# Pregnancies and Time to Pregnancy in Women With and Without a Previous *Chlamydia trachomatis* Infection

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**Background:** A *Chlamydia trachomatis* infection (chlamydia) can result in tubal factor infertility in women. To assess if this association results in fewer pregnant women, we aimed to assess pregnancy incidences and time to pregnancy among women with a previous chlamydia infection compared with women without one and who were participating in the Netherlands Chlamydia Cohort Study (NECCST).

**Methods:** The NECCST is a cohort of women of reproductive age tested for chlamydia in a chlamydia screening trial between 2008 and 2011 and reinvited for NECCST in 2015 to 2016. Chlamydia status (positive/negative) was defined using chlamydia screening trial–nucleic acid amplification test results, chlamydia immunoglobulin G presence in serum, or self-reported chlamydia infections. Data on pregnancies were collected via questionnaires in 2015–2016 and 2017–2018. Overall pregnancies (i.e., planned and unplanned) and time to pregnancy (among women with a pregnancy intention) were compared between chlamydia-positive and chlamydia-negative women using Cox regressions.

**Results:** Of 5704 women enrolled, 1717 (30.1%; 95% confidence interval [CI], 28.9–31.3) women was chlamydia positive. Overall pregnancy proportions were similar in chlamydia-positive and chlamydia-negative women (49.0% [95% CI, 46.5–51.4] versus 50.5% [95% CI, 48.9–52.0]). Pregnancies per 1000 person-years were 53.2 (95% CI, 51.5–55.0) for chlamydia negatives and 83.0 (95% CI, 78.5–87.9) for chlamydia positives. Among women with a pregnancy intention, 12% of chlamydia-positive women had a time to pregnancy of >12 months compared with 8% of chlamydia negatives ($P < 0.01$).

**Conclusions:** Overall pregnancy rates were not lower in chlamydia-positive women compared with chlamydia-negative women, but among women with a pregnancy intention, time to pregnancy was longer and pregnancy rates were lower in chlamydia-positive women.

Trial registration number: Dutch Trial Register NTR-5597.

Studies over the last 20 years show a stable 3% *Chlamydia trachomatis* (chlamydia) infection prevalence among Dutch women in the ages between 16 and 34 years.[1–3] Chlamydia can cause pelvic inflammatory disease, which can then result in tubal scarring due to an intense and chronic inflammatory response.[4] Tubal scarring may lead to tubal factor infertility (TFI).[5] In the Netherlands, TFI was diagnosed in approximately 11% of infertile couples between 2002 and 2004.[6] It is estimated that approximately 1% of women will develop TFI after chlamydia infection.[7,8] In the case of high chlamydia prevalence in a population, this will result in considerable numbers of infertile women. Women with chlamydia-related TFI may need medical assistance, for example, in vitro fertilization (IVF) to become pregnant.[9] However, the mean chance of a live birth after IVF—with large variability because of determinants such as age and cause of infertility—has been estimated at

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only approximately 42% after 3 complete IVF cycles.[10] Hence, we hypothesized that pregnancy rates are lower, or time to pregnancy is longer among women with a previous chlamydia infection versus women without a previous chlamydia infection.

So far, only a few studies have assessed the association between chlamydia infections and subsequent pregnancies or births. A large retrospective study (>20,000 women included) compared birth rates between women who tested chlamydia positive either by serology or by DNA test compared with women with negative test results only.[11] No differences in birth rates between chlamydia-positive and chlamydia-negative women were found. Similar results were found in a historical follow-up study from Denmark in which again >20,000 women were included; birth rates (alive or stillborn) were similar between chlamydia-seropositive and chlamydia-seronegative women.[12] In both studies, exposure time was calculated from a first chlamydia test onward. Factors such as sexual behavior, previous contraceptive use, intention to conceive, and time to pregnancy in months were unknown in these studies and noted as limitations. These limitations need to be addressed to determine true differences in pregnancies between chlamydia-positive and chlamydia-negative women.

One main aim of chlamydia control is to prevent complications like infertility through early testing and treating infections. It is therefore important to focus not only on chlamydial-induced infertility but also on fertility after infection. We investigated differences in pregnancy incidences and time to pregnancy between women with and without a previous chlamydia infection, where time was defined as the months women attempted to conceive and where factors such as sexual (risk) behavior, intention to conceive, and contraceptive use were included.

## MATERIALS AND METHODS

### Study Design and Participants

In the Netherlands Chlamydia Cohort Study (NECCST), Dutch women of reproductive age are followed up until 2022. The design of NECCST has been described previously.[13] Briefly, in 2008 to 2011, women between the ages of 16 to 29 years participated in the population-based chlamydia screening implementation study (CSI).[2] During CSI, women received annual invitations for chlamydia nucleic acid amplification tests (NAATs). Women who participated at least once and gave consent to be approached for further research were invited to participate in NECCST in 2015 to 2016 (Fig. 1). Participants gave online informed consent to comply with study procedures for the collection of repeat questionnaires and biological samples.

