONS

Preconception health screening and use of risk novel risk classification system could effectively identify important risk factors and stratify couples into different risk categories. It is of importance to include male partners in preconception care, as more than half of the male partners were exposed to risk factors during the preconception period. A majority of the risks in our novel amenability-based risk classification model chould be avoidedor prevented, which suggested that integrating PCC in healthcare could be beneficial for improving pregnancy outcomes.

Sero-epidemiological map of TORC infection showed that a significant proportion of women in rural China are susceptible before conception. Therefore, screening for these infections followed by appropriate referral, diagnosis, treatment, counseling, health education and vaccination before pregnancy should be taken into account to reduce the risk of vertical transmission during pregnancy and childbirth. Targeted preventative strategies are needed to tackle significant regional variations in susceptibility to TORC infections among women during preconception period.

14 FUTURE PERSPECTIVES

This project provided some important insights into a model of universal free PCC for couples with clear conception plans. Risk identification and stratification was feasible and use of serological screening was acceptable to couples and effective in establishing a sero-epidemilogical map of TORC infections in preconception period. However, the effectiveness of subsequent interventions on pregnancy outcomes should be further evaluated. There are several challenges, barriers, opportunities, and threats to offering universal free PCC to all couples planning a pregnancy.

14.1 Challenges related to translation of research findings into healthcare practice

Although there is sufficient evidence and consensus that at least some components of PCC are useful, it has not been universally implemented in healthcare practice. Since this project is mainly conducted in rural areas, the preconception risk profile among couples living in urban China is still lacking, and the PCC model of rural China needs to be expanded to urban areas. However, providing free PCC within the framework of national healthcare systems will require substantial resource allocation, and cost-effectiveness should be evaluated. The prepregnancy risks factors evaluated in NFHCP were chosen by experts based on the review of literatures. Importance of these risk factors and whether targeted PCC interventions work well needs further evaluation by comprehensive follow-up at the community level. Addressing wider social determinants of health, the impact of education, income, work and relationships on preconception health also needs to be addressed.

14.2 Opportunities for health promotion during the whole span of reproductive life

The current idea of PCC is to embed it into care pathways throughout a woman's reproductive life. PCC is justified not just before conception, but across the whole span of reproductive years. Planning a pregnancy provides good motivation to improve health and an opportunity to maintain good reproductive as well as general health. The impact of PCC on general health across reproductive years needs to be explored in longitudinal studies.

PCC is helpful for raising health awareness. The high coverage of this nationwide free PCC project has a potential for enhancing awareness about PCC and promoting health behavior among couples as well as their families.

14.3 Threats to feasibility and effectiveness of PCC

To provide an efficient PCC service, caregivers need to be appropriately trained. Before and during this PCC project, community staff and local doctors were specially trained. Also, training guidelines, standards and tools are developed to support them in delivering PCC efficiently. It is important to train new staff and maintain their knowledge and professional skills by providing adequate, continuous education and training, which will require extra resource allocation.

In China, a high uptake of PCC has been achieved using the current community based national healthcare system to deliver free PCC. Continued efforts will be required to maintain and improve the infrastructure at community level for the success of PCC delivery using this strategy.

PCC is expected to provide health benefits resulting in healthier mothers and their offspring. However, its cost-effectiveness should be considered and evaluated before a universal PCC can be recommended. It is also important to evaluate each component of PCC to figure out which items are most beneficial and needed.

13 REFERENCES

1. WHO. Meeting to develop a global consensus on preconception care to reduce maternal and childhood mortality and morbidity: World Health Organization Headquarters, Geneva, 6–7 February 2012: meeting report. 2013.

2. American College of Obstetricians and Gynecologists. Preconceptional care. ACOG technical bulletin 205. American College of Obstetricians and Gynecologists, Washington, DC 1995.

3. Moos MK, Dunlop AL, Jack BW, et al. Healthier women, healthier reproductive outcomes: recommendations for the routine care of all women of reproductive age. Am J Obstet Gynecol. 2008;199:S280.

4. Inskip HM, Crozier SR, Godfrey KM, et al. Women's compliance with nutrition and lifestyle recommendations before pregnancy: general population cohort study. BMJ. 2009;12;338:b481.

5. Johnson K, Posner SF, Biermann J, et al. Recommendations to improve preconception health and health care--United States. A report of the CDC/ATSDR Preconception Care Work Group and the Select Panel on Preconception Care. MMWR Recomm Rep. 2006; 55:1.

6. Cheryl L. Robbins, Loretta Gavin, et al. Preconception Care in Publicly Funded U.S. Clinics That Provide Family Planning Services. Am J Prev Med. 2016;51(3):336-43.

7. Wellings K, Jones KG, Mercer CH, et al. The prevalence of unplanned pregnancy and associated factors in Britain: findings from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). The Lancet. 2013;382;1807-16.

8. Coffey K, Shorten A. The challenge of preconception counseling: Using reproductive life planning in primary care. J Am Assoc Nurse Pract. 2014;26(5):255-62.

9. Bestwick JP, Huttly WJ, Morris JK, Wald NJ. Prevention of neural tube defects: a cross-sectional study of the uptake of folic acid supplementation in nearly half a million women. PloS One. 2014;19;9(2):e89354.

10. Heslehurst N, Lang R, Rankin J, Wilkinson JR, Summerbell CD. Obesity in pregnancy: a study of the impact of maternal obesity on NHS maternity services. BJOG. 2007;114(3):334-42.

11. Shawe J, Delbaere I, Ekstrand M, et al. Preconception care policy, guidelines, recommendationsand services across six European countries: Belgium (Flanders), Denmark, Italy, the Netherlands, Sweden and the United Kingdom. The European Journal of Contraception and Reproductive Health Care. 2015; 20:77-87.
12. Thompson JE, Walsh LV, Merkatz IR. The history of prenatal care: cultural, social, and medical contexts. New perspectives on prenatal care. New York: Elsevier. 1990;9-30.
13. Gortmaker SL. The effects of prenatal care upon the health of the newborn. Am J Public Health. 1979;69:653-60.
14. Quick JD, Greenlick MR, Roghmann KJ. Prenatal care and pregnancy outcome in an HMO and general population: a multivariate cohort analysis. Am J Public Health. 1981;71:381-90.
15. Greenberg RS. The impact of prenatal care in different social groups. Am J Obstet Gynecol. 1983;145:797-801.
16. Centers for Disease Control and Prevention. Recommendations to improve preconception health and health care—United States. MMWR Recommendations and Reports. 2006;55(RR-06):1-23.
17. Czeizel AE. Experience of the Hungarian Preconception Service between 1984 and 2010. European Journal of Obstetrics and Gynecology and Reproductive Biology. 2012;1;161(1):18-25.
18. Czeizel AE. Ten years of experience in periconceptional care. Eur J Obstet Gynecol Reprod Biol. 1999;84(1):43-9.
19. Family-Centered Maternity and Newborn Care: National Guidelines. Canadian Institutes of Health Research. Healthy Life Trajectories Initiative (HeLTI). 2018.
20. Robertson L, Knight H, Prosser Snelling E, et al. Each baby counts: National quality improvement programme to reduce intrapartum-related deaths and brain injuries in term babies. Semin Fetal Neonatal Med. 2017;22(3):193-8.
21. Heijmans BT, Tobi EW, Stein AD, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci USA. 2008;105:17046-9.
22. Vidal AC, Liu Y, Tuli A, et al. Maternal stress, preterm birth, and DNA methylation at imprint regulatory sequences in humans. Genet Epigenet. 2013;6:37-44.

23. Liu Y, Murphy SK, Murtha AP, et al. Depression in pregnancy, infant birth weight and DNA methylation of imprint regulatory elements. Epigenetics. 2012;7:735-46.

24. Huang C, Li Z, Wang M, Martorell R. Early life exposure to the 1959–1961 Chinese famine has long-term health consequences. J Nutr. 2010;140:1874-8.

25. Portrait FRM, van Wingerden TF, Deeg DJH. Early life undernutrition and adult height: The Dutch famine of 1944-45. Econ Hum Biol. 2017;27(Pt B):339-48.

26. Yarde F, Broekmans FJ, van der Pal-de Bruin KM, et al. Prenatal famine, birthweight, reproductive performance and age at menopause: the Dutch hunger winter families study. Hum Reprod. 2013;28(12):3328-36.

27. van Abeelen AF, Elias SG, de Jong PA, et al. Famine in the young and risk of later hospitalization for COPD and asthma. PLoS One. 23;8(12):e82636.

28. Barker DJ, Lackland DT. Prenatal influences on stroke mortality in England and Wales. Stroke. 2003;34:1598-602.

29. Lackland DT, Egan BM, Ferguson PL. Low birth weight as a risk factor for hypertension. J Clin Hyperten (Greenwich). 2003;5:133-6.

30. Kramer MR, Hogue CJ, Dunlop AL, Menon R. Preconceptional stress and racial disparities in preterm birth: an overview. Acta Obstet Gynecol Scand. 2011;90:1307-16.

31. Gomes S, Lopes C, Pinto E. Folate and folic acid in preconceptional period: recommendations from official health organizations in thirty-six countries worldwide and WHO. Public Health Nutrition. 2015;19 (1):176-89.

32. Chris Crawford. AAFP urges family physicians to integrate preconception care into patient visits. Ann Fam Med. 2016;14:180-1.

33. De-Regil LM, Fernández-Gaxiola AC, Dowswell T, Peña-Rosas JP. Effects and safety of periconceptional folate supplementation for preventing birth defects. Cochrane Database Syst Rev. 2010; 6(10):CD007950.

34. Public Health England. Rubella: confirmed cases in England and Wales by age and region, 2012 to 2016. PHE 2017.

35. Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries across the UK (MBRRACE-UK). Saving Lives, Improving Mothers' Care. Lessons learned to inform future maternity care from the UK and Ireland Confidential Enquiries into

Maternal Deaths and Morbidity 2009-2012. National Perinatal Epidemiology Unit. December 2014.

36. Seely EW, Ecker J. Clinical practice. Chronic hypertension in pregnancy. N Engl J Med. 2011; 4;365(5):439-46.

37. Bentley-Lewis R. Gestational diabetes mellitus: an opportunity of a lifetime. Lancet. 2009;23;373(9677):1738-40.

38. Black RE, Victora CG, Walker SP, et al. Maternal and Child Nutrition Study Group. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet. 2013; 3;382(9890):427-51.

39. Royal College of Physicians. Passive smoking and children. 2010.

40. Zhou QJ, Zhang SK, Wang QM, et al. China's community-based strategy of universal preconception care in rural areas at a population level using a novel risk classification system for stratifying couples´ preconception health status. BMC Health Services Research. 2016;16:689.

41. Gianicolo EA, Cresci M, Ait-Ali L, Foffa I, Andreassi MG. Smoking and congenital heart disease: the epidemiological and biological link. Curr Pharm Des. 2010; 16(23):2572-7.

42. Pierpont ME, Basson CT, Benson DW, et al. Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. Circulation. 2007;115:3015-38.

43. Waldenstrom U, Cnattingius S, Vixner L, Norman M. Advanced maternal age increases the risk of very preterm birth, irrespective of parity: a population-based register study. BJOG: an international journal of obstetrics and gynaecology. 2017;124(8):1235-44.

44. Ganchimeg T, Ota E, Morisaki N, et al. Pregnancy and childbirth outcomes among adolescent mothers: a World Health Organization multicountry study. Int J Obstet Gy. 2014;121:40-8.

45. Usynina AA, Postoev V, Odland JO, Grjibovski AM. Adverse Pregnancy Outcomes among Adolescents in Northwest Russia: A Population Registry-Based Study. International journal of environmental research and public health. 2018;15(2).

46. Malabarey OT, Balayla J, Klam SL, Shrim A, Abenhaim HA. Pregnancies in Young

Adolescent Mothers: A Population-Based Study on 37 Million Births. J Pediatr Adol Gynec. 2012;25(2):98-102.

47. Simenc GB, Blickstein I, Verdenik I, Bregar AT, Lucovnik M, Tul N. Is forty the new thirty? Population based study of advanced maternal age. J Perinat Med. 2018;25;46(3):247-50.

48. Okmen Ozkan B, Koroglu N, Turkgeldi LS, Cetin BA, Aslan H. Advanced maternal age and risk of non-chromosomal anomalies: data from a tertiary referral hospital in Turkey. J Matern Fetal Neonatal Med. 2019;32(5):749-52.

49. Goetzinger KR, Shanks AL, Odibo AO, Macones GA, Cahill AG. Advanced Maternal Age and the Risk of Major Congenital Anomalies. Am J Perinatol. 2017;34(3):217-22.

50. Ouyang, Y. China relaxes its one-child policy. Lancet. 2013;382 (9907): e28-30.

51. Hafez Ismaili M'hamdi, Sabine F. van Voorst, Wim Pinxten, Medard T. Hilhorst, Eric A. P. Steegers. Barriers in the uptake and delivery of preconception care: exploring the views of care providers. Matern Child Health J. 2017;21(1):21-8.

52. Poels M, Koster MP, Boeije HR, Franx A, van Stel HF. Why do women not use preconception care? A systematic review on barriers and Facilitators. Obstet Gynecol Surv. 2016;71(10):603-12.

53. Feng XL, Guo S, Hipgrave D, Zhu J, Zhang L, Song L, Yang Q, Guo Y, Ronsmans C. China's facility-based birth strategy and neonatal mortality: a population-based epidemiological study. Lancet. 2011;22;378(9801):1493-500.

54. Gu Y, Li L, Zhou C, Yang T, Dong H. Factors influencing voluntary premarital medical examination in Zhejiang province, China: a culturally-tailored health behavioral model analysis. BMC Public Health. 2014; 28;14:659.

55. Schwank SE, Gu C, Cao Z, et al. China's child policy shift and its impact on Shanghai and Hangzhou women's decision-making. Int J Womens Health. 2018; 25;10:639-48.

56. Zhou QJ, Acharya G, Zhang SK, et al. A new perspective on universal preconception care in China. Acta Obstet Gynecol Scand. 2016;95(4):377-81.

57. Zhang S, Wang Q, Sheng H. Design of the National Free Preconception Health Examination Project in China. Zhonghua Yi XueZaZhi. 2015;95:162-5.

58. Landkroon AP, de Weerd S, van Vliet-Lachotzki E, Steegers EA. Validation of an internet

questionnaire for risk assessment in preconception care. Public Health Genomics. 2010;13(2):89-94.

59. Stichting Erfocentrum en Erasmus MC. Preconception questionnaire [ZwangerWijzer]. www.zwangerwijzer.nl.

60. Remington J, McLeod R, Thulliez P, Desmonts G. Toxoplasmosis. In: Remington J, Klein G, Wilson C, Carol B, eds. Infectious diseases of the fetus and newborn infant. 6th ed. Philadelphia: WB Saunders. 2006:947-1091.

61. Olariu TR, Remington JS, McLeod R, Alam A, Montoya JG. Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. Pediatr Infect Dis J. 2011;30:1056-61.

62. Swisher CN, Boyer K, McLeod R. Congenital toxoplasmosis. The Toxoplasmosis Study Group. Semin Pediatr Neurol. 1994;1:4-25.

63. Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: from animals to humans. Int J Parasitol. 2000;30:1217-58.

64. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol. 2009;39:1385-94.

65. Feldman HA. Epidemiology of toxoplasma infections. Epidemiol Rev. 1982;4:204-13.

66. Li HL, Dong L, Li Q, et al. Seroepidemiology of Toxoplasma gondii infection in Bai and Han ethnic groups in southwestern China. Epidemiol Infect. 2015;143(4):881-6.

67. Baril L, Ancelle T, Goulet V, Thulliez P, Tirard-Fleury V, Carme B. Risk factors for Toxoplasma infection in pregnancy: a case-control study in France. Scand J Infect Dis. 1999;31(3):305-9.

68. Georg Kapperud, Pal A. Jenum, Babill Stray-Pedersen, Kjetil K. Melby, Anne Eskild, Jan Eng. Risk factors for Toxoplasma gondii infection in pregnancy results of a prospective case-control study in Norway. Am J Epidemiol. 1996;15;144(4):405-12.

69. Maria João Gargaté, Idalina Ferreira, Anabela Vilares, et al. Toxoplasma gondii seroprevalence in the Portuguese population: comparison of three cross-sectional studies spanning three decades. BMJ Open. 2016;6:e011648.

70. Gilbert R, Gras L. European Multicentre Study on Congenital Toxoplasmosis. Effect of

timing and type of treatment on the risk of mother to child transmission of Toxoplasma gondii. BJOG. 2003;110(2):112-20.

71. El Bissati K, Levigne P, Lykins J, et al. Global initiative for congenital toxoplasmosis: an observational and international comparative clinical analysis. Emerg Microbes Infect. 2018;27;7(1):165.

72. Watson JC, Hadler SC, Dykewicz CA. Measles, mumps, and Rubella--vaccine use and strategies for elimination of measles, Rubella, and congenital Rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 1998; 22;47(RR-8):1-57.

73. Miller E, Cradock-Watson JE, Pollock TM. Consequences of confirmed maternal Rubella at successive stages of pregnancy. Lancet. 1982;9;2(8302):781-4.

74. Fowler KB, Stagno S, Pass RF, et al. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Engl J Med. 1992; 326:663.

75. Zhu Z, Guo XB, Cui AL. Analysis of epidemiological and genetic characteristics of Rubella viruses in China from 2007 to 2008. Zhongguo Yi Miao He Mian Yi. 2009;15(3):201-4.

76. He H, Yan R, Tang X, Zhou Y, Deng X, Xie S. Vaccination in secondary school students expedites Rubella control and prevents congenital Rubella syndrome. BMC Infect Dis. 2016;30;16(1):723.

77. Xu Q, Xu AQ, Song LZ, et al. Retrospective epidemiologic investigation on congenital Rubella syndrome in monitoring region of Shandong province, China. Zhonghua Liu Xing Bing Xue Za Zhi. 2010;31(7):828-9.

