UNIVERSIDAD SAN FRANCISCO DE QUITO

College of Health Sciences

The frequency of infection caused by *Chlamydia trachomatis* in pregnant women attended at Hospital Gineco-obstétrico Isidro Ayora, Hospital Vozandes de Quito, and the ClínicaUniversitaria USFQ in Quito, Ecuador

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This thesis is presented as a requisite to obtain degree of Doctor of Medicine

Cumbayá, January 2013

Universidad San Francisco de Quito
College of Health Sciences

APPROVAL OF THESIS

The frequency of infection caused by *Chlamydia trachomatis* in pregnant women attended at Hospital Gineco-obstétrico Isidro Ayora, Hospital Vozandes de Quito, and the ClínicaUniversitaria USFQ in Quito, Ecuador

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RESUMEN:

Introducción: Infección genital con *Chlamydia trachomatis* es una de las infecciones de transmisión sexualmás frecuentes, afectando a la poblaciónfértil en todo el mundo. La infección puede ser asintomática por lo que en algunos países se recomienda realizar tamizaje anual para la población en riesgo. La infección no tratada puede causar complicaciones no solamente en la población no embarazada, sino que también afecta a la población embarazada, produciendo riesgo para rupturaprematura de membranas, partoprematuro, endometritis, conjuntivitis neonatal y neumonía.

Materiales y Métodos: Después de obtener el consentimiento informado, 60 muestras endocervicales fueron obtenidas de mujeres embarazadas cursando hasta 14 semanas de edad gestacional, entre las edades de 18 a 36 años, sin historia previa del uso de antibióticos u óvulos adentro de las seis semanas previas. Las muestras fueron recolectadas de tres centros de salud en Quito, Ecuador. Las muestras endocervicales fueron procesadas usando PCR.

Resultados: Cuatro de 60 sujetos fueron positivos para infección con *Chlamydia trachomatis*. Los sujetos infectados, quienes tenían entre 18 y 23 años de edad, demostraron una frecuencia relativa de 7% de infección con *Chlamydia trachomatis* en la población entre 18 y 24 años de edad. Las participantes infectadas presentaron un ingreso mensual menor que la población general no infectada. Adicionalmente, la población infectada reportó más casos de abortos espontáneos y partos normales que la población no infectada. No se determinó una relación

entre la presencia de infección y el número de compañeros sexuales, infidelidad, dolor pélvico y presencia de secreciones vaginales.

Conclusiones: Este estudio piloto apoya la evidencia previamente conocida que reporta que la frecuencia de infección con *Chlamydia trachomatis* es mayor en la población adolescente y de menores recursos econmicos. Se necesita realizar investigaciones futuras con una población más amplia para determinar la prevalencia de *Chlamydia trachomatis* en participantes de varios grupos étnicos y niveles de educación.

PALABRAS CLAVES: Chlamydia*trachomatis*, embarazada, mujeres, Ecuador.

ABSTRACT:

Background: Genital infection with *Chlamydia trachomatis* is one of the most common sexually transmitted infections affecting reproductive population worldwide. As infection may be asymptomatic in majority of cases, annual screening is vital for population at risk. Untreated infection may cause complications not only in the non-pregnant population, but also in the pregnant population, producing risk for preterm rupture of membranes and labor, endometritis, neonatal conjunctivitis, and pneumonia.

Methods: After obtaining informed consent, 60 endocervical samples were obtained from pregnant women (less than 14 weeks of gestation age) between the ages of 18-36, with no history of use of antibiotics or ovules within the previous 6 weeks. Samples were collected from three health care centers in Quito, Ecuador. Endocervical samples were processed using PCR.

Results: Four of 60 subjects tested positive for *Chlamydia trachomatis*. These subjects ranged in age from 18-23 years, producing a relative frequency of 7% of infection with *Chlamydia trachomatis* in the general population between 18 and 24 years of age. The infected subjects presented lower monthly incomes that did the overall non-infected subjects. Additionally, the infected population reported more cases of miscarriages and previous vaginal deliveries, than did the non-infected population. No relationship was found between infection and number of sexual partners, infidelity, pelvic pain, and vaginal secretions.

Conclusions:This pilot study supports previous evidence that frequency of infection is greater in adolescent population. Future studies needed with larger population size to investigate prevalence of *Chlamydia trachomatis* in participants of various ethnic groups and education levels.

KEY WORDS:*Chlamydia trachomatis*, pregnant, women, Ecuador.

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INTRODUCTION:

Chlamydia trachomatis is an intracellular bacterium that causes genital tract infections that have been related to serious reproductive morbidity. The genotypes of *Chlamydia trachomatis* associated with sexual transmission are D, E, F, G; H, I, J, and K. These genotypes are associated with cervicitis and urethritis, while genotypes L1, L2, L2a and L3 are associated with the systemic infection lymphogranulomavenereum (Lima et al., 2007). While these genotypes are related to specific presentations of infection recent data suggests that chlamydial genital infections are asymptomatic in 50% of the male population and up to 80% in the female population (Lima et al., 2007), which has led to under detection of this sexually transmitted infection. In 2008, it was estimated by the Center for Disease Control and Prevention that there were 2.8 million cases of *Chlamydia trachomatis* infections annually, however, only 1.2 million cases were reported (Satterwhite et al., 2011). This large, undetected, asymptomatic group of carriers is at risk of complications and puts the rest of the general population at risk for transmission of sexually transmitted infections (Lima et al., 2007).

Untreated chlamydial genital infections may cause complications such as pelvic inflammatory disease, ectopic pregnancies, tubal-factor infertility, as well as chronic pelvic pain (Satterwhite et al., 2011). Research suggests that two thirds of the cases of tubal-factor infertility and one third of the cases of ectopic pregnancies are associated with genital chlamydial infections (Peipert, 2003). *Chlamydia trachomatis* infection in the pregnant population may cause premature rupture of membranes and consequently preterm labor, as well as postpartum endometritis (Peipert, 2003). In 2006, it was estimated that 155,000 infants were born to chlamydia-infected mothers

(Rahangdale et al., 2006). It has been estimated that approximately 30 to 50% of these infants born to chlamydia-infected mothers will present conjunctivits, and of those who present conjunctivitis, at least 50% will present a nasopharyngeal infection (Peipert, 2003). Thirty per cent of newborns with nasopharyngeal Chlamydia infections will develop pneumonia (Peipert, 2003).

While the majority of patients have asymptomatic infections, during physical examination, at least one third of women will show local signs of infection, including a mucopurulent discharge from the cervix. In the symptomatic population of infected women, they may present with acute urethral syndrome, urethritis, bartholinitis, cervicitis, upper genital tract infections, perihepatitis, and reactive arthritis (Peipert, 2003). Due to the possible complications and consequences of this sexually transmitted infection, screening programs have been implemented with a focus on sexually active women who present any of the following risk factors: younger than 25 years of age, start of sexual intercourse at an early age, multiple sexual partners, a new sexual partner, black race, previous medical history of coexistent sexually transmitted infection, cervical ectopy, and inconsistent use of barrier contraceptive methods (Peipert, 2003). The United States Centers for Disease Control and Prevention recommends screening for chlamydial genital infections during the first prenatal visit in all women and again in the third trimester for women who are at high risk (including those younger than 25 years of age) (Peipert, 2003). The United States Preventive Services Task Force recommends screening for chlamydial genital infection in all pregnant women aged 24 years and younger. As for pregnant women older than 24 years of age, the USPSTF recommends screening for those who present risk factors such as previous history of sexually transmitted infections, new sex partners, multiple sex partners, or those who

exchange sex for money or drugs. Additionally, the USPSTF recommends screening for all pregnant women, regardless of age, during the third trimester (USPSTF, 2001).

In the United States, the prevalence rates of high risk populations has been found to be anywhere between 6 and 20%, while prevalence rates between 4 and 12% have been reported for women attending family planning clinics (Peipert, 2003). Another study states that about half of the 19 million new sexually transmitted infections reported annually in the United States among women and men between the ages of 15-24, the majority of these cases were caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Berggren et al., 2011). Additionally it in this particular study, it was observed that these two sexually transmitted infections occurred most frequently in adolescent girls between 15 and 19 years of age (Berggren et al., 2011). It was also shown that minorities were more frequently affected than Caucasians (Berggren et al., 2011). Various studies demonstrate the same principle. For example, an investigation carried out in 2004 indicated that the prevalence rate of *Chlamydia trachomatis* in the United States varies according to ethnic group. Caucasian adults illustrated a prevalence rate of 1.94%, while it was six times greater in the population of young African adults (12.54%, 95% CI: 4.68-8.91). Similarly, in a group of young Native-American adults, the prevalence of chlamydia was determined to be 10.4%, while in the Hispanic Americans, the prevalence was only 5.89% (Miller, 2004). With such extreme variations in the prevalence of this sexually transmitted infection, it is important that each country establish adequate screening guidelines and programs to determine the frequency of these infections with means to decrease transmission and consequently decrease the consequences of untreated infections.

JUSTIFICATION OF STUDY:

In today´s world, the adolescent and young adult populations are initiating sexual contact at a younger age, use of barrier contraceptive methodsinconsistently, and have more sexual partners which accounts for the rising incidence of sexually transmitted infections (Macmillan, 1999). For this reason it is crucial to establish guidelines for screening programs that apply to populations at risk for STIs.

It is well known that *Chlamydia trachomatis* infection is the most prevalent sexually transmitted infection worldwide, affecting the pregnant and non-pregnant population. However, routine use of screening programs for chlamydial genital infections in prenatal controls throughout countries of Latin America, such as Ecuador, is not a common practice. Due to inadequate population screening, the actual prevalence of the sexually transmitted infection *Chlamydia trachomatis*in Ecuador is still unknown. In countries surrounding Ecuador, such as Peru, the prevalence rate of *Chlamydia trachomatis* among pregnant women was found to be of 33.8% (Portilla et al. 1999). In Chile, the prevalence rate of Chlamydia was established to be 7% in non-pregnant young women (Huneeus et al., 2009), while in Brazil, the prevalence was reported to be 19% in young, non-pregnant women (Lima et al., 2007). In the United States and Europe the prevalence is 4.19% (95% CI, 3.48% - 4.90%)(Miller, 2004). With such extreme range of prevalence it is vital to determine the situation in Ecuador in order to identify risk groups and implement adequate screening and prevention programs.

This sexually transmitted infection must be promptly detected in order to prevent its gynecological and obstetrical complications, in addition to prevent any neonatal complications that may arise. Early detection through screening programs will minimize the distribution of chlamydial genital infections. Additionally, it will diminish the susceptibility and transmission of other sexually transmitted infections such as Human Immunodeficiency Virus (HIV) and Human Papilloma Virus (Miller, 2004). Populations screening programs have reduced the incidence of PID and other complications arising from Chlamydia (Bohm, 2010).

