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Botany Microbiology Department

Isolation of Chlamydia trachomatis and Mycoplasma from endocervical swabs of unfertile women and women subjected of tubal pregnancy

By

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APROVAL SHEET

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Summary

Summary

Infertility is a worldwide health problem with one in six couples suffering from this condition.

Infertility results from untreated pelvic inflammatory disease (PID), a sexually transmitted infection (STI) or other reproductive tract infection.

Chlamydia trachomatis (C. trachomatis) is a well recognized sexually transmitted pathogen associated with long term complications like PID, ectopic pregnancy, and infertility. Although infection with *C. trachomatis* has been suggested to be a cause of infertility due to the outcomes on the genital tract, many obstetrics and gynecology (OB & GYN) clinics do not perform routine screening for *C. trachomatis*.

There is strong support for the role of *Mycoplasma genitalium* (*M. genitalium*) in the etiology of cervicitis, endometritis, PID, genital-tract disease, and in tubal factor infertility.

Mycoplasma hominis (*M. hominis*) is commonly found in the genital tract of sexually experienced females. Reports of the prevalence of *M. hominis* in women with cervicitis vary widely.

The objective of this study was to determine the occurrence of *C. trachomatis. M. genitalium*, and *M. hominis* infection in infertile women attending infertility clinic and to compare the incidence with fertile women as a control group as such data are lacking.

During the period from October 2012 to July 2013 this study was conducted in King Khalid university hospital (KKUH) and King Abdul-Aziz University hospital (KAUH) with the aid of laboratory facilities at KKUH.

Endocervical specimens were collected from 100 women presenting with primary and secondary infertility and from 100 fertile women as control group. Qualitative and quantitative light-mix real-time PCR assay was performed for the detection of *C. trachomatis, M. genitalium, and M. hominis* from endocervical specimens from patients.

C. trachomatis was the most frequent pathogen detected, *C. trachomatis* was significantly higher among infertility group compared with control group (8% versus 1%, respectively, P-value=0.017). *M. genitalium* was detected in 3 (3%) of infertile women (P-value=0.123). *M. hominis* was detected in 1 (1%) of the infertile and 2 (2%) of the control women (P-value=0.50). A mix infection of *M. genitalium* and *M. hominis* was detected in 1 (1%) of infertile women (P-value=0.4999).

Participants aged 26-32 years and aged 33-39 were significantly higher among infertility group (34%and35%) than the control group (13% and 17%) (P-value<0.0001; and P-value=0.004, respectively*).*

Participants aged 40-46 years were significantly lower among infertility (15%) group than control group (53%) (P-value< 0.0001).

C. trachomatis infection was higher among symptomatic women (6/54, 11%) than asymptomatic women (2/46, 4%). *C. trachomatis* was statistically higher among infertile women with low-seated abdominal pain (19.23 %, P-value = 0.044).

There was no statistically significant difference between infertility and control group concerning signs and symptoms except for signs of cervicitis were presented only among infertile group (6%) with a statistically significant difference (P-value =0.014).

Age groups 19-25 and 26-32 were significantly higher among primary than secondary infertility (P-value=0.009; and, P-value < 0.0001, respectively).

Our findings confirm an association between *C. trachomatis* and infertility, the incidence of *C. trachomatis* among infertile women was higher than expected. Therefore, This finding calls for a national screening program for the early detection of *C. trachomatis* infection among infertile couples.

We found no significant differences among fertile and infertile women for *M. genitalium and M. hominis* infections.

The results demonstrate that real-time PCR prove to be a rapid alternative to the traditional methods. Information on bacterial load in genital swabs can be obtained.

Table 1.1 List of Abbreviations.

Abbreviations	Meaning
AIDs	Acquired immunodeficiency syndrome
BV	Bacterial vaginosis
bp	Base pair
C	Cytosine
C. trachomatis	*Chlamydia trachomatis*
C_p	Crossing point
DFA	Direct fluorescent antibody
EBs	Elementary bodies
EIA	Enzyme immunoassay
G	Guanine
HIV	Human immunodeficiency virus
IC	Internal control
KAUH	King Abdulaziz University Hospital
KKUH	King Khalid University Hospital
LPS	Lipopolysaccharide
M. genitalium	*Mycoplasma genitalium*
M. hominis	*Mycoplasma hominis*
MGPs	Magnetic glass particles
mL	Microliter
MOMP	Major outer membrane protein
mPCR	Multiplex PCR
N. gonorrhoeae	*Neisseria gonorrhoeae*
NAATs	Nucleic Acid Amplification Tests
NGU	Non-gonococcal urethritis
OB & GYN	Obstetrics and gynecology
PCR	Polymerase chain reaction
PHC	Primary health care
PID	Pelvic inflammatory disease
P-value	Predictive value
RB	Reticulate body
SD	Standard deviation
STIs	Sexually transmitted infections
TC	Tissue culture
T_m	Melting temperature
TMA	Transcription mediated amplification

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Chapter (1)

Introduction

Introduction

1.1 Infertility

Infertility is a worldwide health problem with one in six couples suffering from this condition and with a major economic burden on the global health care industry. Estimates of the current global infertility rate suggest that 15% of couples are infertile [Zegers-Hochschild *et al.*, **2009**].

1.1.1 Causes of Infertility

The exact incidence of the various etiologic causes for infertility varies with the population studied. In the broadest of terms, 15% to 20% of the causes of infertility are the result of ovulatory dysfunction; 30% to 40% are caused by pelvic causes such as endometriosis, adhesions, or tubal disease; 30% to 40% are because of male causes, increased semen viscosity, decreased sperm motility, or decreased semen volume; fewer than 5% are because of abnormal sperm-cervical mucus penetration or anti-sperm antibodies. In approximately 10% to 15% of couples no direct cause of their infertility can be found, but on further evaluation and treatment, occasionally factors such as poor sperm penetration, abnormal-appearing Oocytes, are elucidated. This group is referred to as unexplained infertility (Figure 1.1).

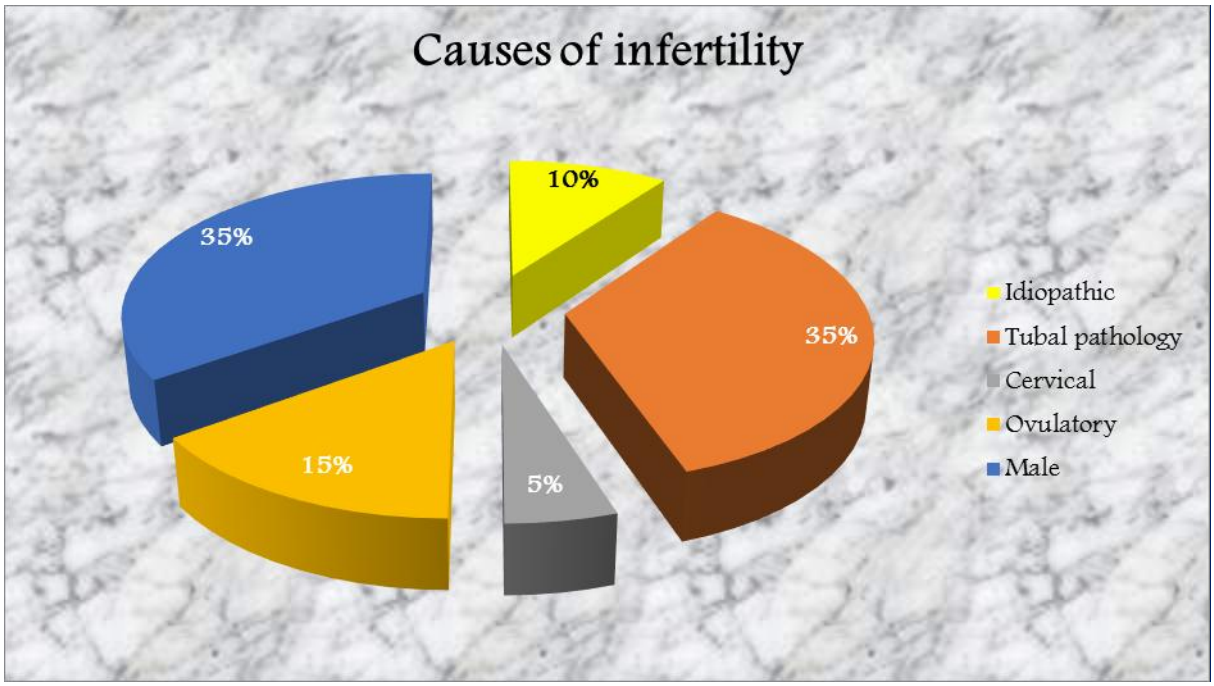

Figure. 1.1 Cause and incidence of infertility in couples [Bradshaw and Carr, **1998**].

In addition, 20%–40% of couples may have multiple factors for infertility [Bradshaw and Carr, **1998**].

1.1.1.1 The Role of Infection

About 35% of women with an infertility problem are afflicted with post-inflammatory changes of the oviduct or surrounding peritoneum that interfere with tubal-ovarian function. Most of these alterations result from infection. In most cases, especially role caused by *C. trachomatis*, signs and symptoms are often slight or nonexistent, the percentage of women with upper genital tract infections is underestimated. Symptomatic, asymptomatic, or latent infections or their outcomes may also contribute to chronic inflammation of the cervix and endometrial, changes in reproductive tract secretions, induction of immune

mediators that interfere with gamete or embryo physiology, and disorders. Infection is also a major factor in male infertility [Westrom, **1994**].

For a great many women in developing countries, infertility results from untreated PID, of a STI or other reproductive tract infection. STIs are a public health problem in all countries and a major health problem, especially in parts of the developing world [Adler, **1996**].

In developing countries, STIs and their complications rank in the top five disease categories for which adults seek health care, untreated STIs can have critical implications for reproductive, maternal and new-borns health. STIs are an important preventable cause of infertility for men and women.

According to the World Health Organization, there are an estimated 448 million new cases of STIs which are acquired worldwide annually [World Health Organization, **2011**].

More than 30 bacterial, viral and parasitic pathogens are transmissible sexually [Holmes *et al*., **1999**]. While STIs are mostly transmitted through sexual intercourse, transmission can occur also from mother to child during pregnancy and childbirth, and through blood products or tissue transfer, every year worldwide, up to 4000 new-born babies become blind every year because of eye infections attributable to untreated maternal *Gonococcal* and *Chlamydial* infections.

Including human immunodeficiency virus (HIV) infection that leads to acquired immunodeficiency syndrome (AIDS), they have been recognized as a major public health problem for many years, occur every year throughout the world in men and women aged 15–49 years, with the largest proportion in the region of south and south-east Asia, followed by sub Saharan Africa, and Latin American and the Caribbean [Global prevalence and incidence of curable STIs, **2001**].

STIs are the main preventable cause of infertility, particularly in women. Between 10% and 40% of women with untreated *Chlamydial* infection develop symptomatic PID.

Post-infection tubal damage is responsible for 30% to 40% of cases of female infertility [Simms and Stephenson, **2000**].

Furthermore, women who have had PID are 6 to 10 times more likely to develop an ectopic (tubal) pregnancy than those who have not, 40% to 50% of ectopic pregnancies can be attributed to previous PID [Westrom, **1980**].

STIs, by their transmissible nature, affect not only individuals, but their partners as well. Surveillance is often poor and data on STIs in Islamic countries are limited.

Although Saudi Arabia is a traditional Islamic society, recent globalisation and rapid economic development have had their own impact on the health and lifestyle of the population, holiday travel has also increased over the last decade in the community.

In Saudi society, discussing STIs is considered taboo where ethics and social factors give rise to many obstacles. Due to these reasons there is a possibility that STIs might pose a significant public health threat.

Data of *C. trachomatis, M. genitalium, and M. hominis* infections are limited in Saudi Arabia, hence the **main objectives of the present study target the followings:**

1. Determining the occurrence of *C. trachomatis, M. genitalium*, and *M. hominis*, among infertile women attending infertility units and to compare it with fertile control women attending OB-GYN units in KKUH and in KAUH in Riyadh.
2. Evaluating the association of these microorganisms with a particular type of infertility (primary or secondary) and to evaluate as well their

association with different infertility factors (hormonal factor, ovulation factor, tubal factor, male factor, and unexplained factor).

3. Determine if screening for *C. trachomatis*, *M. genitalium*, and *M. hominis*, necessary as part of routine family planning.
4. Evaluating the association between the microorganisms investigated with symptomatic and asymptomatic women with infertility problems.
5. Assessing the age-dependent occurrence of *C. trachomatis*, *M. genitalium*, and *M. hominis* infections.
6. Establishing a convenient method for rapid detection and identification of *C. trachomatis*, *M. genitalium*, and *M. hominis* infections from women attending infertility units.

Chapter (2)

Literature Review

Literature Review

2.1 *C. trachomatis*

2.1.1 Classification

Chlamydia was first considered as protozoa and later as viruses, they are gram-negative obligate intracellular bacteria. Originally, they were taxonomically categorised into their own order *Chlamydiales*, with one family, *Chlamydiaceae*, and a single genus, *Chlamydia*. The genus included four species: *C. trachomatis*, *C. psittaci* [Moulder *et al.,* **1984**] *C. pneumonia* [Grayston *et al*., **1989**] and *C. pecorum* [Fukushi and Hirai, **1992**].
In 1999, it was recommended by Everett *et al.,* [**1999]** that the genus *Chlamydia* should be divided in two genera, *Chlamydia* and *Chlamydophila*, containing altogether nine species. However, the proposal to change the taxonomic nomenclature for the *Chlamydiaceae* family has not been generally accepted in the field [Schachter *et al*., **2001**].

Two species, *C. trachomatis* and *C. pneumoniae*, are common human pathogens, whereas the other species occur mainly in animals.

C. trachomatis has been isolated only from humans and comprises two human serotypes (trachoma and Climatic bubo), including a total of 15 serotypes [Everett *et al.,* **1999**].

C. trachomatis is an obligate intracellular human pathogen, *C. trachomatis* is a Gram negative bacteria, therefore its cell wall components retain the counter-stain safranin and appear pink under a light microscope. It can appear as either coccoid or rod shape. Classification of *C. trachomatis* is in table 2.1.

Table 2.1 The classification of *C. trachomatis* [Everett *et al.*, 1999].

C. trachomatis	
Scientific classification	
Domain:	Bacteria
Phylum:	*Chlamydiae*
Class:	*Chlamydiae*
Order:	*Chlamydiales*
Family:	*Chlamydiaceae*
Genus:	*Chlamydia*
Species:	*C. trachomatis*

C. trachomatis is a well-recognized bacteria and one the most frequently reported sexually transmitted infection. Besides its potential to produce genital tract infection, *C. trachomatis* is increasingly being associated with long-term complications such as infertility. According to a World Health Organization [**2001**] report, *C. trachomatis* is responsible for the most common sexually transmitted bacterial infection worldwide, affecting more than 90 million people.

2.1.2 Aetiology of *C. trachomatis*

C. trachomatis has a unique life cycle, which differentiates it from all other microorganisms (Figure 2.1). Infection begins when elementary bodies (EBs) attach to specific receptors found on non-ciliated columnar epithelium of the host. This type of epithelium is located in the endocervix, endometrium, fallopian tube, and urethra, making those sites vulnerable to infection. The host cell ingests the organism by a

Chlamydia-specific phagocytic process (the mechanism was illustrated in Maree thesis on the site of higher education). After phagocytosis, the EB exists within a cytoplasmic vacuole or phagosome, where it is protected from host defence systems. Within the phagosome, the EB transforms into a reticulate body (RB) in order to multiply. It multiplies by binary fusion after duplicating its own DNA, RNA, and proteins by using host ATP. The RBs then reorganize back into EBs, the infectious form of the organism. Ultimately, the host cell undergoes either lyses or exocytose with release of the EBs, which infects adjacent cells and restart the cycle. This process takes 2–3 days. *C. trachomatis* has a cell wall like Gram-negative bacteria but it cannot synthesize its own ATP or grow on artificial media, hence its similarity with a virus is one of its main characters.

The chemical composition of the cell wall of the EB is quite similar to that of Gram-negative bacteria. The cell wall of the RB contains less phospho-lipid than the EB; thus, RBs are highly labile and do not survive outside of the host cell. However, the EB is relatively stable in extracellular environments because its envelope is strengthened owing to cysteine proteins that are cross-linked by disulfide bonds, providing the EB structural integrity and resistance [Hatch, **1999**].

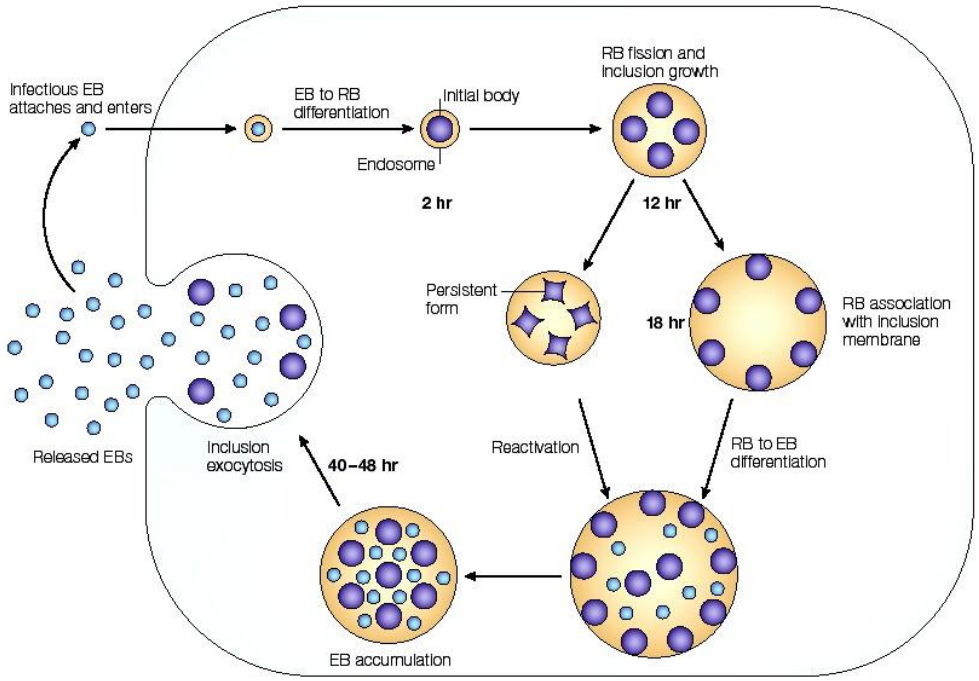

Figure 2.1 The life cycle of *C. trachomatis* [Brunham and Rey-Ladino, 2005].

C. trachomatis is currently classified into 15 serotypes: A, B, Ba (AP-2), C, D, E, F, G, H, I, J, K, L1, L2, and L3 [Morre *et al*., **2000**]. Classification is based on the major outer membrane protein (MOMP) using polyclonal and monoclonal antibodies. Typically, different serotypes are associated with specific clinical diseases (table 2.2).

Table 2.2 Serotypes of *C. trachomatis* diseases [Dimitrakov, 2002].

Species	Acute Diseases	Sequelae/Chronic Diseases
C. trachomatis		
serotype A-C	Conjunctivitis	Trachoma
serotype D-K (Men)		
	Urethritis	Chronic urethritis
		Prostatitis
		Epididymitis
		Infertility
		Reiter's syndrome
		Proctitis
serotypes D-K (Women)		
	Acute urethral syndrome	
	Bartholinitis	
	Cervicitis	Infertility, ectopic
	Endometritis	pregnancy and PID
	Salpingitis	
	Periappendicitis	Chronic abdominal
	Perihepatitis	pain
Serotype LGV	Lymphogranulomavenereum	

2.1.3 Genome structure

C. trachomatis has a genome that consists of 1,042,519 nucleotide base pairs (bp) [Stephens *et al*., **1998**]. *C. trachomatis* strains have an extra chromosomal plasmid, which was sequenced to be a 7493-bp. The plasmid of *C. trachomatis* is a favoured target for DNA-based diagnosis of *C. trachomatis* because there are approximately 7-10 copies of the plasmid present per chlamydial particle [Kalman *et al*., **1999**].

2.1.4 Epidemiology

2.1.4.1 In men

1. **Urethritis** *C. trachomatis* is the most common cause of non-gonococcal urethritis (NGU) in men. The proportion of cases that are asymptomatic vary by population and range from 40 to 96 % [Schwartz and Hooton, **1998**; Cecil *et al.*, **2001**; Kent *et al*., **2005**; Stamm *et al*., **2005**; Bradshaw *et al*., **2006**; Takahashi *et al*., **2006**].
2. **Epididymitis** *C. trachomatis* is one of the most frequent pathogens in epididymitis among sexually active men <35 years of age, Asymptomatic urethritis frequently accompanies sexually transmitted epididymitis [Workowski and Berman, **2010**].
3. **Prostatitis** *Chlamydia* is also a potential cause of prostatitis in men [Wagenlehner *et al*., **2006**; Cunningham and Beagley, **2008**; Hirano and Hoshino, **2010**].

2.1.4.2 In Women

1. **Bacterial Vaginosis (BV):** BV is an inflammation of the vagina. The cause is usually a change in the normal balance of vaginal bacteria or an infection; it is caused by bacteria including *M. hominis, C. trachomatis* [Bacterial Vaginosis].

2. **Endometritis:** Endometritis refers to inflammation of the endometrial, the inner lining of the uterus. Endometritis is present in 40% of women with cervicitis. *C. trachomatis* and, to a lesser extent, *Neisseria gonorrhoeae* (*N. gonorrhoeae)* infections are closely associated with endometritis [Paavonen *et al*., **1979**; Kiviat *et al*., **1990**; Hillier *et al*., **1996**].
3. **Cervicitis:** Cervicitis is an inflammation of the uterine cervix [Centers for Disease Control and Prevention, **2003]**. Infectious cervicitis caused mostly by *C. trachomatis* and *N. gonorrhoeae [Marrazzo and Martin, **2007**; Lusk and Konecny, **2008]***. Cervicitis can progress to PID with severe reproductive problems, even in asymptomatic cases [Kreiss, **1994**; Zunt, **2002**].
4. **PID:** PID is an infection of the female reproductive organs. PID is thought to occur as microorganisms ascend from the lower genital tract, infecting and causing inflammation of the uterus, fallopian tubes, and ovaries [Paavonen *et al*., **2008**]. Genital infection with *C. trachomatis*, the most common reportable disease in the United States, [Geisler, **2011**] can lead to serious conditions among women, including PID, and chronic pelvic pain [Brunham *et al*., **1988**; Westrom *et al*., **1992**]

   C. trachomatis, *N. gonorrhoeae, M. genitalium,* and microorganisms associated with BV are frequently isolated from the lower and upper genital tracts of women with PID [Simms *et al*., **2003**; Haggerty *et al*., **2004**].

   Although *C. trachomatis* is among the most frequent pathogens associated with symptomatic PID, [Heinonen and Miettinen, **1994**; Ness *et al*., **2002**; Ness *et al*., **2004**], isolated in the upper genital tract of up to a quarter of these patients [Heinonen and Miettinen, **1994**; Haggerty *et al*., **2003**; Haggerty *et al*., **2004**], it has also been associated with a wide spectrum of upper genital tract pathology ranging from asymptomatic endometritis

[Bevan *et al*., **1995**; Wiesenfeld *et al*., **2005**] to symptomatic, laparoscopically confirmed salpingitis [Heinonen and Miettinen, **1994**].

This highlights the importance of this pathogen in the aetiology of both acute PID and subclinical upper tract disease. The reproductive and gynaecologic consequences of PID, including infertility, ectopic pregnancy [Brunham *et al*., **1988**; Safrin *et al*., **1992**; Stagey *et al*., **1992**; Westrom *et al*., **1992**], recurrent PID [Buchan *et al*., **1993**], and chronic pelvic pain [Lepine *et al*., **1998**].

5. Tubal Damage, Ectopic Pregnancy and infertility

Microbial infection of the fallopian tubes is one reason for alterations in the tubal epithelial lining. Recent reviews have reported that infection reduces cilia motion and even destroys cilia within the fallopian tubes [Lyons *et al*., **2006**; Shaw *et al*., **2010**]. Reduced cilia function can be a cause of infertility and can result in ectopic pregnancy since the embryo relies on cilia to facilitate its propulsion through the fallopian tubes into the uterus. In addition, inflammation of the lumen of the fallopian tubes results in tubal occlusion and tubal factor infertility [Lyons *et al*., **2006**]. Tubal disease directly causes from 36% to 85% of all cases of female factor infertility in developed and developing nations respectively [Hafner and Pelzer, **2011**]. Studies using in situ hybridization, immune-cyto-chemistry, culture, PCR, and electron microscopy demonstrate persistence of *C. trachomatis* in the fallopian tubes of women with tubal infertility. [Patton *et al.,* **1994**; Paavonen and Eggert-Kruse, **1999**; Ohman *et al*., **2009**]. Prolonged exposure to *Chlamydia* due to a chronic persistent infection or frequent re-infection has been associated with tubal factor infertility [Brunham and Peeling, **1994**; Mardh, **2004**; Ness *et al*., **2008;** Soper, **2010**].

6. Cervical cancer

Cervical cancer is the most common cancer in women worldwide. An association between *C. trachomatis* and cervical cancer has been suggested [Risch *et al*., **1994**; Dean *et al.*, **1995**; Anttila *et al*., **2001**]. Previous case-control studies have found cytological or serological evidence of the role of *C. trachomatis* in cervical neoplasia [Paavonen *et al*., **1979**; Kiviat *et al*., **1985**]. Other studies also shown that *C. trachomatis* infection is associated with cervical carcinoma [Hakama *et al*., **1933**; Lehtinen *et al*., **1996**; Anttila *et al*., **2001**].

7. C. trachomatis infection during pregnancy

Many studies have shown a relationship between miscarriage and *C. trachomatis* infection [Rastogi *et al*., **2000**; Baud *et al*., **2007**; Wilkowska-Trojniel *et al.*, **2009**; Baud *et al*., **2011**; Nigro *et al.*, **2011**].

Studies have also suggested an association between *C. trachomatis* infection in the mother and premature uterine activity, premature birth of infants with extremely low birth weight, and death [Harrison *et al*., **1983**; Kovacs *et al*., **1998**; Gencay *et al*., **2001**; Rours *et al*., **2011**].

8. Neonatal *C. trachomatis* infection

a. Conjunctivitis

Vaginal delivery is the usual route of transmission of *C. trachomatis* from infected mother to infant, as the eyes are coated with *Chlamydia* as the baby passes down the birth canal. Maternally-acquired infection with *C. trachomatis* is the commonest cause of neonatal conjunctivitis is a significant in the developing world [Zar, **2005**].

b. Trachoma

It is a contagious eye infection caused by *C. trachomatis* and is the leading infectious cause of blindness worldwide [Thylefors *et al*.,

1995]. Active infection with *C. trachomatis* is mostly seen in young children with a peak incidence around four to six years, while subsequent scarring and blindness is seen in adults [Turner *et al*., **1993**; Schwab *et al*., **1995**; West *et al*., **2004**].

2.1.5 Prevalence

2.1.5.1 Globally

According to the World Health Organization, [**2001**] new cases of *Chlamydia* infection have been estimated globally to be 92 million. In 2010, the overall rate of *Chlamydial* infection in the United States among women (610.6 cases per 100,000 females) reflecting the large number of women screened for this disease.

In 2010, *Chlamydia* test ranged 3.8% to 13.7% among women aged 15–24 years who were screened at selected family planning clinics in all 50 states, the District of Columbia, Puerto Rico, and the Virgin Islands.

At selected prenatal clinics in 16 states, Puerto Rico, and the Virgin Islands, the median state-specific Chlamydia ranged 2.7% to 21.2%.

The prevalence of infection was greater among women aged 16–24 years in 2010 in 44 states [Centers for Disease Control and Prevention, **2010**].

In some parts of the developing world, over 90% of the population is infected. It is estimated that more than 500 million people still are at high risk of infection; over 140 million persons are infected Africa, Central and South-East Asia, and countries in Latin America [Centers for Disease Control and Prevention, **2010**].

In Seattle, USA nearly 50% of women have been exposed to *C. trachomatis* by age 30 [Stamm, **1999**].

In Amsterdam, The prevalence was between 3 and 5% [Sweet and Gibbs, **2002**]. In North Carolina the prevalence of *Chlamydial* infection ranged from 1.94 to 12.54% [Miller *et al*., 2004]. In other studies *C. trachomatis* prevalence were 9%, 9.9-27%, 6%, 10.3% respectively [Miller *et al*., **1999**; Cameron *et al*., **2003**; Bernstein *et al*., **2006**; Joesoef and Mosure, **2006**].

At University of Washington, 70%–90% of endocervical infections in women caused by *C. trachomatis* were asymptomatic [Stamm and Holmes, **1990**].

C. trachomatis test was done using PCR-based assays, *C. trachomatis* prevalence ranged between 0.2% in Spain and 5.6% in Nigeria [Franceschi *et al*., **2007**]. The prevalence of *C. trachomatis* in asymptomatic women in Europe ranges from 1.7 to 17% [Wilson *et al*., **2002**].

2.1.5.2 In Asia and the Middle East

In Karachi Pakistan, *Chlamydial* infection in asymptomatic antenatal patients was 2% and in women attending a family planning clinic the rate was 6 times greater as 12% tested positive [Wasti *et al*., **2008**].

In India, positive *C. trachomatis* was 27.03% with primary infertility, and 30.56% with secondary infertility [Malik *et al*., **2006**].

The Middle East region continues to be perceived as a region with very limited *C. trachomatis* epidemiological data, raising many controversies about the status of the epidemic in this part of the world. Investigation the prevalence of *C. trachomatis* in the cervical canal in unexplained infertile women and compare it to healthy controls in the Turkish population, *Chlamydial* infection was isolated in one case each in infertile and control patients [Guven *et al*., **2007**]. In a case-control Iranian study, PCR results revealed that 12.4% of the infertile and 8.5% of the fertile women were positive for *C. trachomatis* infection [Rashidi *et al*., **2013**]. Similar findings were in Chamani-Tarbriz *et*

al., **[2007]** study where 12.6% tested positive by PCR. Also, in another Iranian cross-sectional study the prevalence of *C. trachomatis* was 22.26% in women [Afrakhteh *et al.*, **2008**].

2.1.5.3 In Arabian countries

The true incidence of *Chlamydia* infection in Arabian countries is difficult to establish because of limited data, as well as management of any positive cases would create legal and social problems, non-availability of facility to detect the organism in many health units and the largely asymptomatic nature of the disease, most of the published data came from point prevalence studies in clinics and hospitals as noted in Table 2.3. In these studies, the prevalence of *C. trachomatis* varied extensively depending on the health care setting, screened population, high- or low-risk groups, and method of testing.

The highest prevalence rate of *C. trachomatis* in the Arabian region has so far come from Egypt with rates of 4.2% in one study [Sullam *et al*., **2001**] to as high as 33.3% to 82.6% among high-risk groups [El-Shoubagy *et al.*,**1996**].

Ghazal-Aswad *et al.,* [**2004**] reported a prevalence of 2.6% in Abu Dhabi, same prevalence rate from Kuwait revealed that the prevalence was 2.6% [Al-Fouzan and Al-mutairi, **2004**]. A finding slightly higher than another Kuwaiti study (2.1%) [Al-Sweih *et al*., **2011**].

However, in Jordan the prevalence varied depending on the population studied. In a study conducted in a teaching hospital, the prevalence of *C. trachomatis* infection was 0.5% and 0.6% among asymptomatic and symptomatic women, respectively [Maha Fazah *et al*., **2008**]. Whereas in other hospitals the prevalence of *C. trachomatis* ranged from 3.9% to 4.6% among infertile women and women attending urology clinic [Al-Ramahi *et al*., **2008** and Awwad *et al*., **2003**] respectively. A much higher prevalence rate of 40% in presumed infected women has been reported by another group in Jordan

[Mawajdeh *et al*., **2003**]. In Palestine, rate relatively high (20.2%) were reported among women in high-risk groups [El Qouqa *et al*., **2009**].

Table 2.3 Comparative prevalence of *C. trachomatis* in the Middle East [Al-Sweih *et al*., **2011**].

Country	Setting	Method	Prevalence of C. trachomatis	Reference
Egypt (1996)	University Hospital	EIA	33.3% to 82.6%	El-Shoubagy et al., **1996**
Egypt (2001)	Mobile clinic	ELISA	4.2%	Sullam *et al*., **2001**
Jordan (2003)	Urology clinic	PCR	4.6%	Awwad *et al*., **2003**
Jordan (2003)	Community survey	Clinical presumption	40%	Mawajdeh *et al*., **2003**
UAE (2004)	PHC	EIA	2.6%	Ghazal-Aswad *et al*., **2004**
Kuwait (2004)	Community	Overview	NGU–2.6%	Al-Fouzan and Al-Mutairi, **2004**
Jordan (2008)	Infertility clinic	PCR	3.9%	Al-Ramahi *et al*., **2008**
Jordan (2008)	OB & GYN	mPCR	0.6%–symptomatic 0.5%–asymptomatic	MahaFazah *et al*., **2008**
Palestine (Gaza: 2009)	OB & GYN	EIA & PCR	20.2%–symptomatic	El-Qouqa *et al*., **2009**
Kuwait (2011)	PHC	ProbeTec	2.1%	Al-Sweih *et al*., **2011**

PHC: primary health care; PCR, polymerase chain reaction; mPCR, multiplex PCR; EIA: Enzyme immunoassay

2.1.5.4 In Saudi Arabia

Although *Chlamydia* infection is not yet a fully reportable infectious disease in Saudi Arabia, the following studies providing strong estimates of the prevalence of this infection:

Using EIA, the prevalence of *C. trachomatis* infection among asymptomatic healthy-looking Saudi women was 8.5% in Riyadh City [Bakir *et al*., **1989**].
In Al Ali General Hospital, Riyadh. Antibodies for *C. trachomatis* were found in 72 (35%) of 200 female [Massoud *et al*., **1991**].

In Makah City, using ELISA; *Chlamydial* IgG antibodies were detected in 8.7% of pregnant Saudi women [Ghazi *et al*., **2006**].
A study showed the prevalence of *C. trachomatis* among women with chronic cervicitis, abortion, full term pregnancy and infertile women, the prevalence of *C. trachomatis* in this study groups was 30% in women with abortion, 25% in infertile women, 15% in women with chronic mucopurulant cervicitis, 10% in full term pregnancy [Al-Sharif, **2011**].

The latest Saudi study was a community-based study carried out at the OB & GYN clinic at Jazan General Hospital. The study group included 640 Saudi infertile women and randomized control group included 100 women. All recruited women were screened for *Chlamydia* infection by ELISA and then retested by the McCoy cell culture technique. The prevalence of *C. trachomatis* infection among infertile women was high, at 15.0%. The rate of *Chlamydia* infection detected by ELISA was 9.84%, and 12.03% by the culture method [Kamel, **2013**].

2.1.6 Diagnosis

2.1.6.1 Tissue culture (TC)

C. trachomatis cannot be cultivated in non-living or cell free media. TC techniques vary among laboratories. With no standardised protocol it is difficult to compare inter-laboratory performance. TC detects only viable organisms, and hence, as with any other bacterial investigation the specimen collection and transport to the laboratory has to be optimal, irrespective of which laboratory method is to be used. Even under ideal conditions the sensitivity is probably no more than 75% [Robinson and Ridgway, **1996**].

2.1.6.2 Direct Fluorescent Antibody (DFA)

Specimen material is obtained with a swab or brush, which is then rolled over the specimen well of a slide. Once air dried and fixed the specimen can be stained using either a MOMP or Lipopolysaccharide (LPS) fluorescein-labelled monoclonal antibody that binds to *C. trachomatis* EBs [MMWR CDC, **2002**].

2.1.6.3 Enzyme –Immuno- Assay (EIA)

There are many commercially available EIA tests on the market for detecting *C. trachomatis* infection. They detect *Chlamydial* LPS with a monoclonal or polyclonal antibody that has been labelled with an enzyme. The enzyme converts a colourless substrate into a coloured product, which is detected by a spectrophotometer. As the EIA detects LPS, there is a potential that cross reaction occurs with other microorganisms causing a false positive reaction, hence it is vital that confirmation either by DFA or blocking antibody test is performed. [Sexually Transmitted Infections, **2006**]. Sensitivity has been shown to be lower than for molecular techniques [Robinson and Ridgway, **1996**].

2.1.6.4 Nucleic Acid Amplification Tests (NAATs)

The role of the nucleic acid amplification technology in the routine diagnosis of *C. trachomatis* infections is evolving rapidly. Three commercial assays are now available for routine use [Sexually Transmitted Infections, **2006**; Nadala *et al*., **2009**].

• PCR

• Strand displacement amplification (SDA)

• Transcription mediated amplification (TMA)

These sensitive and specific tests have become the "gold standard", and are the preferred diagnostic method that may facilitate *Chlamydia* screening programs [Schachter and Stamm, **1995**; Black, **1997**]. Although these commercial assays differ in their target sequence and their method of amplification, it is their ability to produce a positive signal from a single copy of the target DNA or RNA that has led to the reported increased sensitivity of NAATs [Watson *et al*., **2002**]. A major advantage of NAATs is their performance on specimens that do not require a pelvic examination or the necessity to obtain a urethral swab. These tests can be applied to urine or vaginal swabs in addition to endocervical and urethral swabs [Chamani-Tarbriz, **2001**; Geisler, **2011**].

2.1.6.5 Real time PCR; hybridization probe format

With the recent development of Light Cycler technology, PCR amplification and hybridization probe detection occur simultaneously in homogeneous solution. That is, both amplification and hybridization analysis can proceed in the same reaction. Because the Light Cycler Instrument uses rapid cycling techniques [Wittwer *et al.*, **1994**], the entire process is finished in 15–30 min.

All reagents for both amplification and detection are added before temperature cycling is begun. Sequence-specific probe hybridization occurs during amplification, allowing real-time product identification, quantification, and mutation detection [Wittwer *et al*., **1997**; Wittwer *et al*., **1998**] (Figure 2.2).

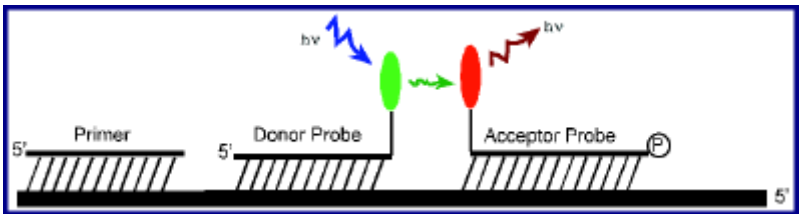

Figure 2.2: Hybridization probes produce fluorescence when both are annealed to a single strand of amplification product. The transfer of resonance energy from the donor fluorophore (3'-fluorescein) to the acceptor fluorophore (5'-LC Red 640) is a process known as fluorescence resonance energy transfer [Caplin *et al*., **1999**].

2.1.7 Sites for testing

1. First catch urine
2. Cervical [Sexually Transmitted Infections, **2006**; Nadala *et al*., **2009**].

2.2 *Mycoplasma*

Mycoplasma refers to a genus of bacteria that lack a cell wall. Without a cell wall, they are unaffected by many common antibiotics such as penicillin or other beta-lactam antibiotics that target cell wall synthesis. They can be parasitic or saprotrophic. *Mycoplasma* is the smallest living cells known, which can survive without oxygen and is about 0.1 µm in diameter [Ryan and Ray, **2004**]. *Mycoplasmas* are known to consist of just plasma membrane which makes them good models for membrane studies. The membrane mostly consists of 60% to 70% of proteins and rest 20% to 30% of lipid. Dependence on exogenous supplies of fatty acids, and cholesterol serves as advantage to conduct further studies on these organisms [Shmuel and Leonard, **2010**]. Classification of *Mycoplasma* in table 2.4.