78. Semerikov VV, Lavrentyeva IN, Popov VF, Fletcher MA, Kolotov ME. Rubella in the Russian Federation: epidemiological features and control measures to prevent the congenital Rubella syndrome. Epidemiol Infect. 2000; 125(2):359-66.

79. Aksit S, Egemen A, Ozacar T, et al. Rubella seroprevalence in an unvaccinated population in Izmir: recommendations forRubella vaccination in Turkey. Pediatr Infect Dis J. 1999;18(7):577-80.

80. Onyenekwe CC, Kehinde-Agbeyangi TA, Ofor US, Arinola OG. Prevalence of Rubella-IgG antibody in women of childbearing age in Lagos, Nigeria. West Afr J Med. 2000; 19:23-6.

81. Reef SE, Frey TK, Theall K, et al. The changing epidemiology of Rubella in the 1990s: on the verge of elimination and new challenges for control and prevention. JAMA. 2002; 287(4):464-72.

82. Reef SE, Redd SB, Abernathy E, Zimmerman L, Icenogle JP. The epidemiological profile of Rubella and congenital Rubella syndrome in the United States, 1998-2004: the evidence for absence of endemic transmission. Clin Infect Dis. 2006;43 Suppl 3:S126-32.

83. http://www.gov.cn/gzdt/2008-02/19/content_893572.htm.

84. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007;17(4):253-76.

85. Fowler KB, Stagno S, Pass RF. Maternal immunity and prevention of congenital cytomegalovirus infection. JAMA. 2003; 26;289(8):1008-11.

86. Staras SA, Dollard SC, Radford KW. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis. 2006;1;43(9):1143-51.

87. Revello MG, Zavattoni M, Furione M, Fabbri E, Gerna G. Preconceptional primary human cytomegalovirus infection and risk of congenital infection. J Infect Dis. 2006;15;193(6):783-7.

88. Zhang S, Hu L, Chen J, Xu B, Zhou YH, Hu Y. Cytomegalovirus seroprevalence in pregnant women and association with adverse pregnancy/neonatal outcomes in Jiangsu Province, China. PLoS One. 2014;11;9(9):e107645.

89. Reichman O, Miskin I, Sharoni L, Eldar-Geva T, Goldberg D, Tsafrir A, et al. Preconception screening for cytomegalovirus: an effective preventive approach. Biomed Res Int. 2014;135416.

90. Plotkin SA, Boppana SB. Vaccination against the human cytomegalovirus. Vaccine. 2018; S0264-410X(18)30288-3.

91. Workowski KA, Bolan GA, Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015;64:1.

92. Brown ZA, Wald A, Morrow RA, et al. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. JAMA. 2003;289:203.

93. Brown ZA, Benedetti J, Ashley R, et al. Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor. N Engl J Med.

1991;324:1247.

94. Bernstein DI, Bellamy AR, Hook EW 3rd, et al. Epidemiology, clinical presentation, and antibody response to primary infection with herpes simplex virus type 1 and type 2 in young women. Clin Infect Dis. 2013;56:344.

95. Delaney S, Gardella C, Saracino M, et al. Seroprevalence of herpes simplex virus type 1 and 2 among pregnant women, 1989-2010. JAMA. 2014;312:746.

96. Brown ZA, Benedetti J, Selke S, et al. Asymptomatic maternal shedding of herpes simplex virus at the onset of labor: relationship to preterm labor. Obstet Gynecol. 1996;87:483.

97. Nahmias AJ, Josey WE, Naib ZM, et al. Perinatal risk associated with maternal genital herpes simplex virus infection. Am J Obstet Gynecol. 1971;110:825.

98. Brown ZA, Vontver LA, Benedetti J, et al. Effects on infants of a first episode of genital herpes during pregnancy. N Engl J Med. 1987;317:1246.

99. Watts DH, Brown ZA, Money D, et al. A double-blind, randomized, placebo-controlled trial of acyclovir in late pregnancy for the reduction of herpes simplex virus shedding and cesarean delivery. Am J Obstet Gynecol. 2003;188:836.

100. Pinninti SG, Angara R, Feja KN, et al. Neonatal herpes disease following maternal antenatal antiviral suppressive therapy: a multicenter case series. J Pediatr. 2012;161:134.

101. Zhou Q, Wang Q, Shen H, et al. Seroepidemiological map of Toxoplasma gondii infectionand associated risk factors in preconception periodin China: A nationwide cross-sectional study. JOGR. 2018;44(6):1134-9.

102. Zhou Q, Wang Q, Shen H, et al. Rubella virus immunization status in preconception period among Chinese women of reproductive age: A nation-wide, cross-sectional study. Vaccine. 2017;25;35(23):3076-81.

103. Cutts FT, Hanson M. Seroepidemiology: an underused tool for designing andmonitoring vaccination programmes in low- and middleincomecountries. Tropical Medicine and International Health. 1016;21 (9):1086-98 .

104. Bernstein DI, Bellamy AR, Hook EW 3rd, et al. Epidemiology, clinical presentation, and antibody response to primary infection with herpes simplex virus type 1 and type 2 in young women. Clin Infect Dis. 2013;56:344.

105. Delaney S, Gardella C, Saracino M, et al. Seroprevalence of herpes simplex virus type 1 and 2 among pregnant women, 1989-2010. JAMA. 2014;312:746.

106. Brown ZA, Benedetti J, Selke S, et al. Asymptomatic maternal shedding of herpes simplex virus at the onset of labor: relationship to preterm labor. Obstet Gynecol. 1996;87:483.

107. Nahmias AJ, Josey WE, Naib ZM, et al. Perinatal risk associated with maternal genital herpes simplex virus infection. Am J Obstet Gynecol. 1971;110:825.

108. Brown ZA, Vontver LA, Benedetti J, et al. Effects on infants of a first episode of genital herpes during pregnancy. N Engl J Med. 1987;317:1246.

109. Watts DH, Brown ZA, Money D, et al. A double-blind, randomized, placebo-controlled trial of acyclovir in late pregnancy for the reduction of herpes simplex virus shedding and cesarean delivery. Am J Obstet Gynecol. 2003;188:836.

110. Pinninti SG, Angara R, Feja KN, et al. Neonatal herpes disease following maternal antenatal antiviral suppressive therapy: a multicenter case series. J Pediatr. 2012;161:134.

111. Cutts FT, Hanson M. Seroepidemiology: an underused tool for designing andmonitoring vaccination programmes in low- and middleincomecountries. Tropical Medicine and International Health. 1016;21(9):1086-98.

112. Jill Shawe, Ilse Delbaere, Maria Ekstrand, et al. Preconception care policy, guidelines, recommendations and services across six European countries: Belgium (Flanders), Denmark, Italy, the Netherlands, Sweden and the United Kingdom. The European Journal of Contraception and Reproductive Health Care. 2015;20:77-87.

113. Michelle St. Fleur, Karla Damus, Brian Jack. The future of preconception care in the United States: multigenerational impact on reproductive outcomes. Ups J Med Sci. 2016;19:1-5.

114. Anna Berglunda, Gunilla Lindmark. Preconception health and care (PHC)-a strategy for improved maternal and child health. Ups J Med Sci. 2016;20:1-6.

115. Braspenningx S, Haagdorens M, Blaumeiser B, Jacquemyn Y, Mortier G. Preconceptional care: a systematic review of the current situation and recommendations for the future Facts Views Vis Obgyn. 2013;5(1):13-25.

116. Amie Steel, Jayne Luckec, Rebecca Reida, Jon Adams. A systematic review of

women's and health professional's attitudes and experience of preconception care service delivery. Family Practice. 2016;1-8.

117. Gao XJ, Zhao ZJ, He ZH, et al. Toxoplasma gondii infection in pregnant women in China. Parasitology. 2012;139(2):139-47.

118. Hua HY, Tang F, Liu YX, et al. Survey of Toxoplasma gondii infection among pregnant women in Jiangsu Province, China. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi. 2013;25(1):56-8.

APPENDIX

Paper I-IV

Paper I

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RESEARCH ARTICLE Open Access

China's community-based strategy of universal preconception care in rural areas at a population level using a novel risk classification system for stratifying couples´ preconception health status

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Abstract

Background: Preconception care (PCC) is recommended for optimizing a woman's health prior to pregnancy to minimize the risk of adverse pregnancy and birth outcomes. We aimed to evaluate the impact of strategy and a novel risk classification model of China's "National Preconception Health Care Project" (NPHCP) in identifying risk factors and stratifying couples' preconception health status.

Methods: We performed a secondary analysis of data collected by NPHCP during April 2010 to December 2012 in 220 selected counties in China. All couples enrolled in the project accepted free preconception health examination, risk evaluation, health education and medical advice. Risk factors were categorized into five preconception risk classes based on their amenability to prevention and treatment: A-avoidable risk factors, B- benefiting from targeted medical intervention, C-controllable but requiring close monitoring and treatment during pregnancy, D-diagnosable prenatally but not modifiable preconceptionally, X-pregnancy not advisable. Information on each couple's socio-demographic and health status was recorded and further analyzed.

Results: Among the 2,142,849 couples who were enrolled to this study, the majority (92.36%) were from rural areas with low education levels (89.2% women and 88.3% men had education below university level). A total of 1463266 (68.29%) couples had one or more preconception risk factors mainly of category A, B and C, among which 46.25% were women and 51.92% were men. Category A risk factors were more common among men compared with women (38.13% versus 11.24%; $P = 0.000$).

Conclusions: This project provided new insights into preconception health of Chinese couples of reproductive age. More than half of the male partners planning to father a child, were exposed to risk factors during the preconception period, suggesting that an integrated approach to PCC including both women and men is justified. Stratification based on the new risk classification model demonstrated that a majority of the risk factors are avoidable, or preventable by medical intervention. Therefore, universal free PCC can be expected to improve pregnancy outcomes in rural China.

Keywords: Preconception care, Preconception health, Risk stratification, Reproductive health, Population-based study, Rural China, Universal preconception care, Community-based care

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Background

Preconception care (PCC) is defined as interventions that aim to identify and, when possible, modify the biomedical, behavioral, and social risks to optimize woman's health before pregnancy with the aim of improving pregnancy outcomes [1]; In 2014, Centers for Disease Control and Prevention (CDC) and the Office of Population Affairs published clinical recommendations, "Providing Quality Family Planning Services" (QFP), and recognized PCC as a critical component of health care for women of reproductive age [2].

The purpose of PCC is to optimize a woman's health prior to pregnancy and promote healthy behavior during pregnancy to reduce the incidence of adverse birth outcomes [3]. It is reported that an estimated 300,000 women die globally as a result of pregnancy-related conditions [4]. The prevalence of birth defects in China is around 5.6%, and there are nearly 900 000 new cases annually according to the official Report on Prevention of Birth Defects in China published in 2012 [5]. Health services provided to the couples of reproductive age, such as family planning, folic acid supplementation [6], genetic counseling, chronic disease management, immunizations, treatment of sexually transmitted infections, and interventions promoting healthier lifestyle, including those directed against alcohol, tobacco, and substance abuse [7] seem to have a positive effect. There is growing evidence that effective treatment of maternal diabetes and hypertension during the preconception period reduces adverse maternal and neonatal outcomes [8–10]. Avoiding unintended pregnancy through PCC could avert 44% maternal mortality [11]. Moreover, the effect of PCC on women with a history of previous adverse infant outcome, such as preterm birth, low birth weight, stillbirth or major birth defect, appears to be meaningful [12].

Even though the benefits of PCC have been well established [13, 14], integrating PCC into regular family planning services still remains a challenge for some providers [15]. Poor organization of health services' delivery systems, lack of comprehensive PCC programs, limited awareness among future parents about the availability and benefits of PCC and that of physicians about the necessity and effectiveness of PCC are apparent barriers affecting delivery and uptake of PCC [16, 17].

PCC in China has been insufficient and inadequate, especially in rural areas, despite the fact that facility-based strategy on reducing neonatal mortality had a significant impact on the Millennium Development Goal 4, and with a rapid economic development there have been improvements in population health in recent decades [18]. Therefore, the National Health and Family Planning Commission of the People's Republic of China(NHFPC)launched the "National Preconception Health Care Project" (NPHCP) in 2010, focusing on rural areas and providing free PCC for the couples of reproductive age [19]. In this project, relevant preconception risk factors were classified according to their amenability to prevention and treatment. The objective of our study was to evaluate the impact of strategy and risk classification model of China's NPHCP in identifying risk factors and stratifying the preconception health status of men and women of reproductive age.

Methods

Data source and study design

We conducted a secondary analysis of data collected within the framework of NPHCP during April 2010 to December 2012 to investigate the characteristics of preconception risk factors among married Chinese women and men of reproductive age. Methodological details of the project have been described previously [20–22]. Briefly, the study covered 220 counties in China. Selected rural counties in all provinces and the urban counties that wanted to participate in this project were included in this population-based prospective cohort study.

NHFPC established the implementation and quality control standards for this program [20, 21]. Local community staff investigated the conception plans of the couples, and those planning to conceive within the next six months were enrolled and invited to attend a free health examination. Professional doctors specially trained in obstetrics, genetic and other related specialties provided necessary medical advice to the couples. NHFPC has drafted and published the consultation guide for common preconception health problems. All couples enrolled accepted a free preconception health examination, risk evaluation, health education and medical advice based on the risk factors. A written informed consent was obtained from each participant, and this study was approved by the Institutional Review Board of the Chinese Association of Maternal and Child Health Studies [20, 21].

Preconception examination included (1) a medical history: current medical illness and use of any medication, family history of hypertension, diabetes, congenital or genetic diseases in the first-degree relatives, life style, dietary habits and exposure to environmental and occupational hazards; (2) physical examination: height, weight, blood pressure, heart rate, palpation of thyroid gland, auscultation of the heart and lungs, abdominal palpation, examination of the limbs and the spine; (3) clinical laboratory tests: genital swabs for microbiological culture and sensitivity, gonococcus and chlamydia test, hemoglobin and full blood count, urine for bacteriology and culture, blood type, serum glucose, liver, renal function and thyroid function tests, hepatitis B serology, syphilis test, TORCH (toxopasma, rubella virus, cytomegalovirus, and herpes simplex virus) screen, and gynecological ultrasound; (4) past medical history: hypertension, diabetes, cardiac diseases, immune system diseases, renal diseases and other chronic diseases; (5) past obstetric history including history of induced abortion, spontaneous abortion, live birth, stillbirth, neonatal death, fetal abnormality, preterm birth and multiple pregnancy. Trained staff regularly recorded and entered the information into the NHFPC database.

Preconception risk evaluation and classification model

The aim of the preconception health examination was to identify all the risk factors as far as possible, and treat accordingly. Therefore, instead of assessing the degree of exposure, we developed a preconception risk classification system based on their amenability to prevention and treatment according to Preconception Health Examination and Risk Evaluation Guides (Science and Technology Division of NHFPC) (Table 1). Risk factors were categorized into five preconception risk classes: A-avoidable risk factors, B-benefiting from targeted medical intervention before conception, C-controllable but requiring close monitoring and treatment during pregnancy, D-diagnosable prenatally but the risk factor not modifiable preconceptionally, X-pregnancy not advisable. The couples with category X risk factor were advised to use appropriate contraception and were considered in further analysis. Participants with missing or incomplete records were excluded from analysis.

Statistical analysis

Statistical analysis was performed using SPSS statistical software version 15.0 (SPSS, System for Windows, Chicago, USA). Data are presented as number (%) and mean ± standard deviation (SD). For comparing groups, we used independent samples *t*-test for continuous variable and χ^2 test for categorical variables. All *P*-values were two-tailed, and a $P < 0.05$ was considered to be statistically significant.

Table 1 Definition of "ABCDX" category of preconception risk factors

Category	Definition	Risk factors
A	Avoidable risks, i.e. they could be avoided though health education and eliminating work place hazards etc.	*Maternal:* smoking, alcohol consumption, exposure to toxins, radiation, noise, pesticide, organic solvent, heavy metal, inadequate nutrition (no intake of meat and egg, no intake of fresh vegetables, raw meat eating habit) *Paternal:* smoking, alcohol, consumption, exposure to toxins, radiation exposure, noise, pesticide, organic solvent, high temperature, preputial ring, inadequate nutrition (no intake of meat and egg, no intake of fresh vegetables, raw meat eating habit)
B	Benefiting from targeted medical intervention,	*Maternal:* anemia[a], bacterial vaginitis, candida infection, gonorrhoea, trichomoniasis, Toxoplasma gondii infection (IgM positive), gingival hemorrhage, history of psychological disorder; *Paternal:* abnormal liver function, abnormal renal function, spermatic cord varicocele, hypertension, congenital heart disease, history of chronic renal disease history, cancer, epilepsy, or psychological disorder
C	Controllable risk factors, i.e. diseases and conditions that can't be cured but risk can be modified and ameliorated. Close monitoring and medical supervision is required during the pregnancy	*Maternal:* Thrombocytopenia[b], abnormal liver function, abnormal renal function, abnormal TSH, HBs-Ag positive, HBe-Ag positive, cytomegalovirus IgM positive, chlamydia positive, syphilis screening positive, Rh negative, history of gynecological diseases, preterm birth, diabetes, congenital heart disease, hypertension, malignancy, chronic renal disease, reported epilepsy, tuberculosis, use of narcotics; *Paternal:* HBs antigen positive, HBe antigen positive, syphilis screening positive, use of narcotics, thyroid disease
D	Diagnosable prenatally but risk factor is not modifiable preconceptionally i.e. women with these risk factors may benefit from preconception risk evaluation, counseling and prenatal diagnosis.	*Maternal:* Maternal birth defect, history of previous child with birth defects, mental retardation, history of recurrent abortion, stillbirth, or neonatal death, family history of Mediterranean anemia, G6PD deficiency, Albinism, Down's syndrome, visual impairment; hearing impairment; *Paternal:* Paternal birth defect, mental retardation, family history of neonatal death, Mediterranean anemia, G6PD deficiency, Albinism, Down's syndrome, hemophilia, family history of visual impairment or hearing impairment
X	Women with these risk factors are advised against pregnancy. Pregnancy should be evaluated under specialist after treatment.	Maternal: severe heart failure, severe thrombocytopenia[c], severe anemia[d]

[a]Anemia referred to haemoglobin ranging from 60–109g/L.
[b]Thrombocytopenia referred to platelet ranging from 50 to $100*10^9$/L.
[c]Severe thrombocytopenia referred to platelet less than $50*10^9$/L.
[d]Severe anemia referred to haemoglobin less than 60g/L.