### Procedures

The first NECCST data collection round included an electronic questionnaire followed by a test kit for self-collection of blood via a finger-prick for chlamydia immunoglobulin G (IgG) analysis. The capillary blood samples, collected in tubes (BD Microtainer SST, Franklin Lakes, NJ), were returned to the laboratory via mail. Time between blood collection and storage was a median of 4 days (interquartile range [IQR], 3–5 days), which was previously validated.[14] Serum samples were stored at −20°C until thawed for enzyme-linked immunosorbent assay (Medac CT IgG ELISA plus, Wedel, Germany) with a sensitivity of 71% and specificity of 97%.[14,15] Presence of chlamydia IgG served as a marker for previous infection. In 2017 to 2018, participants were invited to complete the second questionnaire. Both questionnaires retrospectively enquired about chlamydia infections, pregnancy intention, pregnancies, and time to pregnancy in months. In addition, demographic factors were collected such as migration background and education level. Migration background was classified as "Western" if both parents had a Western country of birth, that is, a country from Europe (excluding Turkey), North America, Oceania, Indonesia, and Japan; "non-Western" if at least 1 parent had a non-Western country of birth; and "unknown" if one parent was Western while the country of birth of the other parent was unknown or when the country of birth of both parents was unknown. Education level was defined as low/medium (no education, primary education only, lower general secondary education, and vocational education) and high (higher professional education and university education) at last completed questionnaire. Furthermore, sexual behavior, other sexually transmitted infections, contraceptive use, and health characteristics were addressed. For all events (i.e., chlamydia infections, gonorrhea infections, and pregnancies), timing in calendar year was requested in order to reconstruct a timeline. Subsequently, CSI data about chlamydia NAAT results, self-reported

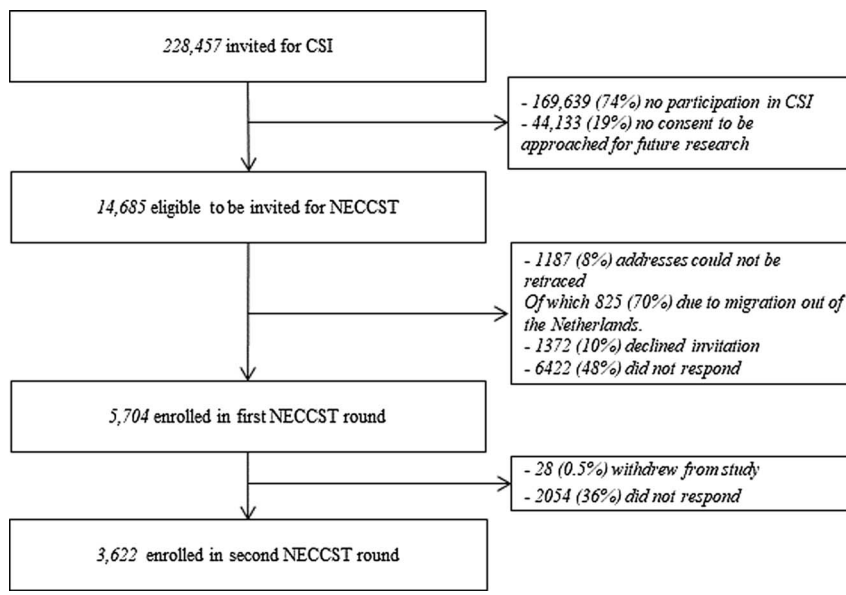


**Figure 1.** Study inclusion flowchart.[8]

chlamydia infections, and age of sexual debut were merged with NECCST data.

## Definitions

### Chlamydia Status

Women were defined as chlamydia positive if they were either chlamydia-NAAT positive in CSI, chlamydia positive by IgG antibody presence, or self-reported an infection during CSI or NECCST; women were considered positive from the year of first infection onward. If women were tested negative by NAAT, were tested negative for chlamydia antibodies, and did not report a previous infection, they were defined as chlamydia negative.

### Overall Pregnancies

Pregnancy was defined as a self-reported pregnancy, which also included miscarriages and induced abortions. Overall pregnancies included planned as well as unplanned pregnancies, and irrespective of fertility treatments.

### Women With a Pregnancy Intention

This includes all women who had at least one planned pregnancy (irrespective of fertility treatments) or who had ever tried to conceive or were still trying.

### Time Period of the Cohort

Women were first invited for the CSI study between 2008 and 2011. Women were invited for NECCST in 2015 or 2016 and reinvited in 2017 or 2018. The cohort period was shortest from 2011 to 2015 and longest from 2008 to 2018. The last received questionnaire was dated August 21, 2018.

Exposure time in Cox regression was defined in 2 ways.

-First, to assess the chance of getting pregnant for chlamydia-positive women compared with chlamydia-negative women, exposure time was defined as the years since sexual debut until last completed questionnaire. This starting point was chosen because from sexual debut on, women were "at risk" for chlamydia infection and (unplanned) pregnancies.

-Second, to determine the chance of getting pregnant over time for chlamydia-positive women compared with chlamydia-negative women, time to pregnancy was defined as the number of months women attempted to become pregnant until first planned pregnancy, until she stopped attempting to get pregnant, or until the last completed questionnaire.

## Statistical Analyses

Population characteristics, pregnancy proportions, and time to pregnancy were compared between chlamydia-positive women and chlamydia-negative women using Student *t* test, Mann-Whitney *U* tests, and $\chi^2$ tests. In addition, pregnancy incidence rates (calculated as the number of pregnancies divided by total person-years at risk) were given.