Ecuador´s predominantly Catholic population and their religious beliefs may negatively influence the practice of safe sex throughout the country. Many do not acknowledge the use of barrier conceptive methods due to cultural and religious viewpoints. With this said, a population that is beginning their sexual lives at an earlier ageand practices inconsistent use of barrier contraceptive methods is at risk for sexually transmitted infections. The purpose of this investigation is to establish the frequency of *Chlamydia trachomatis* infections in a small population of pregnant women in the country´s capital. If necessary, this pilot study will provide pertinent information for future screening programs among other Ecuadorian subpopulations, with the hopes to reduce the transmission of the infection and to properly educate the population about preventative measures.

OBJECTIVES:

GENERAL OBJECTIVE:

- To contribute to the control of infections caused by *Chlamydia trachomatis* in pregnant women in Quito, Ecuador.

SPECIFIC OBJECTIVES:

1) To determine the frequency of infections caused by *Chlamydia trachomatis* in pregnant women in Hospital Gineco-Obstétrico Isidro Ayora (HGOIA), Hospital Vozandes de Quito, and in the ClínicaUniversitaria USFQ.

2) To identify the risk factors associated with infections caused by *Chlamydia trachomatis.*

3) To establish the necessity of screening for infections caused by *Chlamydia trachomatis* in pregnant women.

4) To establish a platform to perform studies in larger populations with the intention to reduce chronic complications of infections caused by *Chlamydia trachomatis.*

BIOETHICAL ASPECTS:

A research protocol and informed consent were written and all possible risks that may or may not affect the participants were identified. This was then presented before the Bioethics Committee of theSan Francisco de Quito University who approved the study, as well as all informed consent forms. Before obtaining any information from the participants of the study, it was explained that all information received and results obtained are confidential. This point was stressed various times during the interview, as personal questions were asked about the participants' sexual history and their planning or lack of planning of their actual pregnancy. Additionally, all participants were required to sign informed consent forms in order to participate. Gynecologists and Obstetricians first examined all participants before obtaining any samples. Participants were instructed that the test itself does not pose any danger to their pregnancies. Additionally, they were educated that in the case that the test results come back positive, they would be contacted so that during their next prenatal control, they could be given treatment. All participants were asked if they have any known allergies to medications. For those who were given treatment, possible side effects of the antibiotics were explained to the participants. The participants were informed during every step of the investigation. All information obtained was kept strictly confidential.

MATERIALS AND METHODS:

STUDY POPULATION AND INSTRUMENTS:

1. **Participant selection:**

   60 pregnant women from three hospitals in Quito, Ecuador were selected to be participants in this study. These hospitals included Hospital Gineco-Obstetrico Isidro Ayora (a public maternity hospital), Hospital Vozandes de Quito (middle class private hospital), and the ClínicaUniversitaria USFQ. The participants of the study were not collected in a randomized or consecutive manner. All patients who attended these hospitals for prenatal control between August 2011 and March 2012 and were eligible were presented the opportunity to participate in the study.

   Inclusion criteria: The study included Ecuadorian women of the reproductive age (18-36 years); nulliparous or multiparous, with a pregnancy less than 14 weeks.

   Exclusion criteria: Women who suspect pregnancy but do not have tests to confirm; international pregnant women; the possibility of miscarriage; use of antibiotics or antifungals within six weeks of the collection of the samples; women who did not sign the informed consent forms.

2. **INFORMED CONSENT:**

Previous to completing the surveys and collecting the endocervical samples, the problem of not having information about the prevalence of genital infections caused by *Chlamydia trachomatis* in pregnant women in Ecuador was explained to the participants. Additionally, it was explained to each participant, the methodology for collecting the samples along with the risks and benefits of performing such testing. The participants were informed that in the case that they would test positive for an infection with *Chlamydia trachomatis*, she and her sexual partner would receive antibiotic treatment. It was made clear that all of the information obtained during this study remains 100% confidential and that the participant has the right to withdraw from the study at any point during the investigation. Participants who accepted to be part of the study signed informed consent forms before any samples were collected (Annex B).

3. SURVEY:

Once participants agreed to be part of the study and signed the informed consent forms, they were asked questions about their gynecological history (age of menarche, date of last menstrual period, characteristics of menstrual periods, sexual partners, use of contraceptives, presence of vaginal secretions, presence of pelvic pain, etc). At the end of the surveys, participants were asked to sign their name with their identification numbers to assure that all of the information collected is correct (Annex A).

4. COLLECTION AND PROCESSING OF ENDOCERVICAL SAMPLES:

Before taking the endocervical samples, all of the materials were shown to the participants, ensuring that all of the instruments are sterile. Previous to taking the samples, it was explained to each participant that the speculum would be inserted into the vaginal canal and a two small swabs would be introduced to collect endocervical cells. One swab was inserted into the vaginal fornix without coming in contact with the vaginal walls and was guided around the circumference of the cervix. It was then minimally introduced into the endocervix. The procedure was repeated with the second swab. Once the sample was obtained, the swabs were placed in a sterilized tube with a transport medium (RemelMicroTest M4RT Transport) and labeled with the patients name, the date, and the hospital where the sample was collected. The samples were then placed into a small cooler and transported to the microbiology laboratory at the Universidad San Francisco de Quito where they were processed using PCR (polymerase chain reaction). If the samples were not able to be delivered to the laboratory within two hours of their collection, they were refrigerated at 4 degree Celsius until they could be transported. In the laboratory, the High Pure PCR Template Preparation Kit (Roche) was used for the DNA extraction from the cervical-endocervical samples. Previous to the DNA extraction, the two swabs were diluted in a lysis buffer. The LightCycler® FastStart DNA Master PLUS HybProbe (MOLBIOL) kit was used for the detection of *Chlamydia trachomatis*. Every three months the equipment was calibrated using the LightCycler® Color Compensation Set.

5. CREATING A DATABASE:

All of the information obtained in the surveys were coded and entered into a database using Microsoft Excel 2010. Based on the results of the screening for *Chlamydia trachomatis*, the results were also coded and entered into the database.

6. TREATMENT OF PARTICIPANTS WITH INFECTION:

Participants who tested positive for *Chlamydia trachomatis* were contacted and given treatment for the infection. In the initial surveys, patients were asked about the existence of medical allergies. As long as the patients had no previous medical history of allergies, they were treated with a single dose of one gram of azithromycin.

STATISTICAL ANALYSIS:

In order to obtain a precision of 5%, this pilot study should use 62 subjects, assuming that the prevalence of *Chlamydia trachomatis* in Quito, Ecuador is similar to that of the United States.

$N = (Z\alpha^2)x(p)x(1-p)/d^2$

Where :

$Z\alpha^2$: 1.96^2

P: 4.19%

d^2 : 5%

So:

$$N = (1.96^2)x(0.0419)x(1-0.0419)/0.05^2 = 61.6$$

Due to the fact that the majority of the variables obtained from the survey were qualitative variables, the statistical analysis was performed using pivot tables in Microsoft Excel. The data was then analyzed using three variables, looking for possible associations between them. The presence or absence of the infection was compared with the age of the subjects along with each of the following variables: socio-economic income, number of sexual partners, suspected infidelity, use of contraceptives, pelvic pain, vaginal secretion, number of vaginal deliveries, and number of miscarriages.

RESULTS:

60 pregnant women were asked to participate in the pilot study, with the majority of the subjects presenting between the ages of 25 and 36 years (Table 1). Of these 60 subjects, four subjects tested positive for chlamydial genital infection, producing a 7% relative frequency of infection (Table 1).

AgeRanges	Non-infected	Number of cases with infection (absolute frequency)	Relativefrequency of infection (%)
18-20	9	1	2
21-24	8	3	5
25-36	39	0	0
Total	**56**	**4**	**60**

TABLE 1. Number of subjects who tested positive forinfection with *Chlamydia trachomatis*in various age groups.

The participants used in this study were currently receiving prenatal care in one of three health care centers located in the country´s capital, Quito. These health care centers included one public hospital, one private hospital, and one private clinic. Due to the types of health care centers studied, the socio-economic status of the participants greatly varied from a monthly income of $200 provided by subject and partner to $4000 (Figure 1, Figure 2). Additionally, the infected subjects presented lower monthly incomes, than the non-infected subjects; however, it should be noted that these four subjects were patients at the public maternity hospital.

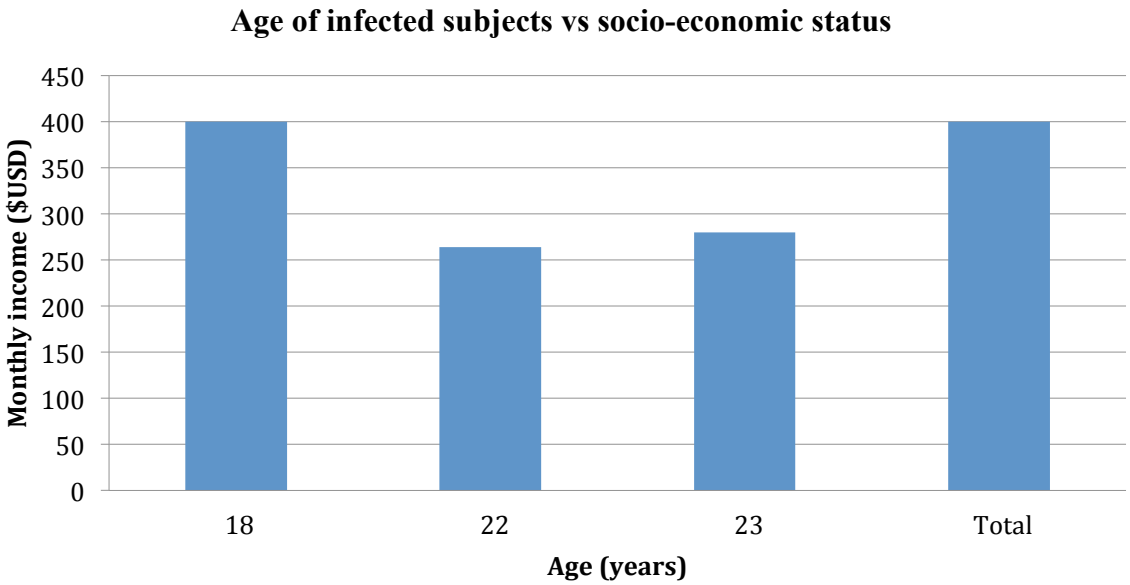

FIGURE 1. Monthly income of the infected subjects, grouped by age.