Table 2 Socio-demographic characteristics of women in different preconception risk factor classification categories

		No risk factors	A	B	C	D	X
Age	≤25	24.7%	21.6%	26.3%	22.4%	14.7%	21.3%
	25–30	46.3%	47.4%	46.7%	44.1%	38.5%	46.0%
	30–35	19.5%	21.2%	18.2%	20.9%	27.1%*	20.1%
	≥35	9.6%	9.8%	8.7%	12.6%	19.8%*	12.6%*
Area	Rural area	93.9%	89.6%	94.0%	94.0%	94.3%	92.2%
Race	Han	92.7%	91.6%	92.5%	88.7%	84.0%*	84.2%*
Education	Secondary school or lower	71.5%	64.8%*	69.4%	71.8%	77.4%	75.5%
	High school	18.7%	19.7%	18.7%	17.1%	13.8%*	16.6%
	College or higher	9.8%	15.5%*	11.8%	11.1%	8.8%	8.0%

*P value <0.05 compared with those women having no risk factors

Results

General characteristics of the study population

During April 2010 to December 2012, a total of 22.42 million married Chinese couples planning to conceive within the six months were recruited to the study from 220 different counties. After excluding those with incomplete medical records and lost to follow-up, data from 2,142,849 couples were available for analysis. NPHCP targeted couples of reproductive age mainly from rural areas, and covered most areas, regions, and ethnicities from all provinces of mainland China. 92.36% couples were from rural areas and 89.2% women and 88.3% men had education below university level. Other socio-demographic details of the participants are presented in Tables 2 and 3.

Preconception risk factor classification

As demonstrated in Tables 2 and 3, category D risk was more common among couples in the age group 30–35 years and >35 years ($P < 0.05$). There were no significant differences between rural areas and cities in both couples in terms of risk factor categories. Proportionally, more women of non-Han ethnicity were classified in category D and X compared to those with no risk factors, while there was no difference in that ratio among men. Women with category A, and men with category B and D risk factors had higher education levels ($P < 0.05$).

Distribution preconception risk factors

Distribution of the participants in different preconception risk categories is presented in Table 4. Among 2,142,849 couples, 46.25% women had preconception risks, mainly of category A, B and C. 9.80% women had category A risks including alcohol consumption (3.4%), inadequate protein intake (1.36%) and exposure to noise (1.18%). 14.83% women were had category B risks, such as anemia (8.40%), gingival hemorrhage (3.57%) and vaginitis (2.29%). Moreover, 23.5% of women had category C risks, such as thyroid dysfunction (6.34%), HBV infection (4.76%), history of gynecological diseases (3.41%) and/or category D risks, such as history of spontaneous abortion (2.66%) and adverse pregnancy history (1.12%). On the other hand, 51.92% of couples had paternal risks, and 38.13% of them had category A risk factors including alcohol (29.61%) and smoking (29.07%) (Table 4).

Table 3 Socio-demographic characteristics of men in different preconception risk categories

		No risk factors	A	B	C	D	X
Age	≤25	9.2%	10.2%	10.3%	9.1%	4.5%*	-
	25–30	43.1%	44.5%	44.9%	45.5%	38.6%*	-
	30–35	28.8%	27.6%	28.0%	28.1%	33.0%*	-
	≥35	18.9%	17.6%	16.8%	17.3%	23.9%*	-
Area	Rural area	92.6%	92.1%	90.1%	92.2%	85.7%	-
Race	Han	92.7%	91.3%	88.4%	92.3%	91.1%	-
Education	Secondary school or lower	69.3%	69.0%	63.1%	66.4%	61.9%	-
	High school	20.0%	19.2%	20.1%	20.2%	16.9%	-
	College or higher	10.6%	11.8%	16.8%*	13.4%	21.3%*	-

*P value <0.05 compared with those men having no risk factors

Table 4 Distribution of preconception risk factors among women and men in different preconception risk categories

Risk factors Maternal	Total number (%)	Risk factors Paternal	Total number (%)
A			
Alcohol consumption	72,808(3.40%)	Alcohol consumption	634,547 (29.61%)
No intake meat and egg	29,126(1.36%)	Smoking	622,834 (29.07%)
Noise exposure	25,267(1.18%)	Preputial ring	84,659(3.95%)
Others[a]	82,739(3.86%)	Noise exposure	48,399(2.26%)
		Pesticide exposure	26,753(1.25%)
		No intake meat and egg	25,857(1.21%)
		Organic solvent exposure	24,211(1.13%)
		High temperature exposure	23,388(1.09%)
		Others[i]	41,492(1.94%)
B			
Anemia[b]	179,941(8.40%)	Abnormal liver function	152,862 (7.13%)
Gingival hemorrhage	76,595(3.57%)	Abnormal renal function	26,107(1.22%)
Vaginitis[c]	49,162(2.29%)	Others[j]	9,973(0.47%)
Others[d]	12,257(0.57%)		
C			
Abnormal TSH	135,958(6.34%)	HBs antigen positive	135,958(5.87%)
HBs-Ag positive	101,970(4.76%)	HBe antigen positive	101,970(1.68%)
Gynecological diseases history	73,107(3.41%)	Others[k]	10,111(0.47%)
Abnormal renal function	57,680(2.69%)		
Abnormal liver function	54,192(2.53%)		
Thrombocytopenia[b]	31,522(1.47%)		
HBe-Ag positive	31,459(1.47%)		
Others[e]	132,582(6.19%)		
D			
Spontaneous abortion history	57,060(2.66%)	Others[l]	4,305(0.20%)
Adverse pregnancy history[f]	23,878(1.12%)		
Others[g]	8,8.73(0.41%)		
X			
Others[h]	5,259(0.25%)		

[a]Including 20,647 not eating fresh vegetables (0.96%), 16,435 pesticide exposure (0.77%), 16,049 organic solvent exposure (0.75%), 12,540 radiation exposure (0.59%), 9,717 smoking (0.45%), 5,582 raw meat eating habit (0.26%) and 1,765 heavy metal exposure (0.08%)
[b]Anemia referred to hemoglobin ranging from 60–109g/L
[c]Vaginitis included 27,657 Candida infection, 11,398 Bacterial vaginitis and 10,107 Trichomonasis
[d]Including 10,107 Trichomoniasis (0.47%), 7,672 Toxoplasma gondii IgM positive (0.36%), 4,545 Gonococcal infection (0.21%) and 40 history of psychological disease (0.00%)
[e]Including 20,705 Rh negative (0.97%), 9,290 Cytomegalovirus IgM positive (0.43%), 9,266 Chlamydia positive (0.43%), 8,482 Syphilis screening positive (0.40%), 4,395 history of preterm birth (0.21%), 14,383 diabetes (0.67%), 1,830 reported hypertension (0.09%), 1,655 reported history of tuberculosis (0.08%), 1,392 anesthetic drug use (0.07%), 1,327 congenital heart disease (0.06%), 1,018 reported tumor history (0.05%), 897 reported chronic renal disease history (0.04%) and 882 reported epilepsy history (0.04%)
[f]Adverse pregnancy history included 16,824 with history of stillbirth and 7,054 with history of birth defects
[g]Including 4,515 with birth defects (0.21%), 1,527 family history of neonatal death (0.07%), 1,416 mental retardation (0.07%), 923 family history of Mediterranean anemia (0.04%), 254 family history of G6PD deficiency (0.01%), 138 family history of Albinism (0.01%), 92 family history of Down's syndrome (0.00%), 5 family history of hearing impairment (0.00%), 2 family history of mental retardation (0.00%) and 1 family history of visual impairment (0.00%)
[h]Including 2,707 severe thrombocytopenia (0.13%) and 2,552 severe anemia (0.12%). Severe thrombocytopenia referred to platelet less than $50*10^9$/L. Severe anemia referred to hemoglobin less than 60g/L
[i]Including 18,726 not eating fresh vegetables (0.87%), 9,734 radiation exposure (0.45%), 9,454 raw meat eating habit (0.44%) and 3,578 exposure to heavy metals (0.17%)
[j]Including 5,325 spermatic cord varicocele (0.25%), 2,432 hypertension (0.11%), 1,052 congenital heart disease (0.05%), 598 chronic renal disease history (0.03%), 404 epilepsy (0.02%), 159 history of cancer (0.01%) and 3 history of psychological disease (0.00%)
[k]Including 7,771 Syphilis screening positive (0.36%), 1,348 anesthetic drug use (0.06%) and 992 reported thyroid disease (0.05%)
[l]Including 2,344 with birth defects (0.11%), 658 family history of neonatal death (0.03%), 603 family history of Mediterranean anemia (0.03%), 274 family history of G6PD deficiency (0.01%), 248 mental retardation (0.01%), 138 family history of Albinism (0.01%), 78 family history of Down's syndrome (0.00%), 4 family history of hearing impairment (0.00%), 1 family history of hemophilia (0.00%) and 1 family history of visual impairment (0.00%)

Discussion

This nation-wide free preconception care project targeting rural areas in China used an integrated model of PCC including both women and men. A novel classification system was used to classify risk factors based on their amenability to prevention and treatment, which stratified couples in five different risk categories. More than 68% of couples with conception plans within the next six months had one or more risk factors, and nearly 40% of these risk factors could be potentially modified by intervention before or during pregnancy. Approximately 23% of risk factors among women were in category A and B, whereas among men the figure was 45%. Avoidable risk factors were more common among men compared with women suggesting that men may have riskier behavior than women, with almost 30% of men reporting consumption of alcohol and smoking.

Our study revealed that preconception risk evaluation in couples with plans to conceive within six months could be meaningful as nearly two-thirds of the recruited couples had preconception risk factors, and 23% maternal risk factors were in category A and B, and thereby potentially avoidable or modifiable preconceptionally by health education, medical intervention and life style changes. More importantly, a similar situation was observed regarding paternal risk factors. Almost 45% of the male partners consumed alcohol or smoked, which may lead to passive smoking by women, a fact often ignored in preconception care. Some European countries have preconception care recommendations for women with chronic diseases, such as diabetes and epilepsy, but guidelines are heterogeneous and recommendations for healthy women and men are fragmented and inconsistent [22]. Our results further enforce the need for an integrated approach to PCC that includes both women and men.

A more innovative and integrated approach to PCC for both women and men is needed for achieving optimal reproductive health status before pregnancy and better pregnancy outcomes [23, 24]. Preconception health promotion may be useful in eliminating some of the Category A and B risk factors before pregnancy. However, some risk factors, such as smoking, alcohol and substance abuse, would require longer term strategies to achieve sustained amelioration. A more comprehensive health promotion strategy during pregnancy would be required for managing other risk categories to achieve better pregnancy outcomes.

The preconception risk classification system used in this big population-based study was practical for stratifying preconception health status of the couples, and helpful in organizing targeted educational and health care interventions, and identifying need for referral. The risk classification was based on existing risk factors during the preconception period and categorized by whether it could be prenatally avoided or modified during the preconception period or prenatally. As preconception risks may vary from prenatal risks, Considering different methods and timing of intervention is important. Nearly half of the risk factors identified were avoidable or preventable by medical intervention during the preconception period in this study, allowing for a window of opportunity for personalized lifestyle modification and health care to achieve better pregnancy outcome. Despite the evidence supporting the value and importance of PCC [25], it is reported that there is lack of sufficient research attention to clinical PCC service delivery, and a more detailed consideration of the practicalities of implementing PCC within contemporary women's health care is required [26]. This integrated universal free PCC service provided in rural China could be a promising model if its positive impact on pregnancy outcomes could be demonstrated in future.

Our study does have some limitations. Follow-up of risk modifications was not included in this study, so the impact of preconception risk classification on the health status of the couple could not be assessed. Prevalence of adverse pregnancy history and chronic disease history in couples planning pregnancy might have been underestimated as this was based on self-reporting and recall bias cannot be excluded.

Conclusions

This project provided new insights into preconception health of Chinese couples of reproductive age. More than half of the male partners planning to father a child were exposed to risk factors during the preconception period, suggesting that an integrated approach to PCC including both women and men is justified. Stratification based on the new risk classification model demonstrated that a majority of the risk factors are avoidable or preventable by medical intervention. Therefore, universal free PCC can be expected to improve pregnancy outcomes in rural China.

Abbreviations

LBW: Low birth weight; NHFPC: The National Health and Family Planning Commission of the People's Republic of China; NPHCP: National Preconception Health Care Project; PCC: Preconception care

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Availability of data and materials
Dataset analyzed in this study was based on the national database and public access to the database is closed. Zhang Shikun gave the administrative permission to access the database on behalf of National Health and Family Planning Commission of the People's Republic of China (NHFPC).

Authors' contributions
ZQ and LX carried out the statistical analysis and drafted the manuscript. GA interpreted data and drafted the manuscript. ZS, WQ, SH and LX participated in the design of the study and coordination. TW and CJ performed the statistical analysis. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study was approved by the Institutional Review Board of Chinese Association of Maternal and Child Health Studies. A written informed consent was obtained from each participant, as consent to participate.

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References

1. Johnson K, Posner SF, Biermann J, Cordero JF, Atrash HK, Parker CS, et al. Recommendations to improve preconception health and health care—United States. A report of the CDC/ATSDR Preconception Care Work Group and the Select Panel on Preconception Care. MMWR Recomm Rep. 2006;55(6):1–23.
2. Robbins CL, Loretta G, Zapata LB, Carter MW, Christina L, Nancy M-S, et al. Preconception Care in Publicly Funded U.S. Clinics That Provide Family Planning Services. Am J Prev Med. 2016;51(3):336–43.
3. Inskip HM, Crozier SR, Godfrey KM, Borland SE, Cooper C, Robinson SM, et al. Women's compliance with nutrition and lifestyle recommendations before pregnancy: general population cohort study. BMJ. 2009;338:b481.
4. Alkema L, Chou D, Hogan D, Zhang S, Moller AB, Gemmill A, et al. Global, regional, and national levels and trends in maternal mortality between 1990 and 2015, with scenario-based projections to 2030: a systematic analysis by the UN Maternal Mortality Estimation Inter-Agency Group. Lancet. 2016;387:462–74.
5. Ministry of Health of People's Republic of China. The report on the prevention of birth defects in China. 2012. Available online at: www.gov.cn/gzdt/2012-09/12/content_2223371.htm. Accessed 17 Jan 2016.
6. De-Regil LM, Fernández-Gaxiola AC, Dowswell T, Peña-Rosas JP. Effects and safety of periconceptional folate supplementation for preventing birth defects. Cochrane Database Syst Rev. 2010;(6)(10):CD007950.
7. Crawford C. AAFP urges family physicians to integrate preconception care into patient visits. Ann Fam Med. 2016;14:180–1.
8. Seely EW, Ecker J. Clinical practice. Chronic hypertension in pregnancy. N Engl J Med. 2011;365(5):439–46.
9. Bentley-Lewis R. Gestational diabetes mellitus: an opportunity of a lifetime. Lancet. 2009;373(9677):1738–40.
10. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and Child Nutrition Study Group. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet. 2013;382(9890):427–51.
11. Saifuddin A, Qingfeng L, Li L, Tsui AO. Maternal deaths averted by contraceptive use: an analysis of 172 countries. Lancet. 2012;380:111–25.
12. Wahabi HA, Alzeidan RA, Bawazeer GA, Alansari LA, Esmaeil SA. Preconception care for diabetic women for improving maternal and fetal outcomes: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2010;10:63.
13. Beckmann MM, Widmer T, Bolton E. Does preconception care work? Aust N Z J Obstet Gynaecol. 2014;54(6):510–4.
14. Luton D, Forestier A, Courau S, Ceccaldi PF. Preconception care in France. Int J Gynaecol Obstet. 2014;125(2):144–5.
15. M'hamdi HI, Voorst SF, Pinxten W, Hilhorst MT, Steegers EA. Barriers in the uptake and delivery of preconception care: exploring the views of care providers. Matern Child Health J. 2016. Epub ahead of print.
16. M'hamdi HI, van Voorst SF, Wim P, Hilhorst MT, Eric AP. Steegers. Barriers in the uptake and delivery of preconception care: exploring the views of care providers. Matern Child Health J. 2016. Epub ahead of print.
17. Poels M, Koster MP, Boeije HR, Franx A, van Stel HF. Why do women not use preconception care? A systematic review on barriers and Facilitators. Obstet Gynecol Surv. 2016;71(10):603–12.
18. Feng XL, Guo GF, Hipgrave D, Zhu J, Zhang L, Song L, et al. China's facility-based birth strategy and neonatal mortality: a population-based epidemiological study. Lancet. 2011;378:1493–500.
19. Zhou Q, Acharya G, Zhang S, Wang Q, Shen H, Li X. New perspective on universal preconception care in China. Acta Obstet Gynecol Scand. 2016;95(4):377–81.
20. Zhang SK, Wang QM, Shen HP. Design, implementation and objective of free "National Preconception Health Care Project in China. Natl Med J China. 2015;95(3):162–5.
21. Wang QM, Zhang M, Zhang SK, Hu M, Liu N, Kang XX, et al. Establishment of quality control system of free "National Preconception Health Care Project in China. Natl Med J China. 2015;95(3):166–8.
22. Jill Shawe ID, Maria E, Hanne Kristine H, Margareta L, Pierpaolo M, et al. Preconception care policy, guidelines, recommendations and services across six European countries: Belgium (Flanders), Denmark, Italy, the Netherlands, Sweden and the United Kingdom. Eur J Contracept Reprod Health Care. 2015;20:77–87.
23. Michelle SF, Karla D, Brian J. The future of preconception care in the United States: multigenerational impact on reproductive outcomes. Ups J Med Sci. 2016;19:1–5.
24. Anna B, Gunilla L. Preconception health and care (PHC)—a strategy for improved maternal and child health. Ups J Med Sci. 2016;20:1–6.
25. Baspenningx S, Haagdorens M, Blaumeiser B, Jacquemyn Y, Mortier G. Preconceptional care: a systematic review of the current situation and recommendations for the future. Facts Views Vis Obgyn. 2013;5(1):13–25.
26. Steel A, Lucke J, Reid R, Adams J. A systematic review of women's and health professional's attitudes and experience of preconception care service delivery. Fam Pract. 2016; Epub ahead of print.

