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Pregnancy Outcome in Swiss-Webster Mice Infected With *Chlamydia trachomatis*

Bryan T. Oshiro, Jack M. Graham, Jorge D. Blanco, Ibrahim M. Seraj, and Karen D. Bishop

Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Texas Health Science Center, Houston, TX (B.T.O., J.M.G., J.D.B., K.D.B.), and Department of Obstetrics and Gynecology, Loma Linda University, Loma Linda, CA (I.M.S.)

ABSTRACT

Objective: **The objective of this study was to observe pregnancy outcomes in mice infected transvaginally with *Chlamydia trachomatis*.**

Methods: **Pregnant mice were inoculated transvaginally with either *C. trachomatis* (CT) or sterile calf serum (CON) on pregnancy day 4. Pregnancy outcomes as well as genital tract histology and culture were compared. Statistical analysis was performed using Fisher's exact test and Student's t-test.**

Results: **Twenty-four of 26 CT mice had positive uterine cultures for *C. trachomatis*. Inflammation occurred in 9 (34.6%) ($P = 0.002$, 95% confidence interval = 1.7–3.5) and intrauterine fetal demise occurred in 5 (19.2%) ($P = 0.05$, 95% confidence interval = 1.6–2.9) of CT mice. No mice in the CON group (0/24) had positive uterine cultures, developed inflammation, or experienced intrauterine fetal demise.**

Conclusions: **Lower genital tract chlamydial infection is associated with intrauterine fetal demise in Swiss-Webster mice.** © 1994 Wiley-Liss, Inc.

KEY WORDS

Chlamydia trachomatis, fetal demise, Swiss-Webster mice

Chlamydia trachomatis is recognized as the most common sexually transmitted organism, with over 4 million cases reported annually.[1] *C. trachomatis* is responsible for causing inclusion conjunctivitis and pneumonia in the neonate and is the leading cause of preventable blindness in the world.[2]

Although *C. trachomatis* has clearly been shown to cause mucopurulent cervicitis,[3] acute salpingitis,[4] and neonatal disease, its effect on pregnancy outcome remains controversial. Martin and colleagues[5] reported that the incidence of prematurity and perinatal death was higher in women with a *C. trachomatis* infection identified before 20 weeks. In contrast, other authors[6,7] have not found an association between *C. trachomatis* infection and adverse pregnancy outcome.

The Swiss-Webster mouse has been used extensively to study the effects of *C. trachomatis* on the female genital tract.[8–10] Rank et al.[11] have reported infecting mice transvaginally with *C. trachomatis*. However, to our knowledge, an animal model has not been developed to study the effects of *C. trachomatis* lower genital tract infection on pregnancy outcome.

To determine whether adverse pregnancy outcome is associated with *C. trachomatis* genital tract infection in an animal model, we studied the effect of lower genital tract *C. trachomatis* infection in pregnant Swiss-Webster mice.

Address correspondence/reprint requests to Dr. Bryan T. Oshiro, Department of Perinatology, McKay-Dee Hospital Center, 3939 Harrison Boulevard, Ogden, UT 84409.

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SUBJECTS AND METHODS

Mice

Seventy-six 6–8-week-old female Swiss-Webster mice (Sasco, Inc., Omaha, NE) with unlimited access to food and water were placed 2 per cage with a male of established fertility over a 24-h period. The mice were then separated into individual cages. Pregnancy was confirmed by the presence of a copulation plug and later by palpation of a pregnant uterus. Twenty-six non-pregnant mice were removed from the study. This experiment was approved by the Animal Welfare Committee of the University of Texas Health Science Center at Houston, and all practices were in accordance with this committee's guidelines.

Chlamydia

The mouse pneumonitis strain of *C. trachomatis* ATCC BR-123 was reconstituted to a concentration of approximately 1×10^6 inclusion-forming units/ml in sterile calf serum as described by Blanco et al.[10]

Experimental Design

The female mice were separated into 2 groups: a group for chlamydial (CT) inoculation and a control group (CON). The inoculation technique used in this experiment has been reported by Rank et al.[11] On pregnancy day 4, the CT group was inoculated transvaginally with chlamydia suspended in 50 μl of sterile calf serum. The CON group was similarly inoculated with 50 μl of sterile calf serum. On pregnancy day 7, lower genital tract cultures were obtained on all mice using a dacron-tipped aluminum swab which was placed in chlamydia transport media and immediately frozen at −70°C. The mice were observed daily for signs of clinical infection or preterm delivery. The mice were sacrificed and a laparotomy was performed on the day of delivery. If delivery did not occur by the normal date of confinement (day 21), the observation period was extended to pregnancy day 24 at which time all undelivered mice were sacrificed and explored. The abdominal cavity and reproductive tract were grossly examined for evidence of inflammation and the uterus was removed. The distal most portion of the uterus was transected and placed in chlamydia transport media and frozen at −70°C. The remainder of the uterus was placed in 10% formalin. The pups were counted and weighed.

Culture

The lower genital tract culture swab specimens were allowed to thaw at room temperature and vortexed for 15 min. The uterine tissue was also thawed, then morcelated and vortexed. The supernatants were removed and placed on McCoy cells which were previously washed with Mg^{2+}, Ca^{2+}, and phosphate-buffered saline. The cells were then centrifuged for 1 h at 3,400*g* and incubated at 37°C for 48 h. A second-pass culture was performed on all samples. The culture technique is standard and has been described elsewhere.[12] The cells were stained with a chlamydia-specific fluorescent antibody stain (Bartels Diagnostic Division, McGaw Park, IL) and examined by indirect fluorescent microscopy in a blinded fashion.