Table 2.4 Classification of *M. genitalium* and *M. hominis* [Tully, **1983**]**.**

Mycoplasma	
Scientific classification	
Kingdom:	Bacteria
Phylum:	*Firmicutes*
Class:	*Mollicutes*
Order:	*Mycoplasmatales*
Family:	*Mycoplasmataceae*
Genus:	*Mycoplasma*
Species	*M. genitalium* *M. hominis*

2.2.1 *M. genitalium*

M. genitalium is a small parasitic bacterium that lives on the ciliated epithelial cells of the primate genital and respiratory tracts. Most of *Mycoplasmas* are spherical because they lack a cell wall but *M. genitalium* is more flask-shaped, with the bottle's neck forming a tip structure [Taylor-Robinson, **1995**; Razin *et al*., **1998**].

2.2.1.1 Genome Structure

In 1995 *M. genitalium* complete genome sequence was published. It is the species with the smallest genome of all *Mycoplasmas* studied so far with a genome of only 580 kb [Fraser *et al*, **1995**].

The small genome of *M. genitalium* made it the organism of choice in 'The Minimal Genome Project', a study to find the smallest set of genetic material necessary to sustain life [Himmelreich *et al*., **1997**; Pace, **2009**].

2.2.1.2 Pathogenesis

M. genitalium has been shown to attach to different cell types, including erythrocytes, Vero cells, fallopian tube cells, respiratory cells and spermatozoa [Jensen, **2006**; Ekiel *et al*., **2009**]. *M. genitalium* has several virulence factors that are responsible for its pathogenicity. These include the ability to adhere to host epithelial cells using the terminal tip organelle with its adhesions [Razin and Jacobs, **1992**; Burgos *et al*., **2006**; Jensen, **2006**], intracellular localization [Tully *et al*., **1983**; Tully *et al*., **1986**; Mernaugh *et al*., **1993**; Razin, **2005**], the release of enzymes, [Dhandayuthapani *et al*., **2001**; Alvarez *et al*., **2003**] and the ability to evade the host immune response by antigenic variation [Razin et *al*., **2005**; Rottem, **2003**; Jensen, **2006**; Svenstrup *et al*., **2008**; Ueno *et al*., **2008**].

There is overwhelming evidence, based on numerous detection studies [Keane *et al*., **2000**; Falk *et al*., **2003**; Falk *et al*., **2004**; Anagrius *et al*., **2005**; Tosh *et al*., **2007**; Thurman *et al*., **2010**], that the human urogenital tract is the preferred site of colonization, the results strongly indicated that *M. genitalium* is sexually transmitted.

2.2.1.3 Epidemiology

2.2.1.3.1 IN MEN

1. Urethritis [Jensen *et al*., **1991**; Horner *et al*., **1993**; Keane *et al*., **2000**; Deguchi and Maeda, **2002**; Taylor-Robinson *et al*., **2003**].
2. Balanoposthitis [Horner and Taylor-Robinson, **2011**], Chronic Prostatitis [Doble *et al*., **1989**; Krieger *et al*., **1996**; Mandar *et al*., **2005**].

2.2.1.3.2 In women

1. Cervicitis [Taylor-Robinson, **2002**; Manhart *et al*., **2003**].
2. Endometritis [Irwin *et al.*, **2000**; Taylor-Robinson,**2002**].
3. Salpingitis [Collier *et al*., **1990**; Clausen *et al*., **2001**; Cohen *et al*., **2005**; Baczynska *et al*., **2007**; Haggerty, **2008**; McGowin *et al*., **2010**].
4. BV [Palmer *et al*., **1991**; Keane *et al*., **2000**; Wroblewski *et al*., **2010**].
5. PID [Irwin et al., **1994**; Irwin *et al.*, **2000**; Cohen *et al*., **2002**; Simms *et al*., **2003**; Jensen, **2006**].
6. Preterm delivery, abortion, ectopic pregnancy and infertility: There is information available on the role of *M. genitalium* in causing adverse pregnancy outcome, either as preterm labour, abortion or stillbirth: *M. genitalium* was detected of mid-trimester from women delivering preterm [Lu *et al*., **2001**; Oakeshott *et al*., **2004**]. *M. genitalium* has been identified among women with tubal factor infertility [Clausen *et al*., **2001**; Svenstrup *et al*., **2008**].

An association between *M. genitalium* and ectopic pregnancy were found [Jurstrand *et al*., **2007**]. Relationship between *M. genitalium* and infertility have been reported [Stephen and Chandra, **2006**; Haggerty, **2008**].

2.2.1.4 Prevalence

2.2.1. 4.1 Globally

In Denmark, the prevalence of *M. genitalium* infection was 2.3% [Andersen *et al*., **2007**], 2.8%in Japan [Hamasuna *et al*., **2008**], 13.6 %In Indiana-USA [Tosh *et al*., **2007**], in Seattle-USA, two NAATs were assessed with genital specimens, *M. genitalium* was detected by the TMA and PCR assays in (13%) and (14%) of vaginal swab specimens [Wroblewski *et al*., **2006**].

In Paris, France, *M. genitalium* was statistically more frequently detected in the vagina (39%) than in the cervix (21%) or urethra (28%) [Anagrius *et al*., **2005**].

The prevalence of *M. genitalium* infection in London, UK was 3.3% [Bjartling *et al.*, **2012**], 9.8%In Greenland, Canada, [Gesink *et al*.,**2012**], 6.3% and 2.1% receptively in Sweden [Anagrius *et al*.,**2005**; Bjartling *et al*.,**2012**], and 1.4% in Norway [Nilsen *et al.*, **2011**].

2.2.1.4.2 In the Middle East

Information on the incidence of *M. genitalium* is still rarely reported in most Middle East countries. In Jordan 3.5% [Shehabi *et al*., **2009**], in Iran it was 1.02% and 5.2% receptively [Haghighi Hasanabad *et al*., **2011**; Mirnejad *et al*., **2011**], in Gaza, Palestine the rate was 1% [Nassar *et al*., **2008]**.

2.2.1.5 Diagnosis of *M. genitalium*

Classical bacteriological tests including morphology, cultural characteristics are very time consuming and slow, taking several months before an isolate is obtained [Tully *et al*., **1981**; Tully *et al*., **1983**; Luo *et al*., **1999**; Jensen, **2006**].

Research workers have noted the unreliability of immunological assays used for detection of *M. genitalium* [Ma *et al*., **2007**; Edberg *et al*., **2008**]. Molecular tests are the prevailing tests in *Mycoplasma* identification [Taylor-Robinson and Jensen, **2011**]. NAATs are the only tools available for detection of *M genitalium* [Barry *et al*., **2007**]. A quantitative real-time Light Cycler PCR assay was developed for the detection of *M. genitalium* in cervical specimens from female patients [Jensen *et al*., **2003**; Jensen *et al*., **2004**]. To determine the bacterial load in patients' specimens, a quantitative real-time Light Cycler PCR was previously developed, and the housekeeping gene *gap* encoding glyceraldehyde-3-phosphate dehydrogenase was chosen as the target gene [Svenstrup *et al*., **2005**].

2.2.2 *M. hominis*

The first human *Mycoplasma* isolated was *M. hominis* [Dienes and Edsall, **1937**]. M. hominis is an intracellular that lacks a typical bacterial peptidoglycan cell wall and is one of the smallest bacteria capable of self-replication, and lacks the genes coding for the cell wall, leading *M. hominis* to a parasitic and saprophytic existence [Waites and Cunha, **2013**], and one of the most commonly reported sexually transmitted bacterial infections in most of the Western countries and a role in PID and post-abortion fever has been suggested [Taylor-Robinson, **2007**].

2.2.2.1 Genome Structure

M. hominis's circular chromosome has been studied and sequencing has taken place to help determine its pathology in humans [Boesen *et al*., **1998**; Pereyre *et al.*, **2009**; Kanehisa Laboratories, **2012**]. The genome has 665,445 bp with a G-C content of 27.1%. There are 537 DNA coding sequences, 345 of which the function has been established and 40 RNA genes [Pereyre *et al.*, **2009**]. *M. hominis* viewed under a light microscope in figure 2.3.

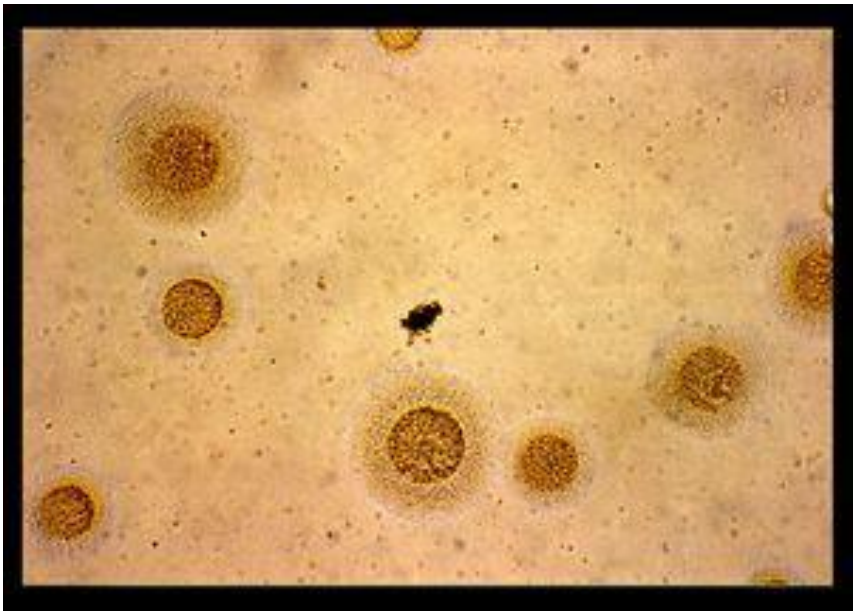

Figure 2.3 *M. hominis* viewed under a light microscope. The coccoid shape of the microorganism was observed [*Mycoplasma hominis*, MicrobeWiki]**.**

2.2.2.2 Ecology

M. hominis can colonize in humans in the urogenital tracts [*Mycoplasma hominis*, **2011**]. *M. hominis's* lies mainly in the reproductive tract of the vagina. It has also been known to thrive in human respiratory tract. The bacterium lives a parasitic life gaining nutrients and molecules necessary for its survival in the urogenital tract [Xiao *et al*., **2006**].

2.2.2.3 Epidemiology

2.2.2.3.1 In men

1. NGU [Møller *et al*., **1990;** Sharma and Khandpur, **2003**].
2. Systematic reduction in motility and sperm concentration [Styler and Shapiro, 1**985**; Rose and Scott, **1994**; Reichart *et al.*, **2000**].
3. Male infertility [Styler and Shapiro, **1985**].

2.2.2.3.2 In women

1. BV [Taylor-Robinson, **2007**; Rajan, **2012**].
2. PID [Moller *et al*., **1985**].
3. Adverse pregnancy outcome: miscarriage [Moller *et al*., **1990**; Badami and Salari, **2001**; Rajan, **2012**], preterm birth [Lamont *et al*., **1987**; Polk *et al*., **1989**; McGregor *et al*., **1990**; Hillier *et al*., **1995**; Kasper *et al*., **2010**], recurrent pregnancy losses [Stray-Pedersen *et al*., **1978**; Harger *et al*., **1983**], Postpartum Fever [Platt *et al*., **1980**].
4. Infertility: In Turkey, study showed that (8%) of infertile women in the study group were *M. hominis* positive [Fenkci *et al*., **2002**]. Among infertility patients, *M. hominis* in cervical swabs was detected only in 2.3% [Stray-Pedersen *et al*., **1982**]. Similar findings on women undergoing *in vitro* fertilization showed the presence of *M. hominis* in 2.1% [Witkin *et al*., **1995**]. *M. hominis* was detected in 11% and 16.5% and 3.7% respectively of the examined infertile women [Yavuzdemir *et al*., **1992**; Di Bartolomeo *et al*., **2002**; Zdrodowska-Stefanow *et al*., **2006**]. To determine the prevalence of positive test for *M. hominis, C. trachomatis* infections when undergoing workup for infertility, a total of 46 patients, three patients were positive for *M. hominis* (1.3%), five patients were positive for *C. trachomatis* (2.2%) [Imudia *et al*., **2008**].

2.2.2.4 Prevalence of *M. hominis*

2.2.2.4.1 Globally

Colonization values worldwide for *M. hominis* range between 20% and 30% [Clegg *et al*., **1997**]. As of September 2012, it is estimated that infection cases are over 2 million annually in the United States alone [Waites and Cunha, **2013**]. In a Czech study 28% *M. hominis* positive compared to 15% among control patients [Kapatais-Zoumbos *et al*., **1985**]. In Japan, *M. hominis rate was* 11.2% [Kataoka *et al*., **2006**]. In Greece the incidence of *M. hominis* was

2.92% [Kechagia *et al.,* **2008**]. In Portugal, 57.4% were positive for *M. hominis* [Domingues *et al.,* **2003**]. In Poland, *M. hominis* was detected in 3.7% women [Zdrodowska-Stefanow *et al.,* **2006**]. Other previous studies have reported less than 30%, of women were colonized with *M. hominis* [Gratlard and Soleihac, **1995**; Keane *et al.,* **2000**; Domingues *et al.,* **2003**]. Other studies using PCR for detecting *M. hominis* in endocervical specimens have reported prevalence rate as high as 20 to 50% for *M. hominis* [Zheng *et al.,* **1997**; Luki *et al.,* **1998**; Domingues *et al.,* **2003**; Stellrecht *et al.,* **2004**]. Isolation rate of *M. hominis* were higher in women under 30 years of age that by other studies [Leon, **1993**; Gratlard and Soleihac, **1995**; Clegg *et al*., **1997**].

2.2.2.4.2 In the middle east

The magnitude of the burden of the prevalence of *M. hominis* is not well known in most countries of the Middle East region due to limited surveillance in the Region, KSA is not an exception to these general conditions. Although there are considerable numbers of research articles published on different aspects of STIs. In Iran, Of the 377 patients studied 31 (26.7%) were PCR positive only for *M. hominis* [Shahin and Sattari, **2006**]. Another Iranian study, *M. hominis* was isolated in 35.6% of infertile females compare with 7.2% of normal population [Badami and Salari, **2001**]. The prevalence rate of *M. hominis* among infertile Jordanian patients showed 27.3% [Al-Daghistani and Abdel-Dayem, **2010**]. Prevalence of *M. hominis* in women varies widely between 2.3% in a Turkish study and 26% in a small Wisconsin college population [Schlich *et al.,* **2004**].

2.2.2.4.3 In Saudi Arabia

In Khamis Mushayt City, Saudi Arabia, investigators compared *Mycoplasma* commercial kits and a conventional culture system, the number of genital

Mycoplasmas detected by commercial kit and culture system was 26% and 17% respectively [Abdul-Wahab, **2010**].

Another study showed that two genital *M. hominis* were detected out of 263 specimens (0.76%) by culture method [Abdul-Wahab and Al Sunaidi, **2013**]. A study showed the prevalence of *M. hominis* was assessed by NAATs as well as culture, results showed that by culture *M. hominis* rate was (4%), by PCR *M. hominis* detection rate was (20%) [El-Feky and Baddour, **2009**].

2.2.2.5 Diagnosis

Mycoplasmas have been included as a subject of investigation for many years, their presence was first based on culture, and because the culture techniques are challenging, this requires technical skill for interpretation of microscopic colonies and takes two to five days, differences in technical expertise between laboratories may have slowed the process of discovery and certainty about the clinical role of these organisms. PCR targeting the genes of glyceraldehydes-3-phosphate dehydrogenase (gap) have been developed to test for the presence of *M. hominis*. This allows for more accurate diagnosis and treatment of *M. hominis* associated illnesses thought by passing the amplicon contamination possibility of conventional cultivation techniques [Baczynska *et al.*, **2004**]. Real-time PCR detection from clinical samples circumvents technical issues related to culture and shortens turnaround time for detection and identification.

Few real-time PCR assays and associated studies have been described for *M. hominis.* A real-time PCR assay targeting *M. hominis gap* identified two positive cervical swabs from women being evaluated for infertility [Baczynska *et al*., **2004**]. 153 urogenital specimens were tested with a real-time PCR assay, of which 10were PCR positive [F´erandon *et al*., **2011**].

Genital *M. hominis* infection was diagnosed in three patients using a real-time PCR assay targeting the *M.hominis* 16S ribosomal RNA gene [Pascual *et al*., **2010**]. mPCR was used to detect *M. hominis* and *Ureaplasma parvuma* and *Ureaplasma urealyticum*; 16% tested positive for *M. hominis* [McIver *et al*., **2009**].

In addition to the advantage of speed, the described assays overcome the challenges of detection of these organisms by culture. Although culture was considered a gold standard method, colonial identification is challenging and subjective because it is done using the human eye and a dissecting microscope. This approach is more user-friendly (and generalizable among assays for various microorganisms) than culture [Cunningham *et al*., **2013**].

2.3 Association between *C. trachomatis, M. genitalium,* and *M. hominis* with other STIs

M. genitalium is similar to *C. trachomatis* in several respects such as preference for the genital tract, mode of transmission, making various adverse gynecologic and reproductive events and cervicitis [Vanessa *et al*., **2010**].

Also, in one study, an association has been reported between *C. trachomatis, M. genitalium* and preterm delivery [Witkin and Ledger, **1992**].

Gaydos *et al*., [2009] while conducting studies on STIs in patients attending STI clinics, showed 5.9 % of them to be co-infected with *C. trachomatis*. In West Africa, Pepin and colleagues showed that almost half of the infections due to *M. genitalium* occurred as co-infections [Pepin *et al*., **2001**].

Co-infections amongst genital *Mycoplasmas* have also been reported. In a study by Amirmozafari *et al*., simultaneous occurrence of *M. genitalium* and *U. Urealyticum* was shown in 1.4 % of women with genital infections, while triple infection of *M. genitalium, U. Urealyticum* and *M. hominis* was seen in 0.5 % of these patients [Amirmozafari *et al*., 2009]. Other authors have also

demonstrated co-existence of *M. genitalium* with other pathogens [Mirnejad *et al*., **2011**; Samra *et al*., **2011**].

Screening of *C. trachomatis* with cell culture as a gold standard is very difficult and requires specimens from urogenital swabs, which are unacceptable to many people. Urine samples have cytotoxic effect and are not suitable for culture. Other routine tests for *Chlamydia*, such as EIAs, have low sensitivity in asymptomatic individuals. Detection of *M. genitalium and M. hominis* by the culture method is also very time-consuming and requires up to 8 weeks for this microorganism whereas the NAATs can detect infectious agents in less than 8 hours [Mardh *et al*., **1980**].

M. genitalium, *M. hominis* and *C. trachomatis* could cause PID, vaginitis, cervicitis, postpartum, and infertility in women. In men, it could lead to NGU, acute epididymitis, and in newborn, septicemia, neonatal pneumonia, neonatal conjunctivitis, meningitis, low birth-weight infants, premature delivery and premature rupture of membranes [Judlin, **2003**; Taylor-Robinson and Jensen, **2011**].

Chapter (3)

Material and Methods

Materials

All equipment's were provided by KKUH, Riyadh, KSA. All kits, chemicals, and reagents were provided from different companies in Riyadh.

Table 3.1 List of kits, chemicals, buffers, reagents used in the study.

Code	Description	Source
DRJ COP 002	Copan Universal Transport Medium (UTM-RT) A combined viral transport medium and transport for *Chlamydiae, Mycoplasma*	Copan Diagnostics Inc. Copan Italia S.P Dar Reayat Aljazira Drajeh Group Alkhobar, KSA Tel: +96638140056.
DRJ COP 003	Copan FLOQ Endocervical Swabs. Copan Flocked Swabs for bacteriology samples, virology culture, DFA testing, rapid direct testing, EIA, PCR and molecular-based assays, as well as for forensic applications	Copan Diagnostics Inc.Copan Italia S.P Dar Reayat Aljazira Drajeh group Alkhobar, KSA Tel:+96638140056
03 730 964 001	MagNA Pure Compact total Nucleic Acid Isolation Kits(Isolate bacterial DNA from different sample types)	Roche Diagnostics Tel**:+41-41-799-6161** Tamer Group Riyadh, KSA Tel:+966114024653
03003248001	Light Cycler Fast Start DNA Master HybProbe Fast Start Enzyme	Roche Diagnostics Tel**:+41-41-799-6161** Tamer Group

	And Reaction Mix for PCR, using HybProbe probes with the Light Cycler Carousel-Based System.	Riyadh, KSA Tel:+966114024653
40-0098-32	Light Mix Kit *C. trachomatis* Kit with reagents for the detection of *C. trachomatis* using the Roche Diagnostics Light Cycler 2.0 Instruments. Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each.	TibMolbiol-Berlin Tel: +49 30 787 994 27 Alhayat Center EST Riyadh, KSA Tel:+966112935096
40-0139-32	Light Mix Kit *M. hominis* Kit with reagents for the detection of *M. hominis* using the Roche Diagnostics Light Cycler 2.0 Instruments. Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each.	TibMolbiol- Berlin Tel: +49 30 787 994 27 Alhayat Center EST Riyadh, KSA Tel:+966112935096
40-0169-32	Light Mix Kit *M. genitalium* Kit with reagents for the detection of *M. genitalium* using the Roche Diagnostics Light Cycler 2.0 Instruments. Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each.	TibMolbioL- Berlin Tel: +49 30 787 994 27 Alhayat Center EST Riyadh, KSA Tel:+966112935096

	Agarose NA	Promega/USA
	Tris/Borate/EDTA (TBE buffer 10%)	Gibco
	Ethedium Bromide10mg/ml	
	100 bp ladder	Qiagen

Laboratory Equipment

Table 3.2 List of equipment used in the study.

Code	Equipment/company
13338	Type 16700 Mixer/Bernstad-Thermolyne
3020532	Centrifuge A 14 14000 rpm/ JOUAN-France
32076	Gel electrophoresis apparatus /Labnet Int.
31942	Gel electrophoresis document system/Biorad-USA
25241	MagNA Pure Compact/Roche Molecular Biochemicals, Mannheim, Germany
25335	LightCycler 2.0 (Roche Molecular Biochemicals, Mannheim, Germany)
25653	Safety cabinet/Bioair Euoro Lone division
20302905	Refrigerator/ Sanyo
30207653	Safety cabinet fume hood maintenance log/Jouan-France
10200920	Biomedical Freezer /Sanyo
25342	LC Carosel Centrifuge 2.0 /Roche Molecular Biochemicals, Mannheim, Germany

3.1 Specimen collection, storage and transportation:

Copan Universal Transport Medium (UTM-RT):
A combined transport medium for *Chlamydiae*, *Mycoplasma.*

Product Information:
Copan Universal Transport Medium (UTM-RT) System is intended for the collection and transport of clinical specimens containing viruses, *Chlamydiae*, *Mycoplasma* or *Ureaplasma* from the collection site to the testing laboratory.

PRINCIPLE
Copan UTM-RT medium consists of modified Hank's balanced salt solution supplemented with bovine serum albumin, cysteine, gelatin, sucrose, and glutamic acid.

The pH is buffered with HEPES buffer. Phenol red is used to indicate pHs Vancomycin, amphotericin B, and colistin are incorporated in the medium to inhibit growth of competing bacteria and yeast. The medium is isotonic and non-toxic to mammalian host cells. The presence of sucrose acts as a cryo protectant which aids in the preservation of viruses and *Chlamydiae.*

3.2 Genomic DNA extraction

DNA was extracted using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Diagnostics).

The MagNA Pure Compact System is the automated bench top solution for nucleic acid purification. With its instrument, extensive integrated features, and a sample throughput of one to eight samples per run, the instrument meets the

demanding nucleic acid isolation needs of research laboratories with low to medium sample throughput.

Contents

1. Reagent Cartridges, 32 sealed cartridges
2. Tip Tray, 32 disposable Tip Trays
3. Sample Tube, 35 tubes, 2.0 ml
4. Elution Tube, 35 barcoded tubes, 2.0 ml
5. Elution Tube Cap, 35 tube caps

3.3Amplification and detection

Amplification was obtained using the Light Cycler Fast- Start DNA Master Hyb-Probe (Roche Diagnostics) and Light Mix Kits (TIB MOLBIOl)

3.3.1 Light Cycler Fast- Start DNA Master Hyb-Probe:

Contents

1. Light Cycler Fast Start Enzyme
2. Light Cycler Fast Start Reaction Mix HybProbe
3. $MgCl_2$ Stock Solution, 25 mM
4. Water, PCR Grade

3.3.2 Light Mix Kit *C. trachomatis* by TIB MOLBIOL

Kit with reagents for the detection of *C. trachomatis* DNA using the Roche Diagnostics Light Cycler 2.0 Instruments.

Contents

1. 3 Vials with green caps containing premixed lyophilized primers and probes for 32 PCR reactions each of *C. trachomatis*
2. 3 Vials with white caps containing the internal control (IC)
3. 1 Standard row with 6 lyophilized cloned plasmid standards of *C. trachomatis* from 10^1 to10^6 target equivalents per reaction.
4. 1 Sealing foil for the standard row

3.3.3 Light Mix Kit *M. genitalium* by TIB MOLBIOL

Contents

1. 3 Vials with green caps containing premixed lyophilized primers and probes for 32 PCR reactions
2. 3 Vials with white caps containing IC.
3. 1 Standard row with 6 lyophilized plasmid standards *M. genitalium* 10^1 - 10^6 target equivalents / reaction
4. 1 Sealing foil for the standard row

3.3.4 Light Mix Kit *M. hominis* by TIB MOLBIOL

Kit with reagents for the detection of *M. hominis* using the Roche Diagnostics Light Cycler 2.0 Instrument

Contents

1. 3 Vials with green caps containing premixed lyophilized primers and probes for 32 PCR reactions
2. 3 Vials with white caps containing the IC.
3. 1 Standard row with 6 lyophilized plasmid standards *M. hominis*10^1 to 10^6 target equivalents / reaction
4. 1 Sealing foil for the standard row

3.3.5 Primers and probes

A 136 bp fragment of the *C. trachomatis* MOMP genome gene (TIB MOLBIOL) was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 (detected in channel 640). The PCR reaction was monitored by an additional PCR product of 278 bp, formed from the IC (detected in channel 705). This control did not interfere with the *C. trachomatis* specific reactions.

A 224 bp long fragment of the glyceraldehyde-3-phosphate dehydrogenase (gap) gene of *M. genitalium* (TIB MOLBIOL) was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 and detected in channel 640, an additional PCR product of 349 bp was formed from the IC DNA.

A 129 bp long fragment of the glyceraldehyde-3-phosphate dehydrogenase (gap) gene from *M. hominis* (TIB MOLBIOL) was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 and detected in channel 640. An additional PCR product of 349 bp was formed from the DNA IC (table 3.3).

3.3.6 Light Cycler real-time PCR

Light Cycler real-time PCR (LightCycler2.0 by Roche, Germany) was performed on 200 clinical swab samples for Qualitative real time PCR assay (used to give either positive or negative results only), positive samples were subjected to absolute quantitative real time PCR assays (used to determine the concentration or copies of a detected target present in the original sample).PCR data are presented by graphical output of assay results including amplification and melting point curves. The amplification curve gives data regarding the kinetics of amplification of the target sequence, whereas the dissociation curve reveals the characteristics of the final amplified product.

Table 3.3 List of primers and probes used for the amplification and detection.

Designation	Sequence (5' to 3')	Target gene	Length (bp)
	C. trachomatis oligonucleotides		
Forward Primer	CTGCTTCCTCCTTGCAAGCT	MOMP	136 bp
Reverse Primer	ACGCATGCTGATAGCGTCA	MOMP	
Probe I	TTCCCACAGAATTCCGTCGATC ATAA – fluorescein	MOMP	
Probe Il	LCred705 CTTGGTTCAGCAGGATTCCCCA C – phosphate	MOMP	
	M. genitalium oligonucleotides		
Forward Primer	GTG CTC GTG CTG CAG CTG T	*Gap*	224 bp
Reverse Primer	GCT TGA TTT ACT TGT TCA ACA GAT GGA C	*Gap*	
Probe I	TGT TGT TCC AGA AGC AAA TGG CAA ACT T-FL	*Gap*	
Probe lI	GGG ATG TCA CTC CGT GTT CCA GTG T-phosphate	*Gap*	
	M. hominis oligonucleotides		
Forward Primer	TTGAAGGAAC TGGAAGATAT GTAACAAAAG AAGGTGCTGA ATTACATATT	*Gap*	129 bp
Reverse Primer	ATAAAATTTT ATCAGGCGCT TCA*T*GTACTA CTAACTGTTT AGCTCCTATT	*Gap*	
Probe I	CAAGCAGGTG CTAAAAAGGT GTTTATTACT GCTCCAGCTA AAAGCGAAGG– fluorescein	*Gap*	
Probe Il	TGTTAAAACA GTTGTTTATT CAGTAAACGA AGATATCATT ACGCCA*G*AAG – phosphate	*Gap*	

Methods

3.4 Setting

During the period October 2012 to July 2013 This case control study was conducted in KKUH and KAUH with the aid of laboratory facilities at KKUH.

The study was ethically approved (table 3.4) by the ethical committee board at KKUH (7-10-2012).

3.5 Participants

1. The study group included a total of 200 married women:

   100 infertile married women(case group) of primary and secondary infertility, aged between 19 and 46 years, who attended the outpatient infertility clinic at KKUH for infertility examination and fulfilled the inclusion criteria during the period of study and agreed to participate (signed informed consent), were enrolled in the study (table 3.5).

   The control group included women who were attending the clinic for gynaecologic purposes (routinely or annual check-up, abnormal uterine bleeding, abdominal pain, abnormal discharge and more).
2. 100 fertile married women(Control group) included fertile married women who attended the OB&Gyne clinic at KAUH , aged between 19 and 46 years and fulfilled the inclusion criteria during the period of study and agreed to participate (signed informed consent), were enrolled in the study.

Relevant medical records were reviewed for any possible present and past medical or surgical diseases, also pregnant women and women who had taken antibiotics in the previous 30 days were excluded from this study.

Each participant from both groups completed a questionnaire form (table 3.6) on demographic information including age, nationality, signs and symptoms were also obtained:

1. Burning sensation during urination
2. Genital bleeding
3. Irregular menstruation
4. Abnormal discharge
5. Low seated abdominal pain
6. Previous abortion
7. PID
8. Ectopic pregnancy
9. Premature delivery
10. Low birth weight infertility duration
11. Type of infertility
12. Signs of cervicitis
13. Signs of vaginosis

Table 3.4 Ethical approval form.

بسم الله الرحمن الرحيم

Kingdom of Saudi Arabia
Ministry of Higher Education
King Saud University
Code 034
College of Medicine
& King Khalid University Hospital

المملكة العربية السعودية
وزارة التعليم العالي
جامعة الملك سعود
رمزها ٠٣٤
كلية الطب
ومستشفى الملك خالد الجامعي

Date: 21.11.1433 / 07.10.2012 التاريخ: No.: 12/3519/IRB الرقم:

Dr. Ali M. Somily
Associate Professor
Department of Pathology – Microbiology Unit
King Saud University College of Medicine
King Khalid University Hospital

Subject: **Research Project No. E-12-672**

"Isolation of Chlamydia trachomatis and mycoplasma from Endocervical swabs of unfertile women and subject of tubal pregnancy"

Dear Dr. Somily,

Thank you for your response to the comments raised by the Board regarding the above-mentioned research project, which was reviewed and re-discussed in the IRB Meeting 01 (Academic Year 1433-1434), held on 03 Dhul'qadah 1433 (19 Sept. 2012). The IRB has reviewed your response and found that you have answered satisfactory and adequately fulfilled the requirements. Therefore, the project is now approved.

We wish you success in your research.

Thank you!

Sincerely yours,

Prof. Ahmed S. BaHammam
Chairman, Institutional Review Board
King Saud University - College of Medicine
P.O.Box 7805 Riyadh 11472 K.S.A.

Cc: Head, Department of Microbiology

P. O. Box 7805, Riyadh 11472 Tel 4670011 Fax.: 4672439 ص .ب ٧٨٠٥ الرياض ١١٤٧٢ هاتف: ٤٦٧٠٠١١ فاكس: ٤٦٧٢٤٣٩
www.ksu.edu.sa

Table 3.5 Consent form.

نموذج إقرار
CONSENT FORM

RESEARCH PROJECT TITLE

Isolation of Chlamydia trachomatis and mycoplasma from endocervical swabs of unfertile women and women subjected to tubal pregnancy

(Control group)

You are being asked to participate voluntarily in a Research Study. If you decide to take part in this study, please sign this consent form and return it.

نرجو منك المشاركة في هذه الدراسة البحثية وعند موافقتك بذلك ترجو منك التوقيع على هذه الورقة وإرجاعها إلينا

STUDY PURPOSE:

To determine the presence of M. genitalium and C. trachomatis in women attending fertility clinics and investigate the rule of *Chlamydia trachomatis*, *Mycoplasma hominis* and *mycoplasma genitalium* in the cervical canal in unexplained infertile women and compare it to healthy controls in the Saudi population.

الغرض من الدراسة:

بسبب محدودية المعلومات محليا فإن الغرض من هذه الدراسة هو الكشف عن بكتيريا الكلاميديا والميكوبلازما من عينات مأخوذة من عنق الرحم لنساء مصابات بالعقم الغير مفسر ومقارنة ذلك بعينات لنساء طبيعيات مراجعات للعيادات الأولية لأسباب لا تتعلق بالعقم

STUDY PLAN:

A total of 500 women presenting with a history of infertility primary infertility, secondary infertility and reproductive women between 20 and 45 years of age will be included into this study. Specimens will be taken from the endocervical canal. CT, MH and MG will be detected with polymerase chain reaction

الهدف من الدراسة:

. أخذ مسحات من عنق الرحم لسيدات تتروح أعمارهن من 20_45مصابات بالعقم الاولي والثانوي مراجعات لعيادة العقم ومسحات لسيدات طبيعيات مراجعات للعيادات الأولية،سيتم الكشف بكتيريا الكلاميديا والميكوبلازما عن طريق تحليل تفاعل البلمرة المتسلسل

BENEFITS: The result of this study may not benefit you directly, but in the future with God's will the patients will benefit from the knowledge acquired.

الاستفادة المرجوة من الدراسة: إن الاستفادة من هذه الدراسة قد لا تعود عليك مباشرة ولكن قد تكون لنتائج هذا البحث تأثيرات على المرضى الآخرين.

SIDE EFFECT: There are no side effects. You participation in this study does not have any further risks or discomfort to you.

الآثار الجانبية: لا توجد هناك أي أضرار جانبية من هذه الدراسة ومشاركتك لا تسبب أي إزعاج أو مخاطر مستقبلاً

REFUSAL: If you refuse to participate, there will be no penalty or loss of benefits.

عدم الرغبة في المشاركة: إذا رفضت المشاركة في هذه الدراسة فإنك لن تتعرض لأي جزاء أو فقدان للمزايا العلاجية

CONFIDENTALITY: Your participation in this study will be kept confidential. The results of this research may be published, however, your identity will never be revealed.

سرية المعلومات: إن مشاركتك في هذه الدراسة ستكون في غاية السرية. قد يتم نشر النتائج هذا البحث لأغراض أكاديمية ولكن لن يتم الكشف عن هويتك في أي حال من الأحوال.

This study for the purposes of research not therapeutic

الهدف من هذه الدراسة بحثي وليس علاجي

APPROVAL: I fully understand the information and the consent form. And I don't mind using sample for further studies.

الموافقة بالمشاركة: استوعبت المعلومات في هذا النموذج, لذا أوافق بالمشاركة في هذه الدراسة. كما أنني لا أمانع من استخدام العينات المتحصل عليها من هذه الدراسة بأن تستخدم في دراسات مستقبلية من قبل الباحثين.

لقد تم شرح هذا النموذج للمتبرع بواسطة أحد الباحثين قبل طلب التوقيع منه.

I sign freely and voluntarily.

أوقع أنا بمحض إرادتي وحريتي.

Investigator or Associate:

أسم الباحث أو من ينوب عنه:

Table 3.6 Questionnaire form.

Questionnaire sheet

PROJECT TITLE : Isolation of Chlamydia trachomatis and Mycoplasma from endocervical swabs of unfertile women and women subjected to tubal pregnancy

NAME _____

AGE _____

NATIONALITY _____

New sexual contact last 2 months?	☐ Yes	☐ No
Have you had STIs in the past?	☐ Yes	☐ No
Are you trying to get pregnant?	☐ Yes	☐ No

Have you had any of the following problems?

Pain during urination?	☐ Yes	☐ No
Bleeding at / after intercourse?	☐ Yes	☐ No
Bleeding from your periods?	☐ Yes	☐ No
Abnormal discharge?	☐ Yes	☐ No
Low-seated abdominal pain?	☐ Yes	☐ No
Pelvic inflammatory disease?	☐ Yes	☐ No
Tubal infertility?	Yes	No
Ectopic pregnancy?	Yes	No
Premature delivery and low birth weight?	Yes	No

To be completed by the examining doctor

Abnormal discharge from the cervix?

Macroscopic signs of vaginosis?

Macroscopic signs of cervicitis?

3.6 Specimen collection

Endocervical swabs by a speculum examination were collected from 100 infertile married women (case group) and 100 healthy productive married women (control group).

Two sterile flocked swabs were used per patient by the attending clinician. The first swab was used before sampling to clean off excess mucus while the second was rubbed over the endocervical cells in the cervical canal the swab were rotated several times to collect samples for assay. Swabs were withdraw without touching the vaginal surface and placed into the Copan Universal Transport Medium (UTM-RT).

According to manufacturer instructions, all swabs were stored at 4°C until transported to the laboratory, stored at 20°C until processed.

3.7 Specimen Analysis

3.7.1 Sample preparation

Before amplification, pathogen-specific DNA was extracted from the specimen. A fully automated specimen preparation instrument was used, Automated DNA extraction by MagNA Pure Compact Nucleic Acid Isolation Kit By Roche Diagnostics (table 3.1) and MagNA Pure Compact system (table 3.2).

The principle steps of MagNA Pure Compact nucleic acid isolation procedure were:

1. The samples were transferred to the MagNA pure compact sample tubes, sample amount 400 µl and loaded into the instrument.
2. The DNA bacteria purification protocol was chosen.
3. The samples were lysed by incubation with Proteinase K and a special lyses buffer containing a chaotropic salt.

4. Magnetic Glass Particles (MGPs) were added and nucleic acids were immobilized on the MGPs surfaces.
5. Unbound substances (*e.g.*, proteins, cell debris, PCR inhibitors) were removed by several washing steps.
6. Purified nucleic acids were eluted from the MGPs.
7. Following extraction procedures, samples were stored at −20°C in the elution buffer provided with the kits, as recommended by the manufacturers.

3.7.2 Qualitative real time PCR

3.7.2.1 Amplification and detection

DNA amplification was performed on all DNA isolates, the commercially available *C. trachomatis. M. genitalium*, and *M. hominis* Real time PCR kits were used according to manufacturer's instructions.