Paper II

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Seroepidemiological map of *Toxoplasma gondii* infection and associated risk factors in preconception period in China: A nationwide cross-sectional study

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Abstract

Aim: The aims of this study were to investigate the regional differences in seroepidemiology of toxoplasmosis in preconception period among Chinese women of reproductive age and to evaluate associated risk factors.

Methods: This national, population-based, cross-sectional serosurvey covered all 31 provinces and province-level municipalities in Mainland China. Married women intending to get pregnant within 6 months between 2010 and 2012 were recruited. Information on demographic characteristics (age, place of residence, occupation, dietary habits and exposure to cat) was obtained using interviews, and venous blood samples were collected to screen for *Toxoplasma gondii* infection.

Results: Of 2 008 561 women recruited to the study, 45 405 (2.3%) were *Toxoplasma gondii* IgG positive, and 6884 (0.3%) were IgM positive. Geographical variation for seropositivity ranged from 0.2% in Heilongjiang to 11.2% in Tianjing for IgG and from 0% in Tibet to 0.9% in Anhui for IgM. Advanced maternal age, occupation of a farmer, vegetarian diet and exposure to cat was significantly associated with *Toxoplasma gondii* IgM seropositivity, and its association with occupation of farmer and exposure to cat was significant after adjusting for province of residence ($P < 0.05$).

Conclusion: There were significant regional variations in *Toxoplasma gondii* seropositivity and associated risk factors among Chinese women of reproductive age during preconception period. This calls for a targeted primary prevention strategy. Screening and treatment before conception and preconception health education may have potential for reducing congenital *Toxoplasmosis* in China.

Key words: China, preconception care, pregnancy, *Toxoplasma gondii* infection.

Introduction

Toxoplasma gondii infects up to one-third of the world's population,[1] with a wide spectrum of prevalence across the globe. *Toxoplasma gondii* seropositivity in pregnant women ranges from above 60% in some countries, such as Brazil, Indonesia and Germany, to less than 10% in other countries such as the

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United Kingdom and Korea.[2] The incidence of congenital infection varies from 0.1% live births in France to 0.01% in the USA.[3] Known avoidable risk factors for *Toxoplasma gondii* infection include eating raw/undercooked meat, vegetables or fruits, poor hand hygiene and contact with cats,[4,5] and level of education and awareness plays an important role in disease transmission. The prevalence of *Toxoplasma gondii* seropositivity is reported to be relatively low in some areas in China,[6] but limited information is available concerning the seroepidemiology and associated risk factors of *Toxoplasma gondii* infection among Chinese women nationwide, especially in rural areas.

Maternal acquisition of *Toxoplasma gondii* infection during pregnancy exposes the fetus to the risk of congenital infection through transplacental transmission of the parasite. Congenital infection in early pregnancy is rare but may lead to miscarriages, stillbirths or the birth of children with signs of central nervous system involvement, such as hydrocephalus, meningoencephalitis and retinochoroiditis.[7–9] Although evidence for a beneficial effect of the timing or type of prenatal treatment on the risk of mother to child transmission is lacking, clinically important effects of treatment cannot be excluded.[10] The majority of childbearing women is susceptible to primary infection and are at risk of congenital toxoplasmosis and its respective sequelae. As no effective vaccine is available against *Toxoplasma*, the improvement of primary prevention strategies constitutes a major tool to avoid infection in susceptible groups.[11] Thus, screening for *Toxoplasma gondii* infection in the preconception period could be a good option for the better primary prevention at least in women who plan to conceive.

The aims of this study were to investigate the regional differences in seroepidemiology of *Toxoplasma gondii* infection among Chinese women of reproductive age planning to get pregnant within 6 months and to evaluate associated risk factors in the preconception period.

Methods

Study design and setting

This study utilized data from the National Free Preconception Health Examination Project (NFPHEP) conducted between 2010 and 2012 in 220 counties located in 31 provinces and province-level municipalities of the mainland China.[12] Detailed design, organization, implementation and quality control of this project are described elsewhere.[12–16] The eligibility criteria for inclusion to this study were: women intending to get pregnant within 6 months and willingness to accept the provided free preconception health care. Women who failed to complete the preconception health examination or did not have *Toxoplasma* infection screening were excluded. The Institutional Review Board of Chinese Association of Maternal and Child Health Studies approved the project, and written informed consent was obtained from each participant before enrollment. Results of *Toxoplasma gondii* serology and related demographic and clinical information were extracted for analysis from the central database.

Procedures

General information about the participants, including their age, place of residence, education and occupation, were collected using a standardized questionnaire. The information on dietary habits (intake of meat and egg, intake of fresh fruits and vegetables and raw meat/fish eating habits) and contact with cat was based on self-reports by the participants. All participants were grouped into six geographical regions (Northern, Northeastern, Eastern, Central southern, Southwestern and Northwestern), and 31 provinces and municipalities (Beijing, Tianjin, Hebei, Shanxi, Inner Mongolia, Liaoning, Jilin, Heilongjiang, Shanghai, Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, Shandong, Henan, Hubei, Hunan, Guangdong, Guangxi, Hainan, Chongqing, Sichuan, Guizhou, Yunnan, Tibet, Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang) according to their residential address.

A total of 5 mL of venous blood was collected from each participant, and samples were stored at −30°C. All serum specimens were analyzed for *Toxoplasma* IgG and IgM using commercially available enzyme immunoassay kits for the detection of IgM and IgG antibodies according to the manufacturer's instructions in local laboratories.[13] The reagent kits approved by the China Food and Drug Administration were selected by the local laboratories based on their preference. The National Center of Clinical Laboratories for Quality Inspection and Detection performed an external quality assessment (EQA) twice yearly as described previously.[13] The cut-off value for seropositivity was based on the manufacturer's recommendation for the enzyme-linked immunosorbent assay (ELISA) kit used in a particular laboratory. *Toxoplasma gondii* serology was interpreted as follows: women with positive IgM and IgG were considered to have acute infection, and a referral to specialist was advised. Additional testing was advised to confirm acute infection in patients with a positive IgM serology with negative IgG titer. Women with negative IgM and positive IgG were considered to have had previous infection and to be at low risk. Those with negative IgG and IgM were susceptible and advised to avoid exposure to cat, not to handle cat litter, wash hands thoroughly before eating, avoid raw and undercooked meat, wash fruits and vegetables thoroughly before eating, not to drink unpasteurized milk and to wear gloves when working with soil in the garden or fields during the preconception period and pregnancy.

Statistical analysis

We calculated means (SD) and proportions to describe sociodemographic and clinical characteristics of the study population as appropriate. Associations between variables were tested using *t*-test and multivariate correlation analysis. SPSS version 22 was used for statistical analysis. A two-sided *P*-value<0.05 was considered statistically significant.

Results

A total of 2 008 561 married women of reproductive age from 220 counties enrolled by the NFPHEP were recruited using a two-stage stratified cluster sampling method. Women who had their *Toxoplasma gondii* serology tested were included in the final analysis. The average age of the participating women was 28.1 ± 4.8 years; 78.2% (1 545 510) of women were involved in agricultural activities, and 89.6% (1 634 003) had senior high school education or lower. Regarding dietary habits, 1.3% (25 850) women were vegetarians (did not eat meat and egg), while 1.0% (19 948) did not eat fresh fruits and vegetables. Only 0.3% (5212) of participants had a raw meat/fish eating habit, and 2.9% (57 385) were exposed to cats (Table 1).

The overall prevalence of *Toxoplasma* seropositivity was 2.3% (45 405) for IgG and 0.3% (6884) for IgM, while 0.04% (859) were both IgG and IgM positive. The regional variations in *Toxoplasma gondii* seropositivity are presented in Table 2. Provincial differences in IgG seropositivity among Chinese women in the preconception period were significant and ranged from 0.2% in Heilongjiang to 11.2% in Tianjing (Fig. 1). The highest IgM seropositivity was 0.9% in Anhui, and the lowest was 0% in Tibet (Fig. 2).

Advanced maternal age, urban residence, occupation of farmer, vegetarian diet (no intake of meat and egg) and exposure to cat were significantly associated with *Toxoplasma gondii* IgM positivity ($P < 0.05$) (Table 1). In addition, women with a lower education level tended to be *Toxoplasma* IgM positive, whereas those who did not eat fresh fruits and vegetables or have a raw meat/fish eating habit were more likely be *Toxoplasma* IgG

Table 1 Baseline demographic and risk factor characteristics of the study population†

		IgG			IgM		
	Total	(+)	(−)	*P*‡	(+)	(−)	*P*§
Age, years	28.1 ± 4.8	28.3 ± 4.1	28.0 ± 4.8	<0.0001	28.7 ± 5.2	28.1 ± 4.8	<0.0001
Residence				0.084			<0.0001
Urban	1.1%	5.6%	5.4%	—	3.9%	5.5%	—
Rural	98.8%	94.4%	94.6%	—	96.1%	94.5%	—
Occupation				0.072			<0.0001
Farmers	78.2%	80.0%	78.2%	—	82.4%	78.1%	—
Workers	8.1%	7.0%	8.2%	—	7.1%	8.2%	—
Others	13.7%	13.0%	13.6%	—	10.5%	13.7%	—
Education				0.078			<0.0001
Uneducated	0.3%	0.4%	0.3%	—	0.3%	0.3%	—
Primary school	4.8%	6.5%	4.8%	—	5.9%	4.8%	—
Junior high school	66.9%	64.9%	67.0%	—	69.1%	66.9%	—
Senior high school	17.6%	16.7%	17.6%	—	15.7%	17.6%	—
College or higher	10.4%	11.8%	10.3%	—	9.0%	10.4%	—
No intake of meat and egg	1.3%	1.5%	1.3%	<0.0001	1.6%	1.3%	0.017
No intake of fresh vegetables	1.0%	1.9%	1.0%	<0.0001	1.0%	1.0%	0.793
Raw meat/fish eating habit	0.3%	0.4%	0.3%	<0.0001	0.2%	0.3%	0.363
Exposure to cat	2.9%	4.0%	2.9%	<0.0001	7.2%	2.9%	<10.000

†Data are presented as mean ± SD or percentage. ‡*P*-value for the comparison between women who were *Toxoplasma gondii* IgG (+) and those who were *Toxoplasma gondii* IgG (−). §*P*-value for the comparison between women who were *Toxoplasma gondii* IgM (+) and those who were *Toxoplasma gondii* IgM (−).

Table 2 Prevalence of *Toxoplasma gondii* by regions

		IgG(+)		IgM(+)	
	Total number	Number	%	Number	%
Northern area	219 051	3294	1.5	367	0.2
Beijing	9253	425	4.6	15	0.2
Tianjing	10 107	1135	11.2	5	0
Hebei	162 594	844	0.5	262	0.2
Shanxi	24 949	140	0.6	67	0.3
Inner Mongolia	12 148	750	6.2	18	0.1
Northeastern area	118 569	2354	2.0	588	0.5
Liaoning	17 267	141	0.8	174	1
Jilin	95 953	2202	2.3	407	0.4
Heilongjiang	5349	11	0.2	7	0.1
Eastern area	355 427	8420	2.4	2061	0.6
Shanghai	2707	195	7.2	4	0.1
Jiangsu	76 846	1524	2.0	171	0.2
Zhejiang	26 657	819	3.1	55	0.2
Anhui	89 679	2163	2.4	842	0.9
Fujian	39 850	622	1.6	252	0.6
Jiangxi	35 873	296	0.8	38	0.1
Shandong	83 815	2801	3.3	699	0.8
Central southern area	840 415	18 240	2.2	2286	0.3
Henan	188 239	7259	3.9	839	0.4
Hubei	215 088	2291	1.1	308	0.1
Hunan	178 210	3062	1.7	358	0.2
Guangdong	186 674	3756	2.0	606	0.3
Guangxi	61 649	1578	2.6	110	0.2
Hainan	10 555	294	2.8	65	0.6
Southwestern area	262 302	8229	3.1	853	0.3
Chongqing	68 580	730	1.1	84	0.1
Sichuan	87 018	2337	2.7	413	0.5
Guizhou	51 268	2515	4.9	217	0.4
Yunnan	55 383	2645	4.8	139	0.3
Tibet†	53	2	3.8	0	0
Northwestern area	212 797	4868	2.3	729	0.3
Shaanxi	107 247	1594	1.5	219	0.2
Gansu	21 700	548	2.5	70	0.3
Qinghai	11 552	57	0.5	11	0.1
Ningxia	6217	113	1.8	20	0.3
Xinjiang	66 081	2556	3.9	409	0.6
Total	2 008 561	45 405	2.3	6884	0.3

†There were only 53 participants from Tibet, and therefore, the low *Toxoplasma* seropositivity rate could be due to selection bias.

positive ($P < 0.05$) (Table 1). In addition, there were regional differences in the prevalence of risk factors, such as maternal age, occupation of farmer, dietary habit and exposure to cat. *Toxoplasma gondii* IgM seropositivity was significantly associated with occupation of farmer ($P < 0.0001$) and exposure to cat ($P < 0.0001$) after adjusting for province of residence.

Discussion

This nationwide study presents the seroepidemiological map and risk factors of *Toxoplasma gondii* infection in Chinese women of reproductive age before conception. To our knowledge, this is the largest study that describes the seroepidemiology of *Toxoplasma gondii* infection in the preconception period in China.

In our study, the *Toxoplasma gondii* seropositivity was relatively low among Chinese women of reproductive age: 2.3% were IgG positive and 0.3% were IgM positive, whereas 0.04% were both IgG and IgM positive. *Toxoplasma gondii* IgG and IgM seropositivity among pregnant women in China has been previously reported to be 3.8% and 1.6%, respectively, in Jiangsu province and 15.2% and 2.9%, respectively, in Qingdao and Weihai cities.[17–19] An IgG seropositivity

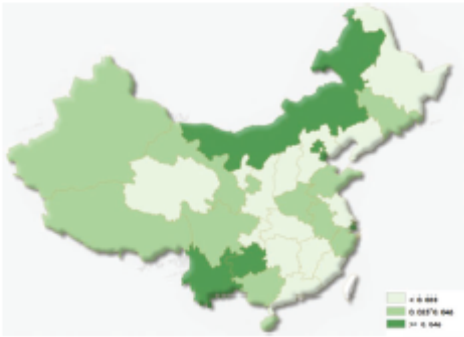

Figure 1 Seropositivity IgG of *Toxoplasma gondii* by regions.

rate of 21.6% has been reported in Dali among all age groups.[6] However, the nationwide seropositivity rate for *Toxoplasma gondii* in the preconception period in China has not been previously reported. In this study, we analyzed the geographical variation in *Toxoplasma gondii* seropositivity in Mainland China. Provincial differences in seropositivity were significant and varied from 0.2% to 11.2% for IgG and from 0.0% to 0.9% for IgM. In a recent meta-analysis, Li *et al*. demonstrated that the pooled rate of vertical transmission is 20% when maternal infection occurs during pregnancy, and the incidence of vertical transmission increases from the first to the third trimester of pregnancy.[20] Therefore, findings of our study underscore the need for more credible targeted primary

Figure 2 Seropositivity IgM of *Toxoplasma gondii* by regions.

prevention strategies for women planning pregnancy to prevent congenital toxoplasmosis.

Our study also revealed that occupation of farmer and exposure to cat are risk factors for *Toxoplasma gondii* infection. It is unsurprising because working as a farmer is likely to be associated with increased exposure to cat, contaminated soil and vegetables. According to Li *et al*., feeding a cat may be the main route of *T. gondii* infection for the Han population.[6] Although eating raw meat and exposure to cat are regarded as the main risk factors,[4,5] it has been demonstrated that educational programs describing hygienic measures will not suffice to significantly reduce the burden of congenital *toxoplasmosis*, suggesting that implementation of systematic serological testing of pregnant women and newborns may be desirable.[21] Prenatal antibiotic therapy after the diagnosis of *toxoplasmosis* during pregnancy had no impact on the maternal–fetal transmission rate, although early start of treatment resulted in a significant reduction in the number of severely affected infants.[22] Identification of susceptible or infected women in the preconception period potentially allows for primary prevention before pregnancy. Our study provides new evidence regarding risk factors associated with *Toxoplasma gondii* infection in Chinese women of reproductive age in the preconception period. These data may be useful for planning and implementing integrated new strategies for preventing congenital toxoplasmosis in Chinese communities.

Our study does have some limitations. The information on dietary habits and exposure to cats was based on self-reporting. The reagents and kits used varied based on local preference, and their sensitivity and specificity might have been slightly different. Furthermore, repeated testing was not performed. Therefore, some cases might have been false positive or false negative. However, the risk of the seropositivity rate being over- or underestimated is likely to be small as quality assurance of laboratories was in place. In addition, only 53 participants were enrolled in Tibet, and thus, the low *Toxoplasma gondii* seropositivity rate in Tibet could be due to selection bias.

There were significant regional variations in *Toxoplasma gondii* seropositivity and associated risk factors among Chinese women of reproductive age during the preconception period. This calls for a targeted primary prevention strategy. Screening and treatment before conception and health education may have potential for reducing congenital *Toxoplasma gondii* infection in China.

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Disclosure

None declared.