### Overall Pregnancies

The association between chlamydia and getting pregnant was assessed through a Kaplan-Meier curve and Cox regression among all participants. To include multiple pregnancies per participant, we used recurrent event analyses based on the method of Prentice et al.,[16] which is known as the conditional risk set model. In this model, the assumption is that a subject is not at risk for a second event until the first event has occurred, and so on. Risks were expressed in (adjusted) hazard ratios, or (a)HRs. Chlamydia status was included as a time-varying variable. Multiple imputations were done to estimate the time of first chlamydia infection if this was unknown, that is, in women with a positive chlamydia IgG result only (a positive IgG result can be a result of an infection from years earlier). With STATA's multiple imputation command using truncated regression analyses with 10 simulation data sets,[17,18] time of first chlamydia infection after sexual debut was estimated based on available data from women with a known year of first infection. This was done for 222 women (4% of all participants and 13% among chlamydia-positive women). The proportional-hazard (PH) assumption was checked using log-log plots and testing Schoenfeld residuals. Effect modification, to identify whether the effect of chlamydia infection was different in groups with different characteristics, was assessed for the following variables: age, sex of the partner, and migration background. In the case of significant effect modification, analyses were stratified. The following variables were considered potential confounders and included in the model if there was a ≥10% change in the regression coefficient: age (time-varying), migration background, and educational level. In addition, smoking behavior and body mass index category at the start of NECCST, gonorrhea infection, number of lifetime sex partners, age of sexual debut, and condom use with casual partner were considered potential confounders.

### Women With a Pregnancy Intention

Timing in months until the first planned pregnancy and chlamydia status was also assessed by Kaplan-Meier curves and Cox regression. All women with a pregnancy intention were included in this analysis. The PH assumption and effect modification were assessed as described previously. Chlamydia status was negative in the case of no positive test outcome or in the case that a first planned pregnancy occurred before the first chlamydia diagnosis. Chlamydia status was defined as positive when the first infection occurred before the first planned pregnancy. The following potential confounders at time of trying to get pregnant, in addition to confounders described previously, were evaluated: age, body mass index, last contraceptive, and smoking behavior. Contraceptive use was categorized as follows: no contraceptive use, hormonal contraceptives, Mirena, copper intrauterine device, or condoms.

**TABLE 1.** Sensitivity Analyses

| Sensitivity Analyses |
|---|
| 1 Analyses were repeated without multiple imputations, i.e., women without a known year of first chlamydia infection were excluded |
| 2 Analyses were repeated selecting CSI-NAAT–positive women versus chlamydia-negative women because of high sensitivity and specificity and no recall bias using CSI-NAAT results. In addition, to understand the impact of measurement bias, an analysis was performed where chlamydia was classified as NAAT negative, NAAT positive, serology positive, or self-reported positive. |
| 3 The analysis was restricted to women who participated in both the first and second questionnaire rounds of NECCST to test for selection bias response. |
| 4 Analyses were repeated where we excluded women with a first chlamydia infection that occurred in the same year as a first pregnancy because of the possibility that the infection occurred after the pregnancy rather than before. |
| 5 Analyses were repeated where we excluded women who had their first infection diagnosed after a planned pregnancy |
| 6 Analyses were repeated in which chlamydia status was categorized as chlamydia negative, one infection, or multiple chlamydia infections. |
| 7 Last, analyses were repeated in which chlamydia status was categorized as chlamydia negative, chlamydia positive, or chlamydia seropositive only. |

## Sensitivity Analyses

Various sensitivity analyses were performed as described in Table 1.

## Medical Ethical Approval

This study was approved by the Medical Ethical Committee VU Medical Center, Amsterdam, the Netherlands (NL 51553.094.14/2015.903[A2019.336]). All participants provided informed consent.

# RESULTS

In total, 5704 women were enrolled in NECCST and completed the initial questionnaire. In 2017/2018, 5676 (99.5%; n = 28 study withdrawals) women were invited to complete the second NECCST questionnaire, of whom 3622 (63.5%) did (Fig. 1). Total exposure time from sexual debut until last questionnaire was 93,854 person-years.