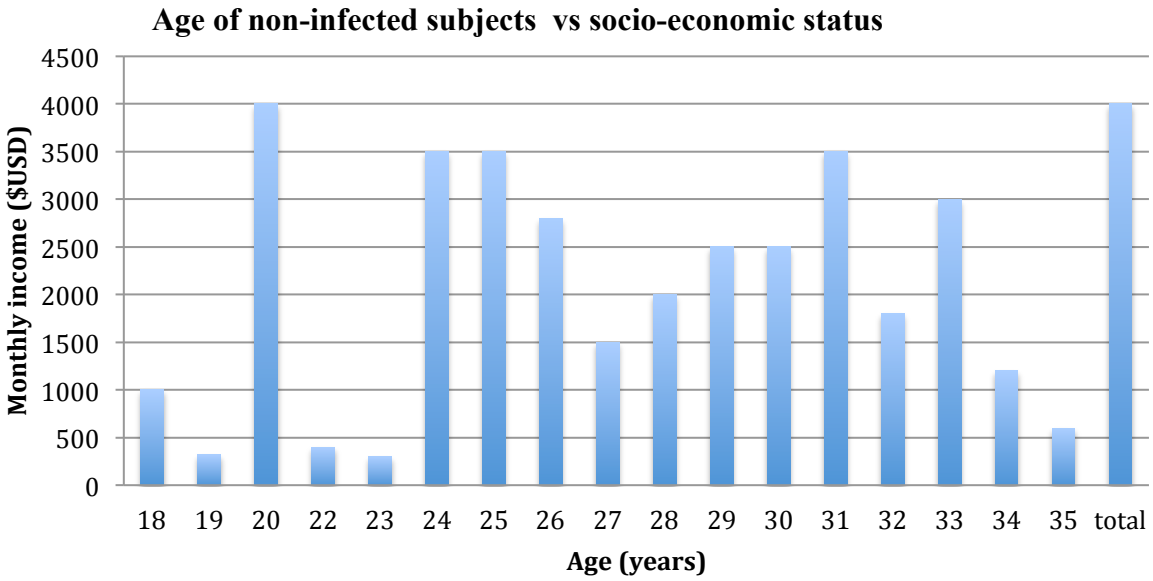

FIGURE 2. Monthly income of the non-infected subjects has been grouped by age.

In terms of sex partners, the majority of the participants have had two sex partners. Analyzing this information based on presence of infection, 75% of the infected subjects

have had only one sex partner (Figure 3). The non-infected subjects presented one to seven sex partners; with the majority reporting two sex partners (42.8%) (Figure 4, Figure 5).

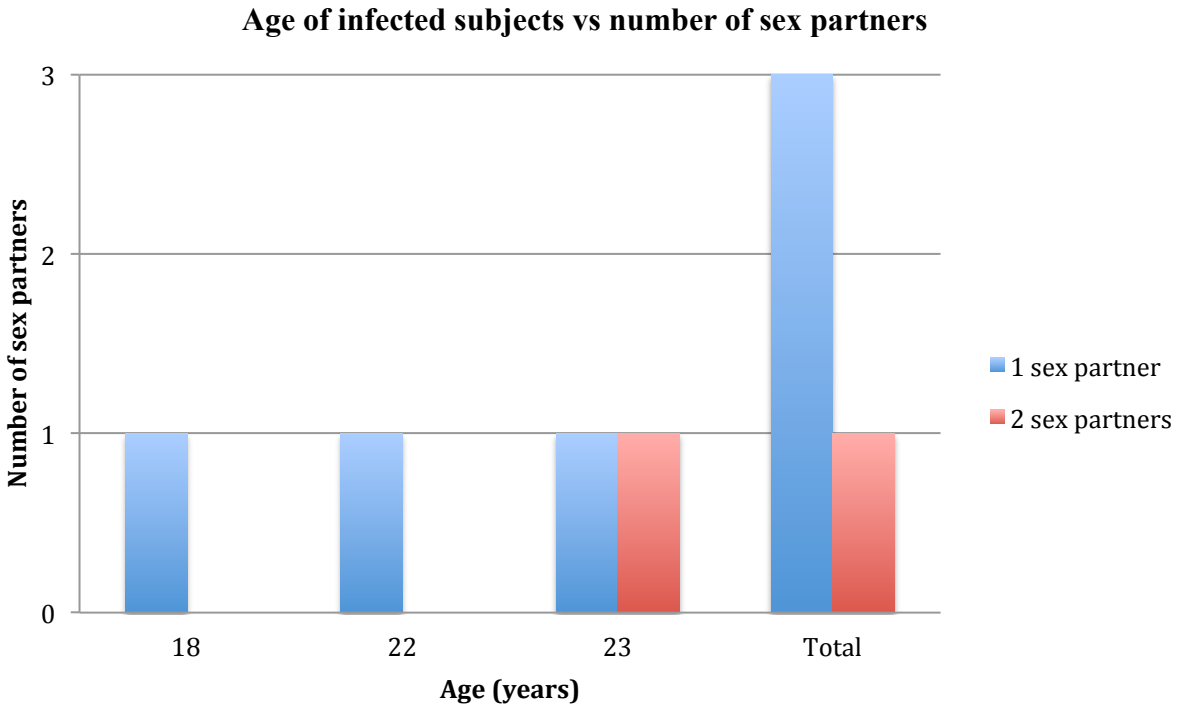

FIGURE 3. Number of sex partners of the subjects infected with *Chlamydia trachomatis*, while grouped according to age.

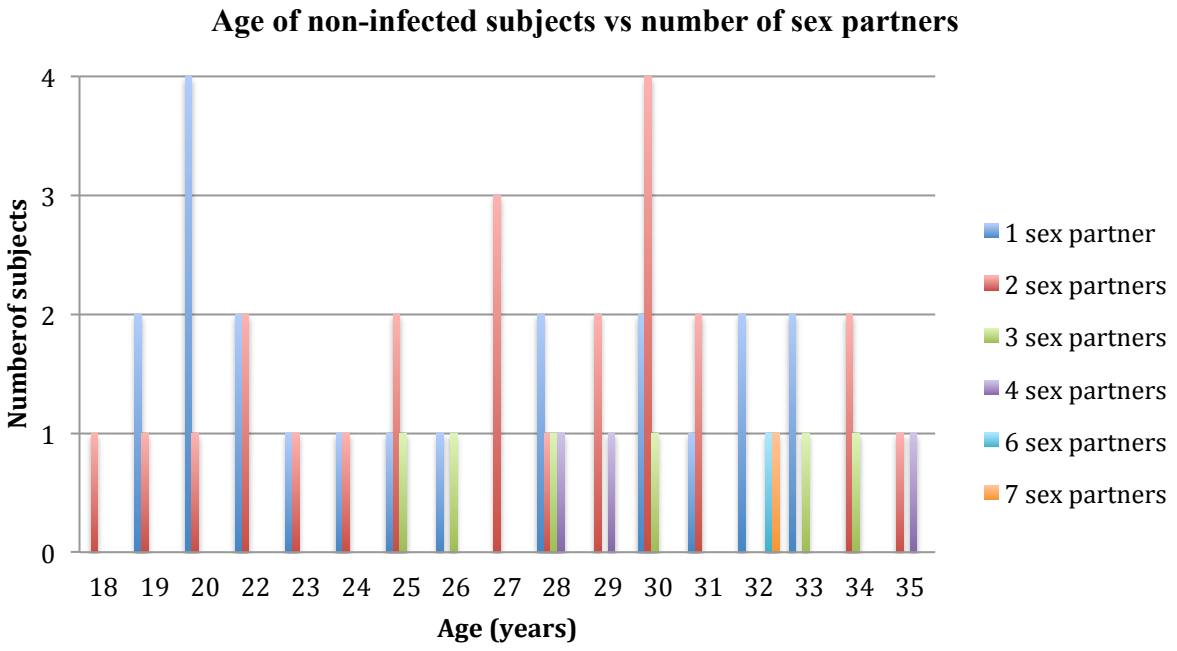

FIGURE 4. Non-infected subjects by age in correlation with the number of sex partners reported.

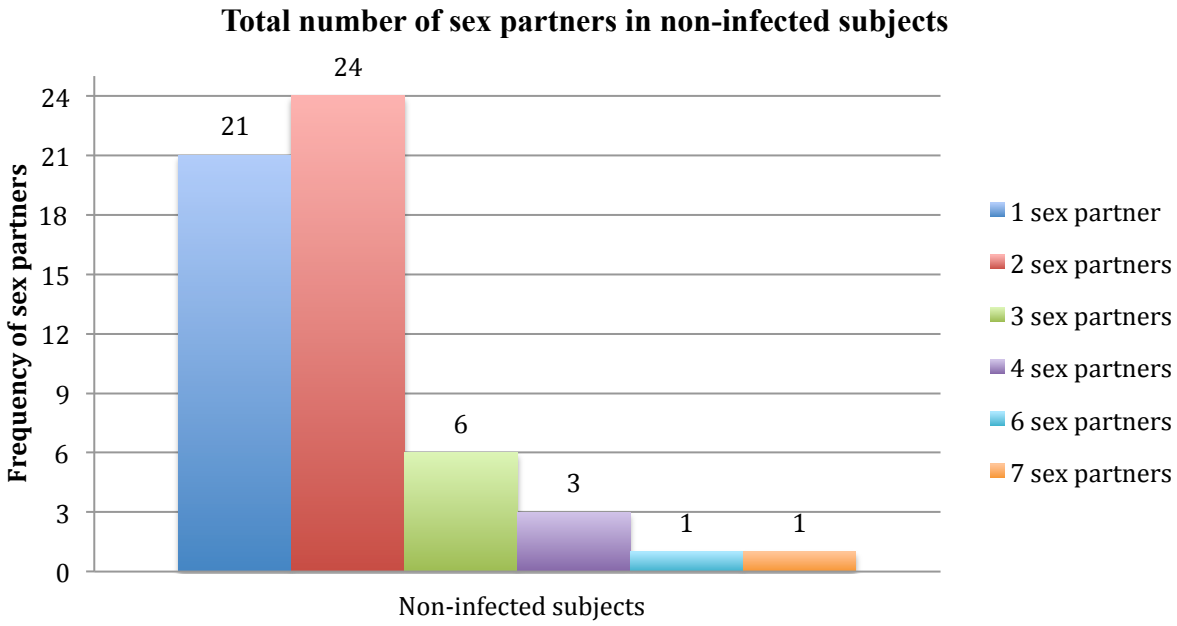

FIGURE 5. Total number of sexual partners in non-infected subjects.

Of the 60 subjects, 29 suspected infidelity from their current partner, where 28 of the 56 non-infected participants (50%) suspected infidelity and only one of the four infected subjects (25%) suspected infidelity (Figure 6).