The master mix was prepared using Light Cycler Fast Start DNA Master HybProbe (table 3.7, 3.8, 3.9).

Then the Light Mix kit for the qualitative detection of *C. trachomatis, M. genitalium, and M. hominis,* data were analysed according to manufacturer's instructions.

3.7.2.1.1 Preparing the master mix :

1. Fast Start Taq DNA polymerase vial 1a (Fast Start Taq DNA polymerase) was ready after pipetting 60 µl from vial 1b (reaction buffer dNTP mix) into vial 1a.
2. 66 µl PCR-grade water was added to the lyophilized primers (reagent mix) and the IC mix.
3. The positive control was ready after adding 40 µl PCR water to the control DNA provided with the light mix kit.
4. The negative control was prepared by replacing the template DNA with water.

Table 3.7 Reaction mixture of light cycler real time PCR for detection of *C. trachomatis.*

Single reaction	Component
6.6 µl	water, PCR-grade (colourless cap, provided with the Roche Master kit)
2.4 µl	Mg^{2+} solution 25 mM (blue cap, provided with the Roche Fast Start kit)
2.0 µl	reagent mix (parameter specific reagents containing primers and probes
2.0 µl	IC mix (IC reagents containing primers, probes and DNA)
2.0 µl	Roche Master (vial 1a)
15.0 µl	Total volume of reaction mix

Table 3.8 Reaction mixture of light cycler real time PCR for detection of *M. genitalium*.

Single reaction	Component
6.6 µl	water, PCR-grade (colourless cap, provided with the Roche Master kit)
2.4 µl	Mg^{2+} solution 25 mM (blue cap, provided with the Roche Fast Start kit)
2.0 µl	reagent mix (parameter specific reagents containing primers and probes
2.0 µl	IC mix (IC reagents containing primers, probes and DNA)
2.0 µl	Roche Master (vial 1a)
15.0 µl	Total volume of reaction mix

Table 3.9 Reaction mixture of light cycler real time PCR for detection of *M. hominis*.

Single reaction	Component
6.6 µl	water, PCR-grade (colorless cap, provided with the Roche Master kit)
2.4 µl	Mg^{2+} solution 25 mM (blue cap, provided with the Roche Fast Start kit)
2.0 µl	reagent mix (parameter specific reagents containing primers and probes
2.0 µl	IC mix (IC reagents containing primers, probes and DNA)
2.0 µl	Roche Master (vial 1a)
15.0 µl	Total volume of reaction mix

The procedures were done according to the manufacturer's instructions with the reaction mixture shown in tables 3.7, 3.8, 3.9, to avoid contamination, mixing of the reagents (except of the DNA template) was performed in a separate room, away from rooms where culturing and DNA purification were done.

5. The reaction mixture was prepared in a cold reaction tube, mixed gently, and spin down.
6. 15 µl of the reaction mix was transferred to a light cycler 2.0 capillary.
7. 5 µl of template DNA was added to each capillary for a final reaction volume of 20 µl.
8. One negative control was always included in each run by replacing the template DNA with water.

9. A positive control was included in each run by replacing the template DNA with one of the provided control DNA from the standard row provided with the light mix kit.
10. The reaction mix was prepared by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

3.7.2.2 Programming (PCR setup):

The protocol consists of four program steps:

1. Denaturation: sample denaturation and enzyme activation
2. Cycling: PCR-amplification of the target DNA
3. Melting: melting curve analysis for identification of the PCR product derived from the target DNA
4. Cooling: cooling the instrument (table 3.10)

Table 3.10 Specification of the cycling parameters.

Program Step:	**Denaturation**	**Cycling**			**Melting**			**Cooling**
Parameter								
Analysis Mode	None	Qualitative mode			Melting curve mode			None
Cycles	1	50			1			1
Target [°C]	95	95	62	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:05	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]	20	20	20	20	20	20	0.2	20
Sec Target [°C]	-	-	55	-	-	-	-	-
Step Size [°C]	-	-	0.5	-	-	-	-	-
Step Delay (Cycles)	-	-	1	-	-	-	-	-
Acquisition Mode	None	None	Single	None	None	None	Cont	None

3.7.2.3 Data Analysis

1. The instructions in the manual of TIB MOLBIOL Light Mix Kit was followed.
2. Data analysis was performed, as described in the Light Cycler Instrument operator's manual.
3. *C. trachomatis, M. hominis, and M. genitalium* data were viewed in channel 640. Melting curves mode was chosen.
4. The melting temperature (T_m) of each sample was calculated automatically.
5. The negative control showed no signal.
6. IC data was viewed in channel 705 to monitor the presence of inhibitors. IC were included in each sample during real time PCR run, all samples (negative samples in particular) must show positive IC signal and any sample showed negative target and negative IC was repeated.

3.7.3 Absolute quantitative real time PCR:

All the positive samples of *C. trachomatis, M. genitalium, M. hominis*, were subjected to confirmatory testing to detect the concentration of the PCR Absolute quantitative real time PCR. Data analysis was Perform, as described in the Light Cycler Instrument operator's manual, the cycle number of the Crossing Point (C_P) and the DNA copy numbers per reaction of each sample was calculated automatically.

3.7.3.1 Quantitative DNA standards used for determining bacterial copy number by real-time PCR.

1. Preparation of the standard row (according to manufacturer's instructions).

2. The target DNA provided was 6 different quantities to yield from 10^1 to 10^6 target molecules in 5 µl once resolved.
3. A pipette tip was used to punch a hole through the ceiling foil, 40 µl PCR-grade water was added to each vial of the row.
4. The target DNA was mixed by pipetting the solution up and down 10 times, 5 µl standard DNA was used for a 20 µl PCR reaction.
5. Each standard DNA control run in the same program separately.

3.7.3.2 Preparing the master mix:

11- Fast Start Taq DNA polymerase vial 1a (Fast Start Taq DNA polymerase) was ready after pipeting 60 µl from vial 1b (reaction buffer dNTP mix) into vail 1a.

12- 66 µl PCR-grade water was added to the lyophilized primers (reagent mix) and the IC mix.

13- The negative control was prepared by replacing the template DNA with water.

3.7.3.2.1 Reaction mixture of light cycler real time PCR for detection of *C. trachomatis*:

9 positive *C. trachomatis* samples + 6 positive controls + 1 negative control + 1 extra = 17 reaction

Table 3.11 Reaction mixture of light cycler quantitative real time PCR for detection of *C. trachomatis.*

Single Reaction	Component	17 reaction total volume
6.6 µl	Water, PCR-grade (provided with the Roche Master kit)	6.6 x 17 = 112.2 µl
2.4 µl	Mg^{2+} solution 25 mM (provided with the Roche Fast Start kit)	2.4 x 17 = 40.8 µl
2.0 µl	Reagent mix (parameter specific reagents containing primers and probes)	2 x 17 = 34 µl
2.0 µl	IC mix (IC reagents containing primers, probes and DNA)	2 x 17= 34 µl
2.0 µl	Roche Master Roche Master (vial 1a)	2x17= 34 µl

1. Reaction mix was mixed gently, spin down.
2. 15 µl each of the reaction mix were transferred to a Light Cycler capillary
3. 5 µl of sample were added to 9 capillaries for a final reaction volume of 20 µl.
4. 5 µl of standard DNA (previously prepared) were added to 6 capillaries for a final reaction volume of 20 µl.
5. The negative control was added by replacing the template DNA with water.

3.7.3.2.2 Reaction mixture of light cycler real time PCR for detection of *M. genitalium*

4 positive *M. genitalium* samples + 6 positive controls + 1 negative control + 1 extra = 12 reaction

Table 3.12 Reaction mixture of light cycler quantitative real time PCR for detection of *M. genitalium*.

Single Reaction	Component	12 reaction total volume
6.6 µl	water, PCR-grade (provided with the Roche Master kit)	6.6x12=79.2 µl
2.4 µl	Mg^{2+} solution 25 mM (provided with the Roche Fast Start kit)	2.4x12=28.8 µl
2.0 µl	reagent mix (parameter specific reagents containing primers and probes)	2 x12= 24 µl
2.0 µl	IC mix (IC reagents containing primers, probes and DNA)	2 x12= 24 µl
2.0 µl	Roche Master Roche Master (vial 1a)	2 x12= 24 µl

1. Reaction mix was mixed gently, spin down.
2. 15 µl each of the reaction mix were transferred to a Light Cycler capillary.
3. 5 µl of sample were added to4 capillaries for a final reaction volume of 20 µl.
4. 5 µl of standard DNA (previously prepared) were added to 6 capillaries for a final reaction volume of 20 µl.
5. The negative control was added by replacing the template DNA with water.

3.7.3.2.3 Reaction mixture of light cycler real time PCR for detection of *M. hominis*

4 positive *M. hominis* samples + 6 positive controls + 1 negative control + 1 extra = 12 reaction

Table 3.13 Reaction mixture of light cycler quantitative real time PCR for detection of *M. hominis.*

Single Reaction	Component	12 reaction total volume
6.6 µl	water, PCR-grade (provided with the Roche Master kit)	6.6x12=79.2µl
2.4 µl	Mg^{2+} solution 25 mM (provided with the Roche Fast Start kit)	2.4x12=28.8µl
2.0 µl	reagent mix (parameter specific reagents containing primers and probes)	2 x12= 24 µl
2.0 µl	IC mix (IC reagents containing primers, probes and DNA)	2 x12= 24 µl
2.0 µl	Roche Master Roche Master (vial 1a)	2 x12= 24 µl

14. Reaction mix was mixed gently, spin down.
2. 15 µl each of the reaction mix were transferred to a Light Cycler capillary.
3. 5 µl of sample were added to 4 capillaries for a final reaction volume of 20 µl.
4. 5 µl of standard DNA (previously provided) were added to6 capillaries for a final reaction volume of 20 µl.
5. The negative control was added by replacing the template DNA with water.

3.7.3.3 Programming (PCR setup):

The protocol consists of four program steps:

1. Denaturation: sample denaturation and enzyme activation
2. Cycling: PCR-amplification of the target DNA
3. Melting: melting curve analysis for identification of the PCR product derived from the target DNA
4. Cooling: cooling the instrument (table 3.14).

Table 3.14 Specification of the cycling parameters (quantitative real time PCR).

Program Step:	**Denaturation**	**Cycling**			**Melting**			**Cooling**
Parameter								
Analysis Mode	None	Qualitative mode			Melting curve mode			None
Cycles	1	50			1			1
Target [°C]	95	95	62	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:05	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]	20	20	20	20	20	20	0.2	20
Sec Target [°C]	-	-	55	-	-	-	-	-
Step Size [°C]	-	-	0.5	-	-	-	-	-
Step Delay (Cycles)	-	-	1	-	-	-	-	-
Acquisition Mode	None	None	Single	None	None	None	Cont	None

3.7.3.4 Data Analysis

1. The instructions in the manual of TIB MOLBIOL Light Mix Kit was followed.
2. Data analysis was performed, as described in the Light Cycler Instrument operator's manual.
3. *C. trachomatis, M. hominis, and M. genitalium* data were viewed in channel 640. Quantification mode.
4. The cycle number of C_P of each sample was calculated automatically.

3.8 Electrophoresis analysis of amplicons

3.8.1 Preparation of Agarose Gel

1. The 1.5% agarose has already been prepared 1.5 gram of Agarose powder dissolved in 100ml 1x electrophoresis buffer.
2. Agarose powder was suspended in the buffer.
3. Gel solution was placed into the microwave oven and was boiled and swirl until all of the small translucent Agarose particles dissolved.
4. The level of the comb was adjusted.
5. The molten Agarose was to 60 °C before pouring an Agarose gel slab.

   2 µg/100 ml ethtidium bromide was added to the molten Agarose
6. When the agarose solution was cooled to 50-60 °C, the molten Agarose was poured on the tray.
7. The gel was allowed to solidify (about 10 minutes); then the comb was removed.

3.8.2 Loading and running the gel

8. Loading dye was added to each sample.
   Loading volume: 3µl loading dye + 7 µl sample
9. The casting tray (with the gel on it) was inserted into the electrophoresis chamber with the wells closest to the negative (black) electrode. DNA is negatively charged.
10. During electrophoresis, it migrated from the negative to the positive electrode.
    1xTBE (Tris-Borate-EDTA; electrophoresis buffer) was gradually add to the chamber until the buffer just covers the top of the gel.
11. Samples were loaded.
12. The power was turned on and the voltage control knob was adjusted to 120 volts.
13. Electrophorese (run) until the migration within ¾ of the positive electrode end of the gel.
14. The gel was removed from the casting tray and placed into Gel electrophoresis document system/Biorad-USA for photographing.

3.9 Conversion factor

The amount of bacteria per sample (bacterial load) is commonly reported as copies /ml while PCR reports copies /reaction .the conversion factor between both depend on sample and extraction volume.

The bacterial load was calculated using the following general formula:
Bacterial Load (BL) = MV x EVF x SF
Where:
BL =Bacterial Load

MV = Measured Volume (copy number per reaction)

EVF = Extracted Volume Factor (final extracted volume / PCR sample volume)

SF = Sample Factor (1000µl / final extracted volume)

So:

BL (copy/ml) =MV x (100/5) x (1000/100)

3.10 Statistical analysis methods

SPSS version 19.0 was used to perform the data analysis. We present mean± SD, median and range for numerical variables (i.e. age and T_m).

We present the frequencies and percentages for different items of nominal variables. We used chi-square and Fisher's exact test to compare between infertile group and control group, Also to compare between primary infertility and secondary infertility with respect to all nominal variables; we also used Z-test of percentages drawn from one sample. Kruskal-walis non parametric statistical test have also been used. We assumed there was a statistically significant when p-value less 0.05.

Chapter (4)

Results

Results

4.1 Patient demographics

A total of 2934 women were offered screening test and 200 agreed to participate, with an acceptance rate of 6.8%.

200 endocervical swabs (100 from infertility group and 100 from control group) from women who fulfilled the inclusion criteria in this study) were subjected to real time PCR for detection of *C. trachomatis*, *M. genitalium*, and *M. hominis*.

The mean age-standard deviation (SD)-for all women enrolled in the study (200) was 34.66 ± 7.72 years.

The mean age-SD-of the infertility group was 32.50 ± 6.397 years compared with 36.82 ± 8.325 years for the control group (table 4.1, figure 4.1).

Table 4.1 The mean age, SD for participates.

Group	N	Mean	SD
Infertility	100	32.50	6.397
Control	100	36.82	8.325
Total	200	34.66	7.72

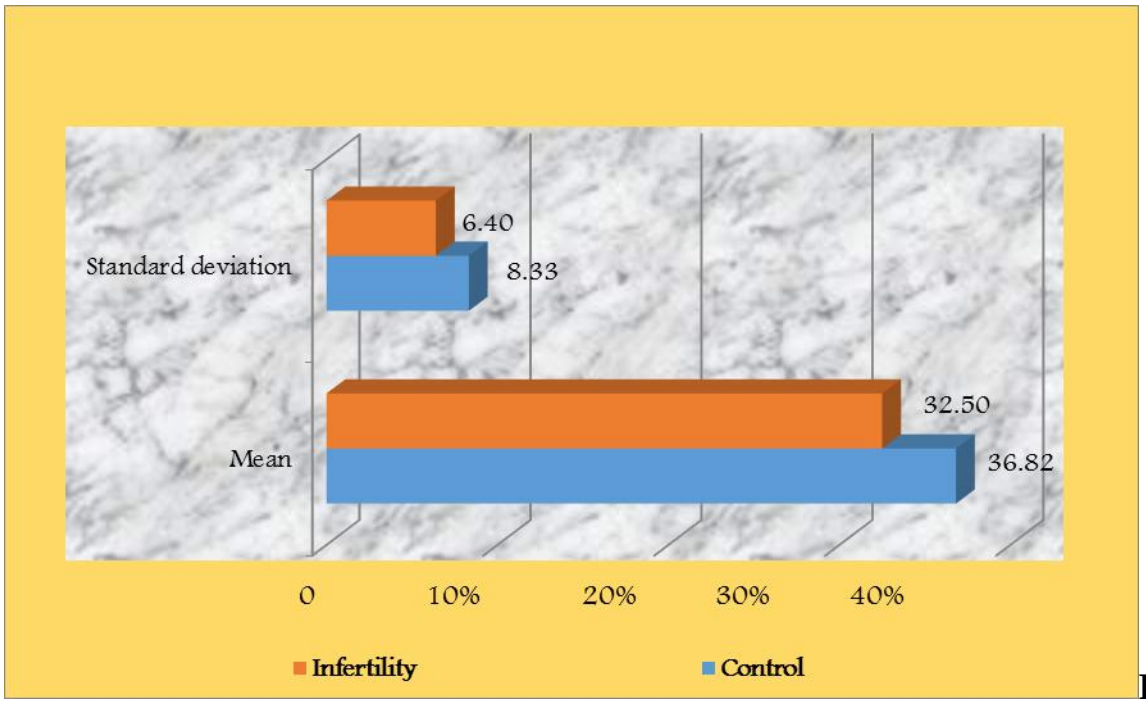

Figure 4.1 The mean age, SD for participates.

The mean age (SD) was significantly lower among the primary infertility group: 29.96 ± 5.735 years, whereas the mean age of the secondary infertile women was 35.86 ± 5.684 years (P-value<0.0001) (Table 4.2, figure 4.2).

Table 4.2 The mean age, SD of primary and secondary cases.

Type of infertility	N	Mean	SD	P-value
Primary	57	29.96	5.735	
Secondary	43	35.86	5.684	<0.0001

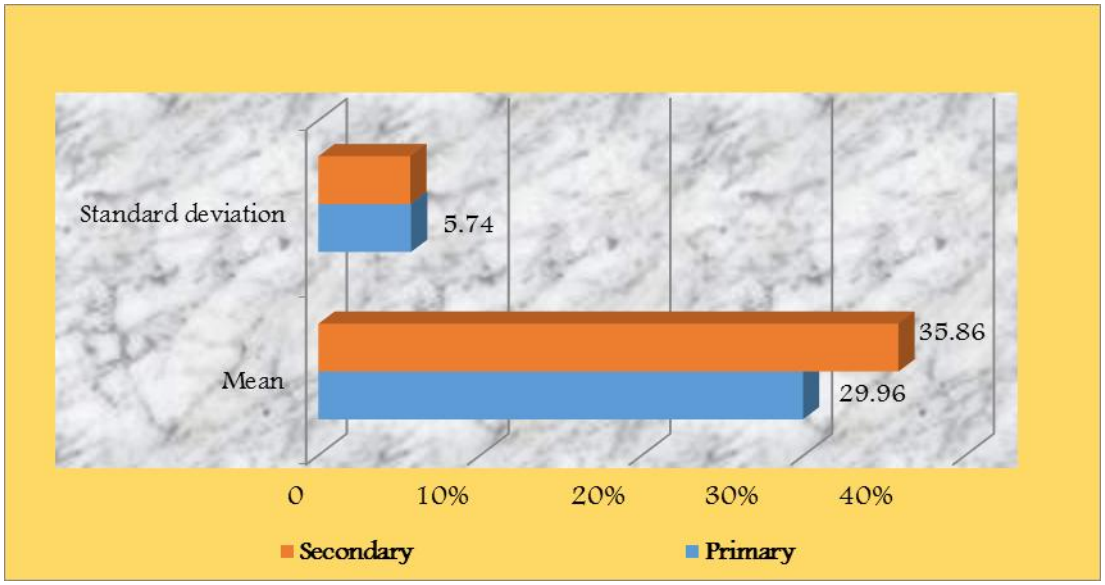

Figure 4.2 The mean age, SD of primary and secondary cases.

The women were stratified into 4 age groups, number of patients in age range 19-25 of the infertility group was 16 compared with 17 for the control group, There was no significant difference concerning this age group (P-value = 0. 849).

Participants aged 26-32 years were 34 among infertility group compared with 13 for the control group, difference was statistically highly significant (P-value<0.0001). Participants aged 33-39 years were 35 among infertility group compared with 17 for the control group, the difference was also statistically significant (P-value=0.004). There was a highly significant difference (P-value<0.0001) as well concerning age group 40-46 years were 15 among infertility group compared with 53 for the control group, (table 4.3, and figure 4.3).

Table 4.3 Age range of the study population.

Age Range		Group		Total	P-value
		Infertility	Control		
19-25	Count	16	17	33	0.849
	% within Group	16%	17%	16.5%	
26-32	Count	34	13	47	< 0.0001
	% within Group	34%	13%	23.5%	
33-39	Count	35	17	52	0.004
	% within Group	35%	17%	26%	
40-46	Count	15	53	68	< 0.0001
	% within Group	15%	53%	34%	
Total	Count	100	100	200	

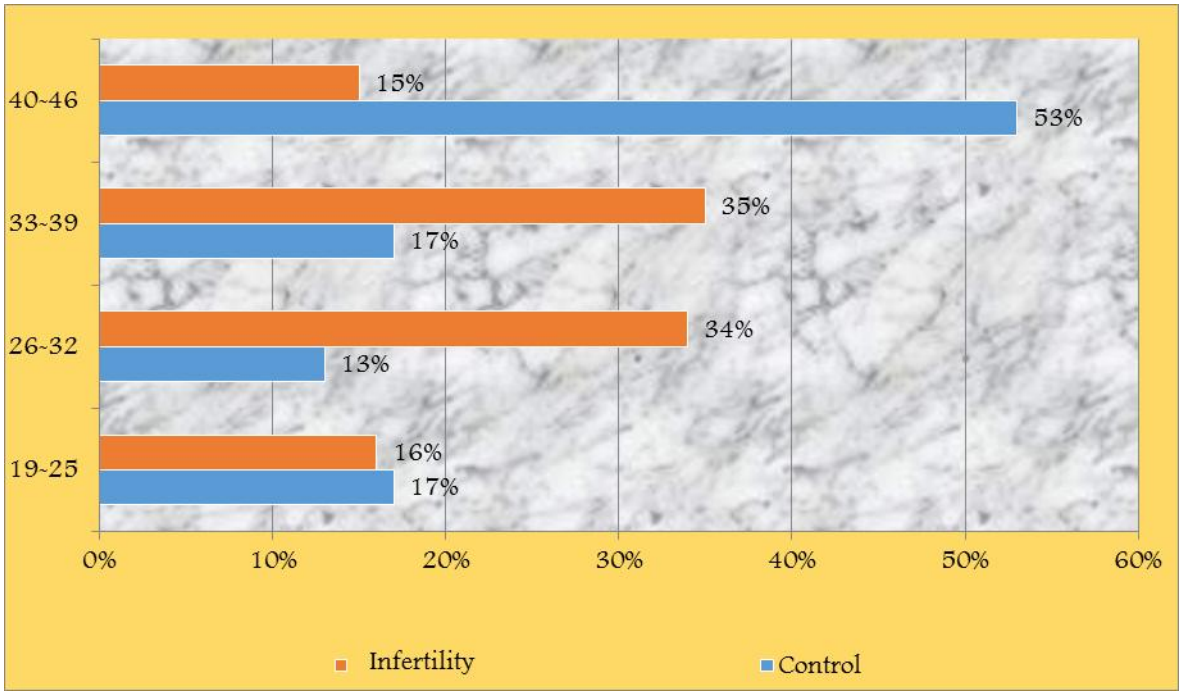

Figure 4.3 Age range of the study population.

We found no significant differences between infertility and control group with respect to Saudis and non-Saudis since P-value=0.756, however, a higher percentage of Saudis compared with non-Saudis were found in both groups (table 4.4, figure 4.4).

Table 4.4 Number (%) of Saudi's and non-Saudi's in both groups.

Nationality	Infertility (%)	Control (%)	P-value
Saudi	95(95%)	94(94%)	0.756
Non-Saudi	5 (5 %)	6 (6 %)	
Total	100 (100%)	100(100%)	

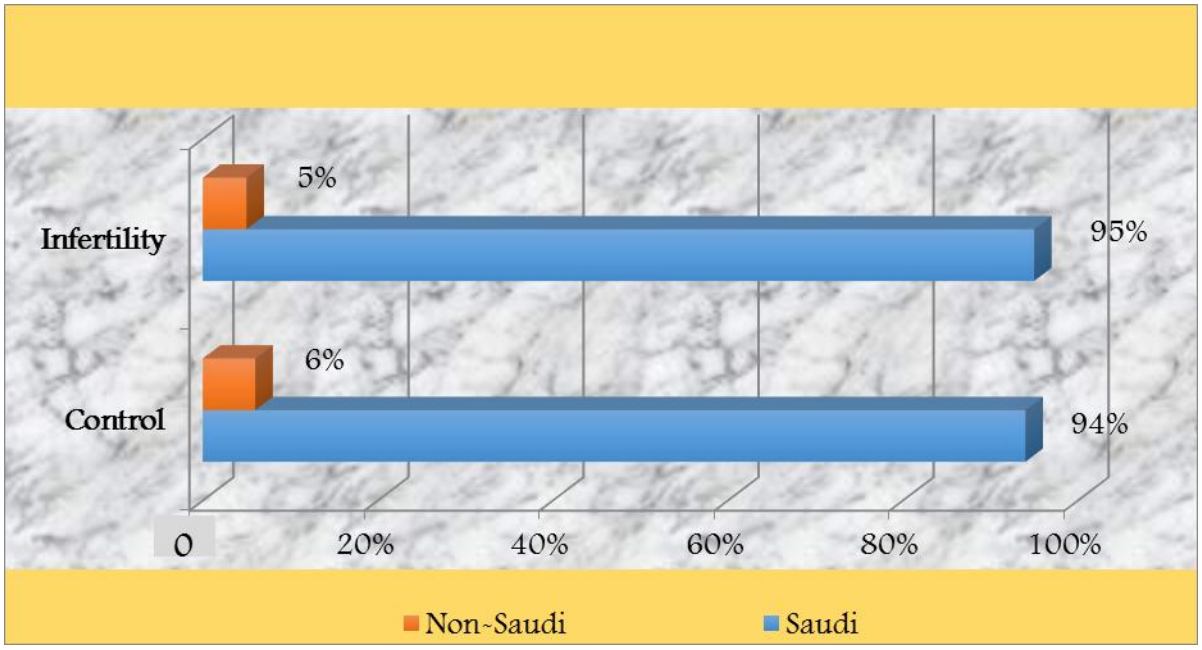

Figure 4.4 Saudis compared to non-Saudi's in both groups.

4.2 Association between Signs- symptoms with infertility and control group

No statistically significant differences were noted between the two groups of women with respect to almost all symptoms except for signs of cervicitis; all respondents reported no prior history of a STIs.

Burning sensation was present in 16 infertile women (16%) compared to24 (24%) control women (P-value=0.157), genital bleeding was present in 11 infertile women (11%) compared to 9 (9%) from control group (P-value=0.637).

Irregular menstruation was present in 8 infertile women (8%) compared to 14 (14%) from control group (P-value=0.175).

Abnormal discharge was present in 21 infertile women (21%) compared to 16 (16%) control women (P-value=0.363).

Low seated abdominal pain was the most frequent symptoms among both groups: present in 26 infertile women (26%) compared to 25 (25%) from control group (P-value=0.871).

Previous abortion was present in one single case of infertile women (1%) and no cases were detected from control group (P-value=0.50).

PID was present in one infertile case as well (1%) (P-value=0.50), 2 infertile women were presented with Ectopic pregnancy (2%) and none from control group (P-value=0.249).

Premature delivery was present in 5 infertile women (5%) compared to 6 cases of premature delivery from control group (6%) (P-value=0.756), Signs of vaginosis was present in one infertile women (1%) and no cases from control group (P-value=0.50). A statistically significant difference was noted between the two groups of women as far as signs of cervicitis (P-value=0.014), where a higher occurrence of infertile group (6 cases 6%) compared with no cases of cervicitis among control group (Table 4.5, figure 4.5).

Table 4.5 Signs & symptoms among all females in the study (case versus control).

Signs & symptoms	Number (%) of 100 patients in the infertility group	Number (%) of 100 patients in the control group	P-value
Burning sensation during urination	16(16%)	24 (24%)	0.157
Genital Bleeding	11(11%)	9 (9%)	0.637
Irregular menstruation	8(8%)	14(14%)	0.175
Abnormal discharge	21(21%)	16(16%)	0.363
Low seated abdominal pain	26(26%)	25(25%)	0.871
Previous abortion	1(1%)	0	0.50
PID	1(1%)	0	0.50
Ectopic pregnancy	2(2%)	0	0.249
Premature delivery and low birth weight	5(5%)	6(6%)	0.756
Signs of vaginosis	1(1%)	0	0.50
Signs of cervicitis	6(6%)	0	0.014

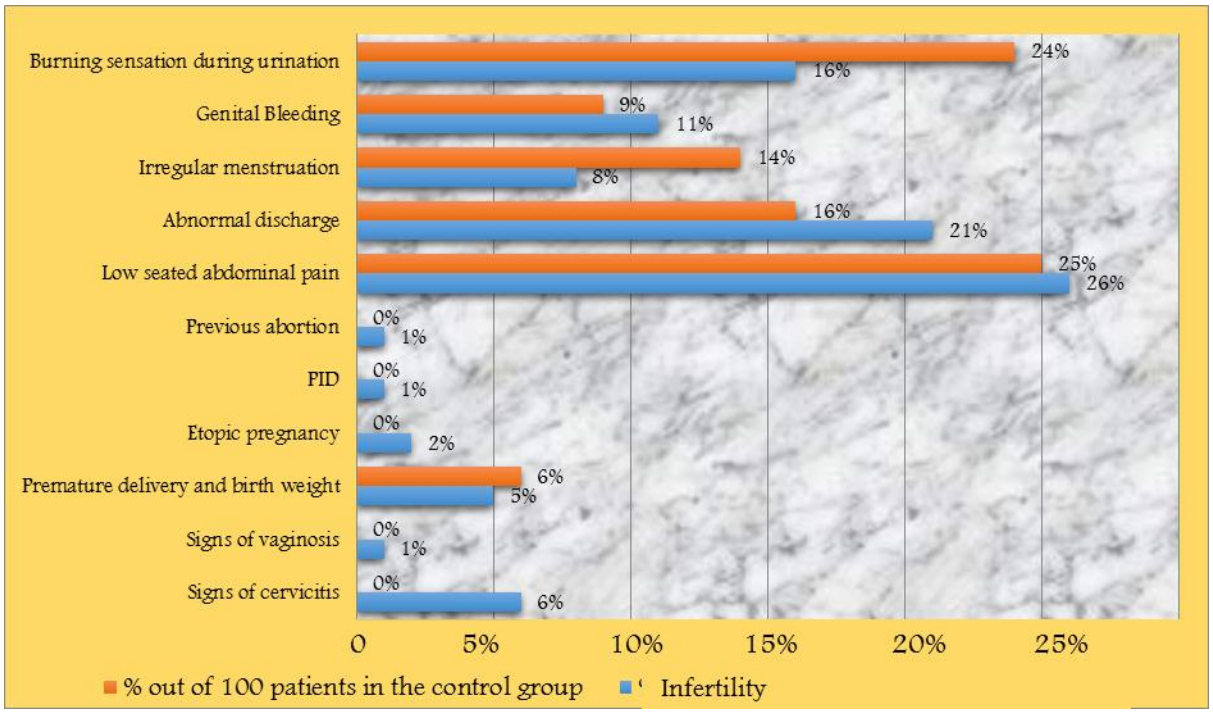

Figure 4.5 Signs and symptoms among all females in the study (case versus control).

4.3 Distribution of infertility group (n=100) according to types of infertility and infertility factors

Out of 100 infertile women, 57 had primary infertility, and 43 had secondary infertility type. The proportion of women with hormonal, ovulation, and unexplained infertility factor in the primary infertility group were:19, 14, and 13 respectively, higher than the proportion of women with the same factors in the secondary infertility group; 10, 9, and 8 (p-value: P- value=0.272, 0.669, and 0.609, respectively), whereas the proportion of women with tubal factor and the male factor in the primary infertility group were 6 and 5 respectively, lower than the proportion of women with the same infertility factors in the secondary infertility group; 9 and 7 (P- value =0.149 and 0.253, respectively) but the differences were not statistically significant (Table 4.6, figure 4.6).

Table 4.6 Distribution of infertility group (n=100) according to types of infertility and infertility factors.

		Infertility factors				
		Hormonal (n=29)	**Ovulation (n=23)**	**Unexplained (n=21)**	**Tubal (n=15)**	**Male factor (n=12)**
Types of infertility	**Primary (n=57)**	19	14	13	6	5
	% within type of infertility	(33%)	(25%)	(23%)	(11%)	(9%)
	Secondary (n=43)	10	9	8	9	7
	%within type of infertility	(23%)	(21%)	(19%)	(21%)	(16%)
	P-value	0.272	0.669	0.609	0.149	0.253

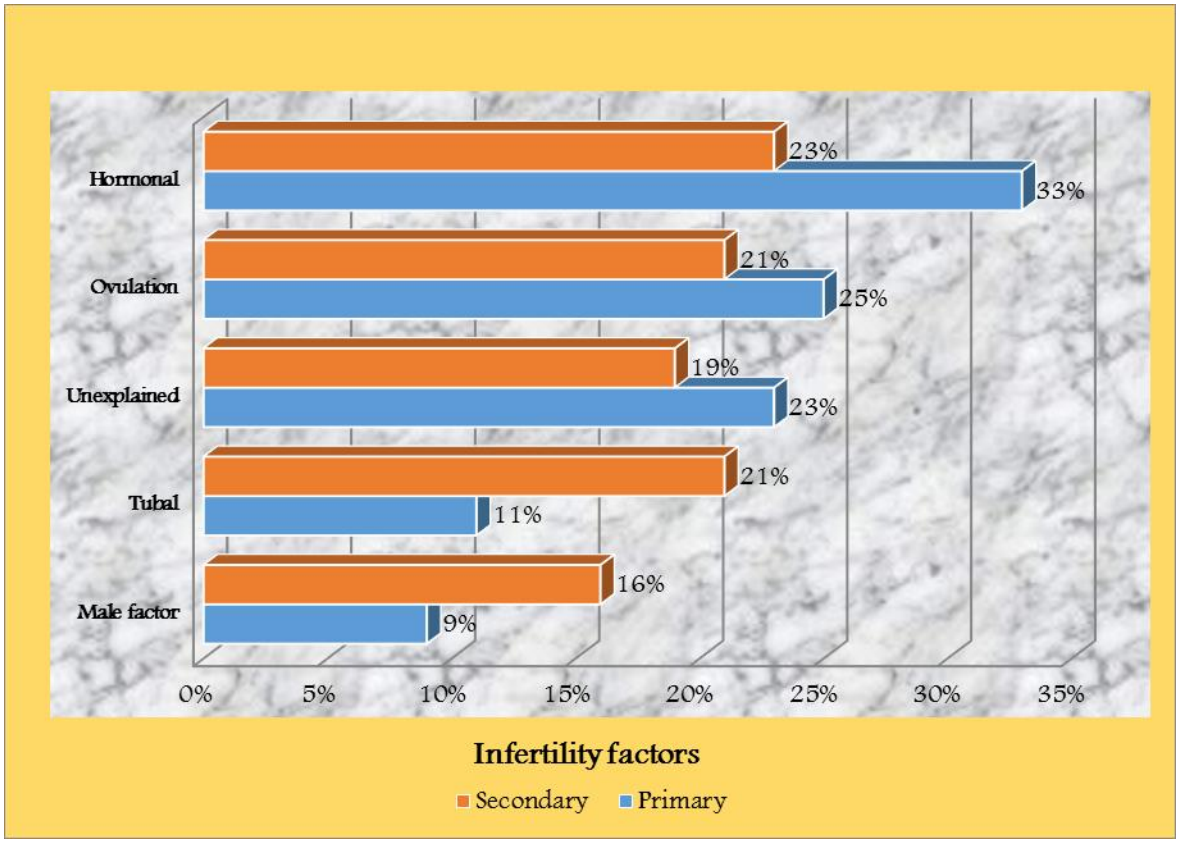

Figure 4.6 Distribution of infertility group (n=100) according to types of infertility and infertility factors.

4.4 Correlation between primary and secondary infertility with respect to age groups

Data analysis showed that there was significantly difference at age range 19-25 between primary infertile women (13/57; 23%) and secondary infertile women (3/43; 7%) (P-value=0.009).

Of the 57 primary infertile women recruited in this study, a statistically significant and high proportion of them were at age group 26-32 (27; 47%) compared to 7 secondary infertile women (7; 16%) from the same age range (P-value< 0.0001). Among participants aged 33-39 years were 13 among primary infertility group (13; 23%) compared with higher number (22; 51%) from the

secondary infertility group, difference was not statistically significant (P-value=0.094). Participants aged 40-46 years were lower as well among primary infertility group (4;7%) compared with 11 for the secondary infertility group (26%), the difference was not statistically significant, yet it was on the border (P- value= 0.06) (table 4.7, figure 4.7).

Table 4.7 Distribution of primary and secondary infertility according to age groups.

			Type of Infertility		Total	P-value
			Primary	Secondary		
Age Range	19-25	Count	13	3	16	0.009
		% within Type of Infertility	22.8%	7%	16.0%	
	26-32	Count	27	7	34	< 0.0001
		% within Type of Infertility	47.4%	16.3%	34.0%	
	33-39	Count	13	22	35	0.094
		% within Type of Infertility	22.8%	51.2%	35.0%	
	40-46	Count	4	11	15	0.06
		% within Type of Infertility	7.0%	25.6%	15.0%	
Total		Count	57	43	100	
		% within Type of Infertility	100%	100%	100%	

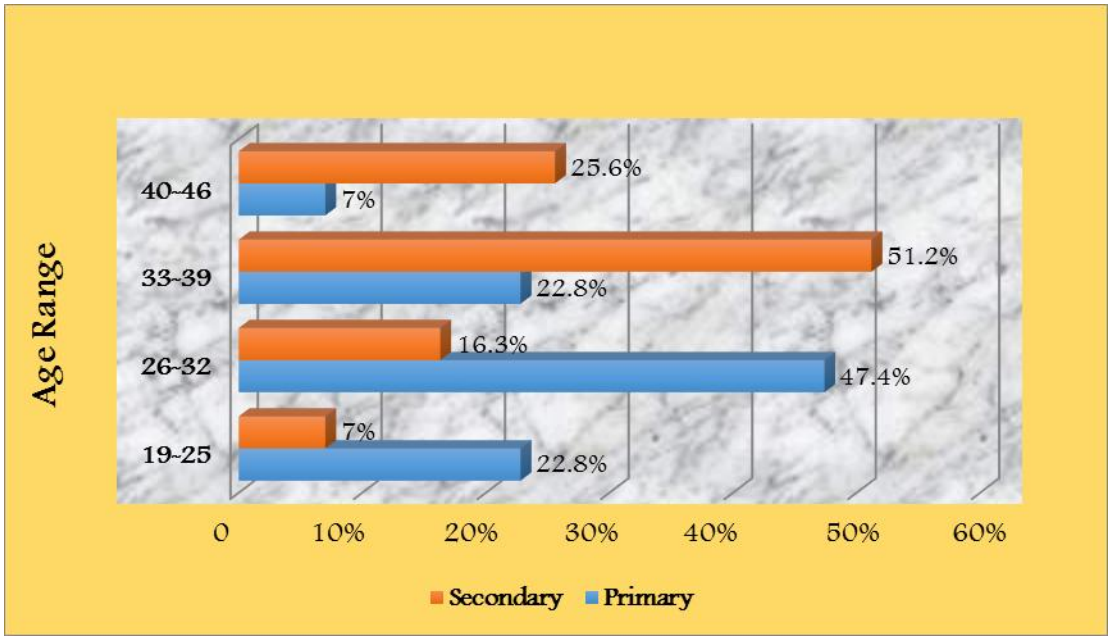

Figure 4.7 Distribution of primary and secondary infertility according to age groups.