**TABLE 2.** Study Population Characteristics by Chlamydia Status at Timing of Last Data Collection Moment

| | Overall, n (%) | Chlamydia Negative, n (%) | Chlamydia Positive, n (%) | *P* |
|---|---|---|---|---|
| Overall | 5704 (100.0) | 3987 (69.9) | 1717 (30.1) | |
| Chlamydia status | | | | |
| Negative | 3987 (69.9) | 3987 (100.0) | 0 (0.0) | <0.01 |
| Positive by category | | | | |
| Self-reported | 900 (15.8) | 0 (0.0) | 900 (52.4) | |
| CSI-NAAT | 4 (0.1) | 0 (0.0) | 4 (0.2) | |
| Chlamydia IgG | 208 (3.7) | 0 (0.0) | 208 (12.1) | |
| Self-reported + CSI-NAAT | 244 (4.3) | 0 (0.0) | 244 (14.2) | |
| Self-reported + chlamydia IgG | 263 (4.6) | 0 (0.0) | 263 (15.3) | |
| CSI-NAAT + chlamydia IgG | 1 (0.0) | 0 (0.0) | 1 (0.1) | |
| All positive | 97 (1.7) | 0 (0.0) | 97 (5.7) | |
| Age, mean (SD), y | 32.4 (3.9) | 32.5 (3.9) | 32.1 (3.9) | <0.01 |
| Migration background | | | | |
| Western | 4565 (80.0) | 3354 (84.1) | 1211 (70.5) | <0.01 |
| Non-Western | 869 (15.2) | 458 (11.5) | 411 (23.9) | |
| Unknown | 270 (4.7) | 175 (4.4) | 95 (5.5) | |
| Educational level*,† | | | | |
| Low/middle | 1163 (20.4) | 648 (16.3) | 515 (30.0) | <0.01 |
| High | 4539 (79.6) | 3338 (83.7) | 1201 (70.0) | |
| Age at sexual debut, mean (SD), y | | | | |
| <16 | 1558 (27.3) | 956 (24.0) | 602 (35.1) | <0.01 |
| 16–17 | 2178 (38.2) | 1507 (37.8) | 674 (30.8) | |
| >17 | 1968 (34.5) | 1524 (38.2) | 444 (25.9) | |
| Lifetime sex partners | | | | |
| <6 | 1306 (22.9) | 1077 (27.0) | 229 (13.3) | <0.01 |
| 6–12 | 2174 (38.1) | 1598 (40.1) | 576 (33.6) | |
| >12 | 2224 (39.0) | 1312 (32.9) | 912 (53.1) | |
| Condom use with casual partners‡ | | | | |
| Never/not often | 340 (6.0) | 242 (6.1) | 98 (5.7) | <0.01 |
| Sometimes | 1807 (31.7) | 1081 (27.2) | 726 (42.3) | |
| Always/mostly | 2610 (45.9) | 1876 (47.2) | 734 (42.8) | |
| No casual partners | 936 (16.4) | 779 (19.6) | 157 (9.2) | |
| Gonorrhea positivity, n (%) | 120 (2.1) | 36 (0.9) | 84 (4.9) | <0.01 |
| Use of IUD | | | | |
| Never | 3507 (61.5) | 2471 (62.0) | 1036 (60.3) | 0.24 |
| At least once | 2197 (38.5) | 1516 (38.0) | 681 (39.7) | |
| Smoking | | | | |
| Never | 2260 (39.6) | 1712 (42.9) | 548 (31.9) | <0.01 |
| Sometimes/in the past | 2919 (51.2) | 1981 (49.7) | 938 (54.6) | |
| Daily | 525 (9.2) | 294 (7.4) | 231 (13.5) | |
| BMI§ | | | | |
| <20 | 730 (12.8) | 536 (13.5) | 194 (11.3) | <0.01 |
| 20–<25 | 3448 (60.6) | 2437 (61.2) | 1011 (59.0) | |
| 25–<30 | 1052 (18.5) | 713 (17.9) | 339 (19.8) | |
| ≥30 | 464 (8.2) | 294 (7.4) | 170 (9.9) | |

Chlamydia-positive was defined as a positive NAAT test outcome in the CSI study (CSI-NAAT), and/or the presence of chlamydia IgG or a self-reported chlamydia infection.

*Educational level: low/medium level of education: no education, primary education only, lower general secondary education and vocational education; high level of education: all other education levels.

†At start of NECCST and based on 5702 observations, 2 missing.

‡Based on 5693 observations, 11 missing and based on first questionnaire data.

§based on 5694 observations, 10 missing.

BMI indicates body mass index; CSI, chlamydia screening implementation. IgG, Immunoglobulin G; IUD, intrauterine device; NAAT, nucleic acid amplification test.

**TABLE 3.** Pregnancies in Chlamydia-Negative and Chlamydia-Positive Women

| | All Women, | | | Chlamydia-Negative Women | | | Chlamydia-Positive Women | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | % | (95% CI) | n | % | (95% CI) | n | % | (95% CI) |
| Never pregnant nor tried | 2558 | 44.9 | (44–46) | 1821 | 44.7 | (43.1–46.2) | 737 | 45.3 | (42.9–47.8) |
| Tried or trying and failed to become pregnant | 291 | 5.1 | (4.5–5.7) | 198 | 4.9 | (4.2–5.6) | 93 | 5.7 | (4.6–7.0) |
| Ever pregnant* | 2855 | 50.1 | (48.7–51.4) | 2058 | 50.5 | (48.9–52.0) | 797 | 49.0 | (46.5–51.4) |
| Planned (at least once) | 2051 | 71.8 | (70.1–73.5) | 1558 | 75.7 | (73.8–77.5) | 493 | 61.9 | (58.4–65.2) |
| Pregnancies per 1000 person-years | | 58.4 | (56.7–60.0) | | 53.2 | (51.5–55.0) | | 83.0 | (78.5–87.9) |

*Thirty-five women who had a previous unplanned pregnancy were also trying to get pregnant without success yet. In this table, they are included in the ever pregnant category.

CI indicates confidence interval.