Reference

1. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: From animals to humans. *Int J Parasitol* 2000; **30**: 1217–1258.
2. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int J Parasitol* 2009; **39**: 1385–1394.
3. Feldman HA. Epidemiology of toxoplasma infections. *Epidemiol Rev* 1982; **4**: 204–213.
4. Baril L, Ancelle T, Goulet V, Thulliez P, Tirard-Fleury V, Carme B. Risk factors for *Toxoplasma* infection in pregnancy: A case-control study in France. *Scand J Infect Dis* 1999; **31**: 305–309.
5. Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A, Eng J. Risk factors for *Toxoplasma gondii* infection in pregnancy results of a prospective case-control study in Norway. *Am J Epidemiol* 1996; **144**: 405–412.
6. Li HL, Dong L, Li Q *et al.* Seroepidemiology of *Toxoplasma gondii* infection in Bai and Han ethnic groups in southwestern China. *Epidemiol Infect* 2015; **143**: 881–886.
7. Remington J, McLeod R, Thulliez P, Desmonts G. Toxoplasmosis. In: Remington J, Klein G, Wilson C, Carol B (eds). *Infectious Diseases of the Fetus and Newborn Infant*, 6th edn. Philadelphia, PA: WB Saunders, 2006; 947–1091.
8. Olariu TR, Remington JS, McLeod R, Alam A, Montoya JG. Severe congenital toxoplasmosis in the United States: Clinical and serologic findings in untreated infants. *Pediatr Infect Dis J* 2011; **30**: 1056–1061.
9. Swisher CN, Boyer K, McLeod R. Congenital toxoplasmosis. The toxoplasmosis study group. *Semin Pediatr Neurol* 1994; **1**: 4–25.
10. Gilbert R, Gras L, European Multicentre Study on Congenital Toxoplasmosis. Effect of timing and type of treatment on the risk of mother to child transmission of *Toxoplasma gondii*. *BJOG* 2003; **110**: 112–120.
11. Gargaté MJ, Ferreira I, Vilares A *et al. Toxoplasma gondii* seroprevalence in the Portuguese population: Comparison of three cross-sectional studies spanning three decades. *BMJ Open* 2016; **6**: e011648.
12. Zhou QJ, Acharya G, Zhang SK, Wang QM, Shen HP, Li XT. A new perspective on universal preconception care in China. *Acta Obstet Gynecol Scand* 2016; **95**: 377–381.
13. Wang Q, Zhang M, Zhang S *et al.* Establishment of quality assurance system of the National Free Preconception Health Care Project in China. *Zhonghua Yi Xue Za Zhi* 2015; **95**: 166–168.
14. Zhang S, Wang Q, Sheng H. Design of the national free preconception health examination project in China. *Zhonghua Yi Xue Za Zhi* 2015; **95**: 162–165.
15. Zhou QJ, Zhang SK, Wang QM *et al.* China's community-based strategy of universal preconception care in rural areas at a population level using a novel risk classification system for stratifying couples' preconception health status. *BMC Health Serv Res* 2016; **16**: 689.
16. Liu J, Zhang S, Wang Q *et al.* Seroepidemiology of hepatitis B virus infection in 2 million men aged 21–49 years in rural China: A population-based, cross-sectional study. *Lancet Infect Dis* 2016; **16**: 80–86.
17. Gao XJ, Zhao ZJ, He ZH *et al. Toxoplasma gondii* infection in pregnant women in China. *Parasitology* 2012; **139**: 139–147.
18. Hua HY, Tang F, Liu YX *et al.* Survey of *Toxoplasma gondii* infection among pregnant women in Jiangsu Province, China. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* 2013; **25**: 56–58, 79.
19. Cong W, Dong XY, Meng QF *et al. Toxoplasma gondii* infection in pregnant women: A seroprevalence and case-control study in Eastern China. *Biomed Res Int* 2015; **2015**: 170278.
20. Li XL, Wei HX, Zhang H, Peng HJ, Lindsay DS. A meta analysis on risks of adverse pregnancy outcomes in *Toxoplasma gondii* infection. *PLoS One* 2014; **9**: e97775.
21. Boyer K, Hill D, Mui E *et al.* Unrecognized ingestion of *Toxoplasma gondii* oocysts leads to congenital toxoplasmosis and causes epidemics in North America. *Clin Infect Dis* 2011; **53**: 1081–1089.
22. Foulon W, Villena I, Stray-Pedersen B *et al.* Treatment of toxoplasmosis during pregnancy: A multicenter study of impact on fetal transmission and children's sequelae at age 1 year. *Am J Obstet Gynecol* 1999; **180**(2 Pt 1): 410–415.

Paper III

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Rubella virus immunization status in preconception period among Chinese women of reproductive age: A nation-wide, cross-sectional study

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ABSTRACT

Objective: Population-based studies on sero-epidemiology of Rubella in women before conception are lacking. The aim of this study was to investigate the sero-prevalence of Rubella in a nationwide survey among Chinese women planning to get pregnant within six months.

Methods: This population-based, cross-sectional, sero-survey of Rubella virus infection was a part of the National Free Preconception Health Examination Project covering all 31 provinces in Mainland China. Women intending to get pregnant within six months was enrolled between 2010 and 12. Information on demographic characteristics (age, residence status, race, education and occupation) and vaccination history was obtained by interviews. Rubella virus IgG sero-positivity was determined using venous blood samples.

Results: Of 2,120,131 women recruited to the study, Rubella virus IgG serology was available in 1,974,188 (99.3%). Participating women were of young age (median = 28 years), mostly engaged in agricultural activities (78%), and the majority (90%) had high school education or lower. The overall prevalence of Rubella virus IgG sero-positivity was 58.4% (1,161,129); geographical variation ranged from 92.5% in Jilin to 20.1% in Qinghai and 0.0% in Tibet. Only 4.6% (n = 91,604) women reported to have had Rubella virus vaccination, and it varied from 18.6% (Guangdong) to 0.2% (Qinghai). Self-reported vaccination status did not correlate with Rubella virus IgG seropositivity.

Conclusions: Prevalence of Rubella sero-positivity is low among Chinese women of reproductive age and there are significant regional differences. Over 40% of women being susceptible to Rubella in preconception period calls for a targeted screening and vaccination strategy.

1. Introduction

Rubella virus causes a self-limited infection in most hosts, but can have potentially devastating effects on the developing fetus. Rubella in pregnancy is known to cause congenital anomalies and is associated with premature labor and spontaneous abortion [1]. Therefore, it is of great importance to screen for antibodies to Rubella as early as possible in pregnancy and obstetricians routinely perform it.

The introduction of routine childhood vaccination with measles, mumps, and Rubella combination vaccine (MMR), and measles, mumps, Rubella, and varicella combination vaccine (MMRV) in recent years, has largely eliminated congenital Rubella syndrome (CRS) in many developed countries. In the United States, the incidence of Rubella had declined from 0.45 per 100,000 in 1990 to 0.1 per 100,000 in 1999 [2,3]. However, the proportion of Rubella susceptible women of childbearing age varies greatly among

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nations, especially in the developing countries. Rubella outbreaks continue to occur in parts of the world, and CRS still remains a public health concern. An estimated 15 percent of women between the ages of 20 and 29 were reported to lack antibodies to Rubella in Turkey [4], and 23 percent of women of childbearing age had negative titers in Nigeria [5]. In the Russian Federation, 16.5 percent of pregnant women were reported to be susceptible to Rubella, and CRS occurred in approximately 3.5 cases per 1000 live births [6]. Fifteen percent of 342 infants with suspected congenital infections had detectable IgM against Rubella in one study from India [7]. In China, according to the National Notifiable Diseases Reporting System (NNDRS), the prevalence of Rubella was 9.11/100,000 in 2008 [8]. The rate of CRS was 9 cases per 1000 live births in Jinan and Yantai in 2007 [9], while the annual incidence rate of Rubella was 0.75 per 100,000 in Zhejiang province in 2013–15 [10].

Even in countries where Rubella vaccination programs are available, the extent of vaccination is not always optimal. A study reported that although more than one-third of countries had a national vaccination policy, 9% had a selective strategy (i.e., vaccination offered only to women or schoolgirls), and 31% reported only childhood immunization [11]. The rationale for the second dose of the MMR vaccine was not to serve as a booster but rather to immunize the 5–20 percent of people who had not responded to the first dose of the vaccine. MR and MMR vaccinations were recommended by Chinese government in 2008 [12]. However reports on the prevalence of Rubella virus IgG sero-positivity among Chinese women of reproductive age, especially in preconception period, are lacking.

Maternal-fetal transmission of Rubella occurs though hematogenous spread and risk varies with gestational age. In the first trimester, fetal infection rates as high as 81 percent have been observed, dropping to 25 percent in the late second trimester and increasing again in the third trimester from 35 percent at 27 to 30 weeks to nearly 100 percent for fetuses exposed beyond 36 weeks [13]. In general, maternal immunity acquired either by vaccine or naturally, is protective against intrauterine Rubella infection. Although cases of CRS resulting from maternal reinfection have been reported [14–16], none of these occurred in women infected after 12 weeks of gestation [17]. However, data concerning the antibodies before conception are lacking.

The objective of this study was to investigate the sero-prevalence and demographic characteristics of Rubella virus infection among women planning get pregnant within six months in different geographic regions of China.

2. Methods

2.1. Study design and setting

This study utilized data from National Free Preconception Health Examination Project (NFPHEP) a population-based, prospective, cross-sectional, nation-wide study that was conducted between 2010 and 2012 in 220 rural counties located in all 31 provinces and province level municipalities of China [18]. The Chinese National Health and Family Planning Commission and Ministry of Finance launched NFPHEP in 2010, providing 19 preconception health service items involving health education, health examination, risk assessment and medical consultation to the married couples of reproductive age (21–49 years) planning a pregnancy within next six months [18–20]. The couples had free access to preconception consultation/counseling, reproductive health education and general health check-up including laboratory tests, and received appropriate medical advice or were referred to specialized hospitals when indicated [18].

The eligibility criteria for inclusion to the study were: women intending to get pregnant within next 6 months and willingness to accept the preconception health care provided. Couples who failed to complete the examination or did not have Rubella virus IgG status or did not answer the question on vaccination history were excluded. The Institutional Review Board of Chinese Association of Maternal and Child Health Studies approved the project, and a written informed consent was obtained from each participant before enrollment. All records are uploaded in a web-based electronic data collection system. Results of Rubella virus IgG serology and related demographic and clinical information were extracted for analysis.

2.2. Procedures

Trained, qualified local community staff enrolled the targeted population by drop-in, telephone calls and free consultations. The participants consenting to the study were given free medical examination and preconception counseling in local hospitals. Trained local health workers collected baseline data from each participant using a questionnaire survey, obtained serum samples, performed a general physical examination to generate a standardized family health file, and entered data into the national database [18–20]. Detailed design, organization, implementation and quality control of this project are described elsewhere [18–22].

Five mL of venous blood was collected from each participant and samples were stored at −30 °C. All serum specimens were tested for Rubella virus IgG in local laboratories. The reagent kits approved by the China Food and Drug Administration were selected by the local laboratories based on their preference. The National Center of Clinical Laboratories for Quality Inspection and Detection performed an external quality assessment (EQA) twice yearly as described previously [20].

Detailed information on each participant was regularly uploaded to the database of the National Data Centre of Preconception Health Care, Beijing. General information regarding the participants, including age, residence status, race, education and occupation were collected using a standardized questionnaire. The recalled Rubella virus vaccination history was based on self-reports and the vaccination records, such as vaccination cards, were not available for examination. All participants were grouped into six geographical regions (Northern, Northeastern, Eastern, Central southern, Southwestern, and Northwestern), and 31 provinces and municipalities (Beijing, Tianjin, Hebei, Shanxi, Inner Mongolia, Liaoning, Jilin, Heilongjiang, Shanghai, Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, Shandong, Henan, Hubei, Hunan, Guangdong, Guangxi, Hainan, Chongqing, Sichuan, Guizhou, Yunnan, Tibet, Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang) according to their residential address.

2.3. Statistical analysis

We calculated means (SD) and proportions to describe socio-demographic and clinical characteristics of the study participants as appropriate. Comparison of proportions between groups were made using chi-squared test. Associations between variables were tested using regression analysis. SPSS version 22.0 was used for statistical analysis. A two sided P-value < 0.05 was considered statistically significant.

3. Results

A total of 2,120,131 women aged 21–49 years from 220 counties were enrolled by the NFPHEP with a two-stage stratified cluster sampling method, which covered 86% of the target population

[17]. Of those, 1,974,188 (99.3%) reporting Rubella virus vaccination history and having serum samples tested for virus IgG serology were included in our final analysis. The median age of the participating women was 28 years (95% confidence interval: 22–45), 1,613,342 (76.7%) were engaged in agricultural activities, 1,884,504 (89.1%) had high school education or lower (Table 1).

The prevalence of Rubella virus vaccination and serological status by regions and provinces are presented in Table 2. The overall prevalence of Rubella virus IgG sero-positivity was 58.4% (1,161,129). Only 4.6% women reported Rubella virus vaccination (n = 91,604). The Rubella virus IgG sero-positivity prevalence among 31 provinces varied from 84.5% in Northeastern area to 45.8% in northwestern area. Provincial differences were substantial: Jilin (92.5%), Beijing (91.9%), Ningxia (82.3%), Guangdong (81.0%), Zhejiang (79.6%) and Hainan (79.6%) had high prevalence (Rubella virus IgG positive ≥80%), while Tibet (0.0%) and Qinghai (20.1%) had low prevalence (Rubella virus IgG positive <25%) (Table 3). The rate of self-reported HBV vaccination ranged from 18.6% (Guangdong) to Qinghai (0.2%).

Prevalence of Rubella virus vaccination history and IgG serological status in the preconception period stratified by age groups is presented in Table 2. The prevalence rate of Rubella virus

Table 1
Demographic and baseline characteristics of participants by age.

Variable	21–29 years	30–39 years	40–49 years	Total	P value
Education	1,470,806	571,188	72,923	2,114,917	0.000
Non-educated	0.2% (2845)	0.3% (1943)	0.8% (579)	0.3% (5367)	
Primary school	3.6% (53,775)	6.2% (35,424)	14.2% (10,476)	4.7% (99,675)	
Junior high school	63.6% (946,466)	67.9% (394,927)	73.5% (54,283)	66.0% (1,395,676)	
Senior high school	20.1% (295,314)	15.1% (86,724)	8.3% (6119)	18.4% (388,157)	
College or higher	11.7% (172,506)	9.8% (56,441)	2.0% (1466)	10.9% (230,413)	
Occupation	1,460,446	571,188	72,447	2,104,081	0.000
Workers	9.4% (137,510)	8.0% (45,931)	5.7% (4094)	8.9% (187,535)	
Farmers	75.6% (1,103,505)	78.3% (446,955)	86.8% (62,882)	76.7% (1,613,342)	
Others	15.0% (219,431)	13.7% (78,302)	7.5% (5471)	14.4% (303,204)	

Data are presented as percentage (n/N).

Table 2
Prevalence of rubella virus vaccination and IgG sero-positivity by regions.

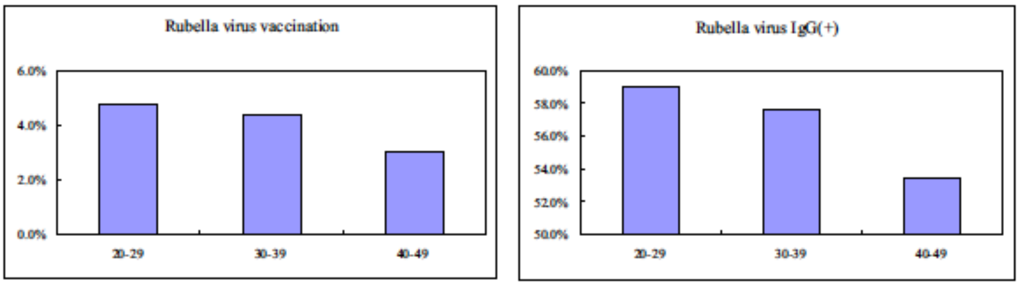
Geographical region	Total number	Number	%	Rubella virus vaccination		Rubella virus IgG(+)	
				Number	%	Number	%
Northern area	218,281	215,698	98.8%	6080	2.8%	137,446	63.0%
Beijing	9236	9213	99.8%	154	1.7%	8490	91.9%
Tianjing	10,107	10,099	99.9%	700	6.9%	7971	78.9%
Hebei	161,868	161,560	99.8%	4095	2.5%	96,803	59.8%
Shanxi	24,934	22,708	91.1%	1073	4.7%	14,000	56.1%
Inner Mongolia	12,136	12,118	99.9%	58	0.5%	10,182	83.9%
Northeastern area	118,069	117,758	99.7%	1549	1.3%	99,748	84.5%
Liaoning	17,048	17,005	99.7%	392	2.3%	8279	48.6%
Jilin	95,689	95,439	99.7%	1092	1.1%	88,468	92.5%
Heilongjiang	5332	5314	99.7%	65	1.2%	3001	56.3%
Eastern area	348,216	344,900	99.0%	15,618	4.5%	194,724	55.9%
Shanghai	2696	2656	98.5%	51	1.9%	1596	59.2%
Jiangsu	72,446	72,195	99.7%	7283	10.1%	43,227	59.7%
Zhejiang	26,324	24,612	93.5%	512	2.1%	20,957	79.6%
Anhui	89,585	88,961	99.3%	1046	1.2%	38,637	43.1%
Fujian	39,732	39,591	99.6%	1964	5.0%	27,625	69.5%
Jiangxi	35,719	35,660	99.8%	1363	3.8%	16,831	47.1%
Shandong	81,714	81,225	99.4%	3399	4.2%	45,851	56.1%
Central southern area	836,691	831,457	99.4%	54,649	6.6%	514,072	61.4%
Henan	188,034	187,566	99.8%	7944	4.2%	136,607	72.7%
Hubei	214,178	210,931	98.5%	7184	3.4%	92,554	43.2%
Hunan	177,539	177,138	99.8%	4127	2.3%	91,567	51.6%
Guangdong	185,267	184,289	99.5%	34,198	18.6%	150,056	81.0%
Guangxi	61,263	61,139	99.8%	762	1.2%	34,997	57.1%
Hainan	10,410	10,394	99.8%	434	4.2%	8291	79.6%
Southwestern area	259,248	258,113	99.6%	6677	2.6%	120,393	46.4%
Chongqing	67,336	66,722	99.1%	1162	1.7%	29,488	43.8%
Sichuan	85,992	85,838	99.8%	4372	5.1%	38,066	44.3%
Guizhou	51,014	50,839	99.7%	393	0.8%	19,270	37.8%
Yunnan	54,853	54,662	99.7%	749	1.4%	33,569	61.2%
Tibet*	53	52	98.1%	1	1.9%	0	0.0%
Northwestern area	206,822	206,262	99.7%	7031	3.4%	94,746	45.8%
Shaanxi	102,489	102,249	99.8%	2335	2.3%	53,181	51.9%
Gansu	21,461	21,407	99.7%	251	1.2%	11,808	55.0%
Qinghai	11,550	11,523	99.8%	25	0.2%	2326	20.1%
Ningxia	5801	5790	99.8%	18	0.3%	4776	82.3%
Xinjiang	65,521	65,293	99.7%	4402	6.7%	22,655	34.6%
Total	1,987,327	1,974,188	99.3%	91,604	4.6%	1,161,129	58.4%

* There were only 53 participants from Tibet. Therefore possibility of selection bias cannot be excluded.