4.5 Results of Real-time PCR for the detection of *C. trachomatis, M. genitalium,* and *M. hominis*

8women tested positive of *C. trachomatis* in the infertility group (8 out of 100; 8%) compared with 1 woman positive in the control group (1 out of 100; 1%). We found a significant differences among infertile and fertile women for *C. trachomatis* infection (P-value=0.017).

3 infertile women were positive of *M. genitalium* (3 out of 100; 3%). No positive cases of *M. genitalium* were detected among control group (P-value=0.123).

One women from infertility group (1 out of 100; 1%) was positive for *M. hominis*, compared to 2 positive results (2 out of 100; 2%) among control group (P-value=0.50). One patient tested positive for *M. genitalium* and *M. hominis* as

a mix or co-infection (1 out of 100; 1%) among infertile group was detected (P-value=0.4999) (table 4.8, figure 4.8).

Table 4.8 Positive cases of *C. trachomatis, M. genitalium,* and *M. hominis* in both infertility and control group.

Positive samples	Infertility group (n=100)	Control group (n=100)	P-value
C. trachomatis	8 (8%)	1 (1%)	0.017
M. genitalium	3(3%)	0	0.123
M. hominis	1(1%)	2(2%)	0.50
M. genitalium and M. hominis	1(1%)	0	0.499
Total	13(13%)	3(3%)	

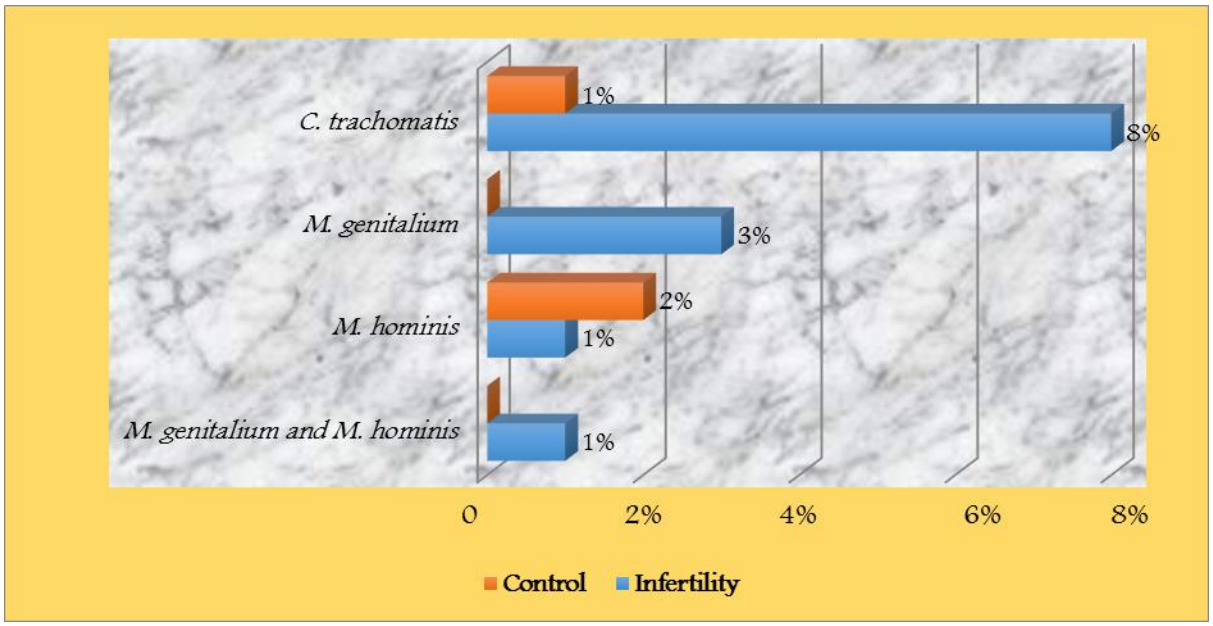

Figure 4.8 Positive cases of *C. trachomatis, M. genitalium,* and *M. hominis* among both infertility and control group.

4.6 Correlation between symptomatic and asymptomatic cases among infertility group

Among 100 infertile women, 54 infertile women were symptomatic 54%, whereas 46 infertile women were asymptomatic 46%, difference was not statistically significant (P-value=0.4242) (table 4.9, figure 4.9).

Results showed that 6 out of 8 *C. trachomatis* positive samples were detected from symptomatic women (6 out of 8; 75%)-(% within symptomatic women 6 out of 54; 11%), whereas 2 out of 8 *C. trachomatis* positive samples were detected from asymptomatic women (25%), % within asymptomatic women 2 of 46; 4%, even if not statistically significant (P-value=0.193), *C. trachomatis* was higher among the symptomatic patients.

The presence of *M. genitalium* was found in 3 patients in the group of symptomatic women (3 out of 3; 100%), % within symptomatic women 3 of 54; 6%, *M. genitalium* was not detected from a symptomatic cases (P-value=0.153).

The presence of *M. hominis* was found in one case in the group of asymptomatic women, within a symptomatic women 1 of 46; 2%, and no positive results from symptomatic cases (P-value=0.460).

A co-infection of *M. genitalium* and *M. hominis* was found in 1 sample in the group of symptomatic women (1 of 54; 2%) (P-value=0.540).

The overall or the total results showed that differences were not statistically significant between positive cases among symptomatic (10/54; 18.5%) and positive cases among asymptomatic patient (3/46; 6.5 %, P-value=0.075) (table 4.10, figure 4.10, figure 4.11).

Table 4.9 Symptomatic and asymptomatic cases among infertility group.

Percentages	P-value
Symptomatic(n = 54)54 %	0.4242
Asymptomatic(n = 46)46 %	

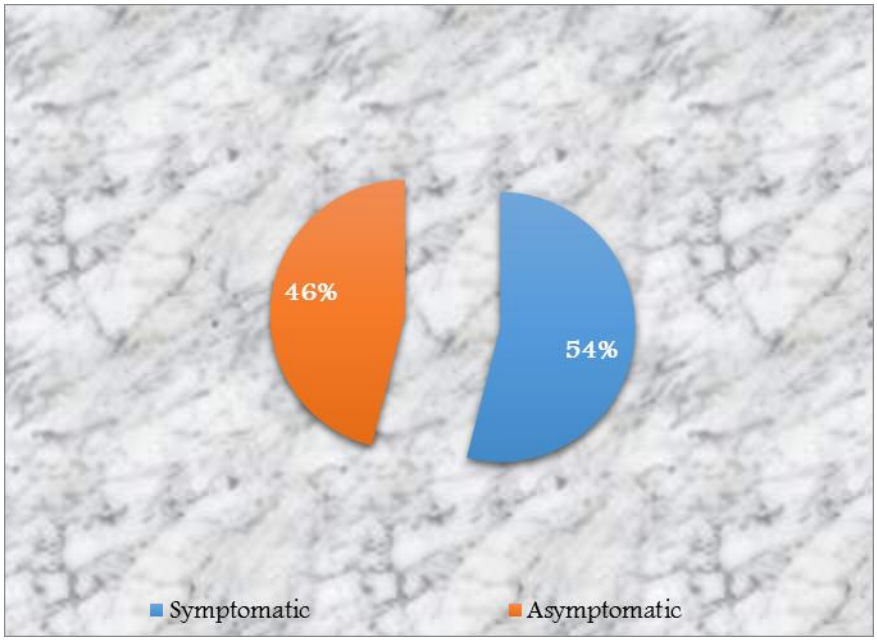

Figure 4.9 Symptomatic and asymptomatic rates among infertility group.

Table 4.10 Correlation between symptomatic & asymptomatic positive cases of among infertility group.

	Microorganism	*C. trachomatis* (n=8)	*M. genitalium (n=3)*	*M. hominis (n=1)*	*M. hominis& M. genitalium (n=1)*	Total (n=13)
Groups	**Symptomatic (n=54) % within group**	6(11%)	3 (6%)	0	1 (2%)	10(18.5%)
	Asymptomatic (n=46) % within group	2(4%)	0	1(2%)	0	3(6.5%)
	P-value	0.193	0.153	0.460	0.540	0.075

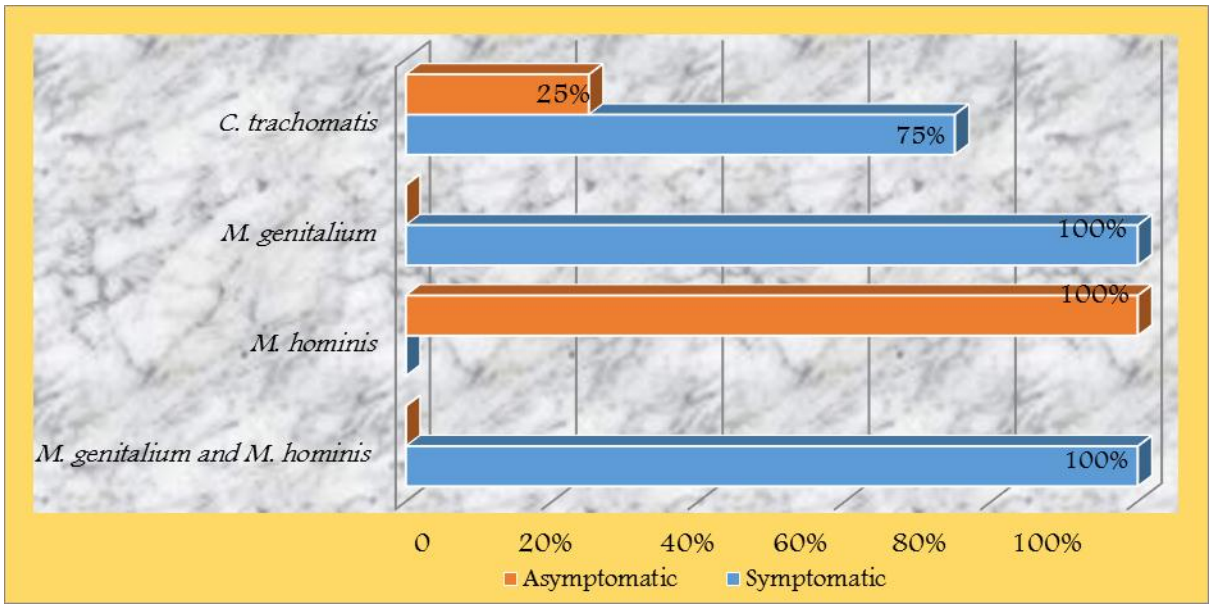

Figure 4.10 Correlation between symptomatic & asymptomatic positive cases among infertility group (% within total number of positive cases).

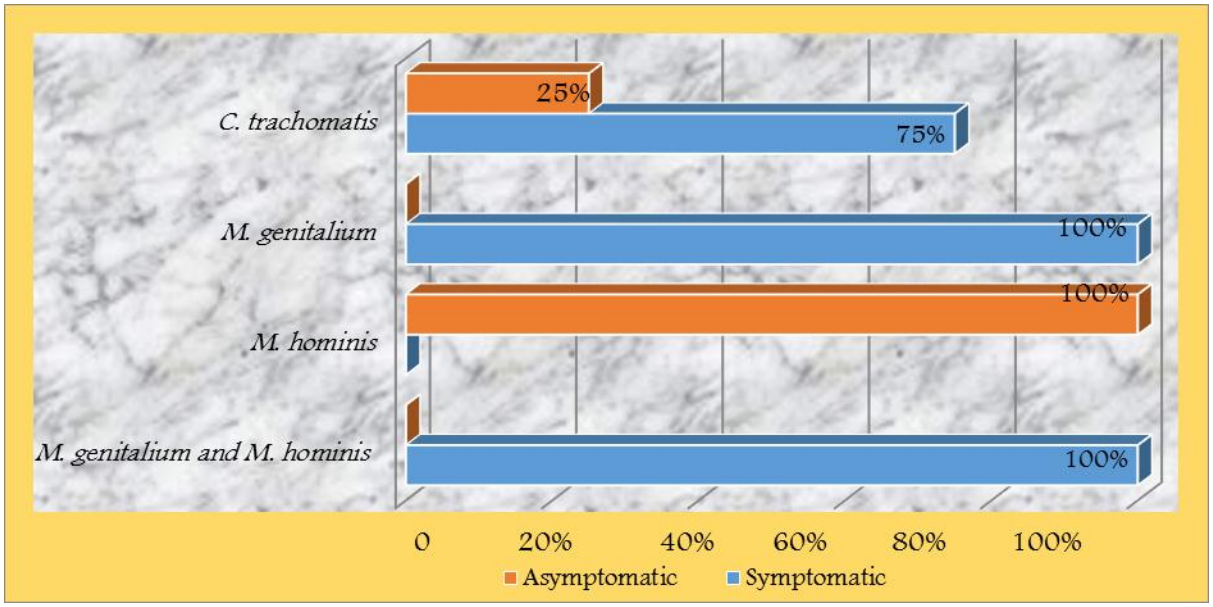

Figure 4.11 Comparing rates of positive and negative cases among symptomatic and asymptomatic patients.

4.7 Correlation between positive cases and the frequency clinical signs and symptoms

We found 5 out of 8 patients positive for *C. trachomatis* complaining low-seated abdominal pain compare to 5 patients positive for *Mycoplasma* and didn't complain low-seated abdominal pain and that is significant difference since P-value = 0.044 (Table 4.11, figure 4.12).

Out of 8 infertile cases with irregular menstruation, one case was positive for *C. trachomatis* (12.5%), and 2 positive cases of *M. genitalium* (25%) (P-value=0.315).

Out of 16 infertile cases with burning sensation during urination, one case was positive for *C. trachomatis* (6%), and 1 positive case of *M. genitalium* (6.25%), and the difference was not statically significant (P-value=0.641).

Out of 21 infertile cases with abnormal discharge, one case was positive for *C. trachomatis* (4.76%), 1 positive case of *M. genitalium* (5%), and the co-infection case of *M. genitalium* and *M. hominis* (5%), the difference was not statically significant (P-value=0.315). The single case of previous abortion was positive for *C. trachomatis* (P-value=0.615).

Out of 6 infertile cases signs of cervicitis, one case was positive for *M. genitalium* (16.67%, P-value =0.385) (Table 4.11, figure 4.12).

Table 4.11 Correlation between positive cases and the frequency of the clinical signs and symptoms.

Clinical signs and symptoms	*C. trachomatis* (n=8)	*M. genitalium* (n=3)	*M. hominis* (n=1)	*M. genitalium* & *M. hominis* (n=1)
Low-seated abdominal pain (n=26) % within symptom	5 (19%)	0	0	0
Irregular menstruation (n=8) % within symptom	1(13%)	2(25%)	0	0
Burning sensation during urination (n=16) % within symptom	1(6%)	1(6%)	0	0
Abnormal discharge (n=21) % within symptom	1(5%)	1(5%)	0	1(5%)
Pervious abortion (n=1) % within symptom	1(100%)	0	0	0
Signs of cervicitis (n=6) % within symptom	0	1(17%)	0	0

Clinical signs and symptoms	*C. trachomatis* (n=8)	Others (n=5)	p-value
Low-seated abdominal pain (n=26) % within symptom	5 (19.23 %)	0	0.044
Irregular menstruation (n=8) % within symptom	1(12.5 %)	2(25 %)	0.315
Burning sensation during urination (n=16) % within symptom	1(6.25 %)	1(6.25 %)	0.641
Abnormal discharge (n=21) % within symptom	1(4.76 %)	2 (9.52 %)	0.315
Pervious abortion (n=1) % within symptom	1(100%)	0	0.615
Signs of cervicitis (n=6) % within symptom	0	1(16.67 %)	0.385

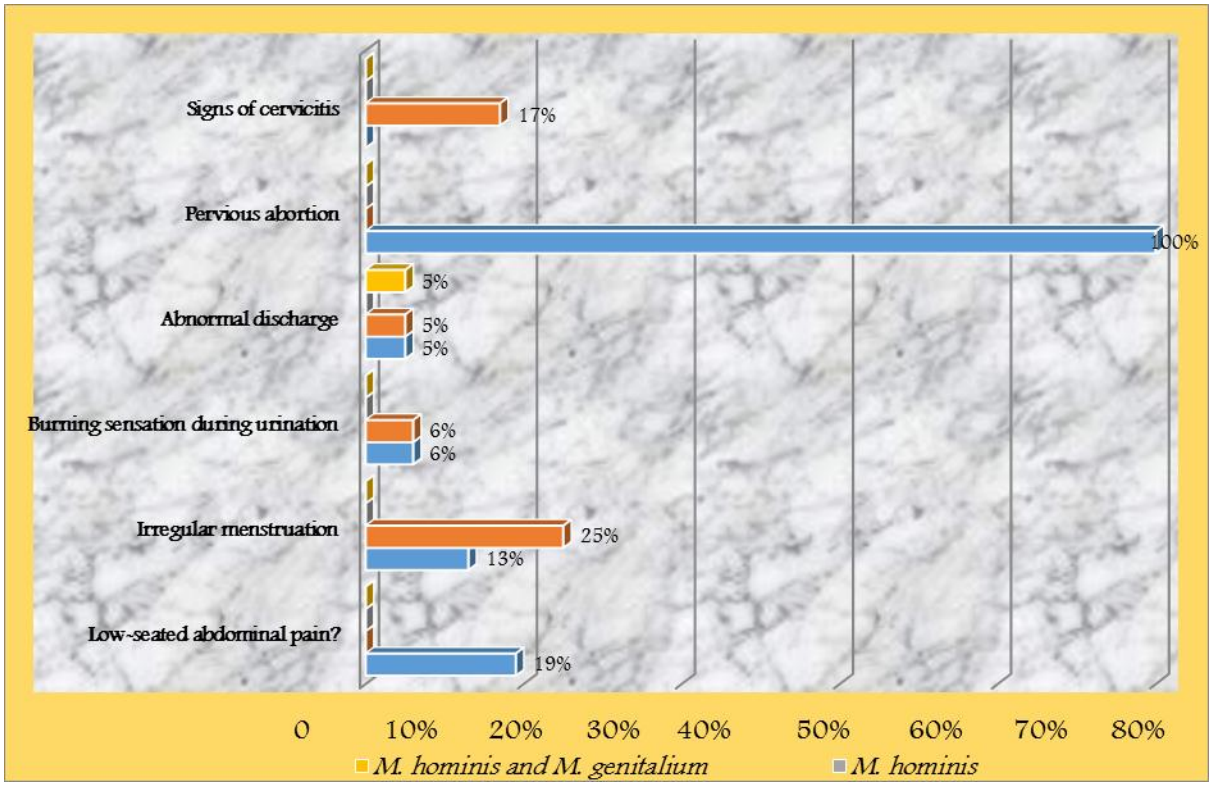

Figure 4.12 Correlation between positive cases and the frequency of clinical signs and symptoms.

4.8 Correlation between positive cases in Saudi's compared to none Saudi's in both control and fertile groups

The rate of *C. trachomatis* infection among infertile group was higher than its rate among the control group (8% versus 1%), and all infected women were Saudis.

M. genitalium was the second most commonly detected pathogen, there were 3 *M. genitalium* positive cases in the infertility group 3%, one of these 3 cases was non-Saudi and it was the only positive case from a non-Saudi reported in this study, and no positive cases from controls.

There was one *M. hominis* positive case among infertility group (1%) compared with 2 positives in the control group (2%) and they were all collected from Saudis.

No significant difference between infertile and control group with respect to nationality of patients for total number of positive results (P-value = 0.867). Table 4.12, figure 4.13.

Table 4.12 Correlation between positive cases in Saudi's compared to none Saudi's in both control and fertile groups.

Positive samples	Infertile (n=100)		Control (n=100)		Total(n=200)	P-value
	Saudi (n=95)	Non-Saudi (n=5)	Saudi (n=94)	Non-Saudi (n=6)		
C. trachomatis **(n=9) % within group**	8(8%)	0	1(1%)	0	9 (4.5%)	
M. genitalium **(n=3) % within group**	2(2%)	1(1%)	0	0	3 (1.5%)	
M. hominis **(n=3) % within group**	1(1%)	0	2(2%)	0	3 (1.5%)	
M. genitalium & M. hominis **(n=1)% within group**	1(1%)	0	0	0	1 (0.5%)	
Total (n=16)	12	1	3	0	16 (8%)	0.867

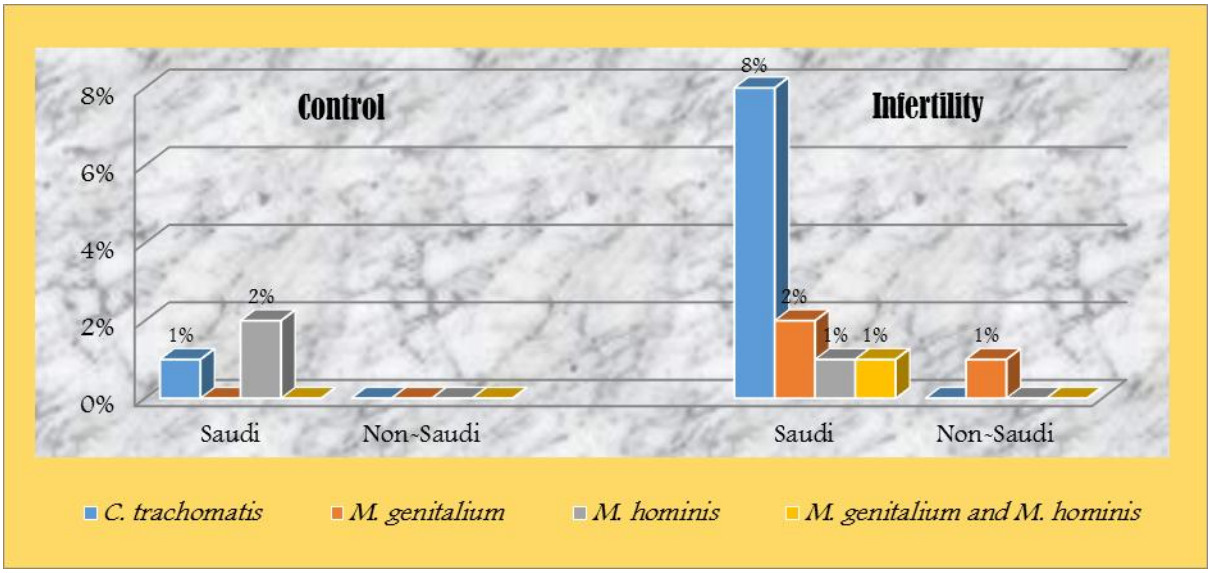

Figure 4.13 Correlation between positive cases in Saudi's compared to none Saudi's in both infertility and control groups.

4.9 Distribution of infertility group according to infertility factors

Among infertile women, causes of infertility (infertility factors) were more frequent than other causes of infertility; hormonal factor was the most frequent factor 29 (29%), followed by Ovulation 23 (23%), Unexplained 21 (21%), Tubal 15 (15%), Male factor 12 (12%), summaries in table 4.13, figure 4.14. There was a significant difference between hormonal factor and tubal factor (P-value = 0.033), Also, there was a significant difference between hormonal factor and male factor (P-value = 0.007) (table 4.14).

Table 4.13 Distribution of infertility group according to infertility factors.

Infertility factor	Number (%)
Hormonal	29(29%)
Ovulation	23(23%)
Unexplained	21(21%)
Tubal	15(15%)
Male factor	12(12%)
Total	100(100)

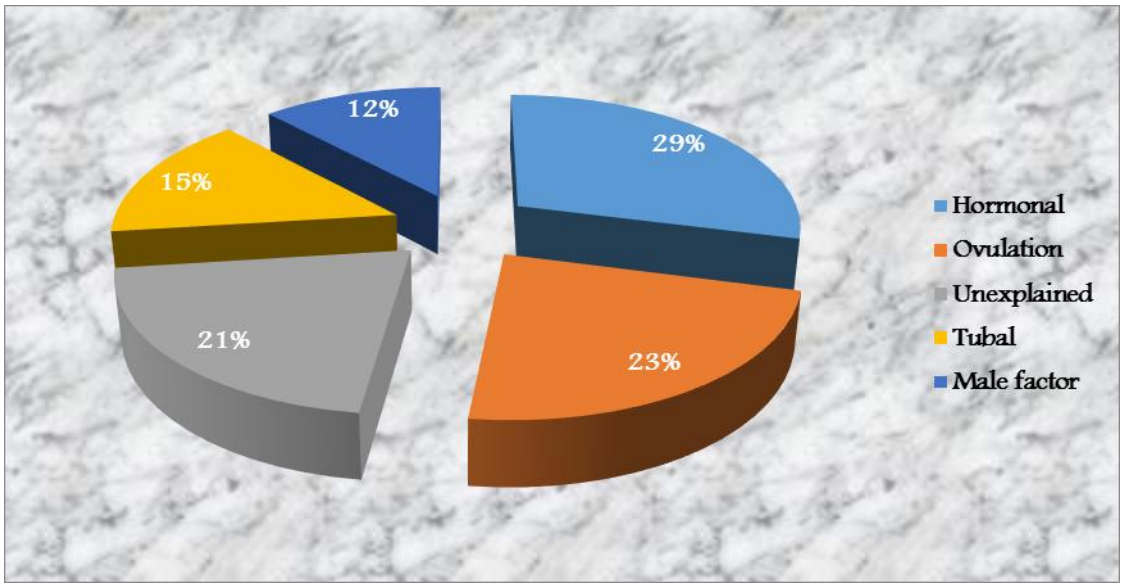

Figure 4.14 Distribution of infertility group according to infertility factors.

Table 4.14 Correlation between tubal and male factor with hormonal factor infertile women.

Infertility factor (%)	Tubal factor	Male factor
Hormonal 29 (29%)	15(15%)	12(12%)
P-value	0.033	0.007

4.10 Positive results based on infertility factors

The detection rate of *C. trachomatis* infection among unexplained infertility cases was the highest (5/21, 24%), whereas the rate of *M. genitalium* infection with the same factor was 10% (2/21), and one case of unexplained infertility was positive for *M. hominis* (1/21, 5%).

C. trachomatis infection rate among tubal factor infertile women was 13% (2/15), compared to one detected tubal factor infertility case of *M. genitalium* (1/15, 7%).

C. trachomatis infection rate among male factor infertility was (1/12, 8%), and a co-infection case of *M. genitalium* & *M. hominis* among male factor infertility was detected (1/12, 8%). We found no significant difference between the three factors of infertility tubal, male factor and unexplained with respect to positive results (P-value = 0.340).

No positive results were detected from hormonal or ovulation factor infertile women. The cause of infertility (infertility factors) and number of positive cases for the infertility group is shown in Table 4.15, figure 4.15.

Table 4.15 Positive cases of *C. trachomatis, M. genitalium, M. hominis* according to infertility factors.

Infertility factor (%)	*C. trachomatis*	*M. genitalium*	*M. hominis*	*M. genitalium & M. hominis*	P-value
Unexplained 21 (21%) **% within infertility factor**	5(24%)	2 (10%)	1(5%)	0	0.340
Tubal factor 15 (15%) **% within infertility factor**	2 (13%)	1 (7%)	0	0	
Male factor 12 (12%) **% within infertility factor**	1(8%)	0	0	1(8%)	

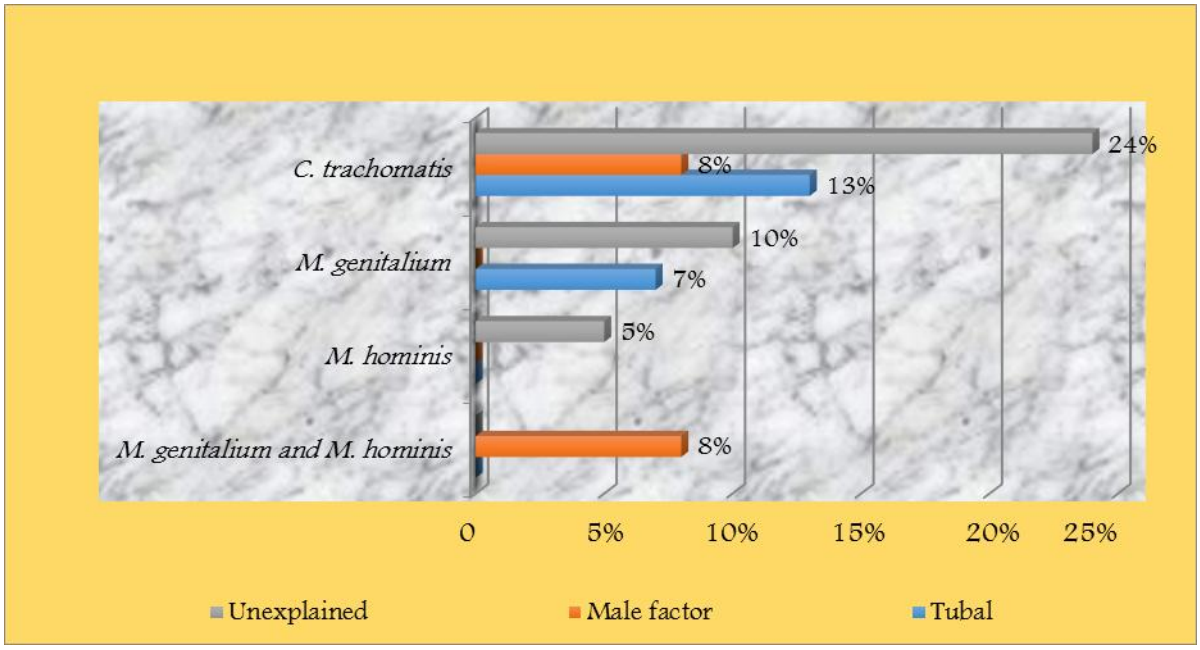

Figure 4.15 Distribution of women tested positive to *C. trachomatis, M. genitalium, M. hominis* according to infertility factors.

4.11 Positive results based on types of infertility.

The proportion of women testing positive for *C. trachomatis* in the primary infertility group (5/57, 9%) was higher than the proportion testing positive in the secondary infertility group (3/43, 7%) with no significant difference (P-value=0.524).

The proportion of women testing positive for *M. genitalium* in the primary infertility group (3/57, 5%), *M. genitalium* was not found among secondary infertility, with a P-value = 0.181. One case of secondary infertility was positive for *M. hominis* (1/43, 2%, P-value=0.430), and the mix infection case of *M. genitalium & M. hominis* in the secondary infertility (1/43, 2%, P-value = 0.430). Data analysis showed no significant difference regarding the types of infertility and positive cases. Results are depicted in table 4.16, figure 4.16.

Table 4.16 Positive cases of *C. trachomatis, M. genitalium, M. hominis* according to types of infertility.

Types of infertility	***C. trachomatis***	***M. genitalium***	***M. hominis***	***M. hominis & M. genitalium***
Primary (n=57) **% within type of infertility**	5 (9%)	3(5%)	0	0
Secondary (n=43) **% within type of infertility**	3(7%)	0	1(2%)	1(2%)
Total(n=100)	8(8%)	3(3%)	1(1%)	1(1%)
P-value	0.524	0.181	0.430	0.430

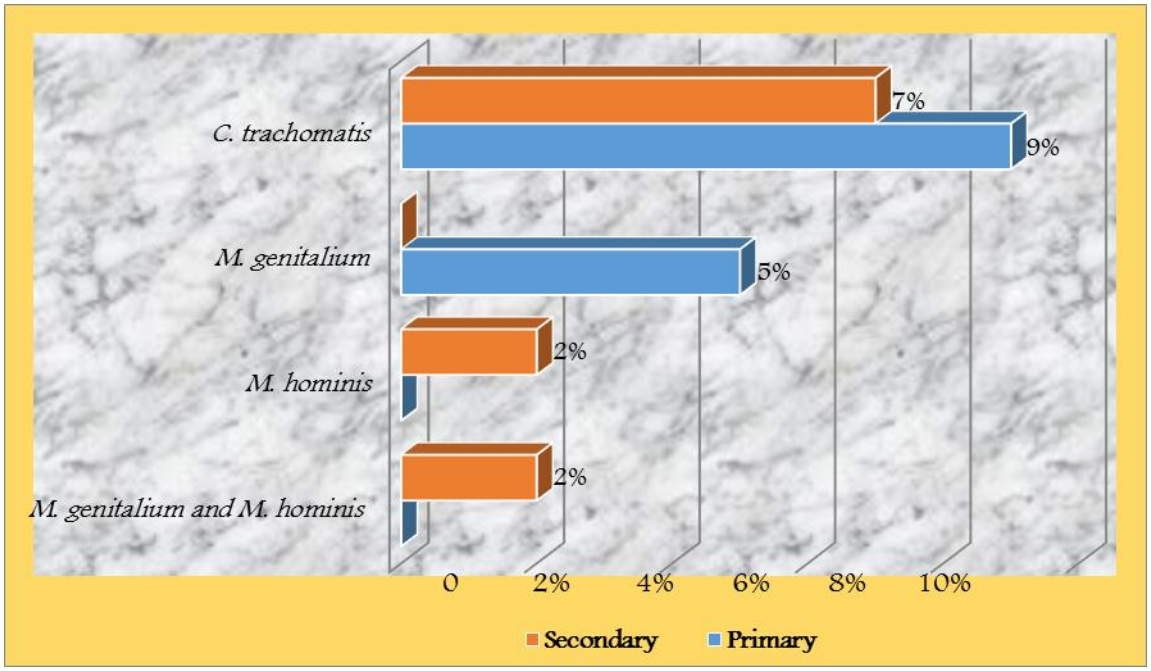

Figure 4.16 Positive cases of *C. trachomatis, M. genitalium, M. hominis* according to types of infertility.

4.12 *C. trachomatis* positive results among infertility group based on infertility factors and types of infertility

There was no significant difference concerning the infertility factors, types of infertility, and *C. trachomatis* positive tests obtained. 2 positive results were isolated from tubal factor primary infertility (2/15; 13%), and no positive results were isolated from secondary infertile with tubal factor (P-value=0.143).

One positive result was isolated from male factor secondary infertility (1/12; 8%), compared with no cases from primary infertility (P-value=0.583).

3 positive cases were detected from unexplained primary infertility (3/21; 14%) compared to 2 cases detected from unexplained secondary infertility (2/21; 10%, P-value=0.656). In general, 5 cases of *C. trachomatis* were

detected from primary infertile women (5%) compared to 3 cases from secondary infertile cases (3%) (P-value=0.524). Table (4.17).

Table 4.17 Positive cases of *C. trachomatis* according to infertility factors and types of infertility.

		Types of Infertility				P-value
		Primary infertility (*n* = 57)		Secondary infertility (*n* = 43)		
		Number tested (%)	Number of positive (%)	Number tested (%)	Number of positive (%)	
Infertility factor	**Hormonal (n=29) %within infertility factor**	19(65%)	0	10(34%)	0	
	Ovulation (n=23) %within infertility factor	14(60%)	0	9(39%)	0	
	Unexplained (n=21) %within infertility factor	13(62%)	3 (14%)	8(38%)	2 (10%)	0.656
	Tubal (n=15) %within infertility factor	6(40%)	2 (13%)	9(60%)	0	0.143
	Male factor(n=12) %within infertility factor	5(41%)	0	7(58%)	1 (8%)	0.583
	Total (n=100)	57(57%)	5 (5%)	43(43%)	3 (3%)	0.524

4.13 *M. genitalium* positive results based on infertility factors and types of infertility

One positive case of *M. genitalium* was isolated from tubal primary infertile patient (1/15; 7%, p-value=0.40), and 2 cases were detected from unexplained primary infertile patients (2/21; 10%) compared to none from unexplained secondary infertility group (p-value =0.371).

In total, *M. genitalium* was detected from 3 primary infertile cases and no positive results from secondary infertile patients (p-value =0.181). Table (4.18).

Table 4.18 Positive cases of *M. genitalium* according to infertility factors and types of infertility.

		Type of Infertility				P-value
		Primary infertility (*n* = 57)		Secondary infertility (*n* = 43)		
		Number tested (%)	Number of positive (%)	Number tested (%)	Number of positive (%)	
Infertility factor	Hormonal (n=29) %within infertility factor	19(65%)	0	10(34%)	0	
	Ovulation (n=23) %within infertility factor	14(60%)	0	9(39%)	0	
	Unexplained (n=21) %within infertility factor	13(62%)	2 (10%)	8(38%)	0	0.371
	Tubal (n=15) %within infertility factor	6(40%)	1(7%)	9(60%)	0	0.40
	Male factor(n=12) %within infertility factor	5(41%)	0	7(58%)	0	
	Total (n=100)	57(57%)	3 (3%)	43(43%)	0	0.181

4.14 *M.hominis* positive results based on infertility factors and types of infertility

Among infertile women one positive case of *M. hominis* was detected and it was isolated from unexplained case of secondary infertile group (1/21; 5%, P-value= 0.381). Table (4.19).

Table 4.19 Positive cases of *M. hominis* according to infertility factors and types of infertility.

		Type of Infertility				P-value
		Primary infertility (*n* = 57)		Secondary infertility (*n* = 43)		
		Number tested (%)	Number of positive (%)	Number tested (%)	Number of positive (%)	
Infertility factor	Hormonal (n=29) %within infertility factor	19(65%)	0	10(34%)	0	
	Ovulation (n=23) %within infertility factor	14(60%)	0	9(39%)	0	
	Unexplained (n=21) %within infertility factor	13(62%)	0	8(38%)	1 (5%)	0.381
	Tubal (n=15) %within infertility factor	6(40%)	0	9(60%)	0	
	Male factor(n=12) %within infertility factor	5(41%)	0	7(58%)	0	
	Total (n=100)	57(57%)	0	43(43%)	1 (1%)	0.430

One case of a mix infection of *M. genitalium & M. hominis* was isolated from secondary infertility with male factor (1/12; 8%, P-value=0.583). Table (4.20).

Table 4.20 *M. genitalium & M. hominis* co-infection according to infertility factors and types of infertility.

	Type of Infertility				P-value
	Primary infertility (*n* = 57)		Secondary infertility (*n* = 43)		
	Number tested (%)	Number of positive (%)	Number tested (%)	Number of positive (%)	
Hormonal (n=29) %within infertility factor	19(65%)	0	10(34%)	0	
Ovulation (n=23) %within infertility factor	14(60%)	0	9(39%)	0	
Unexplained (n=21) %within infertility factor	13(62%)	0	8(38%)	0	
Tubal (n=15) %within infertility factor	6(40%)	0	9(60%)	0	
Male factor(n=12) %within infertility factor	5(41%)	0	7(58%)	1 (8%)	0.583
Total (n=100)	57(57%)	0	43(43%)	1	0.430

4.15 Distribution of positive results of *C. trachomatis, M. genitalium*, and *M. hominis* among age groups

C. trachomatis was the highest occurrence among the age group 19-25 (3/16, 19%), 1 positive case from age group 26-32 (1/43, 3%), 2 positive results were detected from age group 33-39 (2/35, 6%).

2 cases were detected from age group 40-46 (2/15, 13%), and there was no significant differences between *C. trachomatis* positive cases and age groups (P-value=0.661).