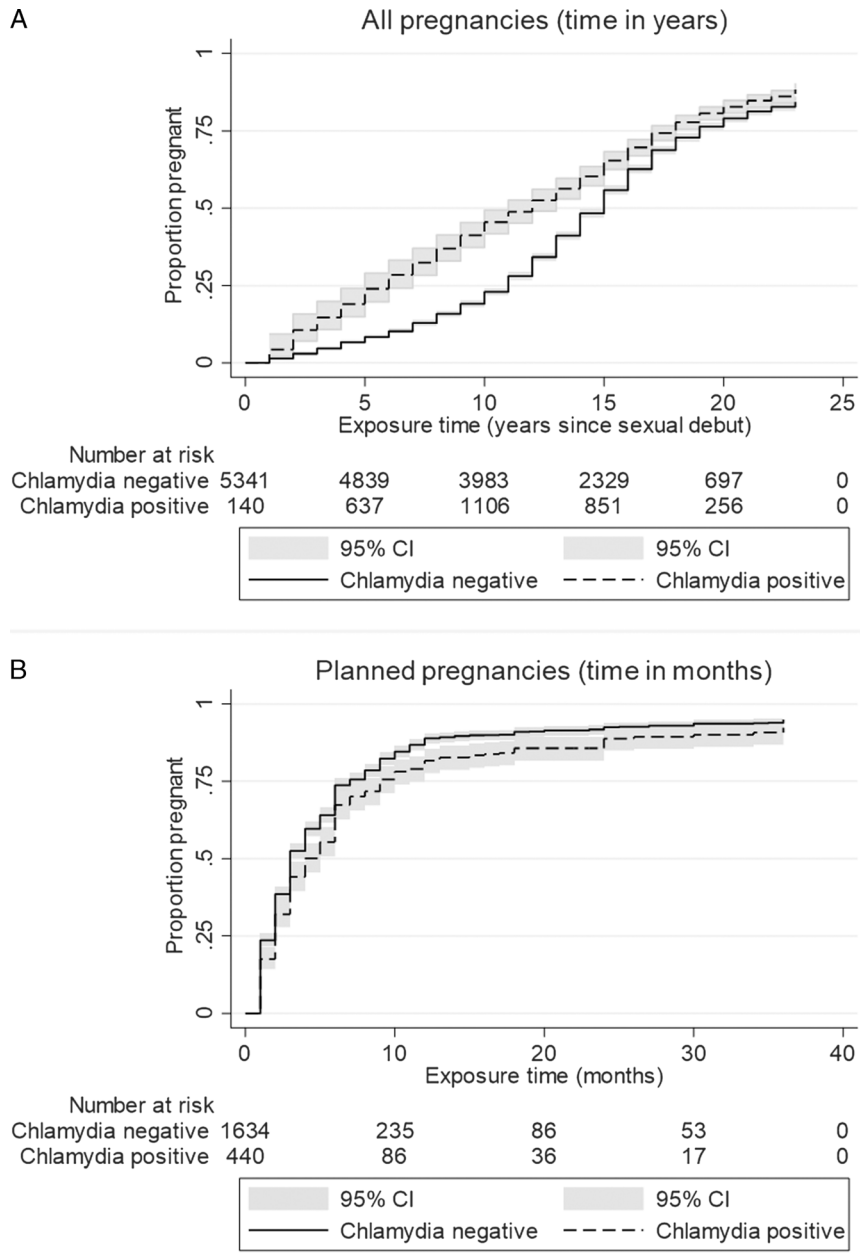


**Figure 2.** Kaplan-Meier plots of years since sexual debut until first pregnancy (A) and time to a planned pregnancy in months among women with a pregnancy intention (B) by chlamydia status. Chlamydia positive was defined as a positive NAAT test outcome in the CSI study (CSI-NAAT), and/or the presence of chlamydia IgG and/or a self-reported chlamydia infection.

## Study Population

Characteristics of the study population by chlamydia status at last data collection round (either the first or second NECCST questionnaire round) are presented in Table 2. Mean (SD) age was 32.0 (3.9) years. Eighty percent of women had a high educational level and a Western migration background. Thirty percent of women were chlamydia positive. Compared with chlamydia-negative women, chlamydia-positive women were younger at sexual debut, had more lifetime sex partners, had a history of gonorrhea more often, and were more often smokers.

## Overall Pregnancies

In total, 3146 (55.2%) women (ever) tried to become pregnant or were pregnant at least once (Table 3). Mean age at first pregnancy was 29.4 years (95% CI, 29.2–29.6 years). There was no significant difference in percentage of women who had ever tried/were trying to become pregnant, had been pregnant, or had never tried to become pregnant between chlamydia-negative women and chlamydia-positive women ($P = 0.316$).

Pregnancy incidences are described in Table 3. The Kaplan-Meier curve is presented in Figure 2A. Time was censored at 23 years after sexual debut because of sample size reduction. The Schoenfeld residuals test ($P = 0.001$) indicated that the chance ratio of getting pregnant was not proportional over time. Therefore, to calculate HRs, the exposure time was stratified in 4 intervals based on graphs and testing the PH assumption. In the first 2 exposure time intervals of 0–6 and 7–10 years, chances for pregnancy were higher in chlamydia-positive women compared with chlamydia-negative women: aHRs, 1.54 (95% CI, 1.26–1.89) and 1.42 (95% CI, 1.22–1.66), respectively. In the 2 last exposure time categories of 11–13 and 13–23 years, no differences were seen: aHRs, 0.93 (95% CI, 0.80–1.07) and 1.00 (95% CI, 0.90–1.10; Table 4).