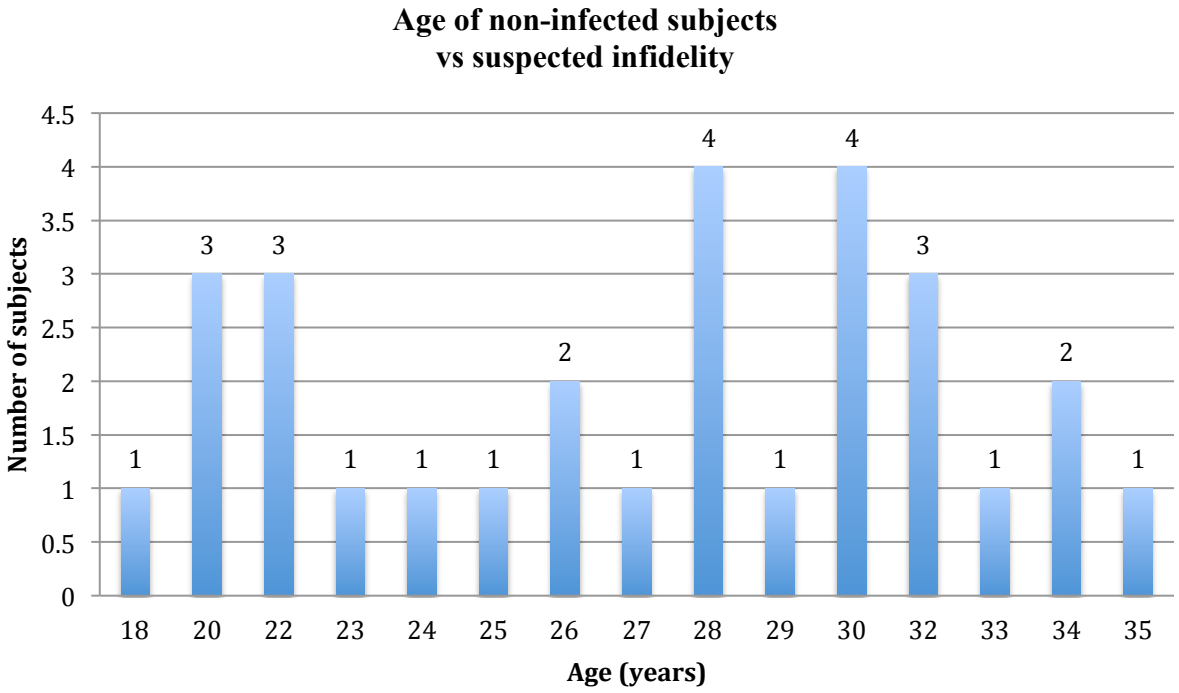

FIGURE 6. Number of non-infected subjects who suspected infidelity is shown and grouped by age.

Along with infidelity, it is important to investigate the use of contraceptives. 37 of 60 participants (61.6%) used some form of contraceptives, previous to actual pregnancy. 50% of the subjects infected with *Chlamydia trachomatis*did not usecontraceptives, while 35 of 56 (62.5%) non-infected subjects did not use any form of contraceptives (Figure 7). The most common method of contraceptives of the remaining non-infected population is oral contraceptive pills (Figure 7).

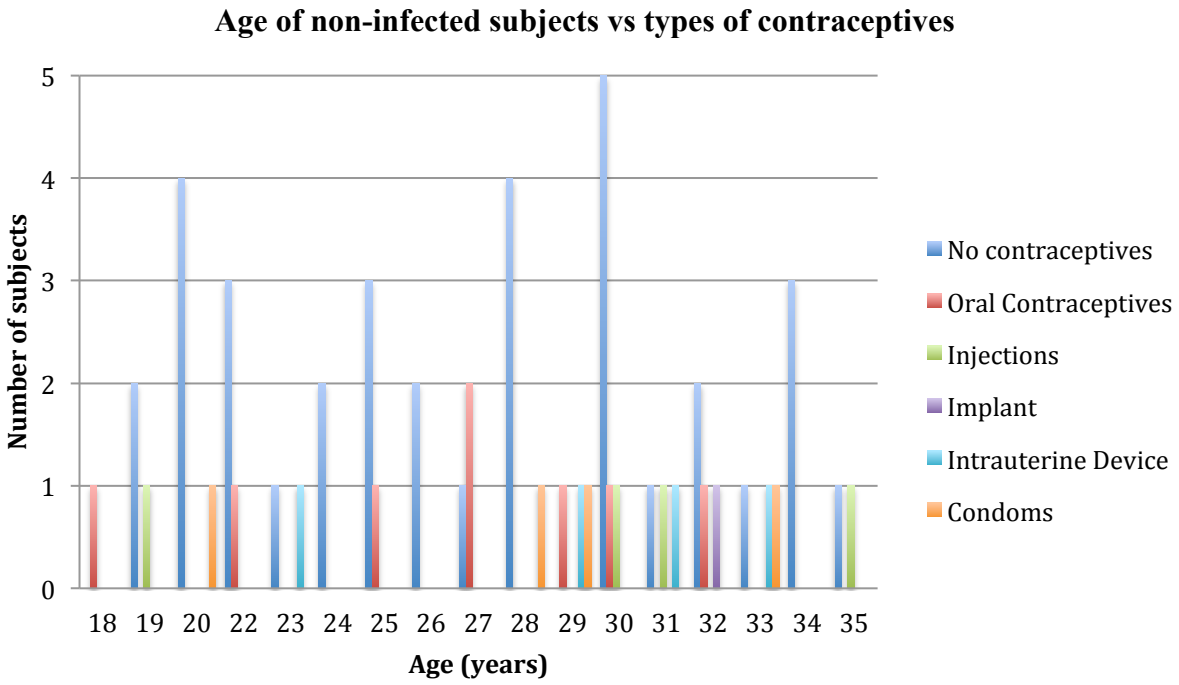

FIGURE 7. Non-infected subjects and types of contraceptives used previous to actual pregnancy.

As far as pelvic pain during the current pregnancy, 45% of the subjects did not present any form of pelvic pain. 50% of the infected population did not present pelvic pain (Figure 8), while 44.6% of the non-infected subjects did not present pelvic pain (Figure 9, Figure 10).

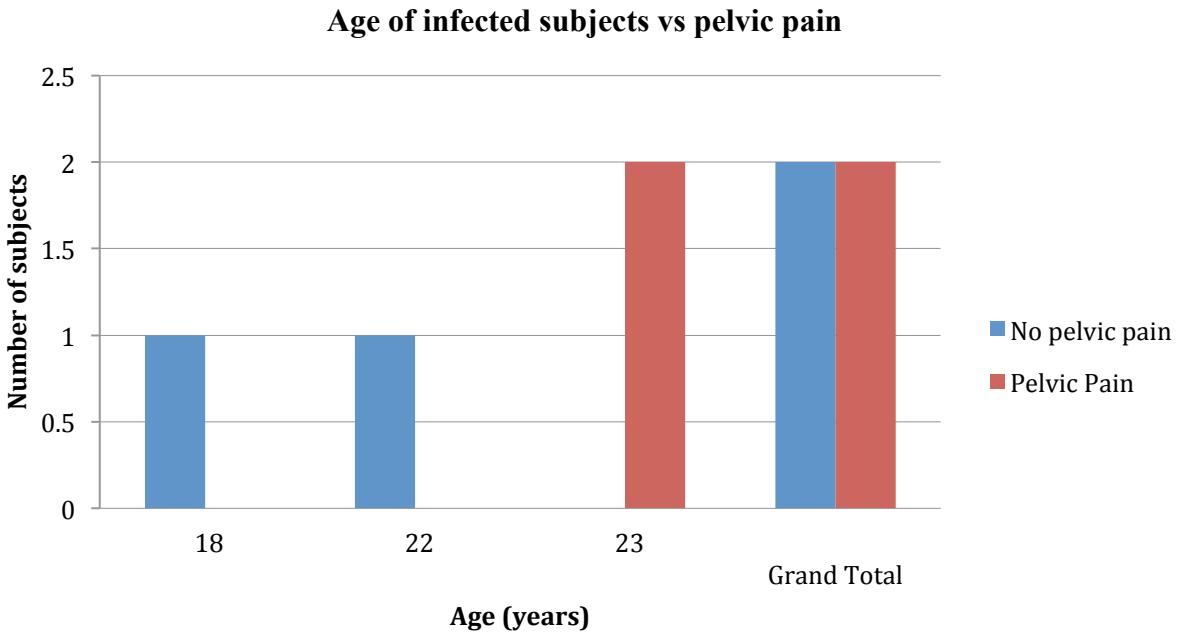

FIGURE 8. Presence or absence of pelvic pain, grouped by age, in subjects infected with *Chlamydia trachomatis*.

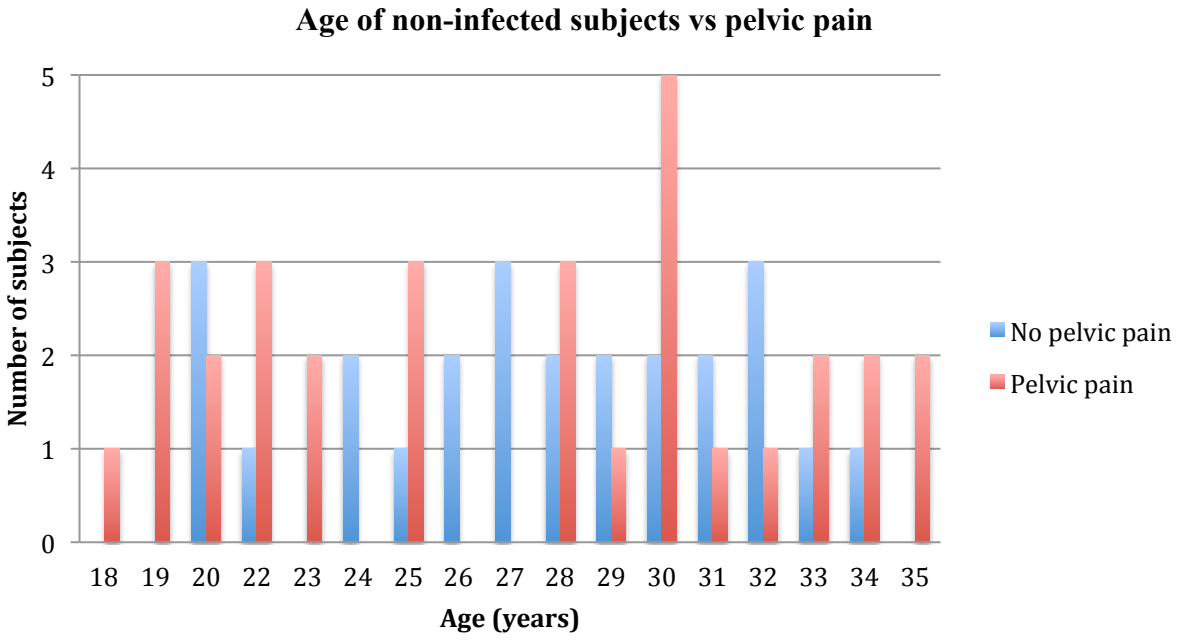

FIGURE 9. Presence or absence of pelvic pain, grouped by age, in non-infected subjects.