Among infertility group, 2 positive cases of *M. genitalium* from age group 19-25 (2/16, 13%) and one positive case from age group 26-32 was detected (1/34, 3%, P-value=0.331).

M. hominis was detected from one case from the age group 40-46(1/15, 13%, P-value=0.487).

One infertility case (age group 40-46) showed a co-infection of *M. genitalium* and *M. hominis* (1/15, 7%, P-value=0.487).

Distribution of positive results of *C. trachomatis*, *M. genitalium*, and *M. hominis* among age groups in table 4.21, figure 4.17.

Table 4.21 Distribution of positive results of *C. trachomatis*, *M. genitalium*, and *M. hominis* among age groups.

Age group	*C. trachomatis* (%)	P-value	*M. genitalium* (%)	P-value	*M. hominis* (%)	P-value	*M. hominis* & *M. genitalium* (%)	P-value
19-25 (n=16)	3 (19%)	0.661	2 (13%)	0.331	0	0.487	0	0.487
26-32 (n=34)	1 (3%)		1 (3%)		0		0	
33-39 (n=35)	2 (6%)		0		0		0	
40-46 (n=15)	2 (13%)		0		1 (7%)		1 (7%)	

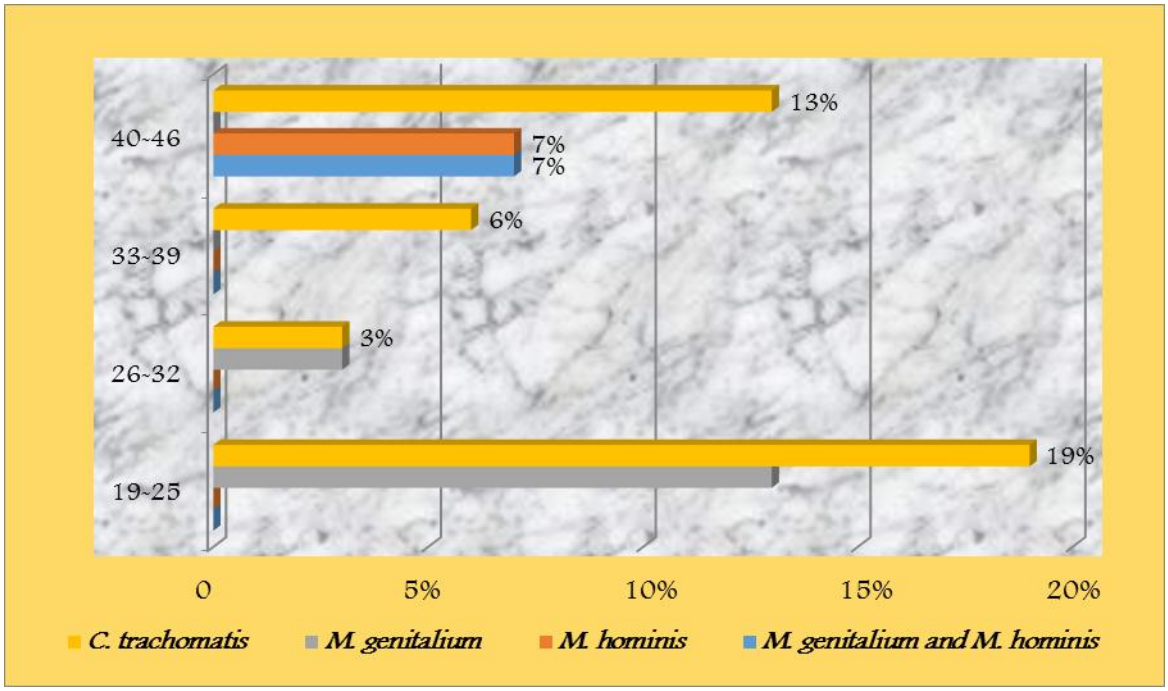

Figure 4.17 Distribution of positive results of *C. trachomatis*, *M. genitalium*, and *M. hominis* among age groups.

4.16 Melting curve analysis of *C. trachomatis* samples

A 136 bp fragment of the *C. trachomatis* MOMP genome gene was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 (table3.3). The PCR products were identified by running a melting curve with a specific melting point (T_m) of 66°C in channel 640, data analysis was performed, as described in the Light Cycler Instrument operator's manual (table 3.10).

PCR results were obtained within 50 minutes (50 cycles and melting curve, table 3.10) The negative control showed no signal, The overall positive samples were 9 samples (8 infertile group samples and 1 sample- number 99-control group sample) identified through the specific T_m . IC were included in each sample during real time PCR run, all negative samples showed positive IC result.

Table 4.22 showing the T_m of all positive samples. Melting curve and melting peak analysis of *C. trachomatis* isolates was performed; isolates were identified through the specific melting points around 66°C. All results are depicted in figures 4.18, 4.19, 4.20, 4.21, 4.22, 4.23, 4.24, 4.25.

Table 4.22 T_m value numbers of *C. trachomatis* positive samples detected by Real Time PCR.

***C. trachomatis* sample number**	**Group**	**T_m value (c°)**
13	Infertility	66.3
22	Infertility	66.3
39	Infertility	66.5
45	Infertility	66.4
63	Infertility	66.2
83	Infertility	66.1
89	Infertility	66.0
93	Infertility	66.3
99	Control	66.3

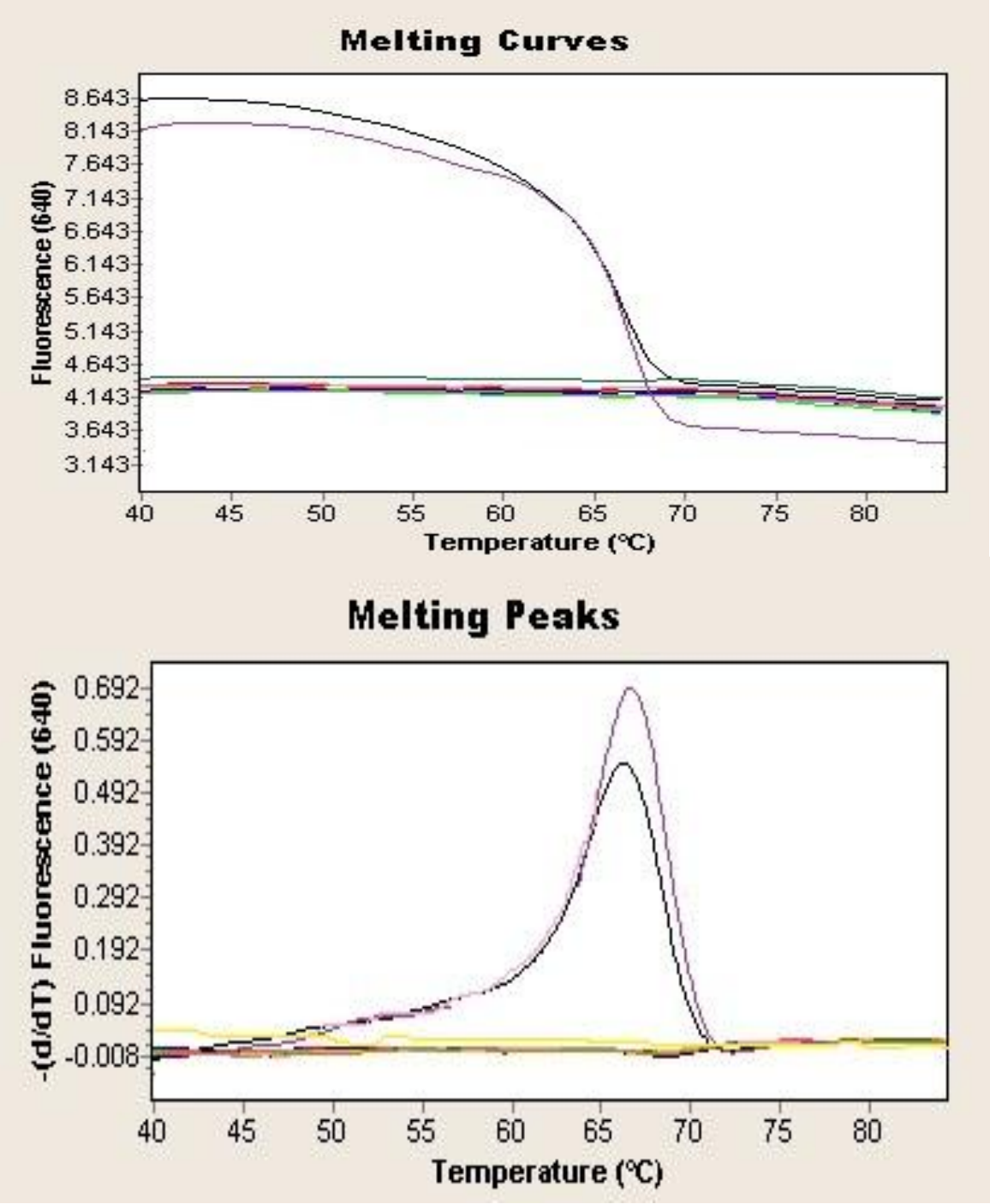

Figure 4.18 Upper panel: Showing the melting curve of *C. trachomatis* sample number13 and positive control - infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

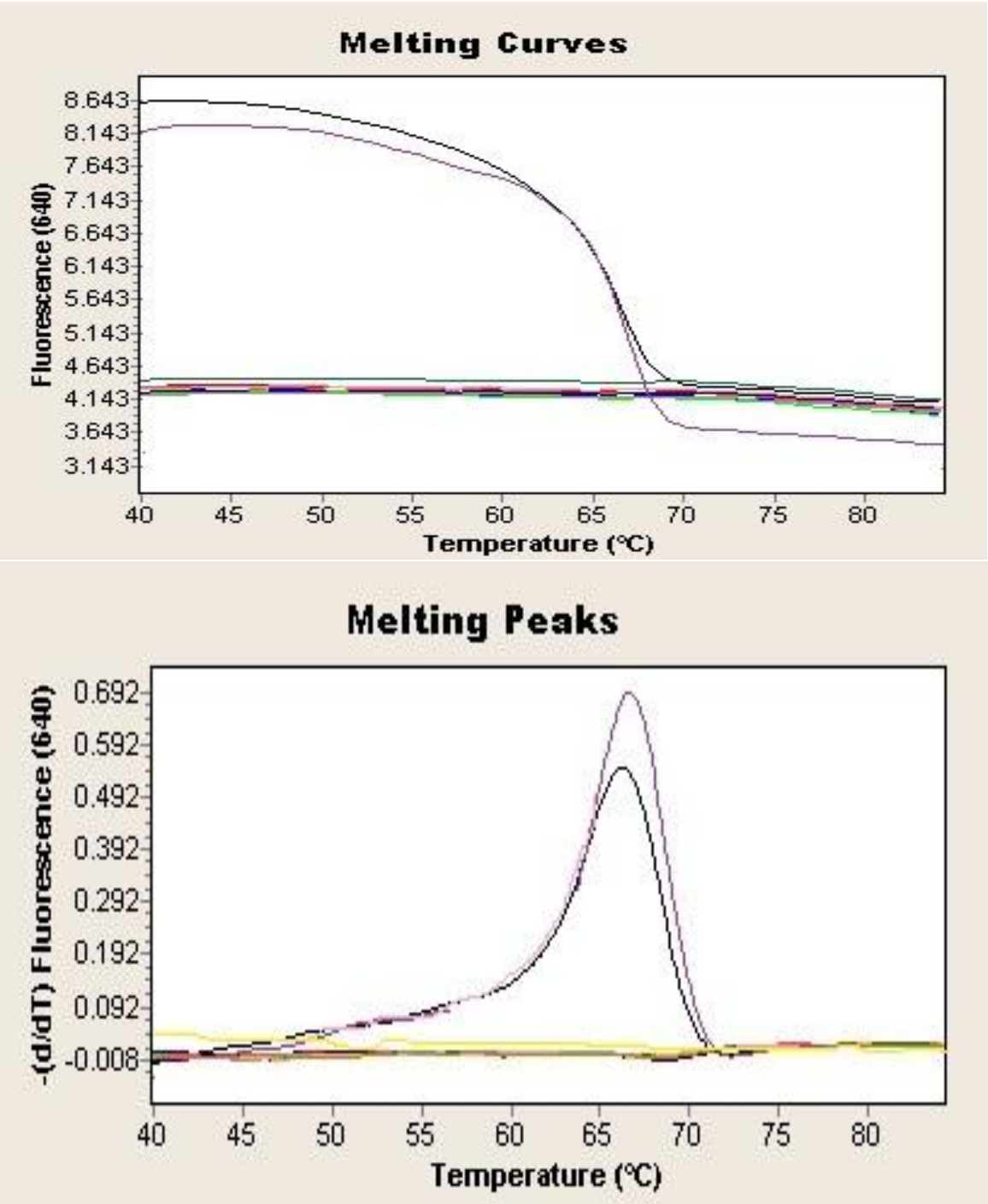

Figure 4.19 Upper panel: Showing the melting curve of *C. trachomatis* sample number 22 and positive control - infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

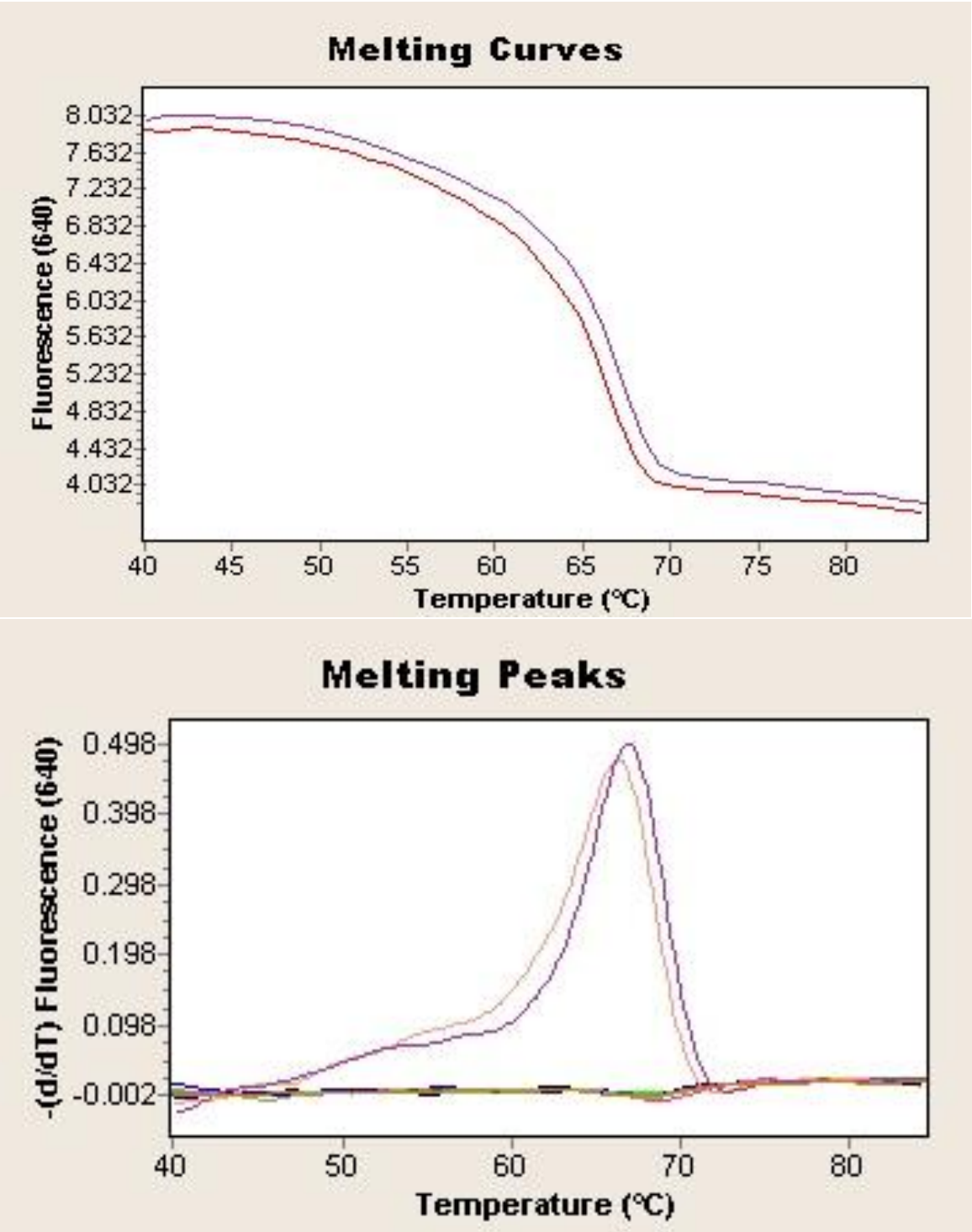

Figure 4.20 Upper panel: Showing the melting curve of *C. trachomatis* sample number 39 and positive control - infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

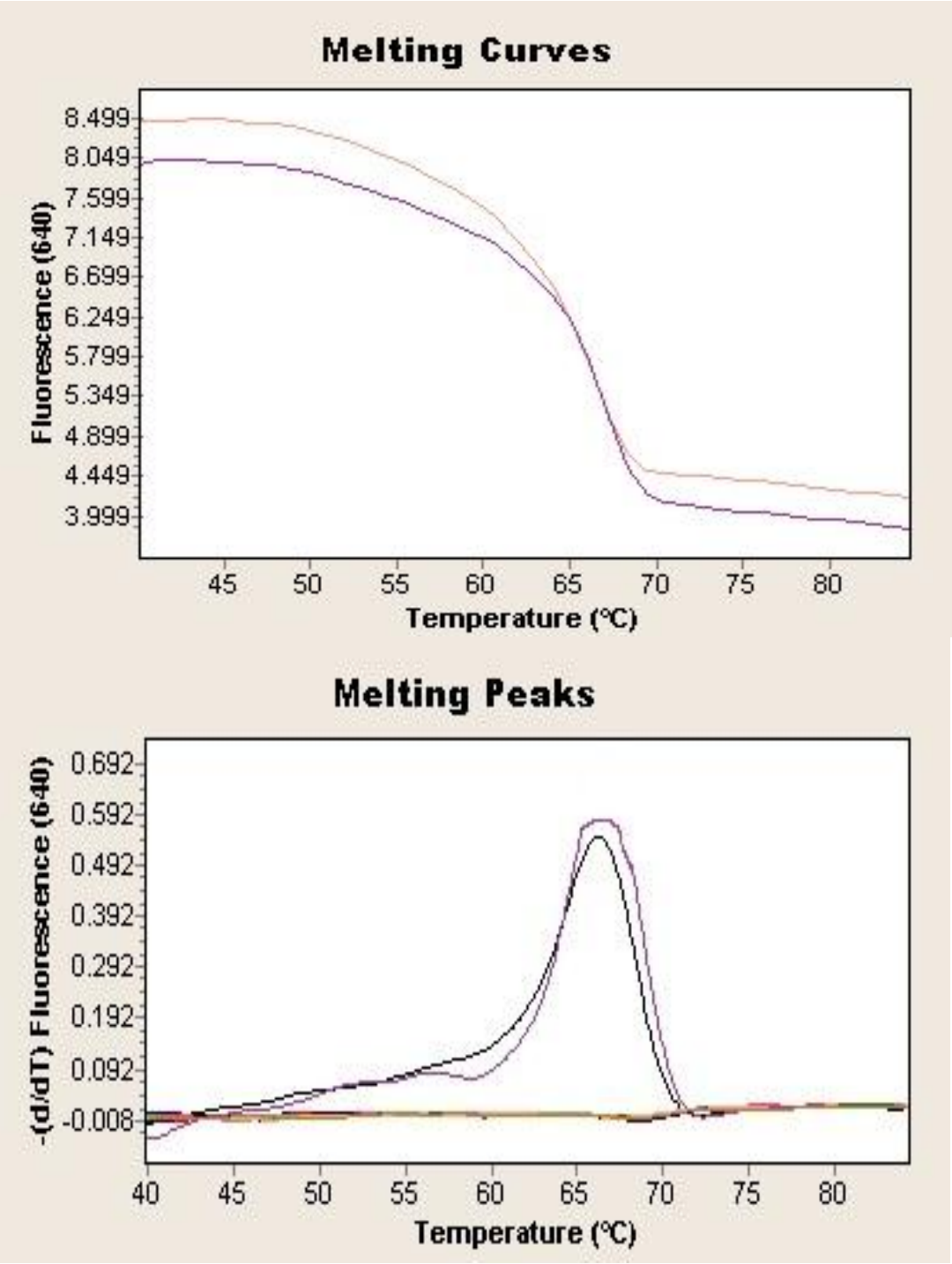

Figure 4.21 Upper panel: Showing the melting curve of *C. trachomatis* sample number 45 and positive control - infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

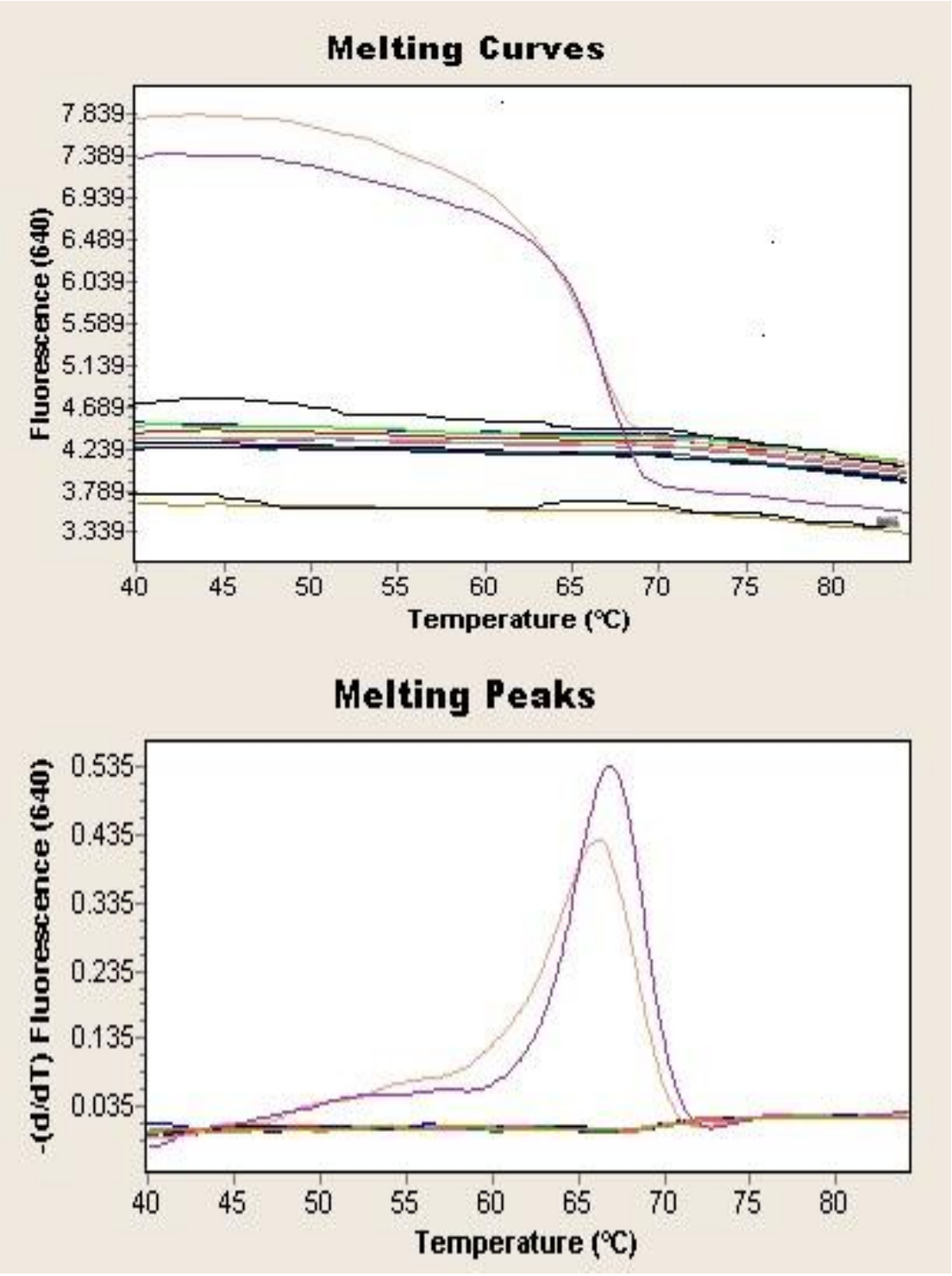

Figure 4.22 Upper panel: Showing the melting curve of *C. trachomatis* sample number 63 and positive control - infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

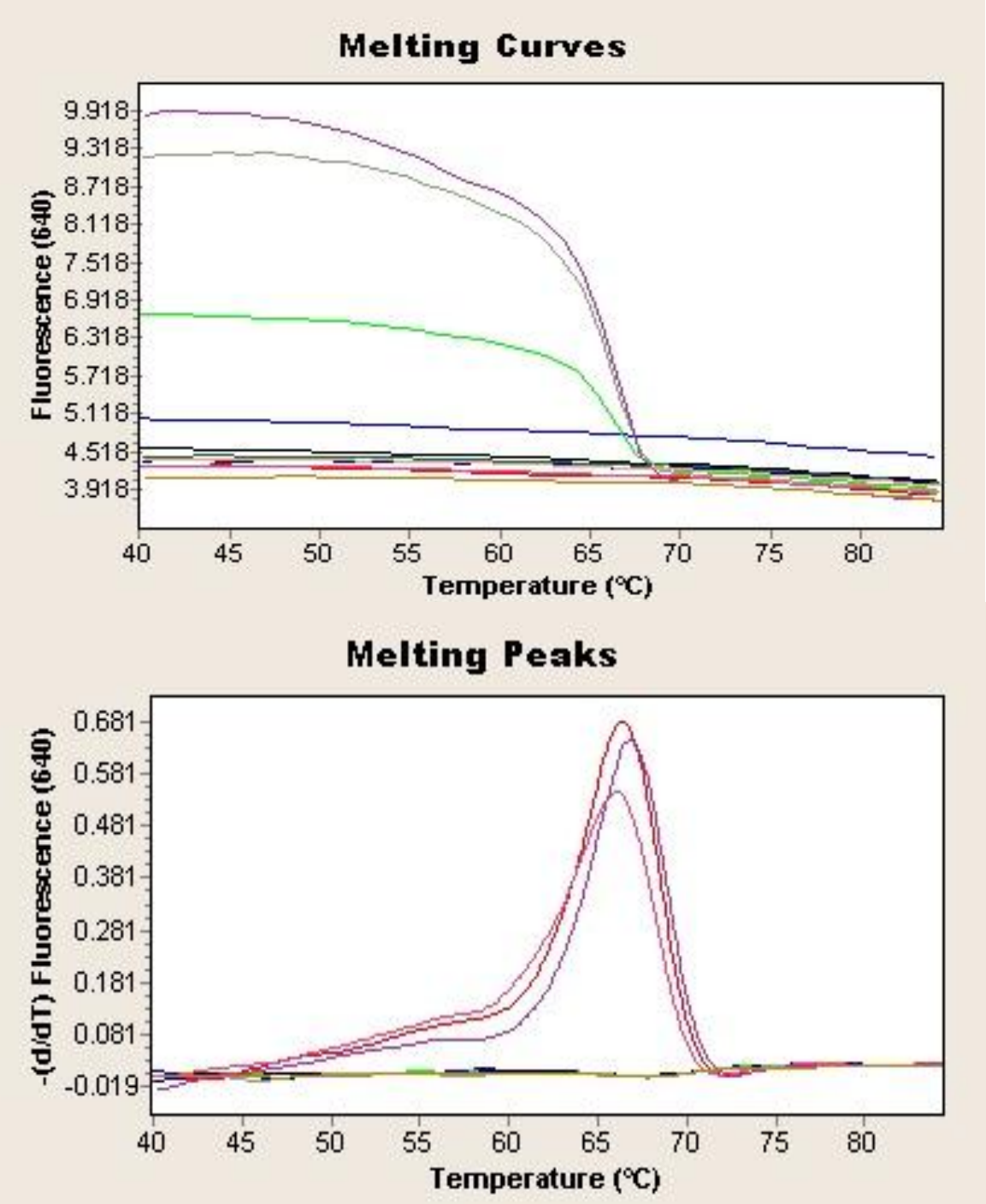

Figure 4.23 Upper panel: Showing the melting curve of *C. trachomatis* sample number 83 & 89 and positive control - infertility group. Lower panel is showing melting peak of the same isolates and the positive control.

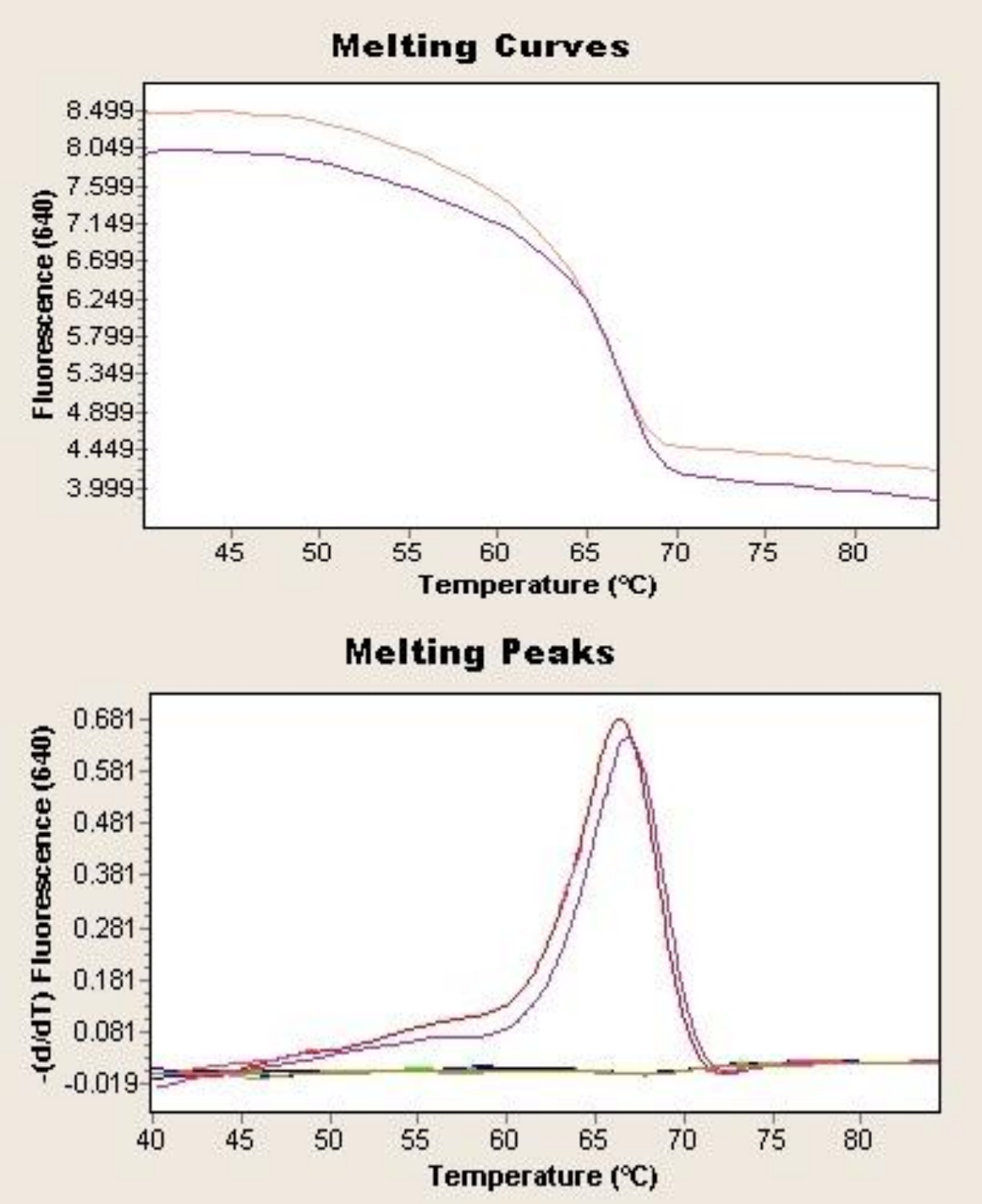

Figure 4.24 Upper panel: Showing the melting curve of *C. trachomatis* sample number 93 and positive control - infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

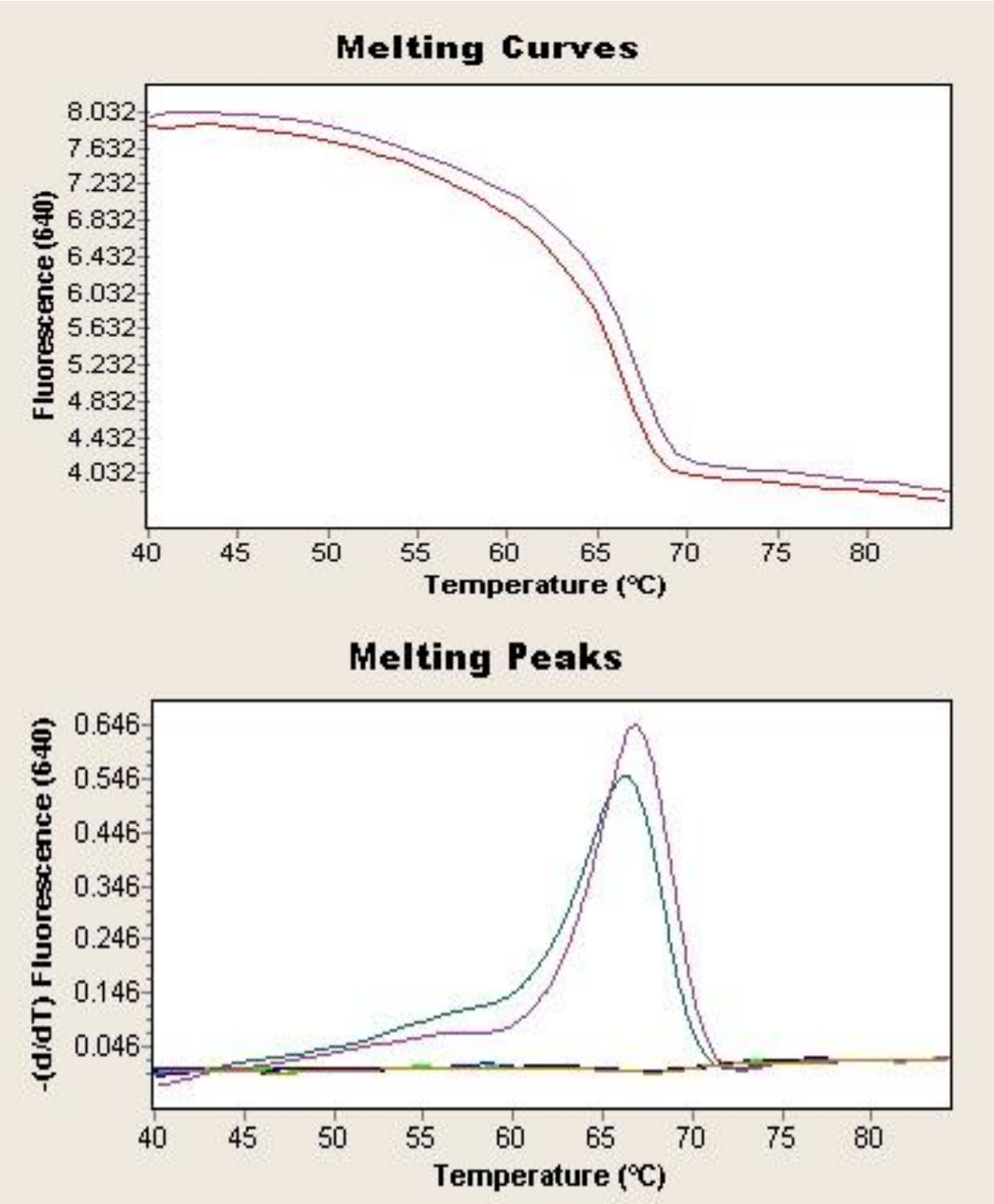

Figure 4.25 Upper panel: Showing the melting curve of *C. trachomatis* sample number 99 and positive control - control group. Lower panel is showing melting peak of the same isolate and the positive control.

4.17 Melting curve analysis of *M. genitalium* samples

A 224 bp long fragment of the glyceraldehyde-3-phosphate dehydrogenase (gap) gene of *M. genitalium* was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 (table 3.3).
PCR results were obtained within 50 minutes (50 cycles and melting curve, table 3.10) *M. genitalium* data was viewed in channel 640. Data analysis was performed, as described in the Light Cycler Instrument operator's manual (table 3.10).

The negative control showed no signal, the positive samples (4 samples detected from infertile group patients) showed specific T_m. IC were included in each sample during real time PCR run, all negative samples showed positive IC result. Melting curve and melting peak analysis of *M. genitalium* isolates was performed, isolates were supposed to be identified through the specific T_m 67-69°C. All results are depicted in Table 4.23 and figures 4.26, 4.27, and 4.28.

Table 4.23 T_m value numbers of *M. genitalium* positive samples detected by Real Time PCR.

***M. genitalium* sample number**	**Group**	**T_m value (c°)**
30	Infertility	68.5
36	Infertility	68.1
58	Infertility	67.1
98	Infertility	68.2

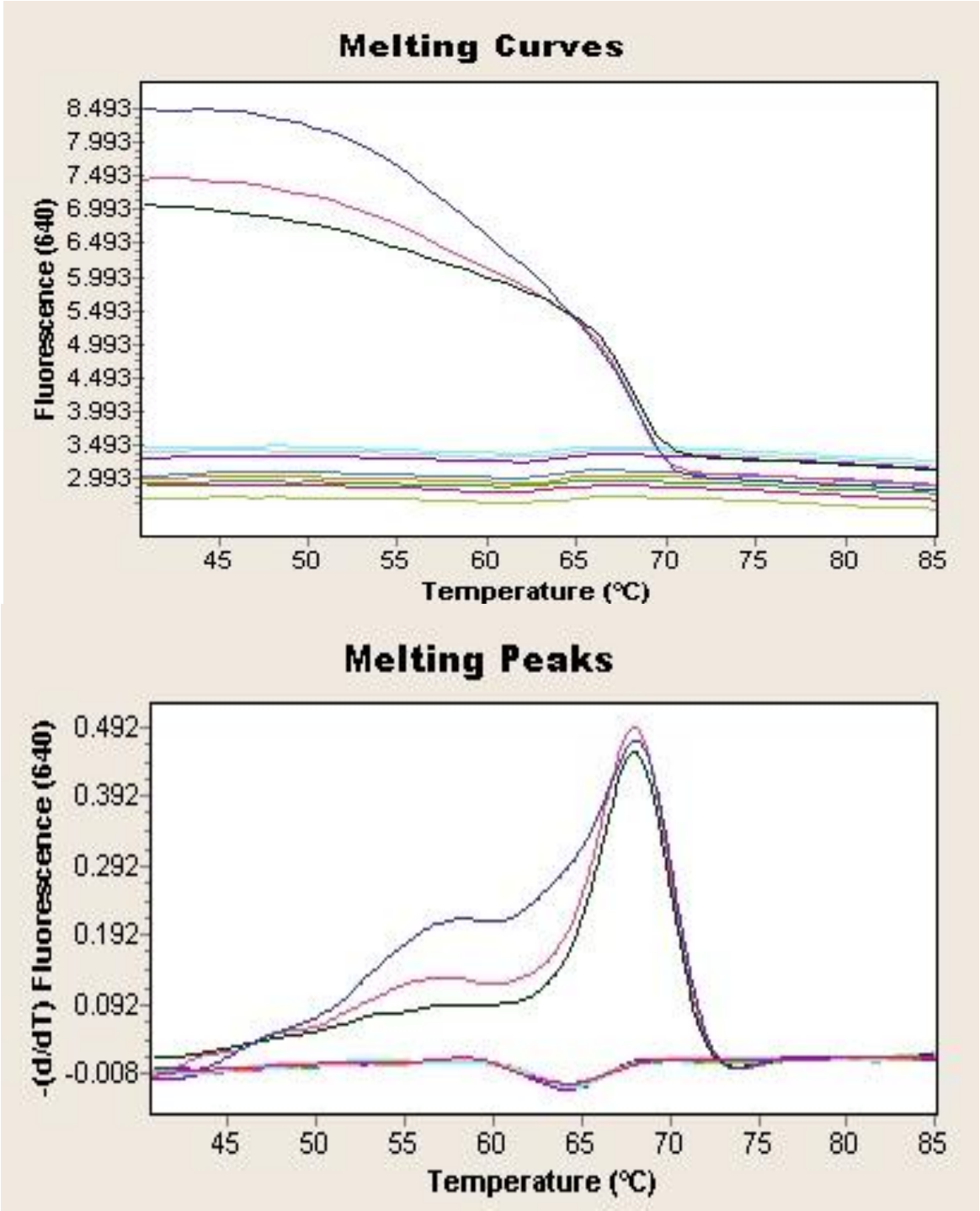

Figure 4.26 Upper panel: Showing the melting curve of *M. genitalium* sample number: 30 & 36 - infertility group. Lower panel is showing melting peak of the same isolates and the positive control.