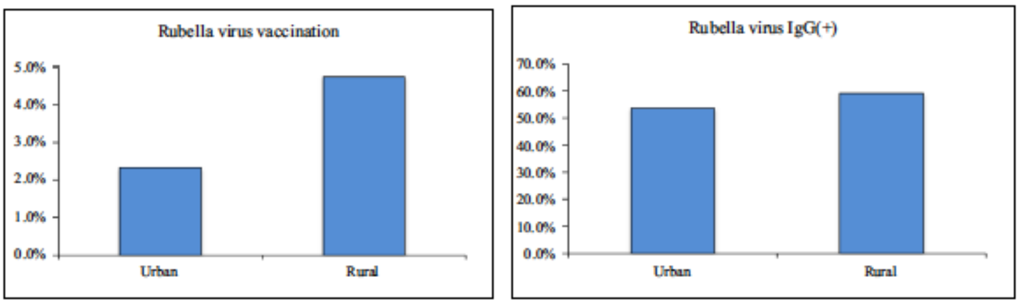
Table 3
Prevalence of rubella virus vaccination and IgG sero-positivity by age and area of residence.

	Age			Area	
	20–29 (N = 1,388,508)	30–39 (N = 530,609)	40–49 (N = 68,210)	Urban** (N = 88,690)	Rural (N = 1,885,498)
Rubella virus vaccination	4.8% (66,178)	4.4% (23,357)	3.0%* (2069)	2.3% (2067)	4.8%* (8,953,798)
Rubella virus IgG(+)	59.0% (819,120)	57.6% (305,559)	53.4% (36,450)	53.6% (47,545)	59.1% (1,113,584)

(A) Prevalence of Rubella virus vaccination and IgG sero-positivity in different age groups.



(B) Prevalence of rubella virus vaccination and IgG sero-positivity in urban and rural areas.



* χ^2 tests for comparing the prevalence rates on Rubella virus vaccination in different ages and areas, were all $p < 0.001$.
** Urban areas referred to Beijing, Shanghai, Tianjing and Chongqing province-level municipalities.

self-reported vaccination among 40–49 years old group was significantly lower compared with 20–29 and 30–39 years old groups ($P < 0.0001$). The prevalence rate self-reported vaccination was lower in urban areas compared to that in rural areas ($P < 0.0001$) (Table 3). However, self-reported vaccination status did not correlate with IgG sero-positivity ($P = 0.07$).

4. Discussion

4.1. Main findings

Our population-based nationwide study showed that the prevalence of Rubella sero-positivity is relatively low among women of reproductive age in Mainland China as more than 40% were susceptible to Rubella in preconception period. We also found significant regional differences, which are possibly due to differences in exposure to Rubella virus and vaccination coverage. To our knowledge, this is the largest study that describes sero-prevalence of Rubella virus in preconception period.

4.2. Interpretation

The high prevalence of Rubella susceptibility and low self-reported vaccination rate with a wide geographical variation suggests that a targeted Rubella virus screening and vaccination strategy for women in childbearing age may be needed for reducing the burden of intrauterine infection and CRS in China. In a previous study, 77.6% female migrant factory workers in Shenzhen, China were reported to be immune to Rubella [23]. However, to our knowledge there are no other similar studies from China or other parts of the world that have reported on Rubella serology in women in preconception period to compare our results.

The low rate of IgG sero-positivity was likely to be a consequence of limited health care and low childhood vaccination rate among women elder than 40 years old. The Chinese government encouraged expanding MR or MMR vaccination coverage during infancy and childhood only since 2005 [12]. Interestingly, the self-reported vaccination rate varied and did not correlate with Rubella virus IgG sero-positivity, suggesting the need and importance of enforcing and expanding the immunization program for young women in rural areas. The national preconception care project provides a window of opportunity to ensure this. In addition, postpartum Rubella vaccination could be considered for sero-negative women in order to prevent occurrence of CRS in future pregnancies as reported previously in Japan and Spain [24,25]. This becomes even more relevant with the recent abolition of one child policy in China.

4.3. Strengths

The major strengths of our study are it's large sample size and nation-wide coverage with less than 1% missing data. This allowed us to analyze the distribution and variation of Rubella prevalence in different Chinese regions and provinces. Moreover, there are no previous studies that specifically targeted women of reproductive age in the preconception period. Identification of Rubella virus susceptibility in preconception period allows timely vaccination before pregnancy preventing infection during pregnancy. In addition, the validity of our data was ensured by the identification and inclusion of almost all eligible women by trained local community staff, use of a uniform questionnaire survey and a standardized family health file, periodic quality controls, and web-based data entry to a centralized database.

4.4. Limitations

Our study does have limitations. The history of Rubella virus vaccination was self-reported, and thus, possibility of recall bias cannot be excluded. Furthermore, there were only 53 participants in Tibet, therefore the very low Rubella sero-positivity rate in Tibet could be due to selection bias.

5. Conclusion

In summary, the prevalence of Rubella sero-positivity is low among women of reproductive age in rural China and there are significant regional differences. Over 40% of women of childbearing age being susceptible to Rubella in preconception period calls for a targeted screening and vaccination strategy.

Disclosure of interest

The authors have no conflicts of interest to report.

Contributions to authorship

ZQ and LX carried out the statistical analysis and drafted the manuscript. GA interpreted data and drafted the manuscript. ZS, WQ, SH, ZY and LX participated in the design of the study and coordination. All authors read and approved the final manuscript.

Details of ethics approval

This study was approved by the Institutional Review Board of Chinese Association of Maternal and Child Health Studies. A written informed consent was obtained from each participant, as consent to participate.

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References

[1] Watson JC, Hadler SC, Dykewicz CA, Reef S, Phillips L. Measles, mumps, and Rubella–vaccine use and strategies for elimination of measles, Rubella, and congenital Rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 1998;47(RR-8):1–57. 22.

[2] Reef SE, Frey TK, Theall K, Abernathy E, Burnett CL, Icenogle J, et al. The changing epidemiology of Rubella in the 1990s: on the verge of elimination and new challenges for control and prevention. JAMA 2002;287(4):464–72.

[3] Reef SE, Redd SB, Abernathy E, Zimmerman L, Icenogle JP. The epidemiological profile of Rubella and congenital Rubella syndrome in the United States, 1998–2004: the evidence for absence of endemic transmission. Clin Infect Dis 2006;43(Suppl 3):S126–32.

[4] Aksit S, Egemen A, Ozacar T, Kurugol Z, Keskinoglu P, Tasbakan M, et al. Rubella seroprevalence in an unvaccinated population in Izmir: recommendations for Rubella vaccination in Turkey. Pediatr Infect Dis J 1999;18(7):577–80.

[5] Onyenekwe CC, Kehinde-Agbeyangi TA, Ofor US, Arinola OG. Prevalence of Rubella-IgG antibody in women of childbearing age in Lagos, Nigeria. West Afr J Med 2000;19:23–6.

[6] Semerikov VV, Lavrentyeva IN, Popov VF, Fletcher MA, Kolotov ME. Rubella in the Russian Federation: epidemiological features and control measures to prevent the congenital Rubella syndrome. Epidemiol Infect 2000;125(2):359–66.

[7] Ballal M, Shivananda PG. Prevalence of Rubella virus in suspected cases of congenital infections. Indian J Pediatr 1997;64:231–5.

[8] Zhu Z, Guo XB, Cui AL. Analysis of epidemiological and genetic characteristics of Rubella viruses in China from 2007 to 2008. Zhongguo Yi Miao He Mian Yi 2009;15(3):201–4.

[9] Xu Q, Xu AQ, Song LZ, Wang CY, Zhang L, Xiao ZK, et al. Retrospective epidemiologic investigation on congenital Rubella syndrome in monitoring region of Shandong province, China. Zhonghua Liu Xing Bing Xue Za Zhi 2010;31(7):828–9.

[10] He H, Yan R, Tang X, Zhou Y, Deng X, Xie S. Vaccination in secondary school students expedites Rubella control and prevents congenital Rubella syndrome. BMC Infect Dis 2016;16(1):723. 30.

[11] Robertson SE, Cutts FT, Samuel R, Diaz-Ortega JL. Control of Rubella and congenital Rubella syndrome (CRS) in developing countries, Part 2: Vaccination against Rubella. Bull World Health Organ 1997;75:69–80.

[12] http://www.gov.cn/gzdt/2008-02/19/content_893572.htm.

[13] Miller E, Cradock-Watson JE, Pollock TM. Consequences of confirmed maternal Rubella at successive stages of pregnancy. Lancet 1982;2(8302):781–4. 9.

[14] Bullens D, Smets K, Vanhaesebrouck P. Congenital Rubella syndrome after maternal reinfection. Clin Pediatr (Phila) 2000;39(2):113–6.

[15] Paludetto R, van den Heuvel J, Stagni A, Grappone L, Mansi G. Rubella embryopathy after maternal reinfection. Biol Neonate 1994;65(5):340–1.

[16] Aboudy Y, Fogel A, Barnea B, Mendelson E, Yosef L, Frank T, et al. Subclinical Rubella reinfection during pregnancy followed by transmission of virus to the fetus. J Infect 1997;34(3):273–6.

[17] Robinson J, Lemay M, Vaudry WL. Congenital Rubella after anticipated maternal immunity: two cases and a review of the literature. Pediatr Infect Dis J 1994;13(9):812–5.

[18] Zhou QJ, Acharya G, Zhang SK, Wang QM, Shen HP, Li XT. A new perspective on universal preconception care in China. Acta Obstet Gynecol Scand 2016;95(4):377–81.

[19] Zhang S, Wang Q, Sheng H. Design of the national free preconception health examination project in China. Zhonghua Yi Xue Za Zhi 2015;95:162–5.

[20] Wang Q, Zhang M, Zhang S, Hu M, Liu N, Zhang SX, et al. Establishment of quality assurance system of the national free preconception health care project in China. Zhonghua Yi Xue Za Zhi 2015;95:166–8.

[21] Zhou QJ, Zhang SK, Wang QM, Shen HP, Tian WD, Chen JQ, et al. China's community-based strategy of universal preconception care in rural areas at a population level using a novel risk classification system for stratifying couples' preconception health status. BMC Health Serv Res 2016;16:689.

[22] Liu J, Zhang S, Wang Q, Shen H, Zhang M, Zhang Y, et al. Seroepidemiology of hepatitis B virus infection in 2 million men aged 21–49 years in rural China: a population-based, cross-sectional study. Lancet Infect Dis 2016;16(1):80–6.

[23] Mou J, Griffiths SM, Fong HF, Hu Q, Xie X, He Y, et al. Seroprevalence of Rubella in female migrant factory workers in Shenzhen, China. Vaccine 2010;28 (50):7844–51. 23.

[24] Hisano M, Kato T, Inoue E, Sago H, Yamaguchi K. Evaluation of measles-Rubella vaccination for mothers in early puerperal phase. Vaccine 2016;34 (9):1208–14. 24.

[25] Vilajeliu A, García-Basteiro AL, Valencia S, Barreales S, Oliveras L, Calvente V, et al. Rubella susceptibility in pregnant women and results of a postpartum immunization strategy in Catalonia, Spain. Vaccine 2015;33(15):1767–72. 8.

Paper IV

Sero-epidemiology of Cytomegalovirus infection and its geographic and socio-economic determinants in preconception period among Chinese women planning a pregnancy within six months: A nationwide study

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Short title: Sero-epidemiology of Cytomegalovirus

Type of article: Original research article

Abstract

Background: The aim of our study was to investigate prevalence of Cytomegalovirus (CMV) sero-positivity, its geographic and socio-economic determinants.

Methods: This national, population-based, cross-sectional sero-epidemiological survey, conducted for women intending to get pregnant within six months in Mainland China between 2010-12. Socio-demographic information, including age, place of residence, education level and occupation, was obtained. Venous blood samples were tested for CMV immunoglobulin G (IgG) and immunoglobulin M (IgM). Associations between CMV serology and geographic variables were tested using multivariate correlation analysis and Spearman correlation test was used to explore the association of CMV serology with economic determinants.

Results: Among 2,019,555 women enrolled, 42.1% (850,592) were CMV IgG positive and 0.4% (9,290) were IgM positive. Geographical variation of IgG positivity ranged from 20.1% in Shaanxi to 97.5% in Zhejiang; the highest IgM positive rate (2.3%) was observed in Liaoning and lowest rate in Heilongjiang (0.1%) and Tibet (0%). CMV sero-positivity was associated with province of residence after adjusting for age, education level and occupation in a multivariate correlation analysis ($P<0.0001$).

Conclusions: More than half of married women planning a pregnancy were susceptible to CMV infection with a significant regional variation. Provincial CMV IgG sero-positivity correlated positively with women´s resident consumption level.

Key words: Cytomegalovirus, Preconception period, Sero-epidemiology, Congenital infection

1. Background

Cytomegalovirus (CMV) is the most common congenital virus infection [1], with 0.64% of infants born with congenital CMV infection (i.e. have virus in urine or saliva within three weeks after birth) in developed countries. Approximately 970000 women of childbearing age experience a primary CMV infection each year in the United States [2]. Sequelae of congenital CMV infection can be serious with substantial risk of perinatal mortality and long-term neurodevelopmental disorders. Maternal immunity is beneficial as the presence of maternal CMV antibodies significantly decreases the risk of fetal infection [3]. It is suggested that women should consider delaying conception for at least 6 months after primary infection to prevent consequences of congenital infection, as effective treatment during pregnancy is still lacking, and prenatal management of CMV infection is confounded by ethical and practical difficulties [4].

CMV immunoglobulin G (IgG) and immunoglobulin M (IgM) are used as serological markers. Women who are positive for CMV IgG are considered at low risk of infection while those who are IgG negative are susceptible to infection. Detection of IgM may indicate recent infection. CMV sero-prevalence differs by race and ethnicity, and substantial regional variations are observed. In the United States, CMV sero-positivity is higher in non-Hispanic black and Mexican Americans compared with their non-Hispanic white counterparts [2]. In China, pregnant women were reported to have 98.7% IgG positivity in Jiangsu province [5]; however, nationwide estimates are not available.

Studies on prevalence of CMV sero-positivity in the preconception period are scarce and whether identification of susceptible women before pregnancy and subsequent preventive strategies could reduce vertical transmission has not been properly explored. A recent study in a group of women undergoing fertility treatment showed that preconception testing and counselling to minimize exposure to CMV by improving personal hygiene [6] may have a positive effect. Also, CMV vaccines could be a future option for eliminating maternal-fetal transmission, though vaccines are not available currently and are still under clinical development [7]. Therefore, preconception screening may be useful in reducing the burden of congenital CMV, at least in areas with high susceptibility rates.

Thus, the objective of our study was to investigate the prevalence as well as geographic and socio-economic disparity of CMV sero-positivity among Chinese married women planning a pregnancy within six months.

2. Methods

Study design and setting

This study utilized data from National Free Preconception Health Examination Project (NFPHEP) conducted between 2010–12 in 220 counties located in 31 provinces and province level municipalities of the mainland China [8]. Detailed design, organization, implementation and quality control of this project were described elsewhere [8-12]. In NFPHEP, women intending to get pregnant within six months were provided free preconception health care. Our study population was extracted from this national database, and those failed to complete the preconception health examination or did not receive cytomegalovirus IgG serology testing were excluded for further analysis. The Institutional Review Board of Chinese Association of Maternal and Child Health Studies approved the project (IRB201001), and a written informed consent was obtained from each participant before enrollment.

Procedures

Results of CMV serology and socio-demographic information were extracted for analysis from the central database. Age, place of residence, education and occupation were collected using a standardized questionnaire. All participants were grouped into 31 provinces and province level municipalities (Beijing, Tianjin, Hebei, Shanxi, Inner Mongolia, Liaoning, Jilin, Heilongjiang, Shanghai, Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, Shandong, Henan, Hubei, Hunan, Guangdong, Guangxi, Hainan, Chongqing, Sichuan, Guizhou, Yunnan, Tibet, Shaanxi, Gansu, Qinghai, Ningxia and Xinjiang) according to their residential address. Considering that the economic level of each province was relatively stable in the last decade and it would take a long time to have an observable effect on CMV prevalence, gross domestic product (GDP) and resident consumption level in 2010, based on National Bureau of Statistics, were used for assessing the provincial economic level. GDP and resident consumption level in 2010 were extracted from the

Chinese national database of the National Bureau of Statistics (http://data.stats.gov.cn/).

Five mL of venous blood was collected from each participant and samples were stored at -30°C. All serum specimens were analyzed for CMV IgG and IgM using commercially available enzyme immuno-assay kits for detection of IgG and IgM antibodies according to the manufacturer's instructions in local laboratories [9]. The reagent kits approved by the China Food and Drug Administration were selected by the local laboratories based on their preference. However, a detailed quality control system was in place to ensure that the diagnostic capability of the test kits was comparable. A series of official documents were published on standards of sampling, storage and transporting of clinical samples, quality control protocols, and standards of clinical testing [9]. The National Center of Clinical Laboratories for Quality Inspection and Detection performed an external quality assessment twice yearly as described previously [9]. Cytomegalovirus serology was interpreted as follows: (1) women with positive IgG and negative IgM were considered to have had previous infection/immunization and to be at low risk; (2) those with negative IgG and negative IgM were susceptible and advised to obtain health education before conception; (3) those with positive IgM and positive or negative IgG were referred to specialist for further diagnostic examination and treatment.