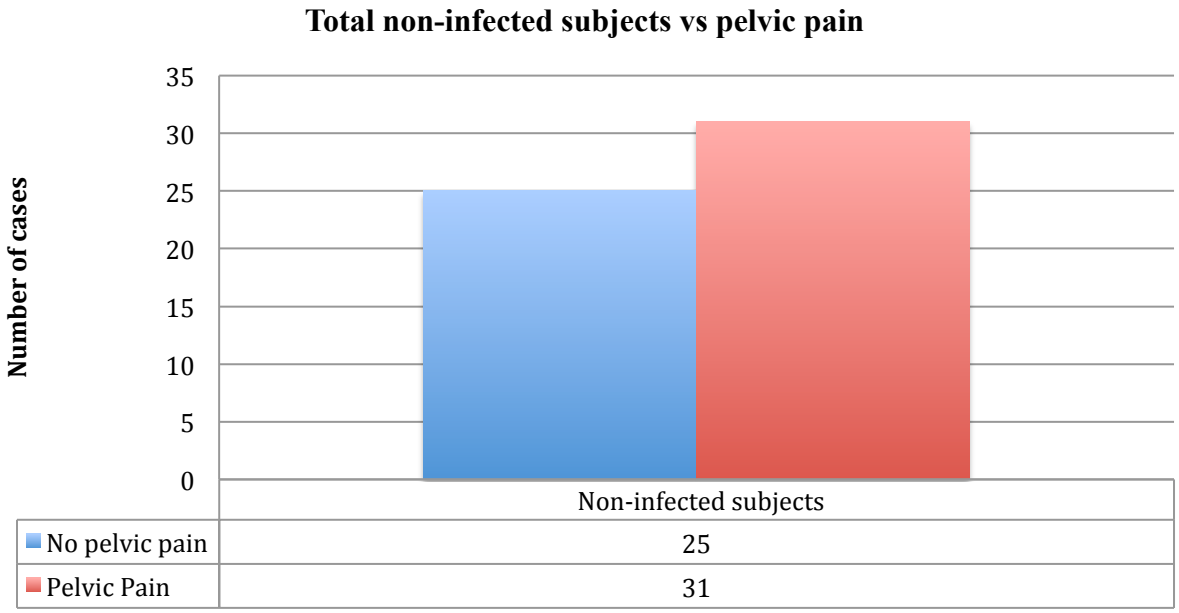

FIGURE 10. Total of pelvic pain and no pelvic pain in non-infected subjects.

The presence of vaginal secretion exists in both the infected and non-infected subjects. 60% of the total subjects (infected and non-infected) complain of vaginal secretions. 75% of the subjects infected with *Chlamydia trachomatis* presented vaginal secretions (Figure 11).

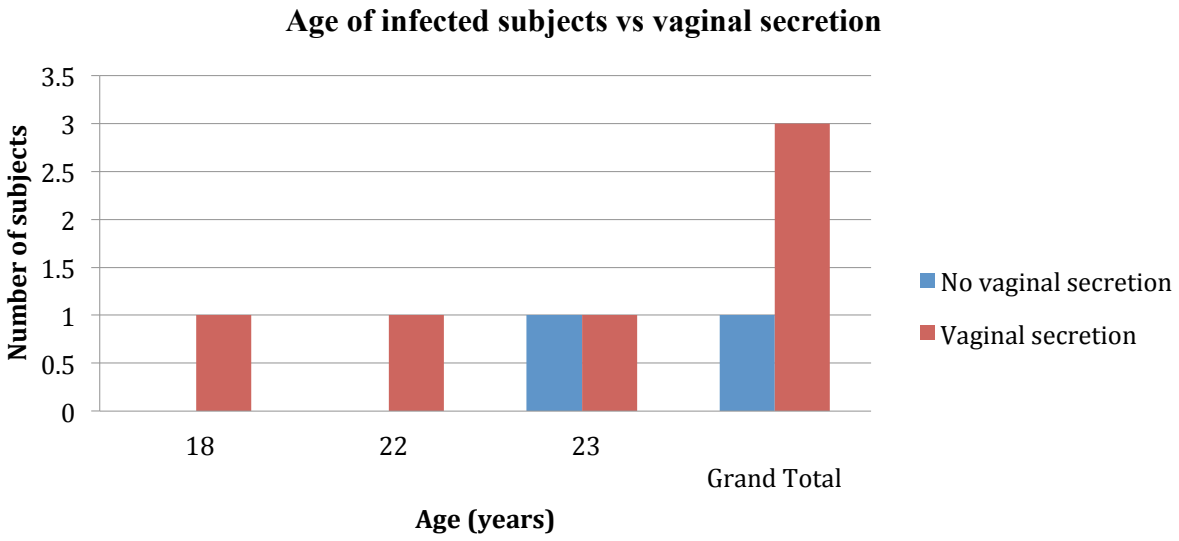

FIGURE 11. Presence or absence of vaginal secretion, grouped by age, in infected subjects.

58.9% of the non-infected subjects presented vaginal secretion (Figure 12, Figure 13). The majority of those who presented secretion were 30 years of age (Figure 12). Of those who did not present secretion, in the non-infected group, the majority were 28 years of age (Figure 12).

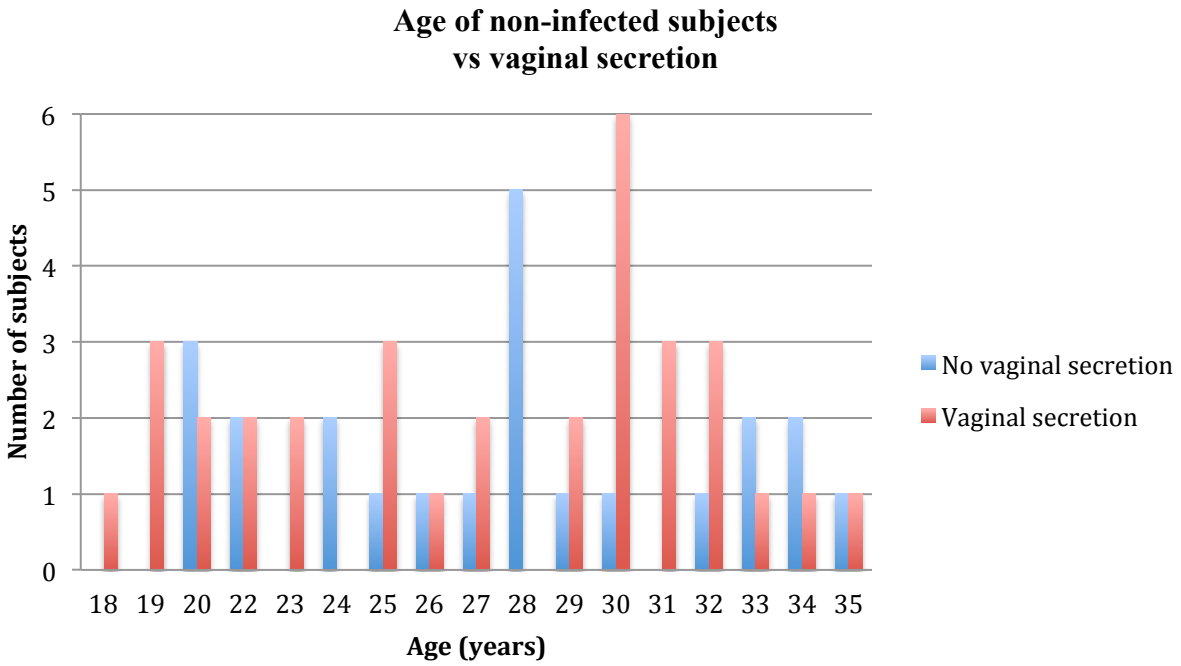

FIGURE 12. Presence or absence of vaginal secretion in non-infected subjects, grouped by age.

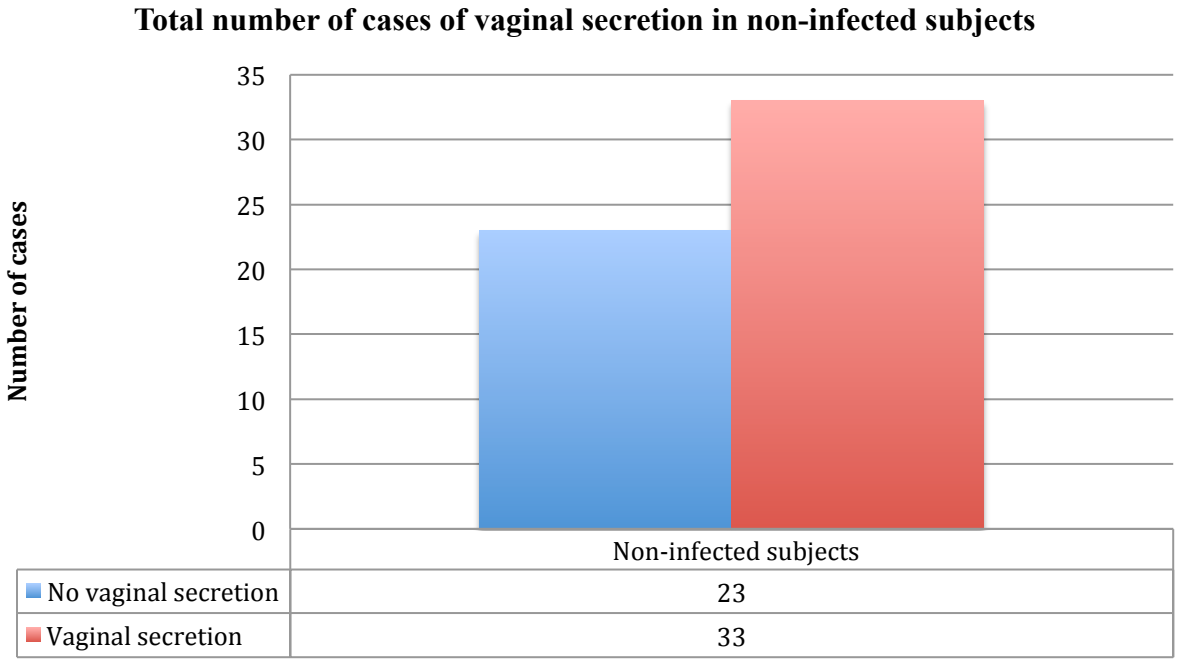

FIGURE 13. Total number of non-infected subjects with vaginal secretion and without vaginal secretion.

Of those who are infected with *Chlamydia trachomatis*, three of the four subjects have had at least one vaginal delivery (Figure 14).