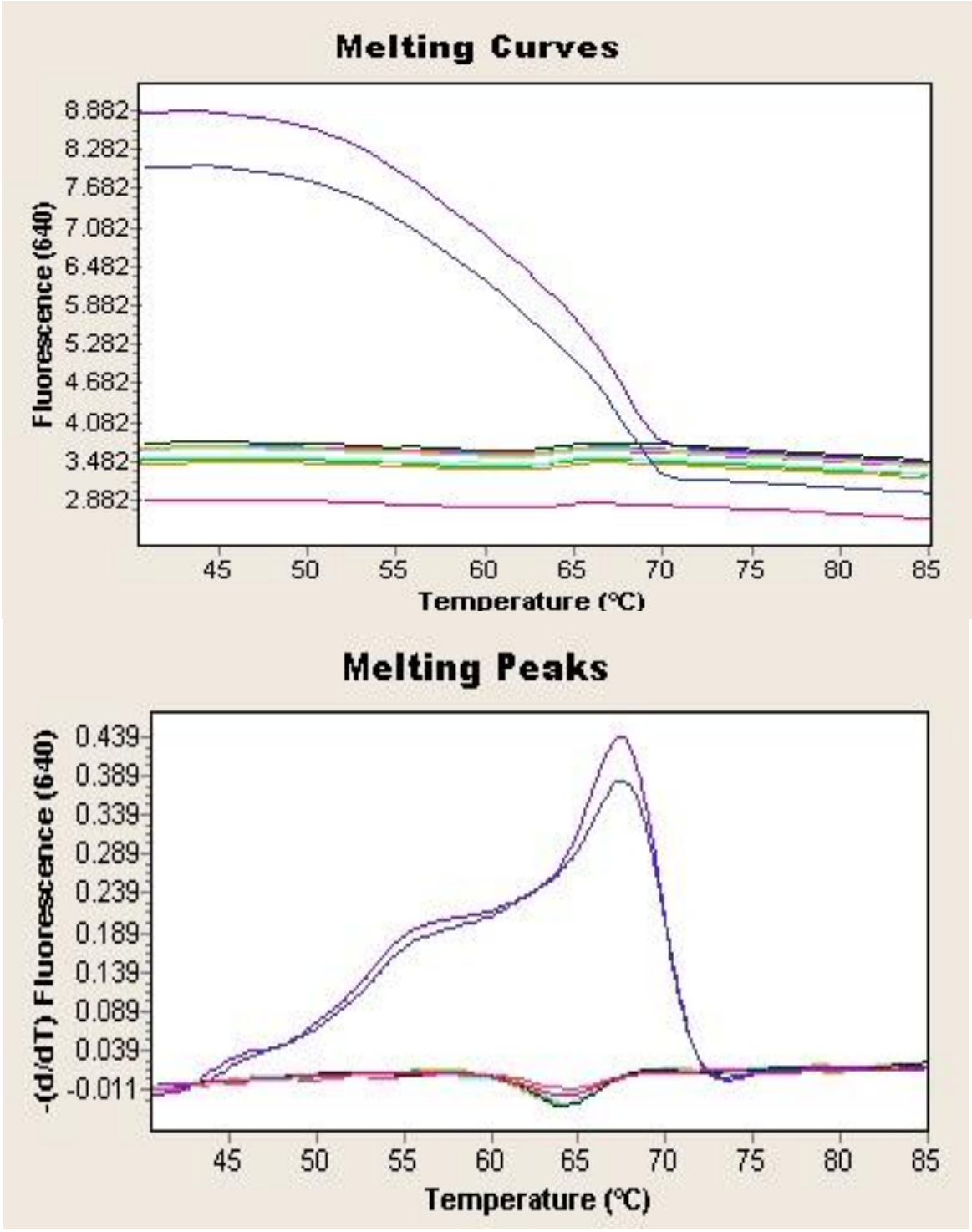

Figure 4.27 Upper panel: Showing the melting curve of *M. genitalium* sample number: 58 with the positive control - infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

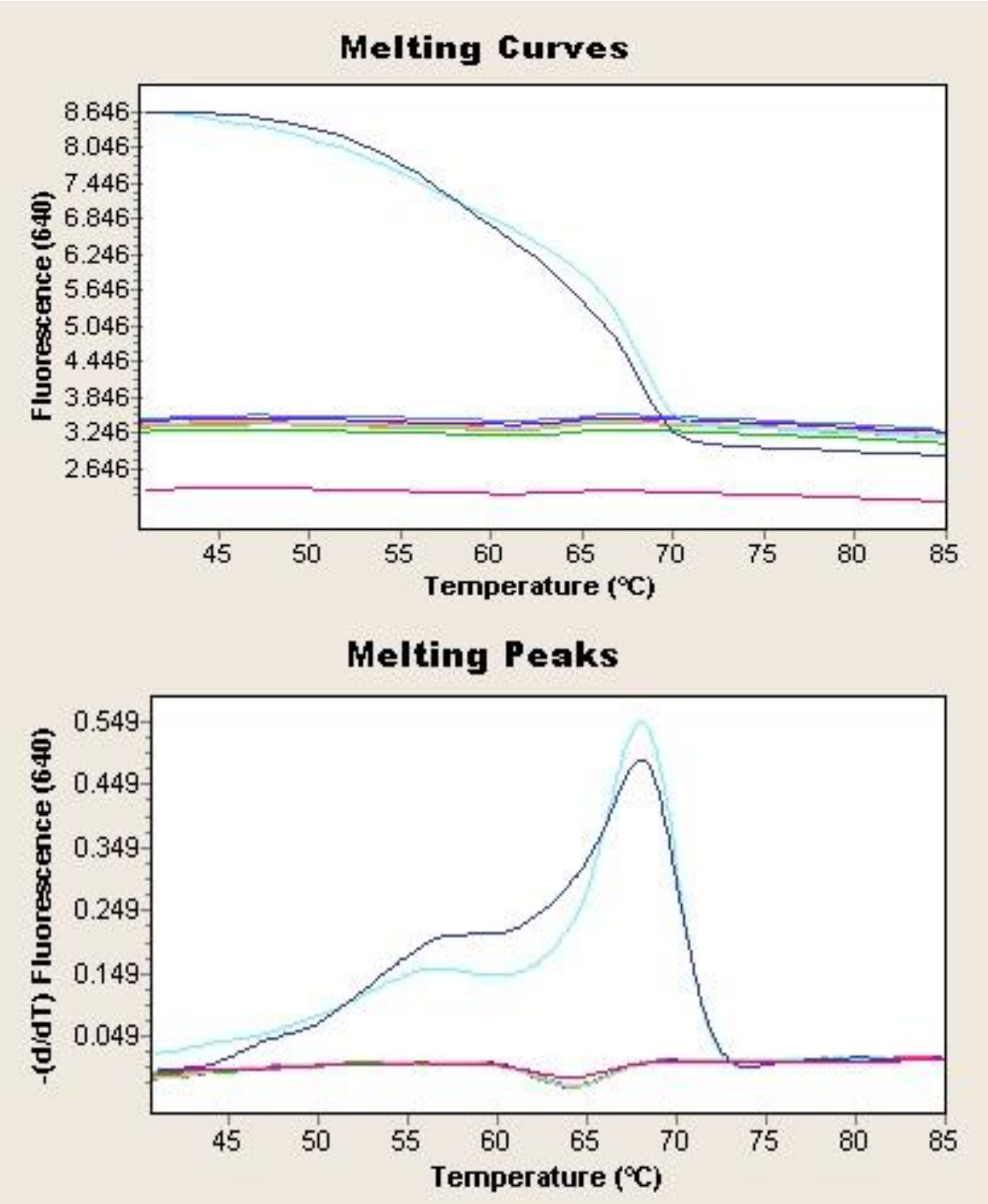

Figure 4.28 Upper panel: Showing the melting curve of *M. genitalium* sample number: 98 - infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

4.18 Melting curve analysis of *M. hominis* samples:

A 129 bp long fragment of the glyceraldehyde-3-phosphate dehydrogenase (gap) gene from *M. hominis* was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 (table3.3). PCR results were obtained within 50 minutes (50 cycles and melting curve, table 3.10) *M.hominis* data was viewed in channel 640. Data analysis was performed, as described in the Light Cycler Instrument operator's manual.

The negative control showed no signal, all positive samples (samples number 7, 36 were detected from infertile group, and samples number 43, 61 were detected from control group) showed T_m of 61.2-62.0°C. IC were included in each sample during real time PCR run, melting curve and melting peak analysis of *M. hominis* isolates was performed. All results are depicted in figures 4.29, 4.30, 4.31, and 4.32. All negative samples showed positive IC result, Table (4.24).

Table 4.24 T_m value numbers of *M. hominis* positive samples detected by Real Time PCR.

***M. hominis* sample number**	**Group**	**T_m value (c°)**
7	Infertility	62.0
36	Infertility	61.5
43	Control	61.2
61	Control	61.9

Melting curve and melting peak analysis of *M. hominis* isolates was performed. All results are depicted in figures 4.29, 4.30, 4.31, and 4.32.

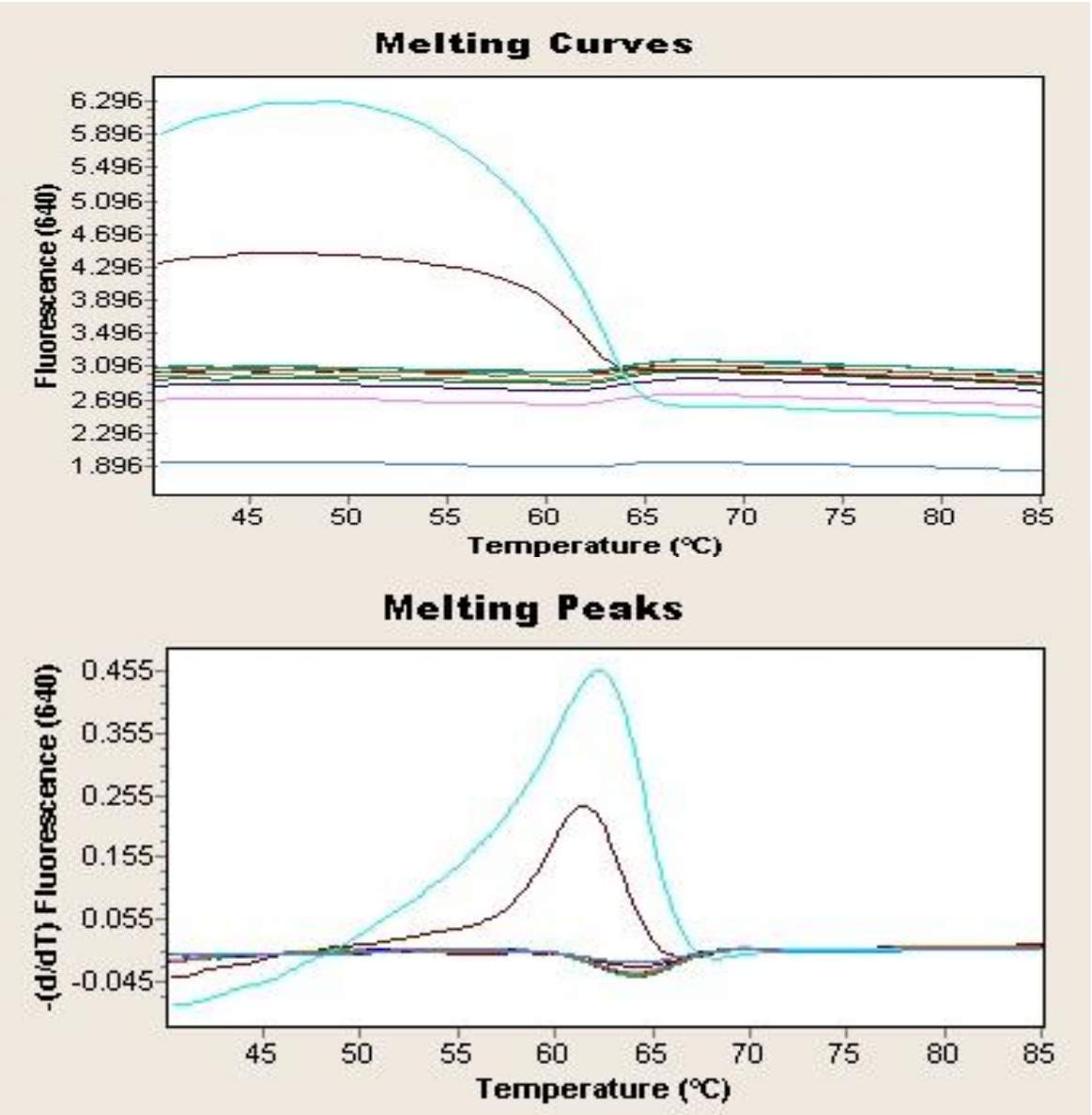

Figure 4.29 Upper panel: Showing the melting curve of *M. hominis* sample number: 7- infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

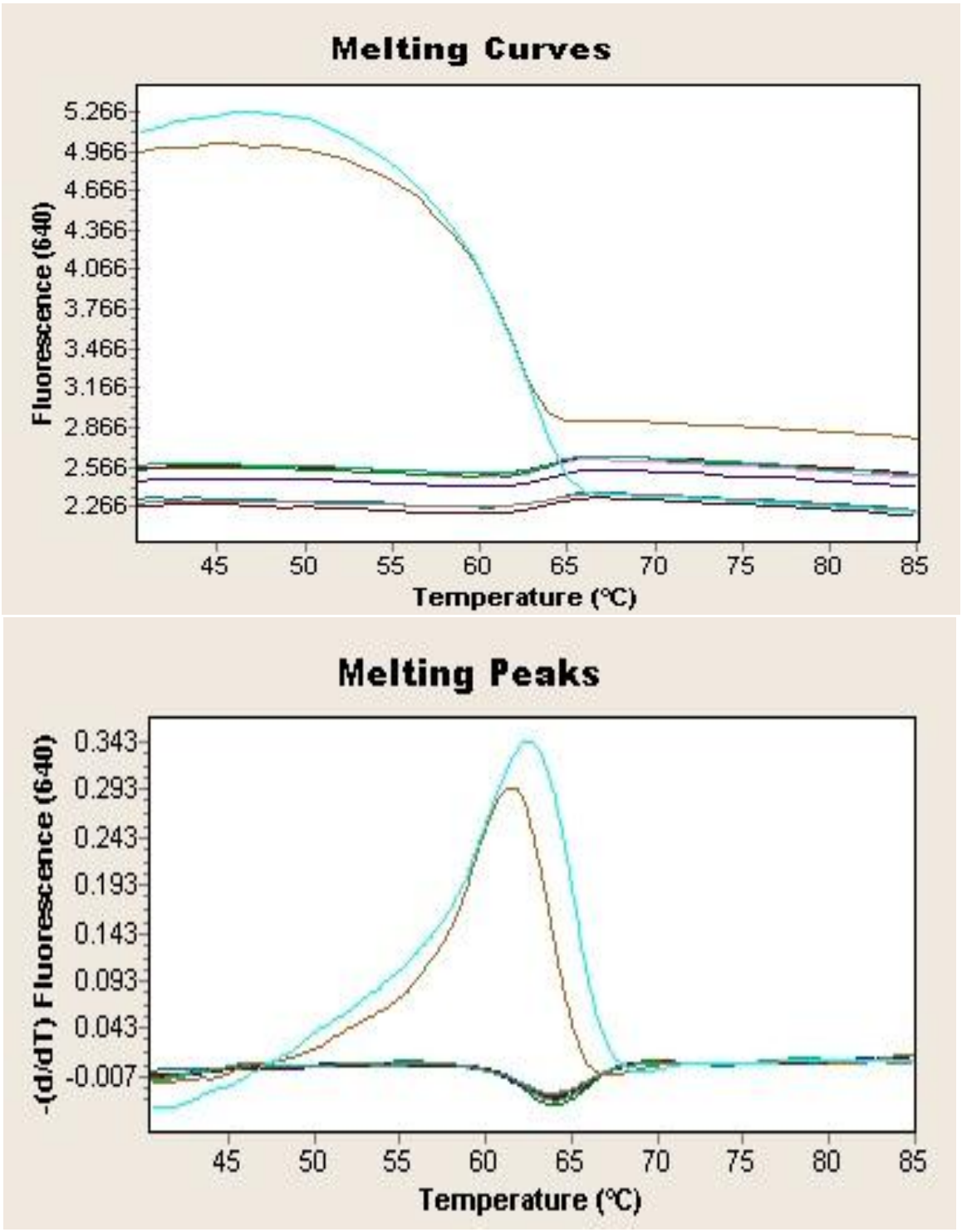

Figure 4.30 Upper panel: Showing the melting curve of *M. hominis* sample number: 36- infertility group. Lower panel is showing melting peak of the same isolate and the positive control.

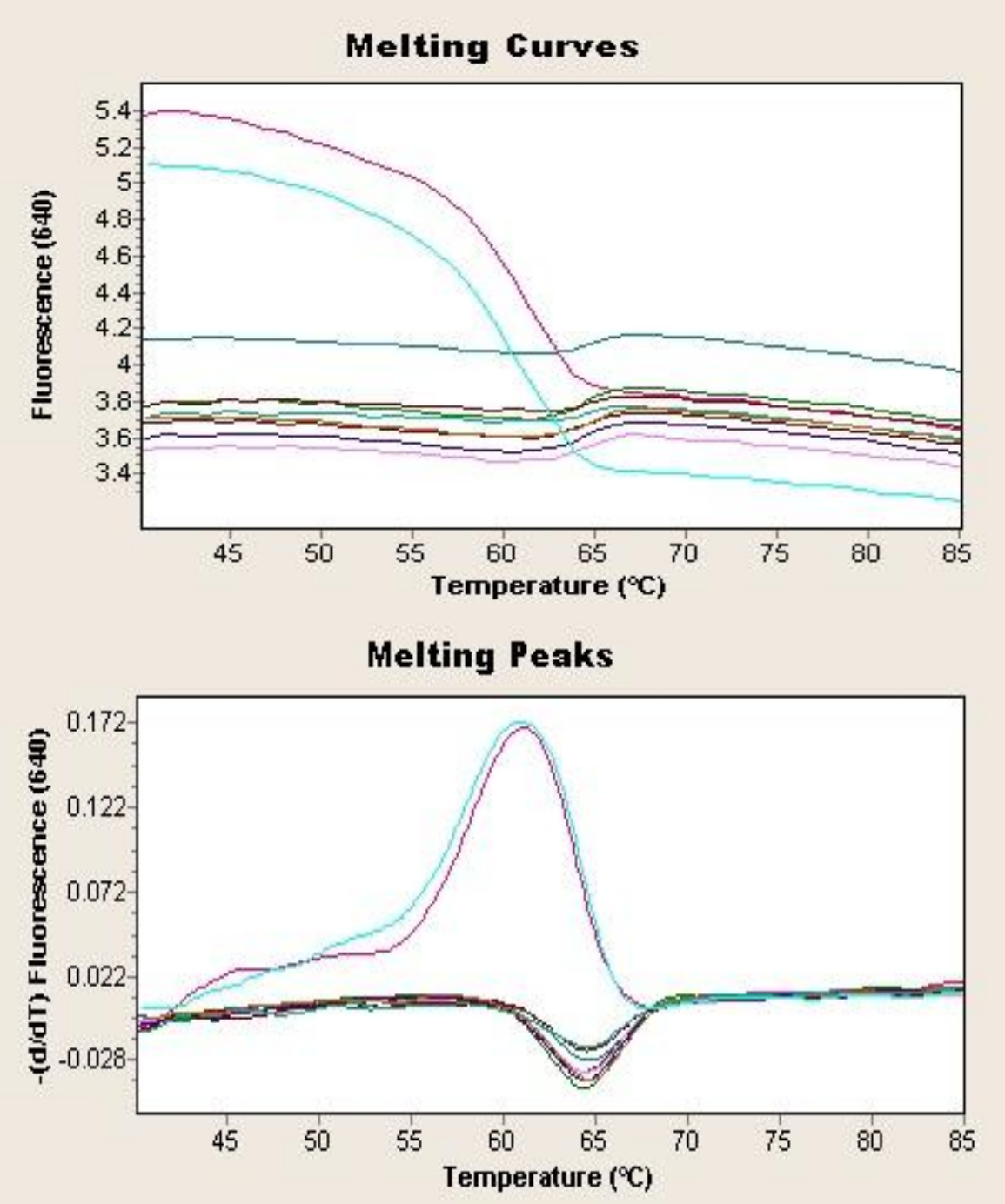

Figure 4.31 Upper panel: Showing the melting curve of *M. hominis* sample number: 43- control group. Lower panel is showing melting peak of the same isolate and the positive control.

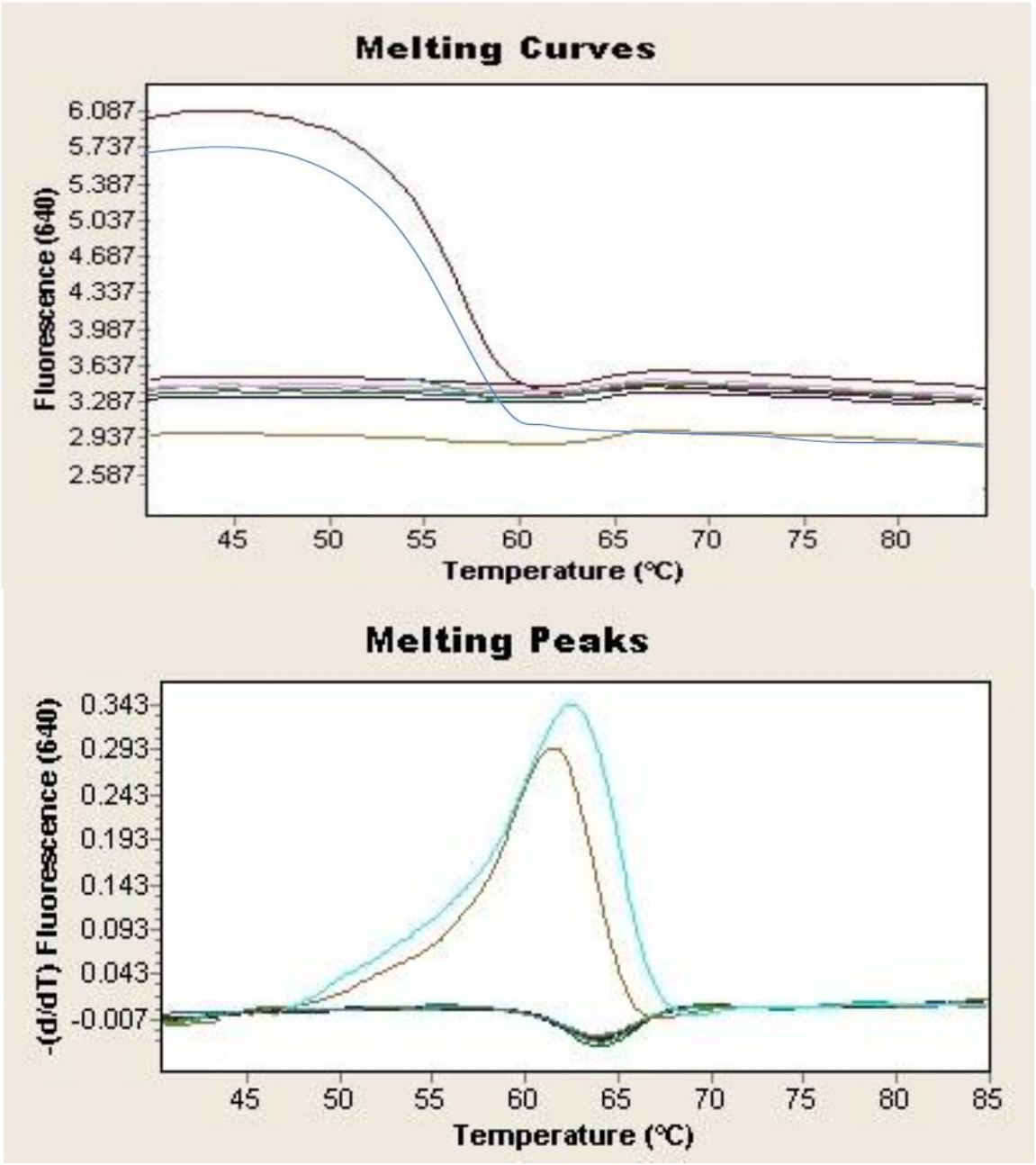

Figure 4.32 Upper panel: Showing the melting curve of *M. hominis* sample number: 61- control group. Lower panel is showing melting peak of the same isolate and the positive control.

We found statistically significant difference between the three different organisms with respect to T_m value numbers of these organisms *C. trachomatis*, *M. genitalium* and *M. hominis* positive samples detected by Real Time PCR since P-value < 0.001. Table (4.25).

Table 4.25 SD of the mean T_m values.

	Mean T_m	SD	Median (minimum – maximum)	P-value
C. trachomatis	66.2667	0.150	66.30 (66.0 – 66.5)	
M. genitalium	67.975	0.6076	68.15 (67.1 – 68.5)	0.001
M. hominis	61.65	0.3697	61.7 (61.2 – 62)	

4.19 Absolute quantitative real time PCR results

All the positive samples of *C. trachomatis*, *M. genitalium*, *M. hominis*, were subjected to confirmatory testing to detect the concentration of the PCR product by Light Cycler 2.0 Instrument with Roche Diagnostics Light Cycler Fast Start DNA Master Hyb-Probe. Data analysis was Perform, as described in the Light Cycler Instrument operator's manual (table 3.14), the cycle number of the Crossing Point (C_P) of each sample was calculated automatically.

4.19.1 Sample data for the *C. trachomatis* detection system

Data analysis was performed, as described in the Light Cycler Instrument operator's manual. The cycle number of the C_p of each sample was calculated automatically. The negative control showed no signal. For the identification of the PCR product *C. trachomatis* data was viewed in channel 640, quantification mode.

The provided standard row of cloned and purified DNA with concentrations in the range from 10^6 copies/ reactionto 10^1 copies/reactions of *C. trachomatis* had C_Ps between cycles 17 and 33. *C. trachomatis* positive samples had C_Ps between cycles 28 and 33.6.

Among *C. trachomatis* samples, the sample with the highest template count (sample number 13, infertility group) showed an amplification curve with C_P at 28.0; and the copy number was $6.88x10^2$ DNA copies/ reaction (1,376,000 copies/ml).

Sample number 39 (infertility group) showed an amplification curve with C_P at 28.1; and the copy number was $4.94x10^2$ DNA copies/reaction (988,000 copies/ml).

Sample number 93 (infertility group) showed an amplification curve with C_P at 28.2; and the copy number was $4.63x10^2$ DNA copies/ reaction (926,000 copies/ml).

Sample number 22 (infertility group) showed an amplification curve with C_P at 29.0; and the copy number was $3.19x10^2$ DNA copies/ reaction (638,000 copies/ml).

Sample number 45 (infertility group) showed an amplification curve with C_P at 29.0; and the copy number was $2.46x10^2$ DNA copies/ reaction (492,000 copies/ml).

Sample number 99 (control group) showed an amplification curve with C_P at 31.2; and the copy number was $5.53x10^1$ DNA copies/reaction. (110,600 copies/ml).

Sample number 63 (infertility group) showed an amplification curve with C_P at 31.2; and the copy number was $5.15x10^1$ DNA copies/ reaction (103,000 copies/ml).

Sample number 89 (infertility group) showed an amplification curve with C_P at 32.1; and the copy number was $2.50x10^1$ DNA copies/ reaction (50,000 copies/ml).

The sample with the lowest template count among infertile group (sample number 83, infertility group) showed an amplification curve with C_P at 33.6; and the copy number was 8.45 DNA copies/ reaction (1690 copies/ml).

Absolute quantitative Real Time PCR run of *C. trachomatis* positive results, their C_P, and the standard dilution series of standard DNA in table 4.26, figures 4.33, 4.34, 4.35, 4.36.

Table 4.26 Absolute Quantitative Real Time PCR run of *C. trachomatis* positive results, C_{Ps}, and the standard dilution series of standard DNA

Standard Positive control dilution		C_p	Observed concentrations (copy/ reaction)	
10^6		17.2	$1.07x10^6$	
10^5		20.8	$9.21x10^4$	
10^4		24.0	$9.44x10^3$	
10^3		27.0	$1.07x10^3$	
10^2		30.2	$1.03x10^1$	
10^1		33.4	9.92	
***C.trachomatis* sample number**	**Group**	**C_p**	**Observed concentrations (copy/ reaction)**	**Observed concentrations (copy/ ml)**
13	Infertility	28.0	$6.88x10^2$	1,376,000
22	Infertility	29.0	$3.19x10^2$	638,000
39	Infertility	28.1	$4.94x10^2$	988,000
45	Infertility	29.0	$2.46x10^2$	492,000
63	Infertility	31.2	$5.15x10^1$	103,000
83	Infertility	33.6	8.45	1690
89	Infertility	32.1	$2.50x10^1$	50,000
93	Infertility	28.2	$4.63x10^2$	926,000
99	Control	31.2	$5.53x10^1$	110,600

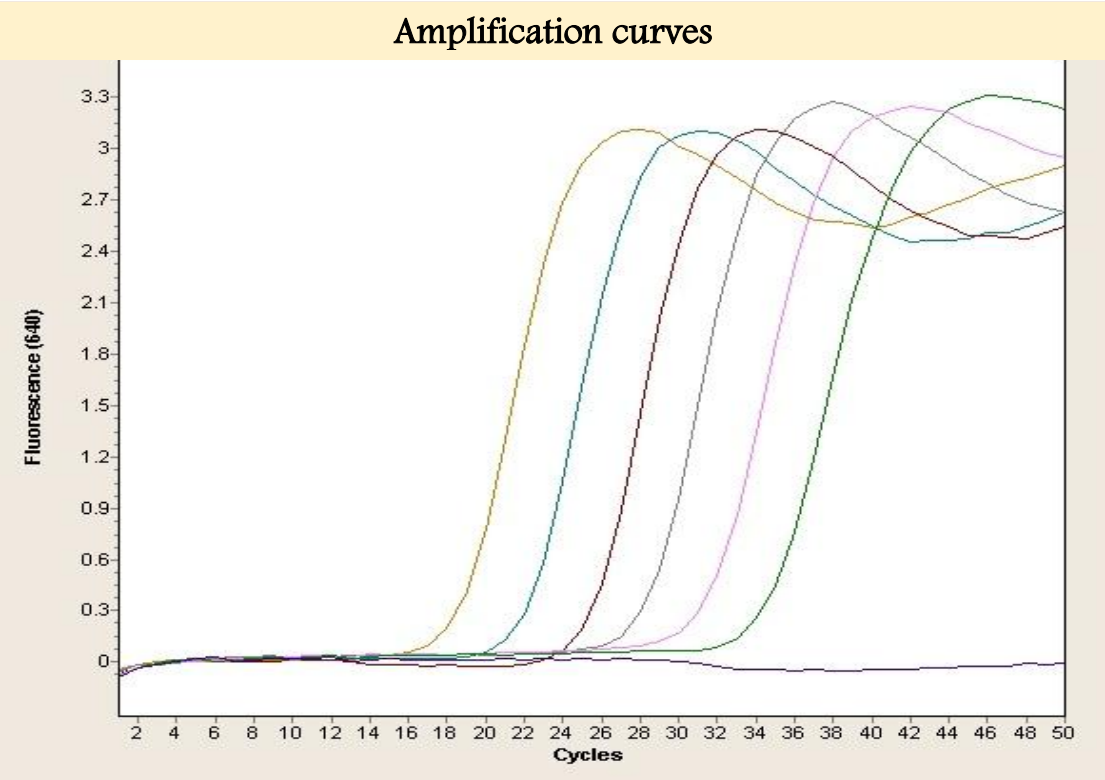

Figure 4.33 Amplification curves of *C. trachomatis* standard dilution series, a negative control was also included.

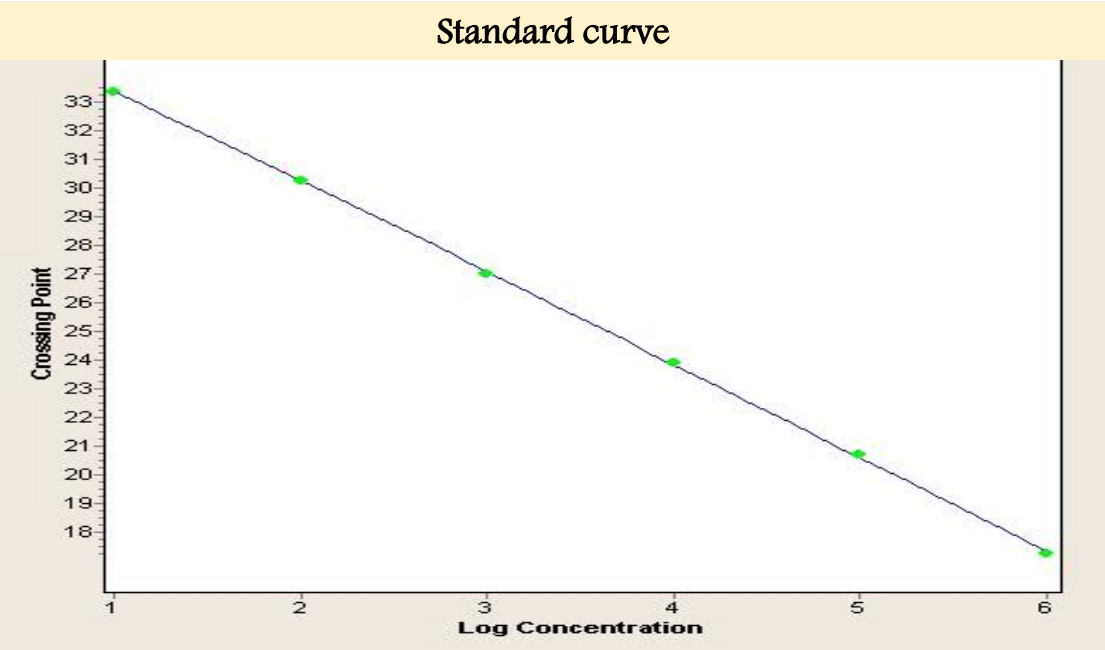

Figure 4.34 Standard curve of *C. trachomatis* standard dilution series 10^6 copies down to 10^1 copy is shown and used to calculate the concentration of *C. trachomatis* DNA in positive samples. C_P is plotted against the log concentration of the standard dilutions.

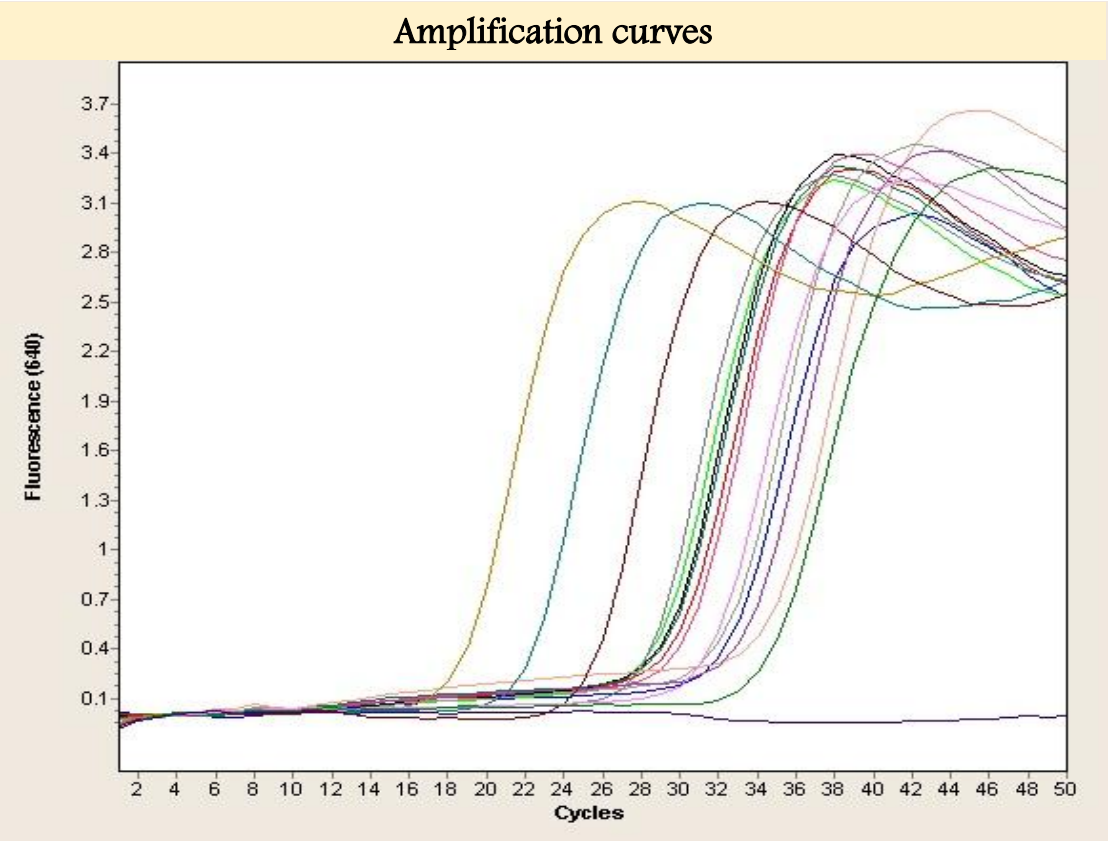

Figure 4.35 Amplification curves of *C. trachomatis* standard dilution series along with *C. trachomatis* positive samples was done (9 positive cases). The fluorescence signal of dilution series from 10^6 to 10^1copy is shown. No reaction was noted in the negative control.