Statistical analysis

We calculated numbers and proportions to describe socio-demographic variables as appropriate. Multivariate correlation analysis was applied for analyzing the association between CMV serology (IgG and IgM) and geographic variable adjusted by maternal age, education level and occupation. The Spearman correlation analysis was used to evaluate the strength and direction of the association of CMV serology (IgG and IgM) status with GDP and resident consumption level, as these variables had skewed distribution. Spearman's rank correlation coefficient and P-value were applied to assess the significance. IBM SPSS Statistics version 24.0. (IBM Corp., Armonk, NY) was used for statistical analysis.

3. Results

During January 1st 2010 to December 31st 2012, a total of 2,142,903 married women with intention to conceive within six months were enrolled. A total of 2,019,555 (94.2%) women who had their CMV serology tested were included in final analysis. The rate of CMV IgG sero-positivity was 42.1% (n=850,592), indicating that 57.9% women were at risk of acquiring CMV infection before conception/during pregnancy or had been recently infected. The rate of CMV IgM sero-positivity was 0.4% (n=9,290). Women who were CMV IgG negative had lower education level (below college), and women who were older than 30 years had higher rates of IgM sero-positivity ($P<0.0001$) (Table 1).

To determine the geographic variation, CMV serology results were grouped into 31 provinces and province level municipalities according to residential address of the participants. Substantial regional differences were noticed: provincial difference in cytomegalovirus IgG sero-positivity ranged from 20.1% in Shaanxi Province to 97.5% in Zhejiang, besides the extreme low rate of 0% in Tibet and 0.2% in Heilongjiang. The highest IgM positive rate was observed in Liaoning (2.3%), and lowest rate was in Heilongjiang (0.1%) and Tibet (0%) (Figure 1). CMV serology results (IgG and IgM) were significantly associated with province of residence after adjusting for maternal age, education level and occupation by multivariate correlation analysis ($P<0.0001$), indicating that geographic factor contributed to CMV serology.

Analysis of correlation between CMV serology and socioeconomic factors demonstrated that provincial CMV IgG sero-positivity was statistically significantly correlated with resident consumption level ($r=0.437$; $P=0.014$), and but not with GDP ($r =0.167$; $P=0.369$). CMV IgM sero-positivity was neither significantly associated with GDP ($r=0.229$; $P=0.216$) nor with resident consumption level ($r=0.049$; $P=0.794$) (Table 2).

4. Discussion

This nationwide study demonstrated geographic variation in CMV sero-positivity in preconception period among Chinese women planning a pregnancy within six months, and resident consumption level was correlated with provincial CMV IgG serology status.

To our knowledge, this is the largest study describing sero-epidemiology of CMV infection in the preconception period in China. The main strength of this study is that it covered all 31 provinces in the Chinese mainland and had 5.8% (123,350/2,142,905) missing data. However, our study does have some limitations. Only 54 women were enrolled in Tibet, and thus the 0% positivity rate in Tibet could possibly be due to selection bias. The reagents and kits used varied based on local preference, which may have caused some variations in prevalence of sero-positivity, although only the kits that were approved by the China Food and Drug Administration were used, and several quality control mechanisms were in place to ensure that appropriate procedures were followed for sampling, transport and storage of clinical samples as well as their laboratory testing and reporting in accordance with the national standards. Therefore, we believe that the detection rate of sero-positivity of CMV IgG and IgM as a categorical variable was comparable across different laboratories even if the quantitative levels of antibodies and cut-off values used might have varied.

Women who are susceptible to CMV infection can be identified by serological testing before pregnancy. In this study, we found that 58% women were at risk of CMV infection before conception. Avoiding CMV exposure during pregnancy is difficult. Therefore, preconception screening might be useful as preventive strategies such as counseling to minimize exposure to CMV by improving personal hygiene, reducing contact with children in day care etc, could be implemented. It also allows postponing pregnancy when acute infection is diagnosed. As viral transmission from susceptible mothers is the main source of congenital infection, vaccine administered to adult women could reduce the burden of CMV by making them immune prior to pregnancy [13,14]. There is growing evidence that CMV vaccination can protect adults and children from infection, with largely known vaccination targets and defined path for licensure [13-15]. Offering effective vaccination to susceptible women before conception could be possible in near future.

Our study indicated a significant geographic disparity in the prevalence CMV sero-positivity. Cultural and social values associated with personal habits regarding hygiene, unbalanced economic development, population density, and disparity in access to health care could explain some of these differences. Interestingly, in our study, the rate of IgG sero-positivity in preconception period in Jiangsu province was 47.6%, which is substantially lower than that reported among pregnant women (98.7%) from the same province [10]. Although this difference can be explained by differences in the populations studied (women planning pregnancy vs pregnant women), it does suggest that the risk of sero-conversion during pregnancy is high.

Our study showed that the CMV serology status is associated with socio-economic factors. We found a significant correlation between provincial CMV IgG sero-positivity and resident consumption level, and GDP correlated with both CMV IgG and IgM sero-positivity. To our knowledge, this is the first study reporting on the socio-economic determinants of CMV infection in China. Further research should focus on developing preventive strategies taking into account geographic variation as well as resident consumption level.

5. Conclusions

In summary, overall prevalence of CMV sero-positivity in preconception period in rural China was 42.5% with a substantial geographic variation and socio-economic determinants. More than half of the women planning a pregnancy within six months were susceptible to CMV infection indicating that the risk of congenital CMV infection is high in rural China. Provincial CMV IgG sero-positivity correlated positively with women´s resident consumption level. Whether identification of sero-negative women before pregnancy and applying preventive strategies could reduce congenital CMV infection merits further investigation.

Declarations

Ethics approval and consent to participate

The Institutional Review Board of Chinese Association of Maternal and Child Health Studies approved the project (IRB201001), and a written informed consent was obtained from each participant before enrollment.

Consent for publication

All the authors are consent for publication.

Availability of data and material

Dataset analyzed in this study was based on the national database and public access to the database is closed. Zhang Shikun gave the administrative permission to access the database on behalf of National Health and Family Planning Commission of the People's Republic of China (NHFPC).

Competing interests

The authors report no conflict of interest.

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Authors' contributions

ZQ and LX carried out the statistical analysis and drafted the manuscript. GA interpreted data and drafted the manuscript. ZS, WQ, SH and LX participated in the design of the study and coordination. All authors read and approved the final manuscript.

Acknowledgments

The views expressed in this report are those of the authors and do not necessarily reflect the official policy or position of the Department of Maternal and Child Health of National Health and

Family Planning Commission (NHFPC) in China. We thank health workers in 220 counties of 31 provinces for their help in recruiting study participants and data collaboration.

Disclosure of interest

The authors report no conflict of interest.

References:

1. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007;17(4):253-76.
2. Staras SA, Dollard SC, Radford KW, et al. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis. 2006;1;43(9):1143-51.
3. Fowler KB, Stagno S, Pass RF. Maternal immunity and prevention of congenital cytomegalovirus infection. JAMA. 2003;26;289(8):1008-11.
4. Revello MG, Zavattoni M, Furione M, et al. Preconceptional primary human cytomegalovirus infection and risk of congenital infection. J Infect Dis. 2006;15;193(6):783-7.
5. Zhang S, Hu L, Chen J, et al. Cytomegalovirus seroprevalence in pregnant women and association with adverse pregnancy/neonatal outcomes in Jiangsu Province, China. PLoS One. 2014;11;9(9):e107645.
6. Reichman O, Miskin, I, Sharoni, L, et al. Preconcpetion screening for Cytomegalovirus: an effective preventive approach. Biomed Res Int. 2014;2014:135416.
7. Pass RF, Zhang C, Evans A, et al. Vaccine prevention of maternal cytomegalovirus infection. N Engl J Med. 2009;19;360(12):1191-9.
8. Zhou QJ, Acharya G, Zhang SK, et al. A new perspective on universal preconception care in China. Acta Obstet Gynecol Scand. 2016;95(4):377-81.
9. Wang Q, Zhang M, Zhang S, et al. Establishment of quality assurance system of the National Free Preconception Health Care Project in China. Zhonghua Yi XueZaZhi. 2015;95:166-8.
10. Zhang S, Wang Q, Sheng H. Design of the National Free Preconception Health Examination Project in China. Zhonghua Yi XueZaZhi. 2015;95:162-5.
11. Zhou QJ, Zhang SK, Wang QM, et al. China's community-based strategy of universal

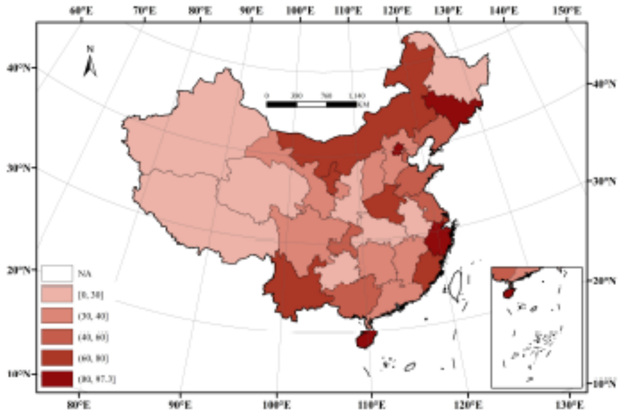
preconception care in rural areas at a population level using a novel risk classification system for stratifying couples´ preconception health status. BMC Health Services Research. 2016;16:689.

12. Liu J, Zhang S, Wang Q, et al. Seroepidemiology of hepatitis B virus infection in 2 million men aged 21–49 years in rural China: a population-based, cross-sectional study. Lancet Infect Dis. 2016;16(1):80-6.
13. Plotkin S. The history of vaccination against cytomegalovirus. Med Microbiol Immunol. 2015;204(3):247-54.
14. McCormick AL, Mocarski ES. The immunological underpinnings of vaccinations to prevent cytomegalovirus disease. Cell Mol Immunol. 2015;12(2):170-9.
15. Krause PR, Bialek SR, Boppana SB, et al. Priorities for CMV vaccine development. Vaccine. 2013;17;32(1):4-10.
16. Mustakangas P, Sarna S, Ammälä P, et al. Human cytomegalovirus seroprevalence in three socioeconomically different urban areas during the first trimester: a population based cohort study. Int J Epidemiol 2000; 29:587

Figure legends

Figure 1. Cytomegalovirus serology by provinces in China.

(A) CMV IgG;



(B) CMV IgM

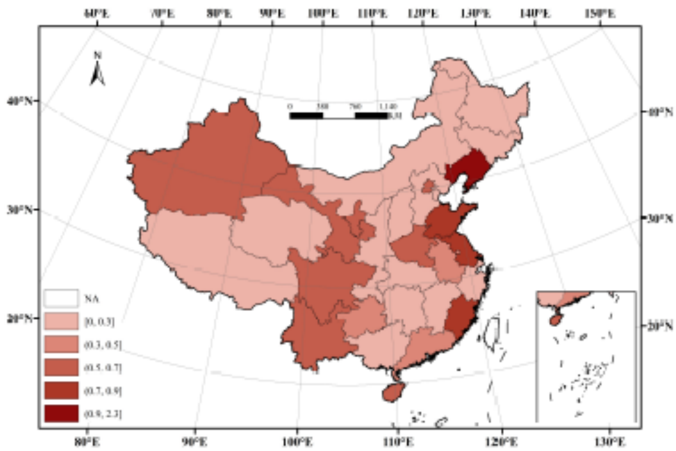


Table 1. Demographic characteristics of women with positive and negative cytomegalovirus serology.

	IgG		IgM	
	(-) n=1,168,963	(+) n=850,592	(-) n=2,010,265	(+) n=9,290
Age (years)				
21-29	70.1% (804,671)	69.5% (583,837)	69.9% (1,383,120)	66.1% (5,388)
30-39	26.4% (303,366)	27.0% (227,243)	26.7% (528,210)	29.4% (2,399)
40-49	3.4% (39,085)	3.5% (29,125)	3.4% (67,841)	4.5% (369)
Total	1,147,122	840,205	1,979,171	8,156
Occupation				
Farmers	79.1% (908,136)	77.1% (645,582)	78.2% (1,555,217)	77.1% (7,050)
Workers	8.5% (97,916)	7.6% (63,719)	8.1% (162,097)	8.6% (788)
Others	12.4% (142,712)	15.3% (127,899)	13.7% (272,208)	14.3% (1,308)
Total	1,148,764	837,200	1,989,522	9,146
Education				
Non-educated	0.3%(3,109)	0.2%(2,063)	0.3%(5,175)	0.4%(38)
Primary school	4.9% (57,171)	4.6% (39,056)	4.8% (96,428)	5.5% (507)
Junior high school	68.2% (788,380)	65.2% (549,901)	66.9% (1,339,186)	64.8% (5,972)
Senior high school	17.9% (98,447)	17.1% (144,586)	17.6% (352,765)	17.8% (1,639)
College or higher	8.6% (100,177)	12.7% (107,636)	10.4% (209,097)	11.5% (1,055)
Total	1,047,284	843,242	2,002,651	9,211

Data were presented as percentage (number).

Table 2. Socio-economic determinants of cytomegalovirus serology by provinces in China.

	Total number	IgG (+)		IgM (+)		GDP*	Resident consumption level**
		Number	%	Number	%		
Beijing	9,272	8137	87.7%	59	0.6%	14,113.58	24,982
Tianjing	10,108	4270	42.2%	16	0.2%	9,224.46	17,852
Hebei	162,966	55704	34.2%	286	0.2%	20,394.26	8,057
Shanxi	25,018	9663	38.6%	49	0.2%	92,00.86	8,447
Inner Mongolia	12,160	9,543	78.5%	33	0.3%	11,672	10,925
Liaoning	17,430	8,532	49.0%	409	2.3%	18,457.27	13,016
Jilin	96,137	91,742	95.4%	323	0.3%	8,667.58	9,241
Heilongjiang	5,365	10	0.2%	7	0.1%	10,368.6	9,121
Shanghai	2,744	1,726	62.9%	9	0.3%	17,165.98	32,271
Jiangsu	77,169	35,214	47.6%	717	0.9%	41,425.48	14,035
Zhejiang	27,702	27,000	97.5%	54	0.2%	27,722.31	18,274
Anhui	89,874	25,724	28.6%	329	0.4%	12,359.33	8,237
Fujian	40,099	28,501	71.1%	360	0.9%	14,737.12	13,187
Jiangxi	36,227	11,895	32.8%	115	0.3%	9,451.26	7,989
Shandong	84,408	41,511	49.2%	726	0.9%	39,169.92	11,606
Henan	188,636	122,585	65.0%	1,244	0.7%	23,092.36	7,837
Hubei	215,468	51,321	23.8%	436	0.2%	15,967.61	8,977
Hunan	178,949	61,038	34.1%	550	0.3%	16,037.96	8,922
Guangdong	187,525	56,608	30.2%	885	0.5%	46,013.06	17,211
Guangxi	62,093	34,766	56.0%	155	0.3%	9,569.85	7,920
Hainan	10,635	9,448	88.8%	73	0.7%	2,064.5	7,553
Chongqing	68,723	27,837	40.5%	170	0.2%	7,925.58	9,723
Sichuan	87,563	26,478	30.2%	624	0.7%	17,185.48	8,182
Guizhou	51,491	12,310	23.9%	242	0.5%	4,602.16	6,218
Yunnan	55,471	33,489	60.4%	332	0.6%	7,224.18	6,811
Tibet***	54	0	0.0%	0	0.0%	507.46	4,469
Shaanxi	107,756	21,627	20.1%	321	0.3%	10,123.48	8,474
Gansu	24,407	9,286	38.0%	210	0.7%	4,120.75	6,234
Qinghai	11,564	2,807	24.3%	18	0.2%	1,350.43	7,326
Ningxia	6,234	4,651	74.6%	43	0.7%	1,689.65	8,992
Xinjiang	66,305	17,169	25.9%	495	0.7%	5,437.47	7,400
Total	2,019,555	850,592	42.1%	9,290	0.5%	410,354.1	10,919

*GDP (100 million Yuan) data from http://data.stats.gov.cn/ for 2010.

** Resident consumption level (Yuan) data from http://data.stats.gov.cn/ for 2010. Resident consumption level = total consumption of residents in GDP/ average annual population in 2010.

***Few participants in Tibet and there possibly exists selective bias for the low Cytomegalovirus IgG (+) and IgM (+) rates.

SUPPLEMENTARY MATERIALS

English-translated Consent Form and Record Sample of the National Preconception Health Examination Project

Informed Consent for the National Preconception Health Examination Project

The Chinese government provides a free preconception health examination for all the married couples in rural areas, aiming for a healthier baby and happier family.

The National Preconception Health Examination Project (NPHCP) is provided 4-6 months before a planning pregnancy. The contents include health education, medical history collecting, physical examination, clinical laboratory tests, imaging test, risk evaluation and medical advice. The purpose is to find the risk factors leading to potential adverse outcomes as early as possible, to help the couples to know their health status and obtain a comprehensive health advise. It is beneficial for the married couples to achieve pregnancy in an ideal psychological and physiological status to prevent of birth defects and have a healthy baby.

NPHCP focuses on identifying the most common and most important risk factors before conception that reflect the couple's current health status. There still exists a possibility of birth defects or other adverse pregnancy outcomes (e.g. miscarriage, stillbirth, etc.), whether the preconception examination is normal or not. You are required to have a regular antenatal care during pregnancy.

If you're willing to participate in this project, please sign your name on this informed consent. Your personal information is will be kept strictly anonymous.

I have read the information described above and understood completely. I am willing to participate in the National Preconception Health Examination Project (NPHCP) and accept the follow-ups.