## Women With a Pregnancy Intention

In total, 2377 (41.7%) women had ever attempted to conceive. After excluding records with missing values in time (n = 29) and with an unclear number of pregnancies (n = 33), 2315 women were included in the analyses. In total, the pregnancy proportion was 87.4%. Overall 83.3% became pregnant within 12 months. These were 85.0% (95% CI, 83.3%–86.7%) among chlamydia-negative women and 77.9% (95% CI, 73.9%–81.5%) among chlamydia-positive women. Of the remaining chlamydia-negative women, 7.7% had a time to pregnancy >12 months and the remaining 7.3% had time <13 months but did not yet conceive. For chlamydia positives, these were 11.6% and 10.7%, respectively. Median (IQR) and mean (SD) time to pregnancy were 3 (IQR, 1–6) months and 5.6 (9.0) months for chlamydia negatives and 3 (IQR, 1–7) months and 6.7 (11.0) months for chlamydia positives ($P = 0.007$; based on the Mann-Whitney $U$ test). The overall pregnancy rate per person-year was 1.8 (95% CI, 1.7–1.9): 1.9 (95% CI, 1.8–2.0) for chlamydia-negative women and 1.5 (95% CI, 1.3–1.6) for chlamydia-positive women. Figure 2B shows the Kaplan-Meier curve. Time was censored at 36 months because of sample size reduction. In Cox regression analyses, stratified for age because of effect modification by age (i.e., age was divided into tertiles), chances of pregnancy for chlamydia-positive women were lower compared with chlamydia-negative women in the age categories 16–29 and 30–32 years (aHRs, 0.79 [95% CI, 0.67–0.94] and 0.74 [95% CI, 0.60–0.92], respectively; Table 5). In age category

**TABLE 4.** Association Between Chlamydia Status and Getting Pregnant by Exposure Time Intervals

| | Pregnancies | Time | Crude HR | | | aHR | | |
|---|---|---|---|---|---|---|---|---|
| | n* | Person-Years† | HR | 95% CI | *P* | aHR | 95% CI | *P* |
| 1. Years after sexual debut 0–6 | | | | | | | | |
| Chlamydia negative | 536 | 30,207 | 1 | | | 1 | | |
| Chlamydia positive | 148 | 2659 | 2.33 | 1.92–2.81 | <0.01 | 1.54 | 1.26–1.89 | <0.01 |
| No infection | 536 | 30,207 | 1 | | | 1 | | |
| 1 infection | 123 | 2394 | 2.24 | 1.83–2.74 | <0.01 | 1.50 | 1.21–1.86 | <0.01 |
| Multiple infections | 25 | 265 | 3.07 | 2.16–4.37 | <0.01 | 1.88 | 1.24–2.85 | <0.01 |
| 2. Years after sexual debut 7–10 | | | | | | | | |
| Chlamydia negative | 637 | 17,103 | 1 | | | 1 | | |
| Chlamydia positive | 268 | 4047 | 1.37 | 1.18–1.58 | <0.01 | 1.42 | 1.22–1.66 | <0.01 |
| No infection | 637 | 17,103 | 1 | | | 1 | | |
| 1 infection | 203 | 3295 | 1.33 | 1.14–1.56 | <0.01 | 1.37 | 1.18–1.62 | <0.01 |
| Multiple infections | 65 | 752 | 1.50 | 1.15–1.96 | <0.01 | 1.67 | 1.25–2.24 | <0.01 |
| 3. Years after sexual debut 11–13 | | | | | | | | |
| Chlamydia negative | 859 | 10,217 | 1 | | | 1 | | |
| Chlamydia positive | 229 | 3228 | 0.77 | 0.67–0.89 | <0.01 | 0.93 | 0.80–1.07 | 0.32 |
| No infection | 859 | 10,217 | 1 | | | 1 | | |
| 1 infection | 183 | 2536 | 0.79 | 0.68–0.92 | <0.01 | 0.93 | 0.80–1.08 | 0.35 |
| Multiple infections | 46 | 692 | 0.68 | 0.51–0.90 | 0.01 | 0.93 | 0.69–1.25 | 0.63 |
| 4. Years after sexual debut 14–23 | | | | | | | | |
| Chlamydia negative | 1,690 | 12,447 | 1 | | | 1 | | |
| Chlamydia positive | 564 | 4637 | 0.86 | 0.78–0.95 | >0.01 | 1.00 | 0.90–1.10 | 0.96 |
| No infection | 1,690 | 12,447 | 1 | | | 1 | | |
| 1 infection | 443 | 3594 | 0.87 | 0.78–0.97 | 0.01 | 0.99 | 0.89–1.10 | 0.81 |
| Multiple infections | 121 | 1043 | 0.88 | 0.68–1.04 | 0.11 | 1.05 | 0.85–1.30 | 0.65 |

Chlamydia positive was defined as a positive NAAT test outcome in the CSI study (CSI-NAAT), and/or the presence of chlamydia IgG and/or a self-reported chlamydia infection. For these analyses, multiple imputations were used to estimate time of first chlamydia infection in women without a known first year of chlamydia infection. Models were adjusted for age, education level, migration background and number of lifetime partners.