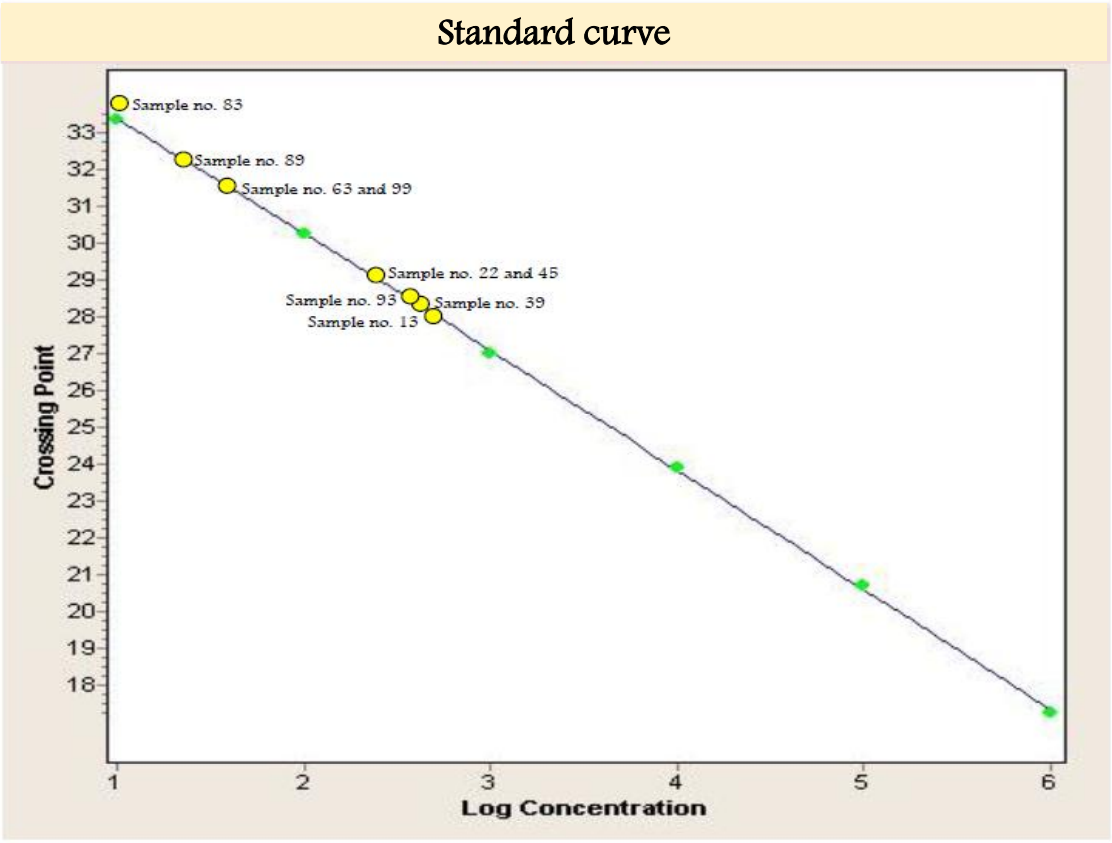

Figure 4.36 Standard curves from standard dilution series 10^6 copies down to 10^1. *C. trachomatis* positive samples (9 results) C_Ps are shown in yellow dots.

4.19.2 Sample data for the *M. genitalium* detection system

Data analysis was performed, as described in the Light Cycler Instrument operator's manual. The cycle number of the C_P of each sample was calculated automatically. The negative control (NTC) showed no signal. For the identification of the PCR product *M. genitalium* data was viewed in channel 640, quantification mode.

The provided standard row of cloned DNA with concentrations in the range from 10^6 copies/reactions to 10^1 copies/reactions of *M. genitalium* had C_p values between cycles 19 and 36.

M. genitalium positive samples had C_Ps between cycles 36.1 and 37.8.
The sample with the highest template count: sample number 58 (infertility group) showed an amplification curve with C_P at 36.7; and the copy number was $1.14x10^1$ DNA copies/reaction (22,800 copies/ml)

Sample number 30 (infertility group) showed an amplification curve with C_P at 36.1; and the copy number was 8.71 DNA copies/ reaction (1,742 copies/ml)

Sample number 98 (infertility group) showed an amplification curve with C_P at 36.7; and the copy number was 6.71 DNA copies/ reaction (1,342 copies/ml)

The sample with the lowest template count (sample number 36, infertility group) showed an amplification curve with C_P at 37.8; and the copy number was 4.04 DNA copies/ reaction (808 copies/ml)

Absolute quantitative Real Time PCR run of *M. genitalium* positive results, their C_P, and the standard dilution series of standard DNA in table 4.27, figures 4.37, 4.38, 4.39, 4.40

Table 4.27 Absolute quantitative Real Time PCR run of *M. genitalium* positive results, C_{Ps}, and the standard dilution series of standard DNA.

Standard Positive control dilution	C_P	observed concentrations (copy/ reaction)
10^6	19.0	$1.12x10^6$
10^5	22.5	$9.36x10^4$
10^4	25.8	$9.04x10^3$
10^3	29.0	$9.45x10^2$
10^2	32.0	$1.12x10^2$
10^1	36.0	$1.00x10^1$

M. genitalium sample number	Group	C_p	observed concentrations (copy/ reaction)	observed concentrations (copy/ml)
30	Infertility	36.1	8.71	1,742
36	Infertility	37.8	4.04	808
58	Infertility	36.7	$1.14x10^1$	22,800
98	Infertility	36.7	6.71	1,342

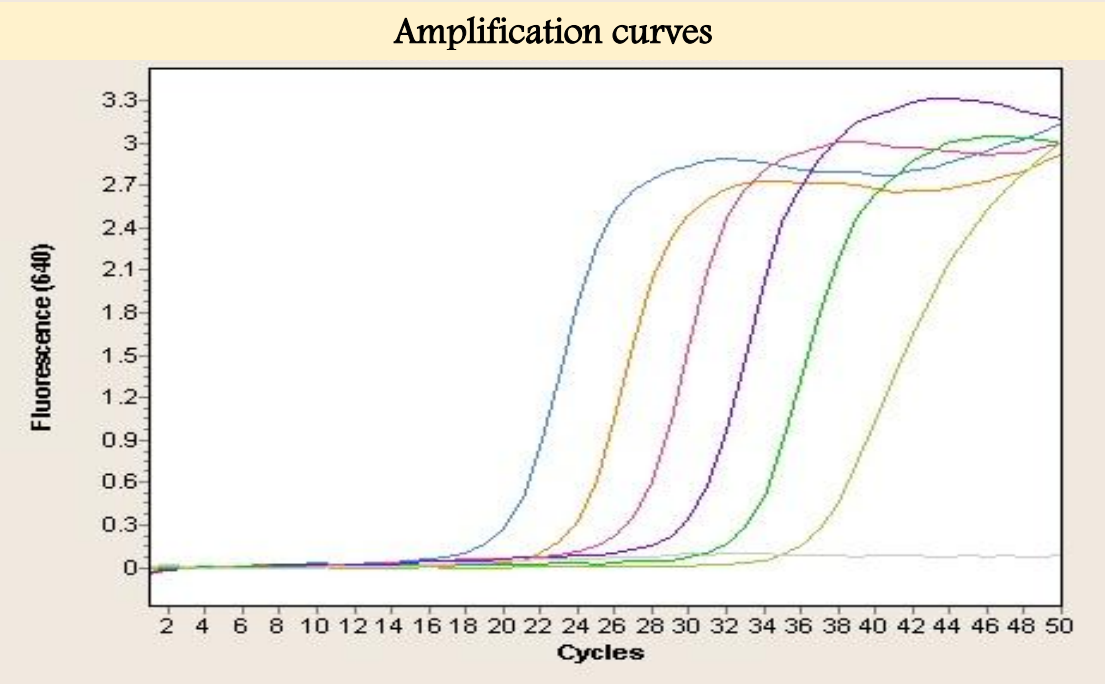

Figure 4.37 Amplification curves of *M. genitalium* standard dilution series, a negative control was also included.

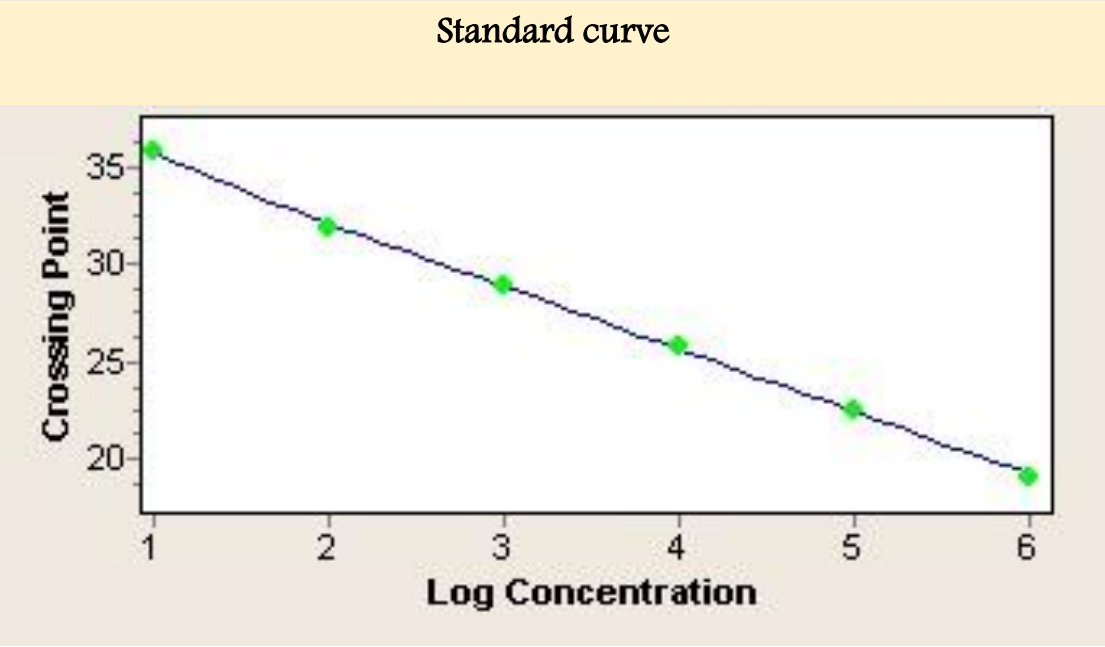

Figure 4.38 Standard curve of *M. genitalium* standard dilution series 10^6 copies down to 10^1 copy is shown used to calculate the concentration of *M. genitalium* DNA in positive samples. C_P is plotted against the log concentration of the standard dilutions.

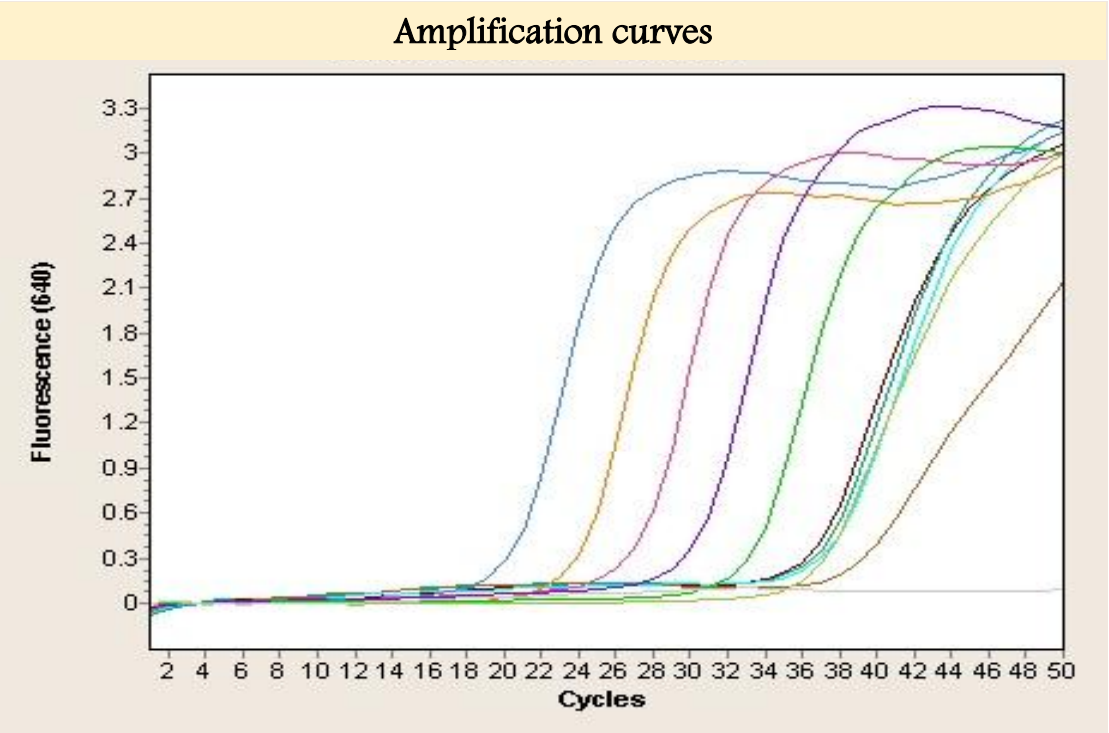

Figure 4.39 Amplification curves of *M. genitalium* standard dilution series along with *M. genitalium* positive *samples* was done (4 positives). The fluorescence signal of dilution series from 10^6 to 10^1 copies is shown. No reaction was noted in the negative control.

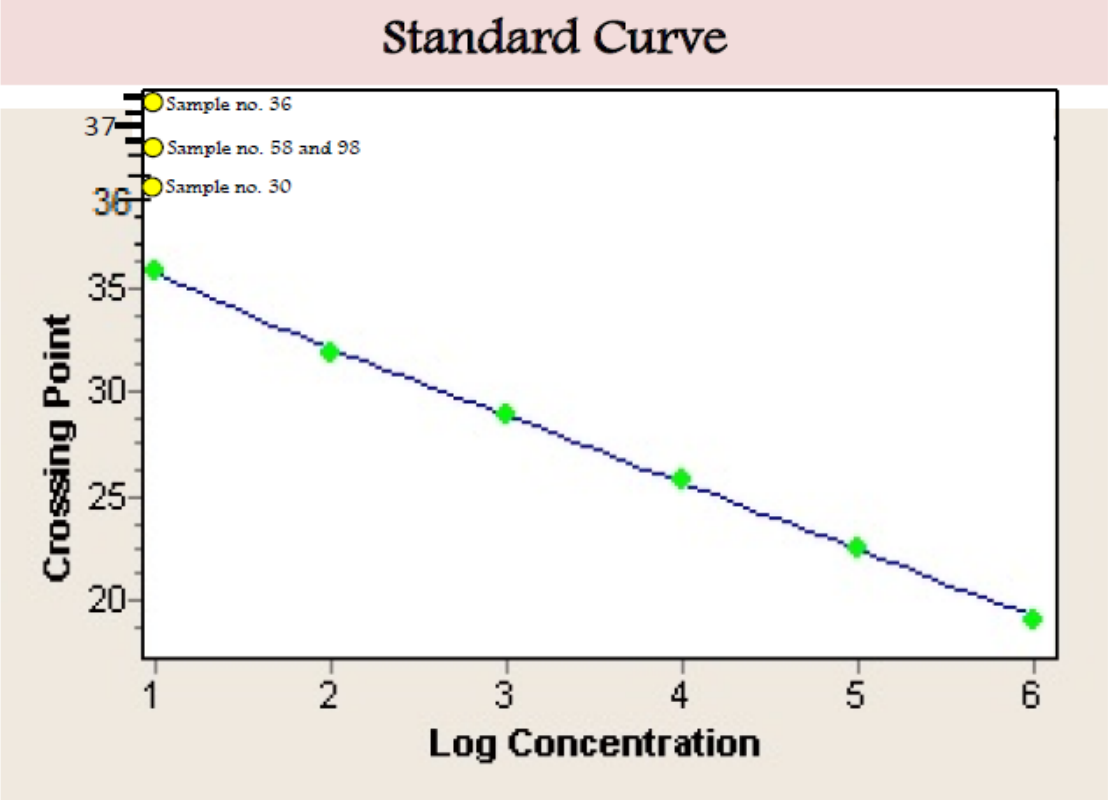

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Figure 4.40 Standard curve of *M. genitalium* standard dilution series 10^6 copies down to 10^1 *M. genitalium* positive samples (4 results) C_Ps are shown in yellow dots.

4.19.3 Sample data for the *M. hominis* detection system

Data analysis was performed, as described in the Light Cycler Instrument operator's manual. The cycle number of the C_P of each sample was calculated automatically. *M. hominis* data was viewed in channel 640, Quantification mode. The negative control showed no signal.

The provided standard row of cloned DNA with concentrations in the range from 10^6 copies/ reaction to 10^1 copies/ reaction of *M. hominis* had C_p values between cycles 20 and 37.9. *M. hominis* positive samples had C_Ps between cycles 20.3 and 37.5.

Among *M. hominis* samples; the sample with the highest template count in our study: sample number 43 (control group) showed an amplification curve with C_P at 20.3; and the copy number was $9.14x10^5$ DNA copies/ reaction (1,828,000,000 copies/ml).

Sample number 36 (infertility group) showed an amplification curve with C_P at 31.3; and the copy number was $5.50x10^2$ DNA copies/ reaction (1,100,000 copies/ml).

Sample number 61 (control group) showed an amplification curve with C_P at 32.1; and the copy number was $3.30x10^2$ DNA copies/ reaction (660,000 copies/ml). The sample with the lowest template count (sample number 7, infertility group) showed an amplification curve with C_P at 37.5; and the copy number was $1.17\ x10^1$ DNA copies/reaction (23,400 copies/ml).

Absolute quantitative Real Time PCR run of *M. hominis* positive results, their C_P, and the standard dilution series of standard DNA in table 4.28, figures 4.41, 4.42, 4.43, 4.44.

Table 4.28 Absolute quantitative Real Time PCR run of *M. hominis* positive results, C_{Ps}, and the standard dilution series of standard DNA.

Standard Positive control dilution	C_p	Observed concentrations (copy/ reaction)
10^6	20.1	$1.10x10^6$
10^5	23.9	$8.82x10^4$
10^4	27.0	$1.04x10^4$
10^3	30.7	$8.84x10^2$
10^2	33.8	$1.11x10^2$
10^1	37.9	$1.00x10^1$

M. hominis sample number	Group	C_P	Observed concentrations (copy/reaction)	Observed concentrations (copy/ml)
7	Infertility	37.5	$1.17x10^1$	23,400
36	Infertility	31.3	$5.50x10^2$	1,100,000
43	Control	20.3	$9.14x10^5$	1,828,000,000
61	Control	32.1	$3.30x10^2$	660,000

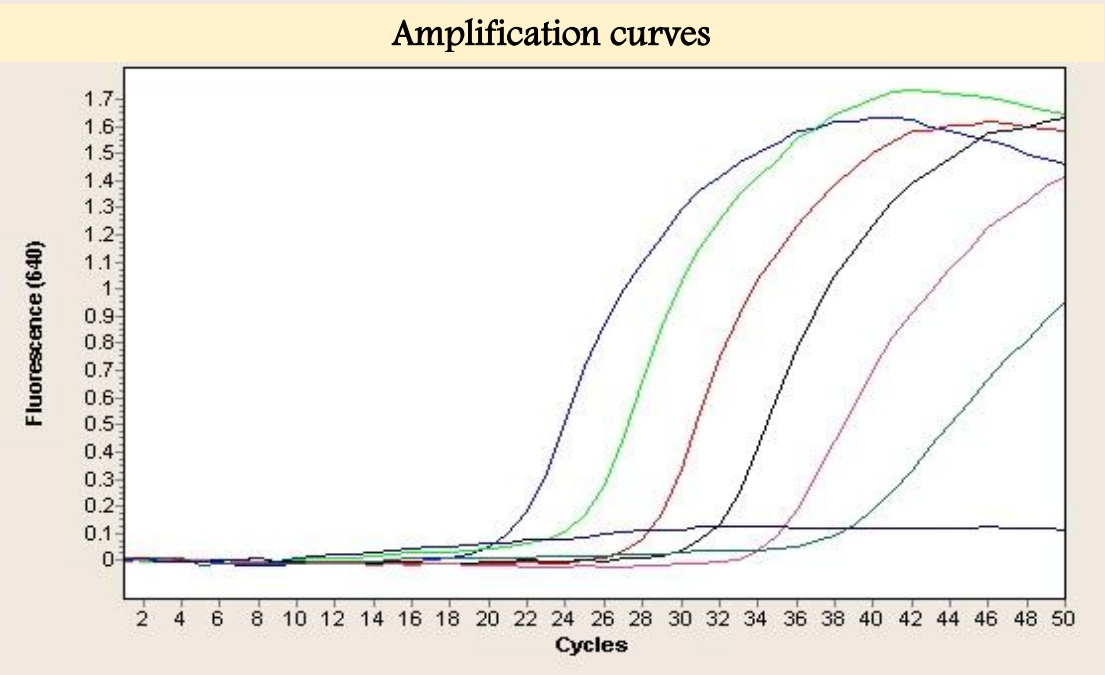

Figure 4.41 Amplification curves of *M. hominis* standard dilution series, a negative control was also included.

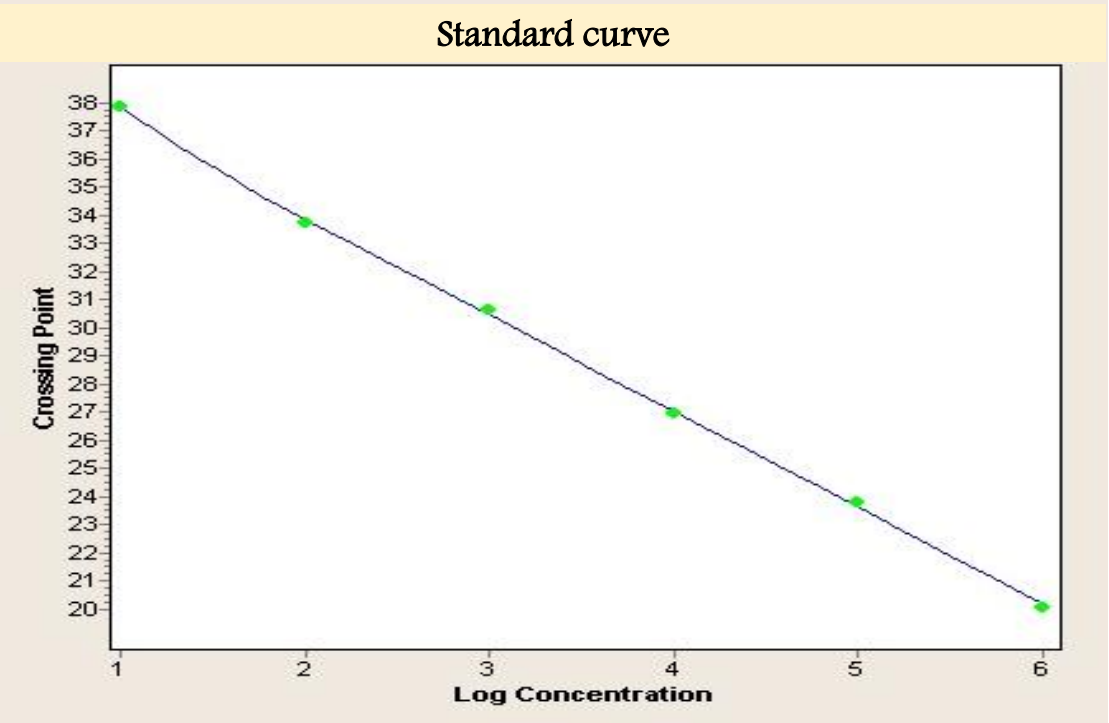

Figure 4.42 Standard curve of *M. hominis* standard dilution series 10^6 copies down to 10^1 copy is shown used to calculate the concentration of *M. hominis* DNA in positive samples. The PCR cycle number C_P is plotted against the log concentration of the standard dilutions.

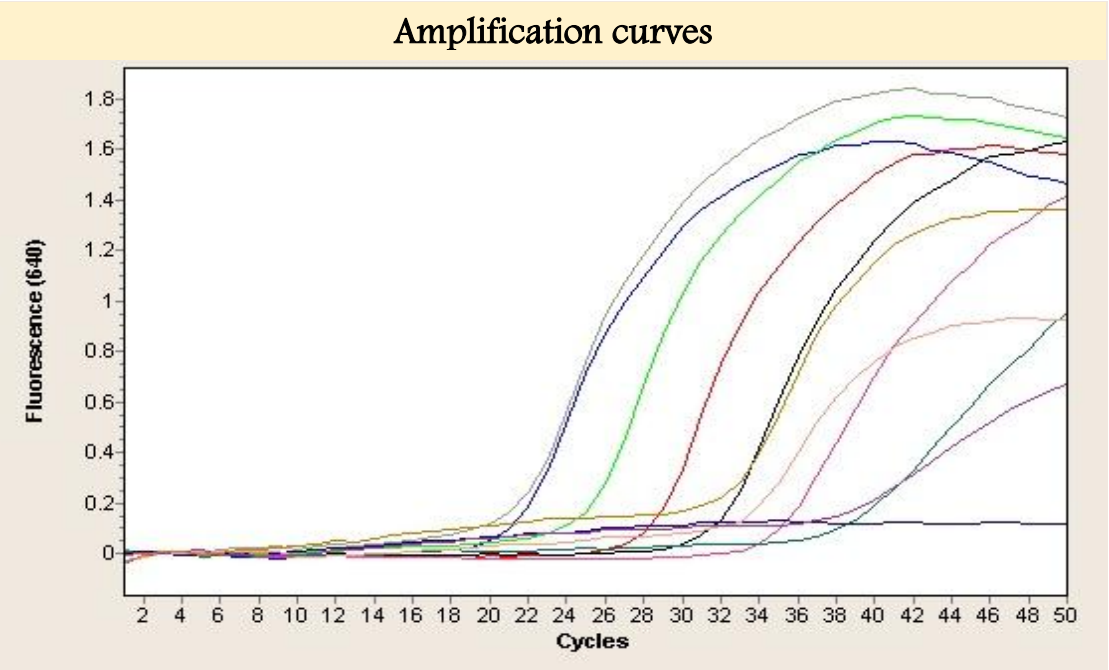

Figure 4.43 Amplification curve of *M. hominis* standard dilution series along with *M. hominis* positive samples were done (4 results). The fluorescence signal of dilution series from 10^6 to 10^1copy is shown. No reaction was noted in the negative control.

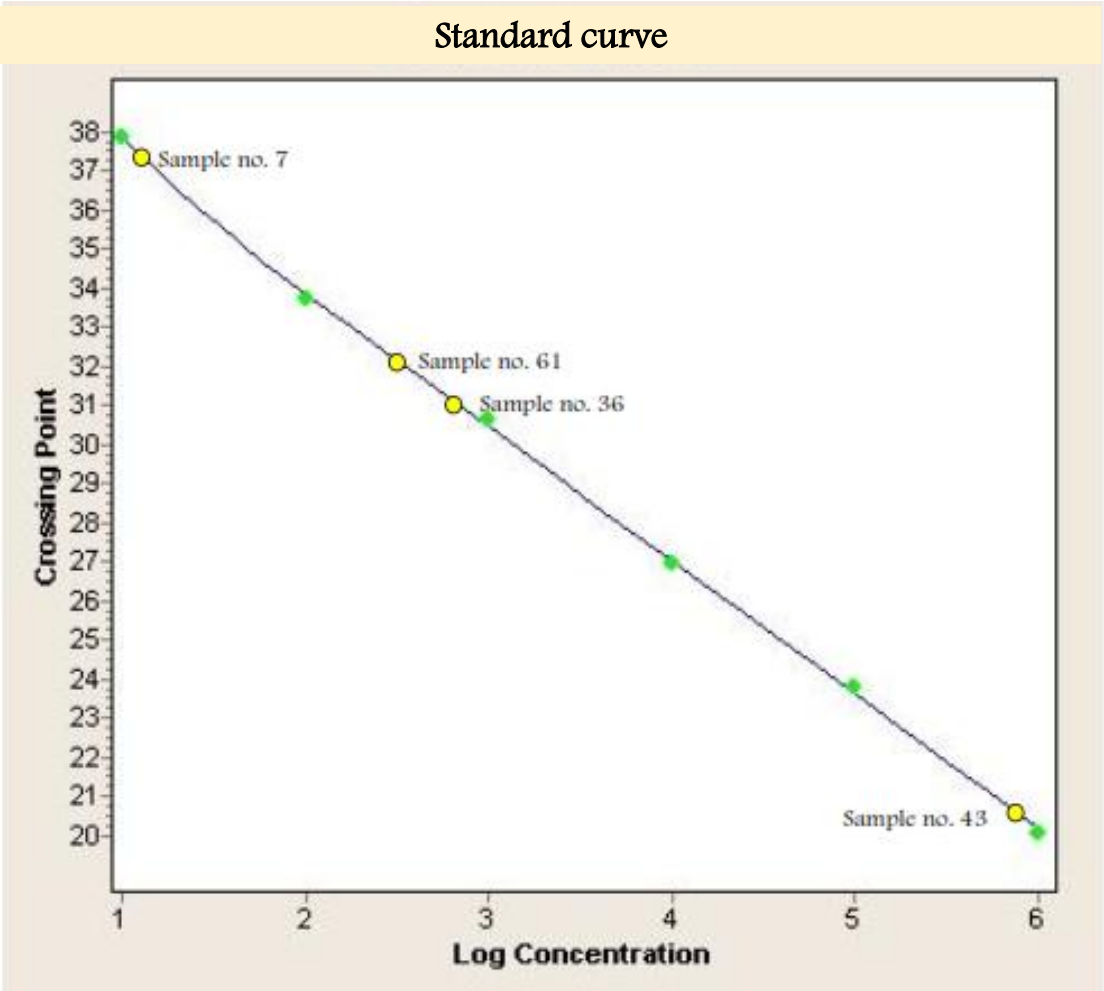

Figure 4.44 Standard curve of *M. hominis* standard dilution series 10^6 copies down to 10^1 *M. hominis* positive samples (4 results) C_{Ps} are shown in yellow dots.

4.20 Electrophoresis analysis of amplicons

Amplicons were electrophoresed on 1.5% agarose gel in 1x Tris Borate EDTA buffer (TBE). Amplicons were compared to a commercial 600 bp molecular weight marker. After staining with Ethidium Bromide, gels were visualized and photographed. All results are depicted in figures: 4.45, 4.46, and 4.47.

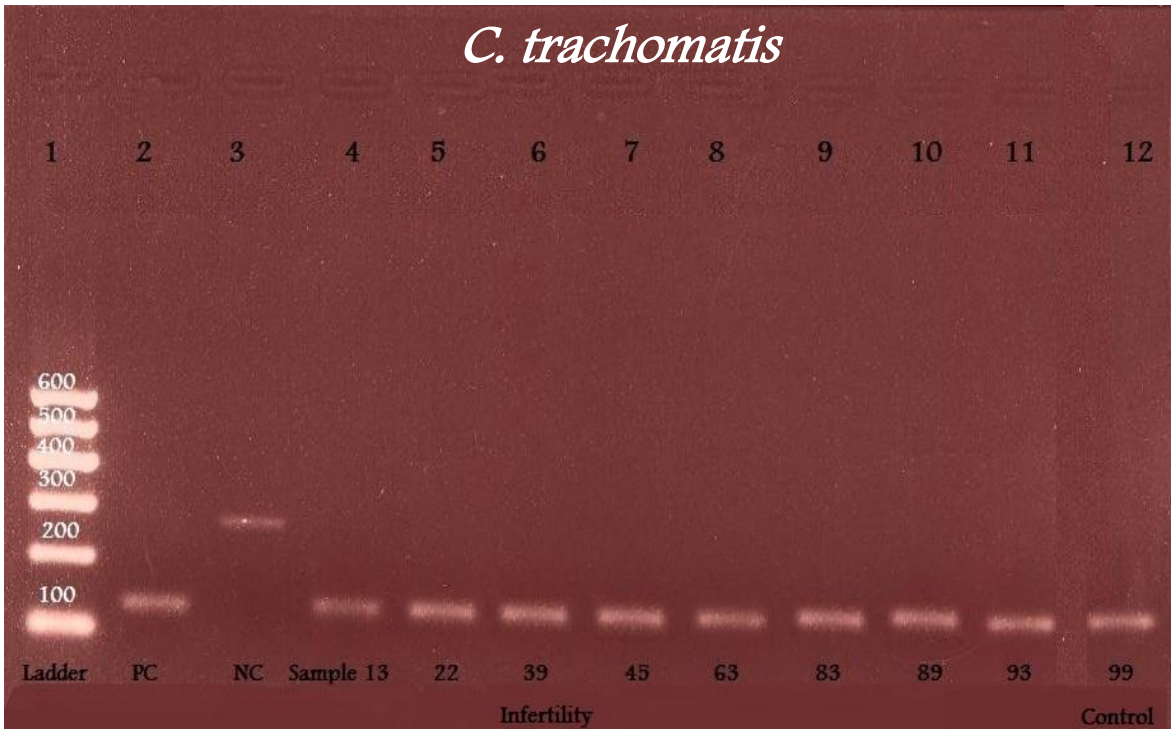

Figure 4.45 Electrophoresis analysis of *C. trachomatis* amplicons (136 bp bands) obtained by real time PCR with primers and probes specific for *C. trachomatis*. Lane 1:600 bp DNA ladder. Lane 2: positive control. Lane 3: negative control showing band at 278bp formed from the IC Lanes 4-11: infertility group positive samples (samples number 13, 22, 39, 45, 63, 83, 89, 93) Lane 12: Control group positive sample (sample number 99) of *C. trachomatis.*

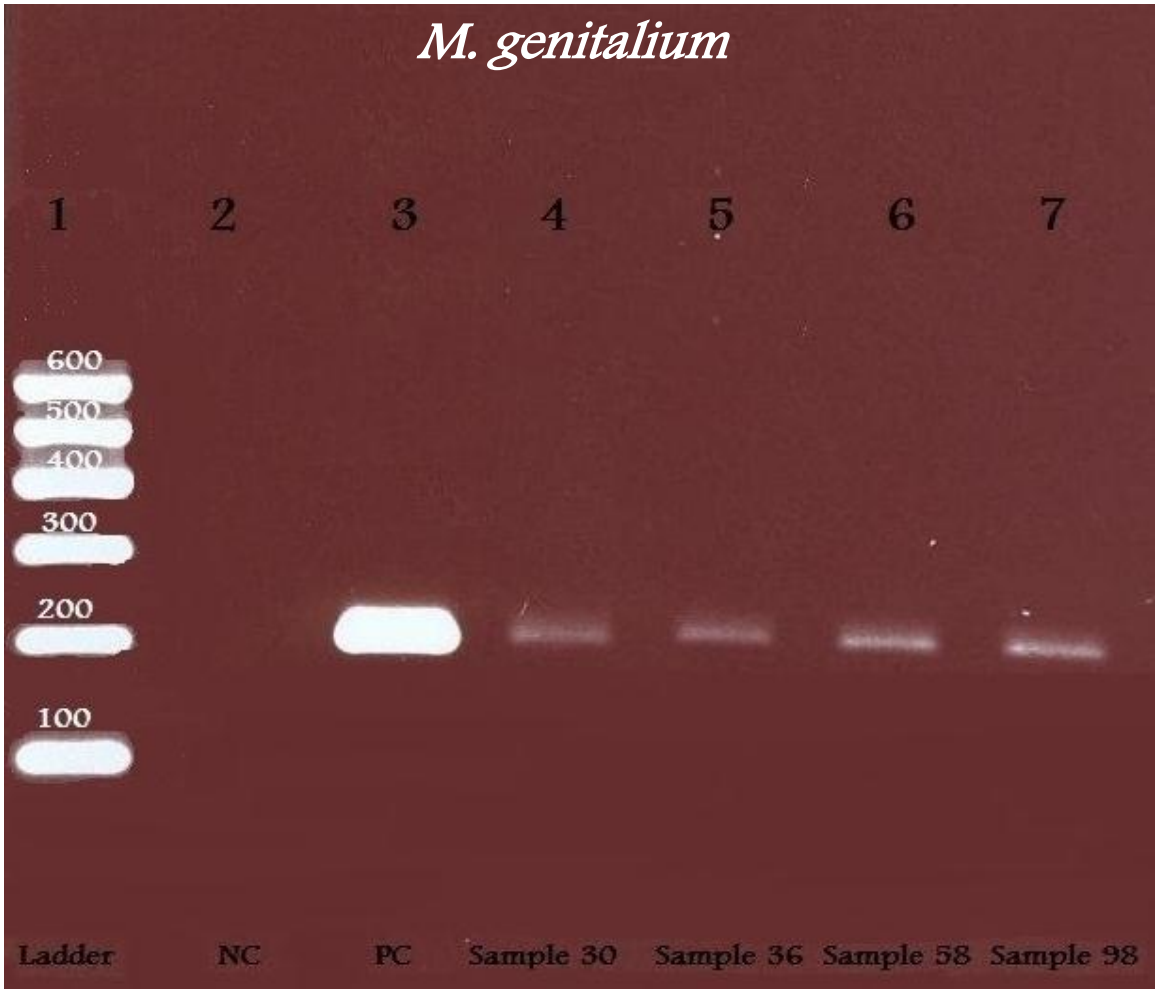

Figure 4.46 Electrophoresis analysis of *M. genitalium* amplicons (224 bp bands) obtained by real time PCR with primers and probes specific for *M. genitalium*. Lane 1:600 bp DNA ladder. Lane 2: negative control. Lane 3: positive control. Lanes 4-7: infertility group positive samples (sample Number 30, 36, 58, 98) of *M. genitalium*.