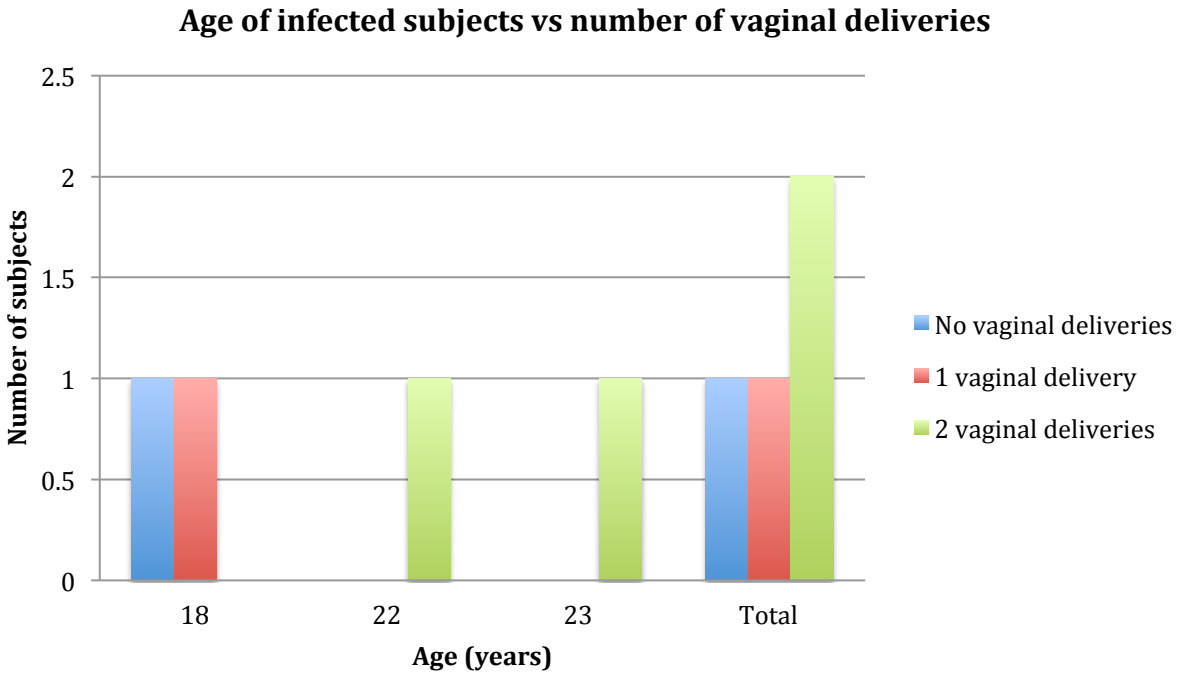

FIGURE 14. Number of infected subjects who have experienced zero, one, and or previous vaginal deliveries.

In terms of the non-infected study population, the number of previous vaginal deliveries ranges from zero to four (Figure 15). The majority of the non-infected participants have had no previous vaginal deliveries (66%) (Figure 16).

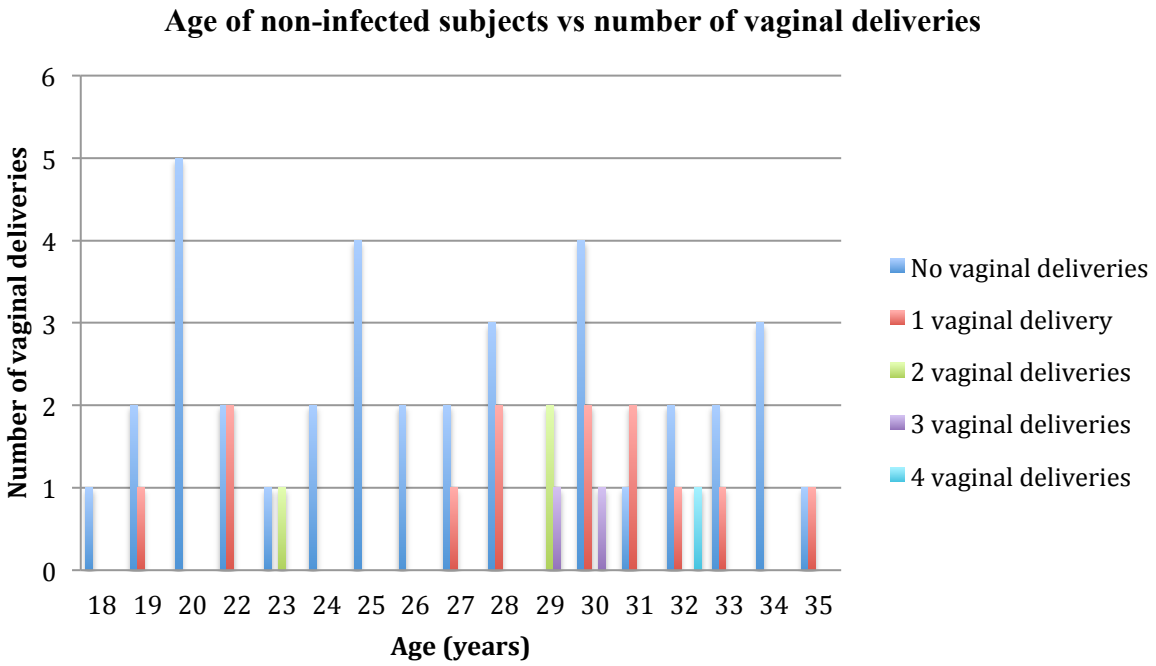

FIGURE 15.Number of vaginal deliveries in non-infected patients, grouped according to age.

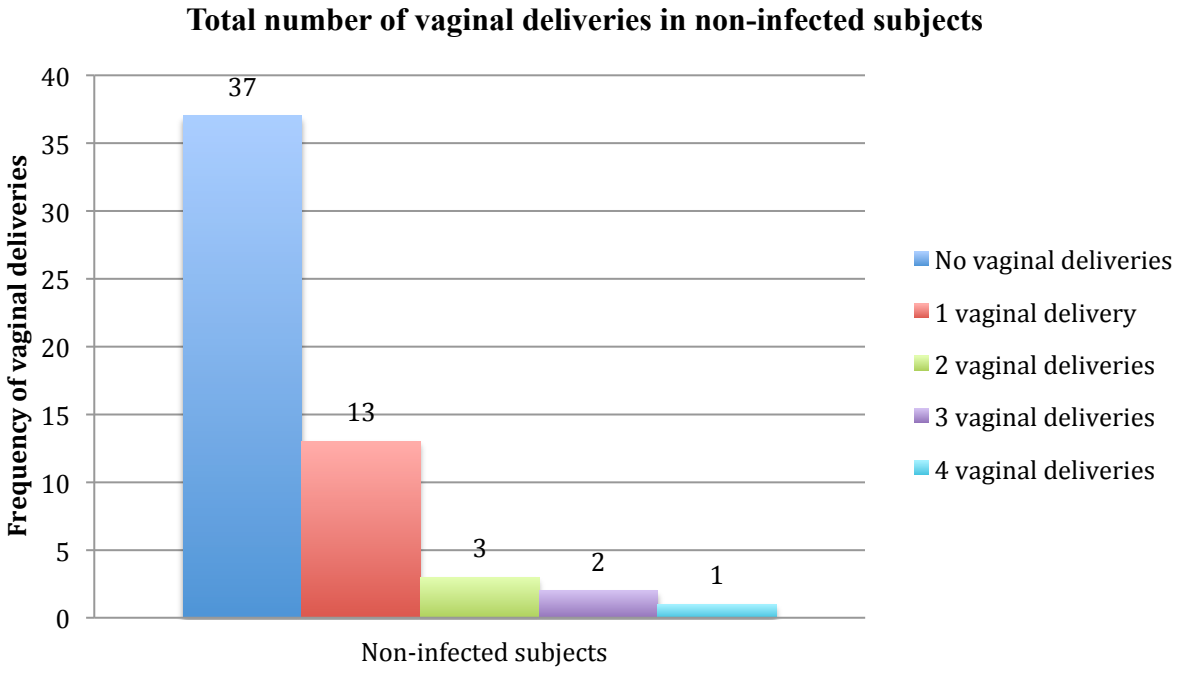

FIGURE 16. Total number of previous vaginal deliveries in non-infected subjects.

Finally, in terms of miscarriages, 78.3% of the total study population has not presented any previous miscarriages (Figure 17, Figure 18, Figure 19). The participants aged 18-20 years of age have not presented any miscarriages (Figure 17, Figure 18). Of the individuals infected with *Chlamydia trachomatis*, only one participant has presented a miscarriage (Figure 17).

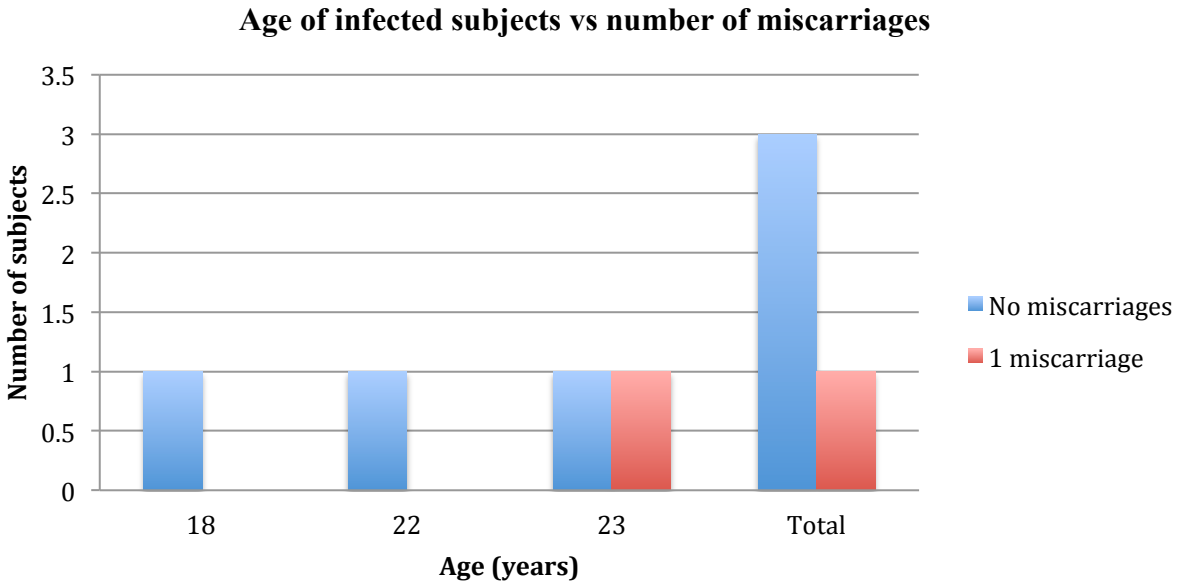

FIGURE 17. Number of infected subjects, by age, in relation to number of previous miscarriages.

The majority of the non-infected subjects, have not reported previous miscarriages (78.5%) (Figure 18, Figure 19); however, of those who have informed a past history of miscarriages, the maximum number of miscarriages presented is three, but was only reported by one subject (Figure 18).