*Complete cases.

†Person-years of complete cases.

aHR indicates adjusted hazard ratio; CI, confidence interval; HR, hazard ratio.

**TABLE 5.** Association Between Chlamydia Status and Getting Pregnant Among Women With a Pregnancy Intention Stratified by Age at Time of Attempting to Conceive

| | Planned Pregnancies | Time | Crude HR | | | aHR | | |
|---|---|---|---|---|---|---|---|---|
| | n* | Person-Months† | HR | 95% CI | *P* | aHR | 95% CI | *P* |
| 1. Age 16–29 y | | | | | | | | |
| Chlamydia negative | 709 | 3871 | 1 | | | 1 | | |
| Chlamydia positive | 162 | 1374 | 0.78 | 0.66–0.92 | <0.01 | 0.79 | 0.67–0.94 | <0.01 |
| No infection | 709 | 3871 | 1 | | | 1 | | |
| 1 infection | 132 | 1069 | 0.80 | 0.67–0.97 | 0.01 | 0.81 | 0.68–0.97 | 0.02 |
| Multiple infections | 30 | 305 | 0.71 | 0.49–1.03 | 0.07 | 0.71 | 0.49–1.03 | 0.07 |
| 2. Age 30–32 y | | | | | | | | |
| Chlamydia negative | 425 | 2162 | 1 | | | 1 | | |
| Chlamydia positive | 98 | 692 | 0.72 | 0.59–0.89 | <0.01 | 0.74 | 0.60–0.92 | <0.01 |
| No infection | 425 | 2162 | 1 | | | 1 | | |
| 1 infection | 79 | 571 | 0.72 | 0.58–0.90 | <0.01 | 0.75 | 0.59–0.94 | 0.01 |
| Multiple infections | 19 | 121 | 0.71 | 0.45–1.13 | 0.15 | 0.72 | 0.45–1.14 | 0.16 |
| 3. Age 33–39 y | | | | | | | | |
| Chlamydia negative | 485 | 3649 | 1 | | | 1 | | |
| Chlamydia positive | 131 | 842 | 1.07 | 0.89–1.28 | 0.48 | 1.07 | 0.89–1.28 | 0.49 |
| No infection | 485 | 3649 | 1 | | | 1 | | |
| 1 infection | 115 | 690 | 1.11 | 0.92–1.34 | 0.29 | 1.10 | 0.90–1.33 | 0.35 |
| Multiple infections | 16 | 152 | 0.81 | 0.49–1.34 | 0.42 | 0.85 | 0.50–1.42 | 0.52 |

Chlamydia positive was defined as a positive NAAT test outcome in the CSI study (CSI-NAAT), and/or the presence of chlamydia IgG and/or a self-reported chlamydia infection. For these analyses, multiple imputations were used to estimate time of first chlamydia infection in women without a known first year of chlamydia infection. Analyses were stratified for age categories based on tertiles. All models were adjusted for age at pregnancy/trying to get pregnant and migration background.

*Complete cases.

†Person-months of complete cases.

aHR indicates adjusted hazard ratio; CI, confidence interval; HR, hazard ratio.

33 to 39 years, no significant difference was found (aHR, 1.07; 95% CI, 0.89–1.28).

### Sensitivity Analyses

Sensitivity analyses on pregnancies and time to pregnancy using data restrictions as indicated in the methodology section yielded comparable results (Supplementary Figs. 1 http://links.lww.com/OLQ/A524 and 2 http://links.lww.com/OLQ/A525, and Supplementary Tables 1 and 2 http://links.lww.com/OLQ/A526).

## DISCUSSION

In this longitudinal cohort study with more than 10 years of follow-up, we assessed pregnancies and time to pregnancy among chlamydia-positive and chlamydia-negative women. Overall pregnancy proportions (either planned or unplanned) in chlamydia-positive and chlamydia-negative women were relatively similar. More chlamydia-positive women were pregnant in the first 10 years after sexual debut, but this difference decreased thereafter. By contrast, among women younger than 33 years with a pregnancy intention, pregnancy rates were lower in chlamydia-positive women compared with chlamydia-negative women and time to pregnancy was longer. No differences in pregnancy rates were seen after 33 years of age.

This unique cohort is a follow-up study from the population-based CSI and included information on previous chlamydia infections based on NAAT results, chlamydia IgG presence, and self-reported positive test results, thereby reducing misclassification. However, in common with other chlamydia studies, it is inevitable that there is misclassification in chlamydia status. Nucleic acid amplification tests can only detect current infections, not past infections, and these were performed only once per year in the CSI.[2] Serology can identify past infections, but sensitivity is not optimal and serology was only done in the first NECCST questionnaire round, not in the second.[14,19] Furthermore, participants were classified as "chlamydia negative" (until first positive test result), which could have been before first test outcome. Last, self-reported infections may be subjected to recall bias. To understand the impact of measurement bias, several sensitivity analyses were performed that yielded similar results. The NECCST is a subset of the population-based CSI study, and selection bias cannot be ruled out. Participants were more often highly educated and from a Western migration background than the general Dutch population. However, our pregnancy results are quite comparable with the general Dutch population. The average age of women when having their first child in the Netherlands in 2013 (i.e., the median year of first pregnancy in this cohort) was 29.4 years, which is equal to the age of first pregnancy in this cohort. Furthermore, in the Netherlands, approximately 83% of women who wish to become pregnant conceive within 1 year, as similarly reported by 82% of women in this study.