Histology

The formalin-fixed tissue was placed in paraffin blocks, sliced, stained with hematoxylin and eosin, and mounted on glass slides. The examiner was blinded as to whether the specimens originated from CT or CON animals. The tissue was classified as inflamed if plasma cells or a polymorphonuclear infiltrate were present on examination of the slides.

Statistical Analysis

Statistical analysis was performed by Fisher's exact test and Student's t-test where appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

Twenty-six mice in the CT group and 24 mice in the CON group became pregnant and were included in the study. All 26 (100%) of the CT mice had positive lower genital tract swab cultures for chlamydia on day 7. Uterine tissue cultures were positive in 24/26 (92.3%) of CT mice. Inflammation of the uterine tissues and intrauterine fetal death were significantly increased in the CT mice (Table 1). In the 5 pregnancies in which fetal death occurred, all of the pups in the litters were dead. Placental histology could not be determined in each pregnancy affected by intrauterine fetal demise because of the various stages of resorption of the pregnancy products, but extensive inflammation

TABLE 1. Adverse pregnancy outcome data in mice infected with *C. trachomatis*

	CT (n = 26)	CON (n = 24)	P	Relative risk	95% Confidence interval
Inflammation	9 (35%)	0 (0%)	0.002	2.4	1.7–3.5
Fetal deaths	5 (19%)	0 (0%)	0.05	2.2	1.6–2.9

TABLE 2. Delivery data in mice with liveborn pups[a]

	CT (n = 21)	CON (n= 24)	Significance
No. live pup-mouse	9.0 ± 1.7	8.9 ± 2.2	NS
Pup weight (g)	1.59 ± 0.24	1.59 ± 0.15	NS
Pregnancy days	21.6 ± 1.1	21.2 ± 1.6	NS

[a]Values are mean ± 1 SD. NS = not significant.

was observed in the placentas that were examined. In 4 of the pregnancies in which fetal death occurred, the gestational sacs appeared to be intact. Because of advanced resorption of pregnancy products in the 5th pregnancy, the status of the sacs could not be determined. There were no instances of preterm delivery in the CT group, and none of the mice with intrauterine fetal death delivered their pups by the end of the normal confinement period. The fetal deaths occurred only in the group of mice with uterine inflammation. None of the mice in the CON group had positive cultures, evidence of an inflammatory reaction in uterine tissues, or intrauterine fetal deaths. Table 2 shows the pregnancy outcome data in mice with liveborn pups. No significant difference was found in the mean duration of pregnancy, the mean number of live pups born per mouse, or the mean weight of the liveborn pups between the CT and the CON groups if fetal death did not occur.

DISCUSSION

The role of *C. trachomatis* in adverse pregnancy outcomes is controversial. In humans, several studies have not shown an association between *C. trachomatis* infection and adverse pregnancy outcomes.[6,7,13–18] However, others have implicated *C. trachomatis* in premature rupture of the membranes, preterm birth, low birth weight, intraamniotic infection, and stillbirth.[5,19–24]

One of the first studies to suggest that *C. trachomatis* was associated with adverse pregnancy outcomes was performed by Martin and colleagues[5] in 1982. In their study, 18 of 268 (6.7%) pregnant women screened before 20 weeks gestation were positive for *C. trachomatis*. Twenty-eight percent of the chlamydia-positive patients delivered prematurely compared with 6% of the chlamydia-negative patients. Most significantly, the perinatal mortality in the infected group was 33% compared with 0.4% in the uninfected group. Gravett et al.[19] found that chlamydial infection of the cervix was associated with low birth weight, premature rupture of the membranes, and preterm birth. Martius et al.[20] and The Johns Hopkins Study Group for Cervicitis and Adverse Pregnancy Outcomes[21] also found that cervical infection with *C. trachomatis* was significantly associated with preterm birth.

Chlamydial infections of the cervix may ascend and infect the membranes and fetus. Thorp et al.[22] reported a case of fetal death attributed to an intraamniotic chlamydial infection across intact membranes. Although chlamydia was never cultured from the fetus, the formalin-fixed lung tissue was highly positive for fluorescent antibody against chlamydia. Other authors[23,24] have also reported in utero chlamydial infection across intact membranes.

In this study, we found that lower genital tract infection with chlamydia caused infection of uterine tissues across intact membranes and furthermore was significantly associated with intrauterine demise in Swiss-Webster mice. We also found that, in contradistinction to humans, intrauterine or intraamniotic chlamydial infection in mice did not result in premature rupture of the membranes or preterm delivery. Rather, the affected pregnancies were retained past their normal term of gestation.

With the exception of the study by Martin and colleagues,[5] human studies have not found an increase in stillbirth associated with chlamydial infection in pregnancy. Many other studies looking at the effect of *C. trachomatis* in pregnant women have had small sample sizes and did not specifically look at stillbirths as an outcome variable. We have shown that *C. trachomatis* is capable of causing intrauterine fetal death in Swiss-Webster mice. These results may not correlate to adverse pregnancy outcomes in humans, but it would be interesting to determine if *C. trachomatis* is a significant

cause of stillbirth in human pregnancy as we have demonstrated in the Swiss-Webster mice.

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