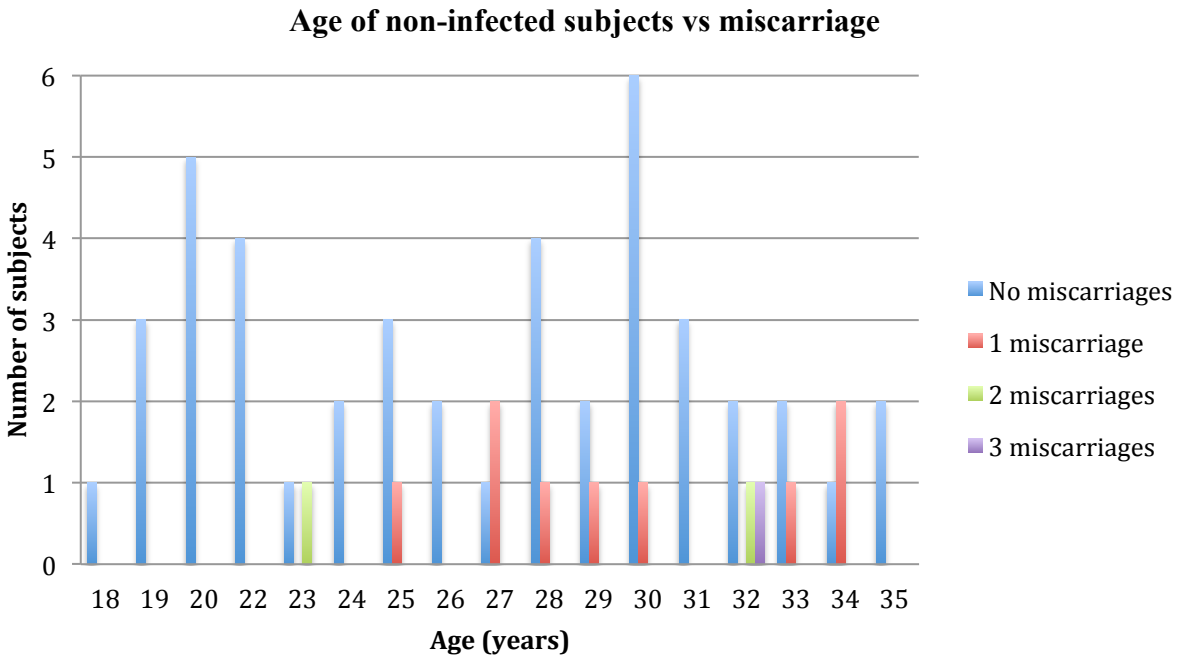

FIGURE 18. Number of miscarriages of the non-infected subjects by age.

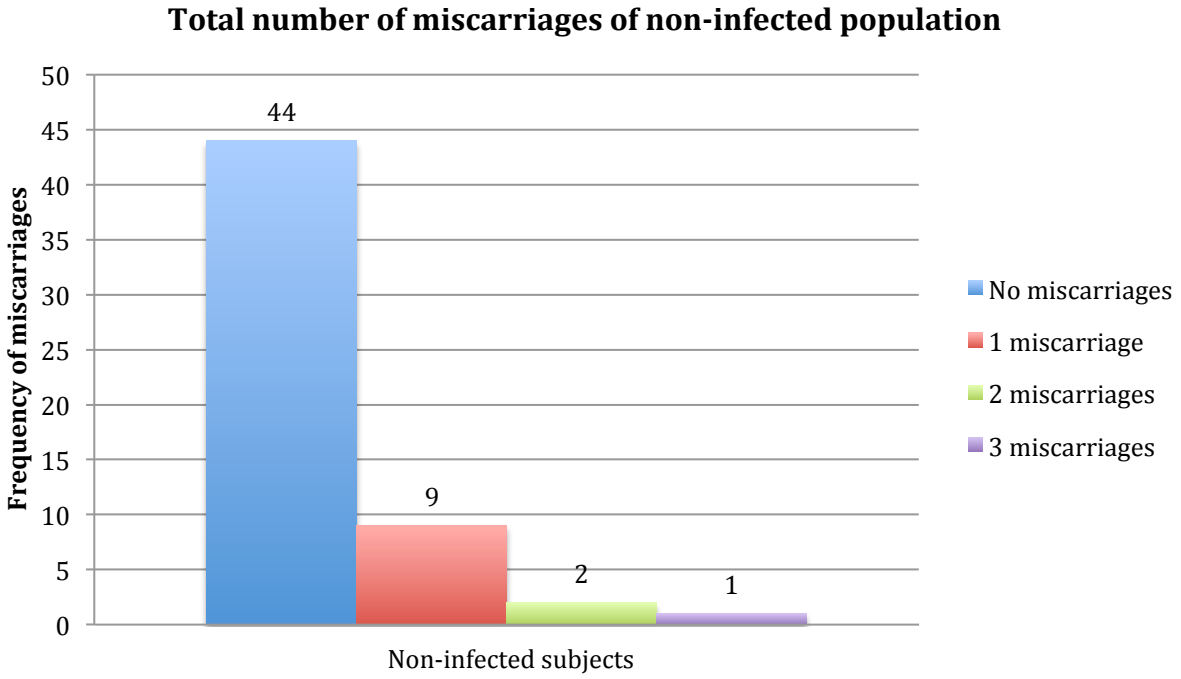

FIGURE 19. Total number of previous miscarriages in non-infected population.

DISCUSSION:

As should be expected based on previous studies, the younger, adolescent population is always at greatest risk for infection with most sexually transmitted infections, such as *Chlamydia trachomatis*. In the United States, the greatest number of sexually transmitted infections by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are found in adolescent girls between the ages of 15 and 19 years of age (Berggren et al., 2011). While this particular pilot study did not investigate the prevalence of chlamydia in young women between the ages of 15-17 years of age, there was still a 7% prevalence rate of *Chlamydia trachomatis* in the population aged between 18-23 years of age. This prevalence rate is high enough to encourage similar investigations including younger subjects.

Additionally, through analysis of the statistical data obtained from the surveys, the subjects who suffered from a genital chlamydial infection presented lower monthly incomes than those who were not infected. The average monthly income of the entire study population was $998.20, while the mode of the monthly income was $300. The maximum monthly income of the infected group was $400, while in the non-infected group; the maximum monthly income was $4000. This could be due to the fact that the infected subjects were actually younger subjects than the non-infected participants and therefore, it might be expected that their sexual partners are also younger. Furthermore, it might be assumed that younger subjects and younger sexual partners do not obtain occupations to provide stable monthly income, thus explaining why the infected population produces less monthly income. It could also be inferred that the lower monthly income is associated with a lower level of education; however, this statement would not be true

seeing as many subjects did not work due to the symptoms of pregnancy and the monthly income presented is only that of their partners, in the case that they are not single mothers. Due to this incomplete information, future studies should investigate level of education of subject and partner, as well as income provided by each member.

In this pilot study, the majority of those who tested positive for genital chlamydial infection reported previous pregnancies. As stated previously, those were tested positive were between the ages of 18 and 23 years of age. This indirectly indicates that the subjects began to have sexual intercourse at an even younger age, putting them at risk for sexually transmitted infections. The same can be said for the number of previous miscarriages. Similar findings were observed between the number of previous pregnancies and miscarriages in the non-infected population. The majority of the non-infected subjects had no previous vaginal deliveries or miscarriages. With this said, it can be deduced that the non-infected population began to have sexual intercourse at an older age than the infected subjects or that the non-infected population effectively used some form of contraceptives. Surprisingly, this was not the case. 50% of the infected population used some form of contraceptives (25% oral contraceptives and 25% injections), while 35 of 56 subjects (62.5%) did not use any form of contraceptives (Figure 7).

Further analysis of the statistical data revealed many relationships that different to the risk factors that have been established by previous studies. For example, it can be expected that the infected subjects would present a sexual history with an increased number of sexual partners, in comparison with the non-infected participants. This however was not the case for this particular study. The participants with a genital infection caused by *Chlamydia trachomatis* presented a

mode of one sexual partner, while the non-infected group presented a mode of two sexual partners. Additionally, only one infected participant suspected infidelity, while 50% of the non-infected population suspected infidelity. Generally, suspected infidelity would increase the number of sexual partners of the sexual companion of the subject, possibly putting them at increased risk for sexually transmitted infections; however, this information is also not as one would presume. In addition, the mode for suspected infidelity in the non-infected population was at 28 and 30 years of age. Typically, one would conclude that the younger the ages of the people in the relationship, the less mature and hence, greater possibility of infidelity; however, this does not appear to be the case in the population used in this pilot study.

Another risk factor that presented with data that was opposite of that expected is the presence of pelvic pain. Chronic pelvic pain is not a very specific symptom, but has been reported in cases of *Chlamydia trachomatis* and other sexually transmitted infections (Satterwhite et al., 2011). 50% of the infected subjects reported pelvic pain, while 31 of 56 (55.3%) non-infected participants presented pelvic pain. Previous investigations report that during pregnancy, pelvic pain is a very common symptom. It has been estimated that 33-50% of pregnant women present pelvic girdle pain before 20 weeks of gestation and that later in the pregnancy, this pain may be present in up to 60-70% of pregnant women (Hilde et al., 2010). This information suggests that pelvic pain is not specific for infection with *Chlamydia trachomatis*, and that there are various factors, which may cause this symptom, such as pregnancy itself.

Another symptom, which may present with pregnancy, is the presence of vaginal secretions. It is well known that during pregnancy, glycogen synthesis increases in the vaginal epithelial cells

due to the effects of estrogen levels along with increased production of lactic acid from glycogenolysis. This produces a lower vaginal pH in pregnant women in comparison with non-pregnant women; consequently producing increased amounts of Lactobacilli in the vagina (Lin et al., 2011). Therefore, the 33 of 56 (58.9%) non-infected subjects who presented vaginal secretions are most likely due to changes in the vaginal pH due to altered hormone levels as a result of pregnancy. Again, infection with *Chlamydia trachomatis* may be asymptomatic in the majority of cases; therefore, there must be another explication of vaginal secretions in the non-infected and possibly even in the infected subjects.

STRENGTHS AND LIMITATIONS:

One of the strengths of this study is that subjects were collected from three different health care facilities, including a public hospital, a private, yet accessible hospital, and a private clinic. By taking samples from participants who attended these three facilities, the overall population of this study is more likely to be a true representation of the Quito population. This is to say that the participants presented a wide range of socio-economic statuses. It could be assumed that those with lower socio-economic statuses are probably related to lower levels of education and thus higher risk candidates for contacting sexually transmitted infections.

As far as the weaknesses of this study, one major weakness is the fact that the Hospital Gineco-Obstetrico Isidro Ayora (HGOIA) changed its policy during the middle of the recollection of samples and instead of accepting all pregnant patients for their first prenatal controls, these patients were sent to their local public health care facilities. Due to this change, fewer healthy patients attended HGOIA during the first trimester of their pregnancy. An additional weakness is that the participants presented a recall bias in which the information that they answered on the surveys could be wrong. For example, some of the patients recalled that their last menstrual period was different than the date that was recorded on their clinical history. Another weakness associated with the response to the survey is the response bias in which the participants may have reported incorrect answers so as to hide any information that may lead one to see her differently. For example, when asked about a history of important illnesses, subjects did not report infection with the human immunodeficiency virus or human papilloma virus. It might also be said that there is a bias of digit preference for those participants who have had multiple sexual partners, but do not wish to reveal the true number, so they round the number

down. Additionally, one might also say that this study possesses a bias in the selection of participants. While the samples were collected from three different health care facilities, the majority of the patients were "*mestizo*." Perhaps the study should take into consideration the various ethnic groups of Ecuador and try to reproduce a population that is representative of the general population. Not many indigenous or afro-Ecuadorian women were attended in any of these three facilities.