In contrast to our hypothesis, overall pregnancy proportions were rather comparable between chlamydia-positive and chlamydia-negative women, both approximately 50% in the total cohort. We expected lower pregnancy proportions because several studies, including a previous study of this cohort, demonstrated a 1.3 to 4 times increased risk for TFI after chlamydia infection.[8,20,21] Our results are, however, in agreement with population-based cohort studies from Denmark and Norway, in which no differences were seen in birth rates between chlamydia-seropositive women and chlamydia-seronegative-tested women.[12] In the population-based study from Denmark, a slightly higher chance of births was seen for chlamydia-positive women, either chlamydia seropositive or by a positive chlamydia DNA test.[11] In a clinical study in Iran in which 250 infertile couples were tested for chlamydia DNA and chlamydia serology, no difference in pregnancy chances was found either.[22] How can this be explained? Although 1.3 to 4 times higher compared with chlamydia-negative women, the risk for TFI after chlamydia infection is low (0.5%–1.0%).[7] This effect might get lost in large cohorts in which many other factors for getting pregnant played a role. We did not have data about frequency of sexual contacts, which may

have had an impact. We found that unplanned pregnancies were significantly more common (14% more) among chlamydia-positive women compared with chlamydia-negative women. Because both are linked to unprotected sexual contact, this is not unexpected.[23] Despite not having information on sexual frequency, our results imply that sexual activity among chlamydia-positive women could have been higher, that is, significantly more lifetime partners, younger age at sexual debut, and more previous gonorrhea infections. In the Netherlands, there is no antenatal chlamydia screening, but it might be that part of unplanned pregnancies was terminated in abortion clinics where women were offered a chlamydia test. More chlamydia tests could have been done, which might have led to an association between chlamydia positivity and pregnancy. However, abortion rates in the Netherlands are low (8.8 per 1000 women in 2018), and only 8% of abortions were done among teenagers.[24] Nevertheless, these factors in combination with less condom use might have resulted in more pregnancies and explain why more chlamydia-positive women were pregnant within the first 10 years after sexual debut.

By contrast, among women with a pregnancy intention, pregnancy proportions and rates per year were lower among chlamydia-positive women. These lower rates can only partly be explained by the known TFI cases, which is only 1% in this cohort. Lower pregnancy proportions were also seen in the study following up infertile women with and without chlamydia antibodies where fewer chlamydia-antibody–positive women became pregnant within 9 months.[25] In that study, other causes of infertility including visible TFI were excluded. The possibility is that even without visible tubal pathology, there might be intratubal microdamage due to a previous chlamydia infection.[25] On the other hand, we cannot completely rule out selection bias for the effect. These lower pregnancy rates were found among women with a pregnancy intention. In this analyses, all women who only had an unplanned pregnancy were excluded. In the questionnaire, women were asked if reported pregnancies were "planned" without the definition of "planned" being explained further. A planned pregnancy could have been interpreted differently between women and wrongly classified as "unplanned," which might explain our high rates of unplanned pregnancies. Although excluding unplanned pregnancies led to more clear analyses, a bias might be introduced because more chlamydia-positive women had unplanned pregnancies, which could have either reduced or underestimated the effect.

It is difficult to put our results in the context of chlamydia control. In the Netherlands, extensive chlamydia control efforts are being made. Youngsters up to the age of 25 years and high-risk groups can be tested and treated for chlamydia free of charge.[26] It is possible that because of the extensive testing and treating of chlamydia infections, pregnancy rates are not lower in chlamydia-positive women compared with chlamydia-negative women. However, we did see lower pregnancy rates among women with a pregnancy intention. In sensitivity analyses in which we estimated risks for women who were positive only by a chlamydia antibody test and had not reported a chlamydia infection, and thus were presumably not treated for chlamydia, pregnancy rates did not differ from chlamydia positives, of which the vast majority had been treated. Although the group of chlamydia-positive women who were presumably not treated for their infection was rather small, it is possible that the effect of chlamydia control in preventing late complications that might result in infertility is rather small.[27]

Using pregnancy rates and time to pregnancy in addition to pelvic inflammatory disease, ectopic pregnancy, and TFI to study reproductive health outcomes after chlamydia infections contributes to the insight in chlamydia disease burden. Pregnancy characteristics are easy to collect with high validity using questionnaires.[28] Nevertheless, in using questionnaires to address events dating back to years ago, recall bias cannot be ruled out. Given that in our study the median number of years between first infection and first pregnancy was 5, we assume that not many first pregnancies were incorrectly reported before first infection; however, this possibility should be taken into account in interpreting the results.

To conclude, our results do not indicate that women with a previous chlamydia infection have lower pregnancy chances compared with women without a chlamydia infection. However, we did find lower pregnancy rates and longer time to pregnancy among women with a pregnancy intention, which could be due to complications of the chlamydia infection caused by either visible or invisible tubal pathology. It is worthwhile to not only focus on chlamydia complications but also include pregnancy as an outcome in surveillance and research.

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