Signatures:

Husband: Date:

Wife: Date:

Doctor: Date:

No. □□□□□□□□□□□□□

Records of the National Preconception Health Examination Project

County authority：____Province____County

Village/town authority：____Province ____County____Village (town)

Form 1. Basic information

Husband's Name____ Race___ Birth of Date___Age___ Education level

ID □□□□□□□□□□□□□□□□□□

Job □ 1.Farmer 2. Worker 3. Service provider 4. Business 5. Househusband 6. Teacher/officer/clerk 7. Others

Residence ___Province__City___County___Town___Village

□ 1.Rural 2. Urban

Wife's Name____ Race___ Birth of Date___Age___ Education level

ID □□□□□□□□□□□□□□□□□□

Job □ 1. Farmer 2. Worker 3. Service provider 4. Business 5. Housewife

6. Teacher/officer/clerk 7. Others

Residence ___Province__City___County___Town___Village

□ 1.Rural 2. Urban

Zip______ Marriage time______ Telephone

Doctor's signature：__________ Date：

Form 2. Wife's preconception Examination

General information

Disease history:

□None □Anemia □Hypertension □Heart disease □Diabetes mellitus

□Epilepsy □Thyroid disease □Chronic renal disease □Tumor □Tubertulous

□Hepatitis virus B □Gonorrhea/trichomoniasis □Psychological or mental diseases

Is there any birth defect, eg: congenital abnormality, genetic disease:

□No □Yes

Is there any gynecological disease:

□No □Infectious disease □Infertility □Others

History of drug use:

Do you take any drug now?

□No □Yes

Have you taken any immunization?

□No □Rubella □HBV □Others

Do you use any contraception?

□No □IUD □Implanon □Oral contraception □Condom

□External medication □Natural contraception □Others

Length of contraception ___ : months   Termination date:

Pregnancy history:

Age of first menstruation___years old   LMP date:

Is menstruation regular? □No □Yes（period_days length____days

Amount of menstruation □Many □Medium □Little

Dysmenorrhea □No □Light □Heavy

Did you ever be pregnant?

□No □Yes: Pregnancy___times, live birth__times (full term____times，preterm birth____times)

Did you have any adverse pregnancy outcome previously?

□No □Stillbirth/death birth □Spontaneous abortion □Artificial abortion

Did you ever deliver a baby with birth defect, eg: abnormalities, genetic disease, Down's syndrome

□No □Yes

Current child/children__ Health status: □healthy □any disease

Family history:

Do you have intermarriage with your husband?

□No □Yes

Did your grandparents or parents have intermarriage?

□No □Yes

Do your relatives have any following disease?

□No □Mediterranean □Albinis □Hemophilia □G6PD anemia m deficiency

□congenital heart disease □Down's syndrome □Diabetes mellitus □Congenital intelligent impairment

□Hearing impairment before 10 years old □Visual impairment before 10 years old

□Neonatal or infant death □Other birth defects

Relationship with me

Nutrition, behavior and environmental factors:

Intake of meat and egg □No □Yes

No intake of fresh vegetables □No □Yes

Raw meat eating habit □No □Yes

Smoking □No □Yes（___cigarettes/day）

Second-hand smoking □No □Sometimes □Often（____minutes/day）

Alcohol drinking □No □Sometimes □Often（____ml/day）

Drug use □No □Yes

Halitosis □No □Yes

Gingival bleeding □No □Yes

Is there any exposure to the following risks?

□No □Radiation □High temperature □Noise □Organic solvent

□Close contact with pets □Shaking □Heavy mental □Pesticide

□Others

Social and psychological factors:

Do you feel any pressure in work □No □Seldom □Sometimes □Often □Always

Do you have a tense relationship with friends or colleagues? □No □Less □To some extent □Often □always

Do you have any economic pressure □No □Less □To some extent □Often □Always

Do you feel ready for a pregnancy □No □Yes

Others

Date：____________ Doctor's signature：

Physical Examination

Height___cm

Weight____Kg

Body mass index____kg/m^2

Heart rate___beats/minute

Blood pressure__/___mmHg

☐ Psychological status 0. Normal 1. Abnormal

☐ Intelligence 0. Normal 1. Abnormal (□common sense □judgment □memory □calculation)

☐ Otorhinolaryngology 0. Normal 1. Abnormal

☐ Special facial features 0. Normal 1. Abnormal

☐ Skin and hair 0. Normal 1. Abnormal

☐ Thyroid 0. Normal 1. Abnormal

☐ Lung 0. Normal 1. Abnormal

□ Heart rhythm 0. Normal 1. Abnormal

□ Auscultation of heart 0. Normal 1. Abnormal

□ Liver and spleen 0. Normal 1. Abnormal

□ Spine and limbs 0. Normal 1. Abnormal

Others

Date： ____________ Doctor’s signature：

Reproductive features：

□ Pubic hair 0. Normal 1. Abnormal

□ Breast 0. Normal 1. Abnormal

Gynecological examination：

□ Vulva 0. Normal 1. Abnormal

□ Vagina 0. Normal 1. Abnormal

□ Vaginal discharge 0. Normal 1. Abnormal

□ Cervix 0. Normal 1. Abnormal

Uterus

□Size 0. Normal 1. Enlarged 2. Smaller

□Mass 0. No 1. Yes

□Ovary of fallopian tube 0. Normal 1. Abnormal

Date： ____________ Doctor’s signature：

Laboratory testing

Leucorrhoea test:

□Clue cell: 0.negative 1.positive 9.suspecious

□Candida infection: 0.negative 1.positive 9.suspecious

□Trichomoniasis: 0.negative 1.positive 9.suspecious

□Cleanness: 0.I 1.II 2.III 3.IV

□Amine odor test: 0.negative 1.positive

□PH value: 0. <4.5 1. ≥4.5

□Gonorrhea screening: 0.negative 1.positive 9.suspecious

□Chlamydia trachomatis screening: 0.negative 1.positive 9.suspecious

Blood routine:

Hb_____ g/L

RBC____ $\times 10^{12}$/L

PLT____ $\times 10^{9}$/L

WBC _ $\times 10^{9}$/L

Neutrophils _%

Eosinophils _%

Basophils _%

Lymphocytes _%

Monocysites _%

□ **Urine routine:**

0. Normal 1. Abnormal

Blood type:

□ABO 1. Type A 2. Type B 3. Type AB 4. Type O

□Rh 0. Positive 1. Negative

Fasting blood sugar level ____mmol/L

Hepatitis virus B 0. negative 1. positive 9. suspicious

□HBs-Ag □HBs-Ab □HBe-Ag □HBe-Ab □HBc-Ab

Liver and renal function tests:

Alanine aminotransferase（ALT）______U/L

Creatitine（Cr）_________umol/L

Thyroid function test:

Thyroid stimulating hormone（TSH） _____________________ulU/ml

□ **Rubella virus**   IgG: 0. negative 1. positive 9. suspicious

□ **Rapid plasma regain** 0. negative 1. positive 9. suspicious

Cytomegalovirus □ IgG: 0. negative 1. positive 9. suspicious   □IgM 0. negative 1. positive 9. suspicious

Toxoplasma □ IgG: 0. negative 1. positive 9. suspicious   □IgM 0. negative 1. positive 9. suspicious

Others:

Date： ____________   Doctor’s signature：

Gynecological imaging examination

□ Gynecological ultrasound

0. Normal 1. Abnormal

Ultrasound No.

Date：____________ Doctor’s signature：

Other examinations

(Based on local authority)

Main Findlings:

Date：____________ Doctor’s signature：

Form 3. Husband's preconception Examination

General information

Disease history:

□None □Anemia □Hypertension □Heart disease □Diabetes mellitus

□Epilepsy □Thyroid disease □Chronic renal disease □Tumor □Tubertulous

□Hepatitis virus B □Gonorrhea/trichomoniasis □Psychological or mental diseases

Is there any birth defect, eg: congenital abnormality, genetic disease:

□No □Yes

Is there any disease of reproductive system:

□No □Infectious disease □Infertility □Others

History of drug use:

Do you take any drug now?

□No □Yes

Have you taken any immunization?

□No □Rubella □HBV □Others

Do you use any contraception?

□No □IUD □Implanon □Oral contraception □Condom

□External medication □Natural contraception □Others

Length of contraception ___ : months   Termination date:

Family disease:

Did your grandparents or parents have intermarriage?

□No   □Yes

Do your relatives have any of following disease?

□No	□Mediterranean anemia	□Albinism	□Hemophilia	□G6PD deficiency
□Congenital heart disease	□Down's syndrome	□Diabetes mellitus	□Congenital intelligent impairment	

□Hearing impairment before 10 years old   □Visual impairment before 10 years old

□Neonatal or infant death   □Other birth defects

Relationship with me

Nutrition, behavior and environmental factors:

Intake of meat and egg □No □Yes

No intake of fresh vegetables □No □Yes

Raw meat eating habit □No □Yes

Smoking □No □Yes（___cigarettes/day）

Second-hand smoking □No □Sometimes □Often（____minutes/day）

Alcohol drinking □No □Sometimes □Often（____ml/day）

Drug use □No □Yes

Halitosis □No □Yes

Gingival bleeding □No □Yes

Is there any exposure to the following risks?

□No □Radiation □High temperature □Noise □Organic solvent

□Close contact with pets □Shaking □Heavy mental □Pesticide

□Others

Social and psychological factors:

Do you feel any pressure in work □No □Seldom □Sometimes □Often □Always

Do you have a tense relationship with friends or colleagues? □No □Less □To some extent □Often □always

Do you have any economic pressure □No □Less □To some extent □Often □Always

Do you get ready for a pregnancy □No □Yes

Others

Date： ____________ Doctor’s signature：

Physical Examination

Height___cm

Weight____Kg

Body mass index____kg/m^2

Heart rate___beats/minute

Blood pressure__/__mmHg

☐ Psychological status 0. Normal 1. Abnormal

☐ Intelligence 0. Normal 1. Abnormal (□common sense □judgment □memory □calculation)

□ Otorhinolaryngology 0. Normal 1. Abnormal

□ Special facial feature 0. Normal 1. Abnormal

□ Skin and hair 0. Normal 1. Abnormal

□ Thyroid 0. Normal 1. Abnormal

□ Lung 0. Normal 1. Abnormal

□ Heart rhythm 0. Normal 1. Abnormal

□ Auscultation of heart 0. Normal 1. Abnormal

□ Liver and spleen 0. Normal 1. Abnormal

□ Spine and limbs 0. Normal 1. Abnormal

Others

Date： ____________ Doctor's signature：

Reproductive features：

□ Pubic hair 0. Normal 1. Abnormal

□ Laryngeal prominence 0. Normal 1. Abnormal

Andrology examination：

□ Penis 0. Normal 1. Abnormal

□ Foreskin 0. Normal 1. Abnormal

□ Testicle 0. Palpable, volume (ml) left__right___1. Unpalpable on left side 2. Unpalpable on right side

□ Epididymis 0. Normal 1. Abnormal

□ Vas deferens 0. Normal 1. Abnormal

☐ Vericocele 0. No 1. Yes, site____degree

Date: ____________ Doctor's signature:

Lab testing

☐ **Urine routine:**

0. Normal 1. Abnormal

Blood type:

□ABO 1. Type A 2. Type B 3. Type AB 4. Type O

□Rh 0. Positive 1. Negative

Hepatitis virus B 0. Negative 1. Positive 9. Suspicious

☐HBs-Ag ☐HBs-Ab ☐HBe-Ag ☐HBe-Ab ☐HBc-Ab

Liver and renal function tests:

Alanine aminotransferase（ALT）______U/L

Creatitine（Cr）_________umol/L

☐ **Rapid plasma regain** 0. Negative 1. Positive 9. Suspicious

Others:

Date: ____________ Doctor's signature:

Other examinations

(Based on local authority)

Main Findlings:

Date: ____________ Doctor's signature:

No. □□□□□□□□□□□□□

Preconception Health Assessment

(1st copy for participants, 2nd copy for county-based health authority, 3rd copy for village/town-based health authority)

Name of wife______ Age ______ Telephone

Name of husband____ Age ______ Telephone

Address____Province___County____Town___Village

□1. Both of the couples have accepted preconception health examination, and there is no pre-existing risk factor. Regular health education and advice is recommended.

Recommendations:

□2. Either of the couple has accepted preconception health examination, and there is no pre-existing risk factor. Your wife/husband is recommended to accept the preconception health care.

Recommendations:

□3.There are some pre-existing risk factors before conception. Further medical care is recommended.

Recommendations:

Date： ____________ Doctor's signature：

Participants： Wife___________Date： ____________ Husband_________Date：

No. □□□□□□□□□□□□□

Follow-up records in early pregnancy

(for county-based health authority)

Name of wife______ Age ______ Telephone

Home Address____Province___County____Town___Village

Follow-up authority:____Province___County

Date of last menstruation period (LMP)

□Is last menstruation period correct 0 No 1 Yes

□Timing of folic acid supplement 0. No 1. at least 3 months before LMP 2. 1-2 months before LMP 3. After LMP

□Method of folic acid supplement 0. No 1. Regularly 2. Irregularly

Intake of meat and egg □No □Yes

No intake of fresh vegetables □No □Yes

□Has your husband stopped smoking? 0. No 1. Stopped 2. Decreased 3. Unchanged 4. Increased

□Have you stopped smoking? 0. No 1. Stopped 2. Decreased 3. Unchanged 4. Increased

□Have you stopped drinking? 0. No 1. Stopped 2. Decreased 3. Unchanged 4. Increased

□Have there existed the following risk factors? 0. No 1. Yes

□exposure to cat or dog

□pesticide

□radiation

□second-hand smoking

□others

☐ Have there existed the following symptoms? 0. No 1. Yes

□vaginal bleeding

□fever equal to 38.5°C or above

□diarrhea

□abdominal pain

□influenza

□viral hepatitis

□others

☐Have you taken any drug after conception? 0. No 1. Yes

☐The medical authority you have confirmed pregnancy with

1. This medical authority

2. Referral to other medical authority

□County-based medical service authority

□County-base family planning service authority

□Village or town based medical service authority

□Village or town based family planning service authority

□Other authority___________________)

☐Urinary pregnancy test 0. I have not taken this test 1. Positive 2. Negative 3. Inconclusive

☐Ultrasound 0. I have not taken this test 1. Pregnant 2. Non-pregnant 3. Other findings

☐Your comments on preconception health examination 0. Very satisfactory 1. Satisfactory 2. Medium 3. Bad 4. Very bad

Date： ____________ Doctor’s signature：

No. □□□□□□□□□□□□□

Follow-up records of pregnancy outcomes

(for county-based health authority)

Name of wife______ Age ______ Telephone

Home Address____Province___County____Town___Village

Follow-up authority:____Province___County

□□□ Pregnancy outcome:

1. Normal live birth

2 Preterm birth

3. Low birth weight

4. Birth defects (please complete the form of birth defects records)

5. Spontaneous abortion

6. Medical abortion

7. Surgically induced abortion

8. Ectopic pregnancy

9. Stillbirth or neonatal death

10. Other

If you have chosen 1, 2, 3, 4, 7, 9 for the question above, please continue to complete the following items:

□Sex of baby 1. Male 2. Female 3. Hermaphroditism 4. Unknown

Birthweight____g

□ Was it a multiple pregnancy? 1.Yes   2. No

Date of delivery

Gestational week at delivery

Delivery site_____Province______County

□ Site of delivery   1. Medical authority   2. At home 3. Other

□ Delivery method 1. Vaginal delivery   2. Vaginal assisted delivery 3. Cesarean section 4. Other

□ Health status of the baby within 42 day postpartum

0. Not live birth   1. Alive 2. Neonatal death within 7 days after delivery 3. Neonatal death 8-28 days after delivery 4. Neonatal death 28 days after delivery

Date：____________   Doctor's signature：

No. □□□□□□□□□□□□□

Follow-up records of birth defects
(for county-based health authority)

1. Family information Father Name______Age____(years) Race______ID No. Mother Name_____Age____(years) Race______ID No. Pregnancy_______ Parity □ Residence 1.Urban 2.Rural Home address Zip Telephone
2． Baby's information Date of birth Sex of baby 1. male 2. female 3. hermaphroditism 4. unknown Delivery week Birthweight____g □Number of baby 1. Singleton 2. Twin pregnancy 3. Multiple pregnancy with triplets or higher order □Outcome 1. Alive 2. Neonatal death within 7 days after delivery 3. Neonatal death 8-28 days after delivery 4. Neonatal death 28 days after delivery Diagnosis criteria □clinical □ultrasound □autopsy □alpha fetoprotein □chromosome □others

□timing of diagnosis 1. Prenatal 2. Within 7 days after delivery 3. After 7 days postpartum
3. Birth defects 01 Anencephaly………………□ 16 Left syndactylia …………..□ 02 Spina bifida……………………□ Right syndactylia …………..□ 03 Encephalocele…………………□ 17 Limb shortening 04 Congenital hydrocephalus……□ Left upper limb………….. □ 05 Cleft palate……………………□ Right upper limb ………….. □ 06 Cleft lip…………………………□ Left lower limb……………..□ 07 Cleft lip and cleft palate……□ Right lower limb …………..□ 08 Microtia…………………………□ 18 Congenital diaphragmatic hernia □ 09 Other abnormality of ear………□ 19 Exomphalus……………… □ 10 Atresia or stricture of esophagus□ 20 Gastroschisis……………… □ 11 Atresia or stricture of anorectal□ 21 Conjoined twins………….. □ 12 Hypospadias……………………□ 22 Down's syndrome………..□ 13 Ectropion of bladder…………□ 23 Congenital heart disease…… □ 14 Left clubfoot…………………□ 24 Others……………………..□ Right clubfoot………………□ 15 Left polydactylism…………..□ Right polydactylism …………..□
4. Health status in early pregnancy

Disease history	Drug use	
□Fever（＞38.5°C）	□Sulfanilamide	Exposure to harmful substance
□Rubella	□Antiboitics	□Pesticide
□Cytomagalovirus	□Contraception	□Radiation
□Hepatitis（type ）	□Sedative	□Alcohol
□Others	□Others	□Chemical substance
		□Others
5．□Diagnosis in		
（1）Provincial hospitals （2）City-based （3）County based （4）others		

Date：_______ Authority:_____ Doctor’s signature：