Finally, another potential weakness is that several subjects denied participation in this study due to the invasive method used to collect the samples. Many patients expressed that they did not have a problem with the collection of blood samples or urine samples; however, the idea of a vaginal examination turned many away.

RECOMMENDATIONS:

After concluding this pilot study, several recommendations can be made for future investigations related to this topic. In future studies, not only should the sample size be increased, but also the testing sites. Samples should be collected from patients in both public and private hospitals. Additionally, samples should be collected in the different geographical regions of Ecuador, as there are various cultural groups within the country. This is to say that samples should be collected in the coast, the sierra, and the rainforest. In addition, it should be made a point to include the diverse indigenous groups of Ecuador as each group may have different customs. Another important area to investigate in the future is the prevalence of certain sexually transmitted diseases amongst various age groups. This particular pilot study included pregnant women from 18-36 years of age; however, pregnant adolescents were discarded from the study due to the guidelines established in the inclusion and exclusion criteria.

The questions posed in the survey should also be changed for future studies. It is important to identify the patient´s ethnic group as well as the level of education of the participant and their sexual partner. Perhaps even identifying the occupations held by both the participant and her sexual partner could help to identify potential risk factors for infection with sexually transmitted infections. Moreover, it is important to ask about the sexual history of the participant and her partner. In this type of question, the names of the sexually transmitted infections should be shown on the survey, as often times, certain infections or conditions are not understood to be sexually transmitted.

Another area that could be examined in future studies could be the participants' general knowledge of sexually transmitted infections and how to prevent said infections. Participants

could be presented questions in the survey asking what are sexually transmitted infections, how does one contact such infections, and how can they be prevented. Further questioning should be made about the correct and incorrect use of condoms. With this information, the investigation could identify areas where public and private health care employees should emphasize sexual education to diminish the prevalence of sexually transmitted infections.

CONCLUSION:

Four of the 60 subjects who were found at the time of screening to be less than 14 weeks pregnant, were found to test positive for a genital infection with *Chlamydia trachomatis*. This is to say that there is an absolute frequency of genital chlamydial infection of 4% in the study population and a relative frequency of 7% of the general population in women between the ages of 18-24 years. There was not a significant relationship between testing positive for *Chlamydia trachomatis* and the presence of pelvic pain, vaginal secretion, number of sexual partners, or suspected infidelity. There was however, a rather important relationship between previous vaginal births and miscarriages in relation to the age of the infected participants. Additionally, there was a significant relationship, which deserves further investigation, between the infected subjects and their reported monthly income. As this is a pilot study, future investigations should be carried out in a much larger population, diverse population including the various geographical regions of Ecuador. Additional investigations may be created with the emphasis of detecting the prevalence of Chlamydia trachomatis in the adolescent population.

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ANNEXES:

Annex A: Survey.

HGOIA

ENCUESTA

Toda la información obtenida en este documento será completamente confidencial. La razón por la que pedimos información demográfica (nombre, teléfono) es para poder contactarnos con usted en caso de requerir tratamiento. Favor llenar la encuesta con letra imprenta

Nombre: _____

Edad: _____ años

Tiene alergia a algún medicamento?_____

Ha tenido tratamiento medico reciente?_____

Estado civil (Encierre en un círculo su respuesta):

Soltera Unión Libre Casada Separada Divorciada Viuda

Ingreso económico mensual del hogar: _____ USD

Cuántas personas viven en su casa? _____ personas

A qué edad tuvo la primera menstruación: _____ años

Duración de ciclo menstrual:

Cada cuanto tiempo viene su menstruación: _____ días
Cuantos días sangra? _____ días
FUM_____

Cuántos partos normales ha tenido? _____

Cuántos partos prematuros ha tenido? _____

Cuántas cesáreas ha tenido? _____

Cuántos abortos ha tenido? _____

Cuándo fue la fecha de su ultima Papanicolaou y cual fue el resultado:
(dd/mm/aa): _____
Resultado: _____

Número de parejas sexuales: _____

Sospecha de infidelidad Sí No

Usted usa anticoncepción Sí No

Cuál? _____

Tiene dolor pélvico? Sí No

Tiene secresion vaginal? Sí No

Si tiene alguna enfermedad de importancia (presión alta, diabetes, cáncer, etc.) por favor, descríbala a continuación:

_____
_____
_____

Escriba a continuación los medicamentos que Usted toma actualmente:

_____
_____
_____

Firma:

No. C.I.: _____

Annex B: Informed consent form approved by the Bioethics Committee of the Universidad San Francisco de Quito.

Comité de Bioética, Universidad San Francisco de Quito
El Comité de Revisión Institucional de la USFQ
The Institutional Review Board of the USFQ

Quito, Ecuador
Agosto 8, 2011

Dr. Luis Suarez
Presente
De mis consideraciones

Estimado Dr. Suarez:

Por medio de la presente, el Comité de Bioética de la Universidad San Francisco de Quito le complace informarle que su estudio **"Determinar la frecuencia de infección por *Chlamydia trachomatis y Neisseria gonorrhoeae* en mujeres embarazadas del *Hospital Gineco-obstétrico Isidro Ayora (HGOIA), del Hospital Vozandes de Quito y de la Clínica Universitaria USFQ* de la ciudad de Quito"** ha sido aprobado con fecha Agosto 8, 2011; específicamente en lo que se refiere al protocolo y el formulario de consentimiento informado. La aprobación es por un periodo de un año. En caso de que el estudio dure más tiempo, seria necesario solicitar una extensión.

En toda correspondencia con el Comité de Bioética, favor referirse al siguiente código de aprobación: 2011-25.

El Comité estará dispuesto a lo largo de la implementación del estudio a responder tanto a los participantes como a los investigadores en cualquier inquietud que pudiera surgir. Asimismo, es importante recordar que cualquier novedad debe ser comunicado con el Comité; específicamente cualquier evento adverso debe ser comunicado dentro de 24 horas.

El Comité de Bioética ha otorgado la presente aprobación en base a la información entregada por los solicitantes, quienes al presentarla asumen la veracidad, corrección y autoría de los documentos entregados. De igual forma, los solicitantes de la aprobación son los responsables de aplicarlos de manera correcta en la ejecución de la investigación, respetando los documentos y condiciones aprobadas por el Comité, así como la legislación vigente aplicable y los estándares nacionales e internacionales en la materia.

Atentamente,
William F. Waters
William F. Waters, Ph.D.
Presidente del Comité de Bioética
Universidad San Francisco de Quito

Casilla Postal 17-12-841
Quito, Ecuador
comitebioetica@usfq.edu.ec

Anexos

Consentimiento Informado

Nombre_____

Teléfono _____

Estudio

Determinar la frecuencia de la infección por *Chlamydia trachomatis, Neisseria gonorrhoeae* y *Streptococcus Beta Hemolítica del grupo B* en mujeres embarazadas del Hospital Gineco-Obstetrico Isidro Ayora, del Hospital Vozandes y de la Clínica Unversitaria USFQ de la ciudad de Quito.

Investigador principal: Dr. Luis Suarez.

Coinvestigadores: Karla Arévalo, Laura Weber, Dr. Gabriel Trueba, Dr. Gustavo Molina, MSc. Veronica Barragan.

I. INTRODUCCIÓN

Usted ha sido invitada a participar en este estudio. Su participación es voluntaria. Antes de decidir si participa o no debemos informales sobre el objetivo y las actividades de este estudio, además de los beneficios y riesgo. Le podemos responder cualquier pregunta que tenga. Si acepta ser parte del estudio, Usted tiene que firmar este documento. Gracias.

II. OBJETIVO DEL ESTUDIO

El objetivo general del estudio es determinar la frecuencia de infección por *Chlamydia trachomatis, Neisseria gonorrhoeae* y *Streptococcus Beta Hemolítica del grupo B* en mujeres embarazadas en el Hospital Gineco-Obstetrico Isidro Ayora, del Hospital Vozandes y de la Clínica Universitaria USFQ de la ciudad de Quito, además existen otros objetivos como son la identificación de factores de riesgo de la infección. Esta información nos permitirá implementar una campaña de tamizaje para embarazadas en el futuro.

agosto 8 2011 12

III. PROCEDIMIENTOS DEL ESTUDIO

Si usted participa, los médicos gineco-obstetras del HGOIA, del Vozandes o de la Clínica Universitaria USFQ tomarán una muestra endocervical (del cuello del útero) para realizar PCR (reacción en cadena de la polimerasa) y determinar la frecuencia de infecciones. Además si usted está infectada se le proveerá con una receta para el tratamiento eficaz a usted y su pareja, así reduciendo el riesgo de complicaciones.

IV. POSIBLES RIESGOS DEL ESTUDIO

No existen posibles riesgos asociados a la toma de muestras endocervical si se realiza con las correctas medidas de esterilidad, como se las realizará en este estudio. El tratamiento antibiótico, que se le proveerá a usted y a su pareja, en caso de infección puede tener varias reacciones adversas, sin embargo no son frecuentes y son seguras durante el embarazo.

V. POSIBLES BENEFICIOS DEL ESTUDIO

Los resultados de éste estudio ayudarán a conocer la frecuencia de infección por estas bacterias, además ayudará a identificar grupos de riesgo. El tratamiento evitará complicaciones para su bebé y para usted y su pareja y también evitará la diseminación de la enfermedad.

VI. COSTO

El costo de realizarse esta prueba es $120 pero si participa en este estudio no tendrá costo para usted.

VII. COMPENSACIÓN

Este estudio no contempla pago por su participación.

VIII. DERECHO A ABANDONAR EL ESTUDIO

Si usted decide, no participar, en cualquier momento no existe problema, solo debe informar a su doctor que ya no desea formara parte del estudio. El investigador puede también pedirle que abandone el estudio.

IX. CONFIDENCIALIDAD DE LOS REGISTROS DE DATOS

Sus datos son confidenciales. Si los resultados de este estudio son publicados en revistas o en reuniones científicas, no se publicarán los nombres de las participantes. No se revelará los datos de ninguna participante a menos que sea requerido por ley.

X. PREGUNTAS

Si tienen cualquier pregunta hágala ahora y si las tiene después puede llamar al 0222898840 o al 098827594 y comunicarse con Karla Arévalo estaremos gustosos en ayudarle.

Usted también puede contactar a William F. Waters, PhD. Presidente del Comité de Bioética de la USFQ, a 297-1775

XI. DOCUMENTACIÓN DEL CONSENTIMIENTO INFORMADO

Nombre de participante: _____

Firma de participante: _____

No. C.C.: _____

Nombre de testigo: _____

Firma de testigo: _____

No. C.C.: _____

Nombre del médico: _____

Firma del médico: _____

Código del médico: _____

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