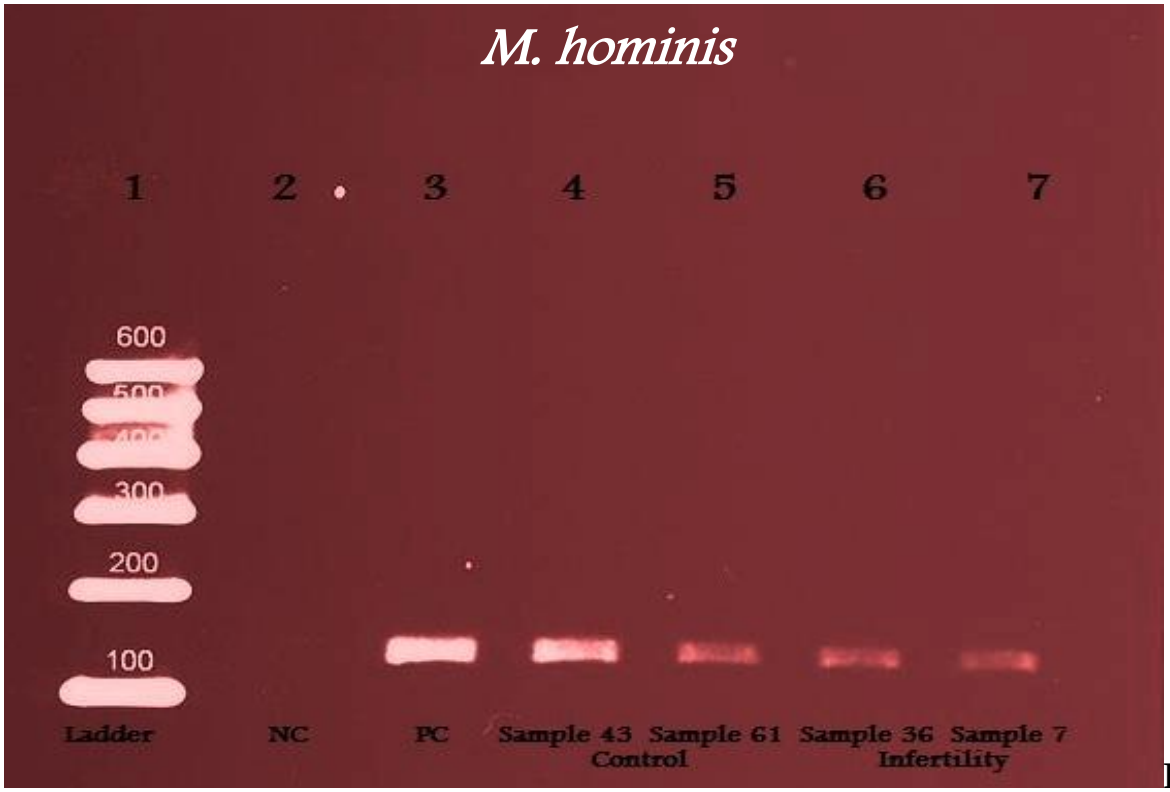

Figure 4.47 Electrophoretic analysis of *M. hominis* amplicons (129 bp bands) obtained by real time PCR with primers and probes specific for *M. hominis*. Lane 1: 600 bp DNA ladder. Lane 2: negative control. Lane 3: positive control. Lanes 4&5: control group positive samples (samples number 43, and 61) of *M. hominis*. Lanes 6&7: infertility group positive samples (samples number 36, and 7) of *M. hominis.*

Chapter (5)

Discussion

Discussion

Infertility is a worldwide health problem with one in six couples suffering from this condition and with a major economic load on the global healthcare industry. Estimates of the current global infertility rate suggest that 15% of couples are infertile [Zegers-Hochschild *et al.,* **2009**].

Approximately 35% of women with an infertility problem are suffering from post-inflammatory changes of the uterine tube that interfere with tub- ovarian function. Most of these alterations result from infection [Westrom, **1994**].

STIs, by their transmissible nature, affect not only individuals, but their partners as well. In developing countries, STIs and their complications rank in the top five disease categories for which adults seek health care, untreated STIs can have critical implications for reproductive, maternal and newborn health. STIs are an important preventable cause of infertility for men and women.

According to the World Health Organization there are an estimated 448 million new cases of STIs which are acquired worldwide annually [World Health Organization, **2011**].

According to information on the status of STI surveillance and control obtained from focal points in Ministries of Health, surveillance is limited or not established in most countries of the region, the magnitude of the problem of STIs is not well known in most countries of the Middle East Region due to limited STI surveillance in the region.

There is clearly an urgent need for all countries to obtain epidemiological information about the load of STIs and their consequences among both the general population and most at risk populations [World Health Organization, **2011**].

C. trachomatis, *M. genitalium*, and *M. hominis* organisms are not screened by routine examination of endocervical specimens in health laboratories in Riyadh, and to our knowledge it is the first study in Riyadh employed real time PCR with Hybridization Probe Format by Light Cycler PCR protocol where detection and quantification detection of these organisms.

Findings of this study showed that PCR testing of endocervix could identify a number of causative microorganisms and should demonstrate to the clinicians the advantage of detection of the fastidious microorganisms from infertile women, when standard cultures fail to detect microbial infection.

Since the detection of those microorganisms should constitute an essential part of the diagnosis and management of patients. The association of infertility with these organisms' infections, however, ought to be discussed.

This study provides some salient points worth highlighting of *C. trachomatis*, *M. genitalium*, and *M. hominis* and their role in the reproductive organs. These microorganisms are associated with various diseases of the genitourinary tract, Infection with these organisms could cause destruction of the cervical endometrial and fallopian tube lining cells may impair fertility, and increase the risk of ectopic pregnancy or damage of a developing pregnancy. It can damage sperm, so they can't swim toward the egg, and it can cause abortions, premature birth and low birth weight.

5.1 Study design and participants demographics

The present study was conducted to detect the incidence of *C. trachomatis*, *M. genitalium*, and *M. hominis* in a case - control study of infertility group and to compare with control group.

A total of 2934 women were offered screening test and 200 agreed to participate, infertile women were more likely to refuse testing. It was possible that they were concerned about being labelled as carriers of sexually transmitted infection; hence there was a higher non-response in this group.

Endocervical samples were collected from 100 married non-pregnant women in the reproductive period presenting with infertility (study group) and from 100 married non-pregnant women in the reproductive period (control group).

The age of all participate ranged from 19-46 years, with a mean of 34.66±7.72 years (table 4.1, figure 4.1).

The mean age (SD) of the infertility group was 32.50±6.397 years, other studies [ElFeky and Baddour, **2009**] also obtained similar findings where the age of the patients ranged from 19- 50 years, with a mean of 32.1 ± 7.5 years. The mean age was significantly lower among the primary infertility group than the secondary infertility group (table 4.2, figure 4.2) : 29.96±5.735 and 35.86±5.684 years respectively (P-value <0.0001), these findings were expected since female age is the most important determinant of treatment-related conception, with a gradual decline in fertility especially after the age of 35 years, also, demographic studies have shown that more women are delaying having siblings at the present time than previously [Botting and Dunnell, **2003**]. This trend is expected to cause a corresponding rise in the mean age at which women first present with infertility.

The women were stratified into 4 age groups (table 4.3, figure 4.3); we excluded women aged less than 19 years. Consequently direct comparison on STIs cannot be made precisely with western studies that include younger women (aged 15–19 years). Moreover, unmarried women were excluded from the present study, as the management of any positive cases would create both legal and social problems.

In our study, there was no significant difference between infertility and control concerning age group 19-25 (P-value = 0. 849).

Participants aged 26-32 years and aged 33-39 were significantly higher among infertile group than control group (P-value<0.0001 and P-value= 0.004 respectively). Whereas Participants aged 40-46 years were significantly lower among infertile group than control group (P-value<0.0001).

Higher numbers of infertile women among age ranges 26-39 may be attributed to the fact that there is an increased awareness of the effects of aging on fertility for patients and health care providers to prevent of age-related infertility, and the fact that a gradual loss of fertility as a function of female age with the rate of decline in fertility becoming more dramatic after the age of 35, resulted in an increase in the absolute number of women seeking pregnancy before the age of 40.

We found no significant deference between infertility and control group with respect to Saudis and non-Saudis since P-value=0.756 (table 4.4, figure 4.4), however, the overwhelming majority of participants were Saudis in both groups (95% infertile and 94% control).

In the current study, each participant completed a questionnaire on demographic characteristics. Information about infertility, type and aetiology of infertility, history of abdominal pain, abortion or ectopic pregnancy were also obtained and a physical examination was conducted by a gynaecologist.

5.2 Signs and symptoms wise findings

This study revealed some correlation between infertility and cervicitis (table 4.5, figure 4.5), signs of cervicitis was presented only among infertile group (6%) with a statistically significant difference (P-value=0.014), this rate was lower than reported from Saudi study where clinical cervicitis was found in 22% among studied population [ElFeky and Baddour, **2009**].

Cervicitis is inflammation of the cervix, the lower end of the uterus that opens into the vagina. Cervicitis is common and may be caused by a number of factors including bacterial infections.

Depending on the trigger of the infection, cervicitis may spread to other parts of the reproductive system. The cervical mucus which occurs with this disease can make it impossible for sperm to pass through and fertilize the egg. The role of cervicitis in infertility have been reported [The New York City Department of Health].

5.3 Correlation between types of infertility and age

Out of 100 infertile women, 57 had primary infertility, and 43 had secondary infertility type. Whereas in Jazan City, Saudi Arabia, out of 640 Saudi infertile women, primary infertility was seen in 425 women, and 215 women had secondary infertility [Kamel, **2013**].

Infertile women aged between 19-25, and 26-32 (table 4.7, figure 5.7), were significantly higher among primary infertility (22.8% and 47.4%, respectively) than secondary infertility (7% and 16%, respectively) (P-value=0.009, P-value < 0.0001, respectively), these findings were expected at these age groups of young

married women trying to conceive for the first time and presented to fertility clinics at a young age.

Rates of infertile participants aged 33-39 and 40-46 were lower among primary infertility group (23%, 7% respectively) compared with higher rates (51%, 26% respectively) from the secondary infertility group, differences were not statistically significant (P-value=0.094, P-value= 0.06, respectively).

Factors influencing the tendency for a woman to delay having siblings are reflected in recent socioeconomic trends including increased level of education, increased percentage of women employed outside the home. In addition, increased divorce rates.

5.4 Infertility factors wise distribution

In this study, some causes of infertility (infertility factors) were more frequent than others (table 4.13, figure 4.14); hormonal factor was the most frequent factor 29 (29%), followed by ovulation 23 (23%), unexplained 21 (21%), tubal 15 (15%), and male factor 12 (12%).

We found a significantly raised rate of hormonal factor comparing with tubal (P-value = 0.033), and with male infertility (P-value = 0.007). Our findings highlight the most common factors influencing fertility, the most common infertility factor was the hormonal factor 29%, Worldwide; hormones are the most common causes of infertility. The process of ovulation depends upon a complex balance of hormones and their interactions to be successful, and any disruption in this process can delay ovulation. Hormonal Imbalances occurs due to many reasons: Altered levels of thyroid hormone, increased levels of prolactin hormone due to a brain tumor called prolactinoma, increased levels of insulin, or adrenal gland dysfunction [What Causes Female Infertility].

Among our case group, ovarian factor was the second most common cause of infertility (23%), whereas in USA, this rate was reported to be up to 50% of female infertility is due to ovulation problems [Harvard health publications].

Ovulation factor rate in our study was lower than UK rates of ovulatory disorders (25%) [Fertility: assessment and treatment, **2012**], and lower than a study in Iran where ovarian infertility rate was 43.2% [Rashidi *et al*., **2013**].

Other factors influencing infertility is unexplained infertility, most practitioners initiate diagnostic after a semen analysis, assessment of ovulation, and, if indicated, tests for ovarian reserve and laparoscopy. When the results of a standard infertility evaluation are normal, practitioners assign a diagnosis of unexplained infertility. Unexplained infertility was found in 21% among our case group, our result was in line with international rates where approximately 15% to 30% of couples were diagnosed with unexplained infertility after their diagnostic workup [World Health Organization, **2011**]. Our result was slightly higher than others where 10%-20% of unexplained infertility was established [Ray *et al*., **2012**].

A higher rates from UK study reported that unexplained infertility rate was 25% [Fertility: assessment and treatment, **2012**]. The fallopian tubes play an integral role in reproduction and undergo cyclical changes in morphology and cilia activity that are dependent upon ovarian hormones [Lyons *et al.,* **2006**].

Tubal disease, one of the many causes of female infertility, is a disorder in which the fallopian tubes are blocked or damaged [Lyons *et al.,* **2006**].

Tubal factor was observed in 15% of infertility cases in the present study, whereas tubal damage was 20% among infertile women in UK [Fertility: assessment and treatment, **2012**]. Our finding was also lower than a similar study in Iran (18.8%) [Rashidi *et al*., **2013**].

Tubal disease directly causes from 36% to 85% of all cases of female factor infertility in developing countries and is associated with poly-microbial-etiologies [Hafner and Pelzer, **2011**].

Infertility is often believed to be a woman's problem. However, studies indicate that 35% of infertility is related to male factor problems such as structural abnormalities, sperm production disorders, ejaculatory disturbances and immunologic disorders [The National Infertility Association].

Male factor was observed in 12% of infertility cases in the present study, comparing to other studies where the rate has been reported to be 20% [Culley, **2013**] and 30% [Fertility: assessment and treatment, **2012**].

5.5 Correlation between symptomatic and asymptomatic women among infertility group.

Screening for STIs is important not only to identify infected symptomatic individuals for the diagnosis and management of their infections but also to identify asymptomatic individuals who serve as reservoirs for these persistent infections. Also, screening of asymptomatic population can be a useful tool to submit patients to antibiotic treatment to avoid the sequelae that undetected infections can produce.

Among our 100 infertile women, 54 % of infertile women were symptomatic, whereas 46% of infertile women were a symptomatic (table 4. 9, figure 4.9). Difference was not statistically significant (P-value=0.4242). Symptomatic women in our study group had an infection rate of 18.5%, whereas infection rate of asymptomatic women was 6.5%, another study showed that most women

were asymptomatic (475 women) with an infection rate of 11.72%, whereas 165 women were symptomatic with an infection rate of 3.28% [Kamel, **2013**].

5.6 *C. trachomatis* findings among infertility and control group

The findings of this study highlight the need for *Chlamydia* screening programs as *C. trachomatis* was the most frequent pathogenic agent of female participants. There was 8/100 women positive among the infertility group (8%) compared with 1/100 women positive among the control group (1%). We found a significant differences between infertile and fertile women for *C. trachomatis* infection (P-value=0.017). Our results revealed that the occurrence of *C. trachomatis* was higher than the occurrence of the other microorganisms detected in this study.
These results suggest that *C. trachomatis* infection should be strongly considered in endocervical samples of infertile women (table 4.8, figure 4.8).

C. trachomatis infection is a worldwide-spread sexually transmitted infection. Prevalence of infection is difficult to estimate without screening, as most of the cases are asymptomatic. The possible effect of *Chlamydial* infection includes pelvic damage, and infertility [Paavonen *et al*., **2008**; Soper, **2010**].

In 2010, the overall rate of *Chlamydial* infection in the United States among women (610.6 cases per 100,000 females) was over two and a half times the rate among men (233.7 cases per 100,000 males), reflecting the large number of women screened for this Disease [Centers for Disease Control and Prevention, **2010**].

Although our finding is compatible with some studies in the United States of America (USA) where estimated to range from 5% to 15% [Mertz *et al*., **1997**], but some other studies have shown greater incidence, in UK the prevalence of *Chlamydial* infection was about 16% [Pimenta *et al.*, **2000**].

In some parts of the developing world, over 90% of the population is infected, it is estimated that more than 500 million people still are at high risk of infection, over 140 million persons are infected and about 6 million are blind in Africa, the Middle East, Central and South-East Asia, and countries in Latin America [World Health Organization, **2011**].
We found that *C. trachomatis* infection rate among our control group was 1%, similar to another study where frequency of *C. trachomatis* was recovered in1% of control group [Franceschi *et al*., **2007**], a low result comparing to other authors [Gorander *et al*.; **2008**] where 6% and 8% of their healthy controls had *C. trachomatis* in their endocervical specimens.

Limited data exist on the prevalence of *C. trachomatis* in the Middle East and Gulf countries, and the true incidence of *Chlamydia* infection in our region is difficult to establish because of several factors. There is combination of social and cultural factors inhibition that prevents women from reporting sexual symptoms, non-availability of service to detect the organism in many health units and the largely asymptomatic nature of the disease.

Most of the published data came from point occurrence studies in clinics and hospitals as noted in table 2.3. In these studies, the prevalence of *C. trachomatis* varied extensively depending on the health care setting, screened population, high- or low-risk groups, and system of testing.

Our study showed higher *Chlamydial* infection rate among infertile and control groups compared to a Jordanian study that compared infertility women with control group, reported 3.9% and 0.7% respectively [Al-Ramahi *et al*., **2008**].
In comparing our *Chlamydial* infection rate across different studies, our results are still lower than that reported in Jordan (40%) [Mawajdeh *et al.*, **2003**], Egypt (31%) [Mohamed and Sharaf, **2001]**, Iran (22%) [Afrakhteh *et a*l, **2008**], India (30.8%) [Singh *et al.*, **2003**].

However, our results is higher than that reported in Jordan (4.6%) [Awwad *et al.*, **2003**], United Arab Emirates 2.6% [Ghazal-Aswad *et al.*, **2004]**, and Ghana 3% [Apea-Kubi *et al.*, **2004**].

The highest rate of *C. trachomatis* in the Arabian region has so far came from Egypt with rates of 4.2% in one study [Sullam *et al*., **2001**] to as high as 33.3% to 82.6% among high-risk groups in another [El-Shoubagy *et al*., **1996**].

This variability of results between different studies compared to ours may be explained by the deviation in sexual activity between our population and other populations, and may be attributed to the fact that these societies are socially and culturally conservative concerning free sexual relations, and among young adult women sexual activity usually starts with legal marriage, also our Islamic religion which restricted sexual action to one partner the husband, while no restriction in some non Islamic societies and there could be several partners.

Variations of *Chlamydia* incidence between countries and studies could also be due to several factors such as, studied population (selection of high risk group, symptoms, education level, sample size, etc.), rate of infection in the study area, hygiene level and social and economic status of the study area, culture of the society whether it is open or traditional, and the screening techniques.

5.6.1 *C. trachomatis* findings among asymptomatic and symptomatic infertile women and the frequency of most related symptoms

Up to 70-80% of *C. trachomatis* infections in women are asymptomatic and can persist for very long periods. Microbial persistence is a state during which the human immune response is not able to eliminate the pathogen that remains silent at focal sites of infection and continues to exercise its virulence, increasing the risk of tubal damage, tubal factor infertility, ectopic pregnancy and chronic abdominal pain [Centers for Disease Control and Prevention, **2006**].

Our results revealed that the occurrence of *C. trachomatis* among symptomatic women was higher (6/54, 11%) than the occurrence of *C. trachomatis* among asymptomatic women (2/46, 4%) and the difference was not statically significant since the P-value=0.193 (table 4.10, figure 4.10). Similar study from Jordan reported that the prevalence of *Chlamydial* infection among symptomatic patients was 4.6%, and no *Chlamydial* infection among asymptomatic patients [Awwad *et al.*, **2003**].

In our study, irregular menstruation was seen in one *Chlamydial* case, burning sensation when urinating in one case and abnormal discharge was seen in one case (table 4.11, figure 4.11).

Also, one of our *Chlamydial* positive cases had a previous two spontaneous miscarriages, this finding confirm the relationship between miscarriage and *C. trachomatis* infection. Several studies have documented an association between *C. trachomatis* and spontaneous or recurrent miscarriage [Rastogi *et al*., **2000;** Wilkowska-Trojniel *et al.*, **2009**; Baud *et al*., **2011**; Nigro *et al.*, **2011**].

To evaluate the occurrence of *C. trachomatis* in women with genital symptoms, and to correlate the presence of this microorganism with specific symptoms and the role played by *Chlamydia* in infections, we found that *C. trachomatis*

infection was statistically higher (P-value = 0.044) among infertile women with low-seated abdominal pain (5, 19%) comparing *Mycoplasmal* positive cases which warent associated with this symptom (Table 4.11, figure 4.12). this association between *Chlamydial* infection and low abdominal pain among our patients could be a sign for PID since untreated infections can spread upward to the uterus and fallopian tubes. PID can be silent, or can cause symptoms such as abdominal and pelvic pain as documented in several studies [Centers for Disease Control and Prevention, **2006**].

5.6.2 *C. trachomatis* findings among different infertility factors.

Unexplained infertility diagnosis is usually made after investigations show normal semen parameters, ovulatory concentrations of serum progesterone, tubal patency, and a normal uterine cavity.

This study explores the relationship between *C. trachomatis* and unexplained infertility, this study recorded that higher incidence of *C. trachomatis* infection was among unexplained infertility (5/21, 24%) than other types of infertility (table 4.15, figure 4.15) out of these 5 positive results, 3 were primary infertile (14%) and 2 were secondary infertile (10%) (table 4.17).

In the 8 cases of *C. trachomatis* infected women, 2 (13%) had documented tubal factor infertility, our finding was higher compared with reported in Iran (4.9%) [Rashidi et *al*., **2013**].

Both *Chlamydial* positive patients with tubal factor infertility complained of low seated abdominal pain, these results may further support the etiologic role of infection with *C. trachomatis* and tubal damage among infertile women. The

association between *C. trachomatis* and tubal damage in infertile women has been recognized [Mei *et al.*, **2009**].

In our study, we detected a case of male factor infertility that was positive for *C. trachomatis*, which may indicate the need for routine screening for the pathogen in males with complaints of infertility so as to rule out this potentially reversible cause of infertility. While women can develop PID after *C. trachomatis* infections, men can develop urethritis [Detels *et al.*, **2011**], epididymitis [Workowski and Berman, **2010**] and proctitis [Cunningham and Beagley, **2008**]. Epididymitis can lead to canalicular system damage and obstructive azoospermia, although such severe postchlamydial outcomes are uncommon. *C. trachomatis* infections more commonly result in the generation of antisperm antibodies and changes in semen quality that diminish rather than prevent male fertility [Hirano and Hoshino, **2010**].

5.6.3 *C. trachomatis* findings among infertility types

Although there wasn't statically significant difference (P-value=0.524), *C. trachomatis* infection had more incidence among primary infertile patients (9%) than secondary infertile patients (7%) in the present study (table 4.16, 4.17).

Comparing to others; a study revealed that *C. trachomatis* infection had the same prevalence in primary and secondary causes of infertility [Rashidi *et al*., **2013**]. From Jordan, *C. trachomatis* in the primary infertility group (2/81, 2.5%) was lower than the proportion testing positive in the secondary infertility group (4/71, 5.6%) [Al-Ramahi *et al*., 2008].

The high rate of *C. trachomatis* found among the primary infertile subjects in comparison with the secondary infertile subjects may be attributed, possibly, to the communal habits which make the married couples seek children immediately after their marriage, and so, they may ask for investigations more than those with secondary infertility whom the social pressure on them is less. Although there were also some reports indicating greater prevalence for the infection in secondary than primary infertility [Malik *et al.*, **2006**; Tukur *et al.,***2006**], opening the door for further investigations.

5.6.4 *C. trachomatis* findings among age groups

In the current study *C. trachomatis* was wide spread among all age groups, the incidence also vary in different age groups of infertile women, the deference between the infection and age ranges wasn't statically significant since P- value = 0.661 (table 4.21, figure 4.16).

The incidence of *C. trachomatis* among all age groups in our study is consistent with previous reviews on the prevalence of *C. trachomatis* in low-risk women for the same age group [Brannstrom *et al.*, **1992**; World Health Organization, **2001**; Franceschi *et al.*, **2007**]. This incidence is also similar to the findings of the studies conducted in primary care settings in Sweden and Greece [Brannstrom *et al*., **1992**; Nelson, **1992**].

In the current study, *Chlamydial* infection rate was 19% for Saudi infertile women aged 19 to 25 years (table 4.21, figure 4.16).

Higher than others where the rate of infection was 7.81% for infertile women with the same age range [Kamel, **2013**]. This rate-age association is largely relating to the higher level of sexual activity among young women, whose squamo-columnar junction of the cervix still presents on ectocervix, which provides a large target area for infection. That is why age is used in many countries as a primary determinant for selective *Chlamydial* screening programs [Centers for Disease Control and Prevention, **2003**].

5.6.5 *C. trachomatis* screening program

C. trachomatis infections are the most prevalent sexually transmitted bacterial infections among women and men worldwide. Screening for this infection is important not only to identify infected symptomatic individuals for the diagnosis and management of their infections but also to identify asymptomatic individuals who serve as reservoirs for *C. trachomatis* infections [White and Perry, **2004**].

Conventional testing methods for *C. trachomatis* can fail to detect asymptomatic infection. In clinical evaluations, nucleic acid tests have been shown to be more sensitive than traditional culture and other non-culture methods.

Several screening tests have been suggested for detecting *C. trachomatis* infection, including DNA-based tests such as PCR [Toye *et al.,* **1996**], gene probe [Ferrero *et al.,* **1998**] and EIA [Chernesky *et al.,* **1994**] using urine or cervical swab samples.

A meta-analysis demonstrated that DNA amplification techniques, including PCR, performed best for urine and cervical swabs in low prevalence populations [Watson *et al.*, **2002**].

Conventional PCR is an open, contamination-susceptible system where it is necessary to transfer the amplified product to other detection systems to confirm a positive result.

5.6.5.1 Qualitative real-time Light Cycler PCR with hybridization probes for detection of *C. trachomatis*

In our study, multiple considerations affected the selection of a screening test, test sensitivity was emphasized to minimize occurrence of false-negative tests, we decided on real-time technology where measurement of the fluorescence emitted during amplicon production is performed during each PCR cycle is considered as a breakthrough in PCR.

Real-time PCR benefits by a closed system in which formation of a product is measured immediately without transfer, Interpretation of Light Cycler PCR results, presented as graphs and calculation of crossing points, introduces many advantages [Jensen *et al*., **2004**].

In this study we elected to screen our patients using endocervical specimens and performing DNA amplification technique using real- time PCR with Hybridization probe format (pair of sequence-specific, single-labelled probes) because of the high sensitivity this method provides in detecting *Chlamydia* infection [Catry *et al.,***1995**].

In the present study Light Mix (table 3.1) was used for the detection of part of *C. trachomatis* MOMP gene using the real time PCR assay. It combines sequence-specific primers and fluorescence-labelled probes.

A 136 bp fragment of the *C. trachomatis* MOMP genome gene was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 (table 3.3). The PCR product was identified by running a melting curve with a specific T_m value of 66°C in channel 640 (according to the manufactures manual). T_m is defined as the temperature at which half of a duplex-DNA becomes single-stranded [Schalasta *et al.*, **2000**].

The PCR reaction was monitored by an additional PCR product of 278 bp, formed from the IC. An IC integrated in this system detects false negative results and invalid PCR conditions.

All our positive samples had melting peaks with T_m values from 66° to 66.5° (Mean T_m=66.2, table 4.25), which determines how well the sequence of probes matches the sequence of template DNA, and it will decrease if mismatched DNA is amplified. Single mismatch between the hybridization probes and their target sequences changes the T_m of the bound probes can decrease the T_m from 1°C up to 30°C depending on many factors, such as pH, duplex length and G + C content [Aboul-ela *et al.*, **1985;** Ke and Wartell, **1993**].

5.6.5.2 Absolute quantitative real time PCR for *C. trachomatis* results.

Absolute quantitation is the process that determines the absolute quantity of a single nucleic acid target sequence within an unknown sample.

To our knowledge it is the first study in the city of Riyadh using Light Cycler PCR with hybridization probes where quantification of *C. trachomatis*, *M. genitalium, and M.hominis* combined with C_p assay.

To additionally confirm that samples which gave a positive fluorescence signal in Light Cycler PCR, In the present study, we showed that the light cycler fast

start DNA master hyprobe was able to specifically quantify concentrations of the *C. trachomatis* MOMP gene. Using the assay, we were able to quantify *C. trachomatis* in endocervical specimens from positive samples.

A quantification of DNA concentrations was based on standard dilution series with known concentrations of genomic *C. trachomatis* DNA. As an external reference standard, concentrations of standard dilution series were: 10^6 10^5, 10^4, 10^3, 10^2, and 10^1. Detection of PCR product was possible for the lowest DNA concentration. The positive results were interpreted with Light Cycler software Vers. 2.0 (Roche Diagnostics).

There was a variation in the DNA copy-number loads, samples with high DNA load had low C_p values, while low DNA load had high C_p values.

Chlamydial positive sample number 13 from infertile group showed the highest template count copy number of 6880 DNA copies/reaction (1,376,000 copies/ml), the sample was detected from a 34 year old primary infertile with tubal factor infertility, complaining from low abdominal pain.

Chlamydial infection may simply be a marker for exposure to other STIs, one possible explanation for these findings is an increased risk of PID related to *C. trachomatis* infections, which are common [Geisler, **2011**]. However, low-copy-number region was clearly demonstrated with sample number 83 from infertile group, which had a copy number of 8.45 DNA copies/reaction (1690 copies/ml). Clinical samples showing the low concentration also had the melting curve with the melting peak around 66°C and were therefore considered as positive.

Our findings were higher comparing to a similar study [ElFeky and Baddour, **2009**], showed that *C. trachomatis* sample with the highest template count had a copy number lower than ours (16,760 DNA copies/ml), also the sample with the

least template count had a copy number lower than ours as well (10 DNA copies/ml). Further studies involving the quantitative assessment of *C. trachomatis* loads in our region will provide useful information for understanding roles of the *Chlamydia* in such subjects.

5.7 *M. genitalium* findings infertility and control group

Our results showed that *M. genitalium* was found in the cervical canal in 4 infertile women (table 4.8, figure 4.8): 3 as a single infection (3%, P-value=0.123) and 1 was detected associated with *M. hominis* as a co–infection case (1%, P-value=0.499). All 4 positive samples were detected from infertile group and no observed cases from the control, indicating an association between this infection and infertility, published studies have evaluated the association of *M. genitalium* infection with infertility [Clausen *et al.,* **2001**].

When compared to other studies from other countries, our results, were considerably lower than reported in Indiana-USA (13.6%) [Tosh *et al*., **2007**], in Seattle-USA (13%) [Wroblewski *et al*., **2006**], and in France (39%) [Anagrius *et al.,* **2005**].

Other studies also obtained similar results. In Jordan 3.5% [Shehabi *et al*., **2009**], Similar rates from UK where the prevalence of *M. genitalium* at baseline was 3.3% [Bjartling *et al.,* **2012**]. Despite different geographic locales and clinical status, these numbers are strikingly similar to ours.

Our rates were higher than reported in in Gaza, Palestine (1%) [Nassar *et al*., **2008**], and in Iran (1.02%) [Haghighi Hasanabad *et al*., **2011**]. It is worth noting that the diagnostic tests varied in the different studies and could contribute to the differences in the reported infection rates.

M. genitalium is an emerging cause of STIs and has been implicated in urogenital infections of men and women around the world. The risk factors for *M. genitalium* infection are typical as for any STI. Asymptomatic carrier state [Williams *et al.,* **2007**] is also a serious epidemiological problem because of transmission to sexual partners and may be vertical transmission from mother to the infant.

M. genitalium urogenital infection in women include cervicitis, PID [Jensen, 2006], endometritis [Cohen *et al.,* 2002], salpingitis [Cohen *et al.,* **2005**], BV [Keane *et al.,* **2000**] and infertility [Svenstrup *et al.,* **2008**].

5.7.1 M. genitalium findings among asymptomatic and symptomatic infertile women and the frequency of most related symptoms.

Although *M. genitalium* is often asymptomatic [Williams *et al.,* **2007**], all positive cases in our study were isolated from symptomatic women (table 4.10, figure 4.10), most importantly, one positive case with signs of cervicitis (sample no 30, table 4.11) comparing to all positive results, and even though the deference was not statically significant, (P-value=0.307), the only infectious case with signs of cervicitis was *M. genitalium* positive (see section 2.2.1.3), supporting an independent role for *M. genitalium* as a cause of cervicitis have been reported [Taylor-Robinson, **2002**; Manhart *et al.*,**2003**].

M. genitalium was also detected from 2 (25%) cases complaining from Irregular menstruation, intermenstrual or irregular bleeding in women with *M. genitalium* have been reported [Jensen, 2006].

The pathogen was also detected from a case complaining from abnormal discharge, and the same symptom has been reported from a co-infection case of *M. genitalium* and *M hominis*. Most people with genital *Mycoplasma* infection have no symptoms. Reports confirmed that occasionally, women develop a discharge and pain or difficulty urinating or discomfort in the genital region, it may contribute to infections caused by *Mycoplasmal* organisms, resulting in vaginal discharge. Pelvic pain may be a symptom of PID brought on by *Mycoplasma* [Keane *et al*., **2000**; Cohen *et al*., **2005**].

5.7.2 *M. genitalium* findings among deferent infertility factors

Two (10%) *M. genitalium* positive cases were obtained from women with history of infertility, due to unexplained reasons (table 4.15, table 4.18, figure 4.15). This finding indicates the role *M. genitalium* plays in cases of unexplained infertility, *Mycoplasmas* implication in several cases of unexplained infertility have been documented [Idriss *et al.*, **1978**].

One positive result was obtained from a case of tubal factor infertility (7%) indicating that previous infections caused by this microorganism may have resulted in permanent damage and occlusion of the fallopian tubes, the presence of *M. genitalium* in women with tubal damage had been determined [Clausen *et al*., **2001**; Svenstrup *et al*., **2008**].

5.7.3 *M. genitalium* findings among infertility types

An interesting finding that all 3 (5%, P-value= 0.181) *M. genitalium* infection cases were isolated from primary infertile women (table 4.16, table 4.18, figure 4.16), which provides strong association and relationship between *M. genitalium* infection with Primary infertility and may suggest that *M. genitalium* is a species having an impact on impaired fertility.

5.7.4 *M. genitalium* findings among age groups

3 positive cases of *M. genitalium* were detected from women under 32 years old (table 4.21, figure 4.16), although this finding did not reach statistical significance (P-value=0.331), but it indicates that younger age is associated with *M. genitalium* positivity.

5.7.5 *M. genitalium* screening program

Although cultivation is regarded as the gold standard in identifying *Mycoplasma* infections, it only enables the detection of living cells which multiply in artificial media. When live microorganisms are not present in the specimen or, owing to various reasons, they are not able to survive outside the host; the results obtained by this method may be falsely negative. Furthermore, positive samples need further testing to determine the species. It was reported that the sensitivity and specificity of cultivation techniques is comparable to the sensitivity and specificity of molecular methods commercial tests currently applied [Biernat-Sudolska *et al.*, **2006**].

The use of other diagnostic method, such as, PCR necessary because it was found to be higher compared with culture in detection of genital *Mycoplasmas* infections. PCR has an advantage of detecting DNA of dead organisms [Shahin and Samimi, **2008**].

5.7.5.1 Qualitative real-time Light Cycler PCR with hybridization probes for detection of *M. genitalium*

A qualitative real-time Light Cycler PCR assay was performed for the detection of *M. genitalium* in endocervical specimens from female patients. The assay was found to be specific, sensitive, fast and had minimal risk of contamination. The clinical performance of the PCR was previously been evaluated and was compared to a conventional PCR [Jensen *et al.*, **2003**; Jensen *et al.*, **2004**].

In our study, the housekeeping gene *gap* encoding glyceraldehyde-3 phosphate dehydrogenase was chosen as the target gene, gene *gap* was chosen in previous a study [Svenstrup *et al*., **2005**].

The hybridization probe format was used for DNA detection and quantitation, providing maximum specificity for product identification.

The specimens were tested for *M. genitalium* by a commercially available (Tib-Molbiol Light Mix Kit and Light Cycler Fast Start DNA Master HybProbe) for detection of DNA from *M. genitalium* (table 3.1).

A 224 bp long fragment of the glyceraldehyde-3-phosphate dehydrogenase (gap) gene of *M. genitalium* was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 (table 3.3). An additional PCR product of 349 bp was formed from the IC DNA.

The Light Cycler PCR reproducibility was able to detect 4 positive samples, (Samples number 30, 36, 58, 98, infertility group).

The species present in the samples were identified through the specific T_m values at 67.1-68.5°C for *M. genitalium* (table 4.23). Melting peaks almost matching T_m values (Mean T_m=67.9) which determine how well the sequence of probes matches the sequence of template DNA (table 4.25).

5.7.5.2 Absolute quantitative real time PCR for *M. genitalium* positive results

To determine the bacterial load in patients' specimens, a quantitative real-time Light Cycler PCR was performed; the provided standard row of cloned DNA with concentrations in the range from 10^6 copies/reaction down to 10^1 copies/reaction, was used as an external reference standard. *M. genitalium* had C_p values between cycles 19 and 36.

After performing data analysis, as described in the Light Cycler Instrument operator's manual. The cycle number of C_ps of each positive sample was calculated automatically.

The sample with the highest template count (sample number 58, infertility group, table 4.27) had a copy number of 114 DNA copies/reaction (22,800 copies/ml).

The sample with the least template count (sample number 36, co-infection with *M. hominis*, infertility group) had a copy number of 4.04 DNA copies/reaction (808 copies/ml), low *M. genitalium* loads of less than 5 copies/reaction might be

because of it is difficult for *M. genitalium*, which requires strict conditions for growth, to colonize and proliferate in the cervix infected with *M. hominis*.

All four clinical samples showing low DNA concentration but when comparing the melting point data, they had melting curves with T_m of 67.1-68.5 (table 4.23) and were therefore considered positives.

Many studies have now found significant associations with lower and upper reproductive tract disease in women. *M. genitalium* should be considered an etiologic agent of cervical inflammation and upper tract disease syndromes, including infertility. Importantly, additional studies with defined diagnostic criteria are necessary in our region to fully understand the relationship between *M. genitalium* and infertility [McGowin and Anderson-Smits, **2011**].

Real-time quantitative PCR is a powerful tool to accurately determine the *M. genitalium* load in clinical samples, because culture is too difficult to be an alternative.

5.8 *M. hominis* findings among infertile and control group

Our result showed that *M. hominis* was present in 4 samples (4%); *M. hominis* was detected from 1 sample as a single infection case from infertile group (1%), 2 cases from control group (2%) (P-value=0.50). Also case as a mix infection with *M. genitalium* was detected among infertile group (1%) (table 4.8, figure 4.8).

The first human *Mycoplasma* isolated was *M. hominis*, [Dienes and Edsall, **1937**] *M. hominis* is commonly found in the genital tract of sexually experienced females and one of the most commonly reported STIs in most of

the Western countries and a role in PID and post-abortal fever had been suggested [Taylor- Robinson, **2007**].

M. hominis is a heterogeneous genital *Mycoplasma* found in at least two-thirds of women with BV, compared to 10% of healthy women. However, its role as a primary pathogen is doubtful since it co-exists with many other bacteria in BV [Taylor-Robinson and Jensen, **2011**].

When comparing our study to studies from other countries, our results are still lower than studies reported in Iran, (26.7%) [Shahin and Sattari, **2006**], and (35.6%) [Badami and Salari, **2001**], Jordan (27.3%) [Al-Daghistani and Abdel-Dayem, **2010**].

From Saudi Arabia, where the incidence of *M. hominis* was assessed by NAATs as well as culture, results showed that by culture *M. hominis* rate was (4%), by PCR *M. hominis* detection rate was (20%) [El-Feky and Baddour, **2009**].

Results of current study are apparently lower than Abdul-Wahab [**2010**] study, in terms of pathogen occurrence, investigators compared *Mycoplasma* commercial kits and a conventional culture system, the number of genital *Mycoplasmas* detected by commercial antibiotic sensitivity kit and culture system was 26% and 17% respectively.

It is noteworthy that our rates were higher than that previously reported in Khamis Mushayt City, Saudi Arabia (0.76%) [Abdul-Wahab and Al Sunaidi, **2013**].

Differences between our study and the others might be due mostly to differences in study population and probably related to the diversity of the detection methods used. These finding deserves further investigation to

determine whether *M. hominis* has a role in disease or just in colonization and to improve the association of infertility with urogenital *M. hominis* infection.

5.8.1 *M. hominis* findings among asymptomatic and symptomatic infertile women and the frequency of most related symptoms

Interestingly, the two positive cases of *M. hominis* from control group were detected from symptomatic patients, whereas the only infertile case with *M. hominis* (2%, P-value= 0.460) was asymptomatic (table 4.10, figure 4.10).

The positivity rate of *M. hominis* infection in asymptomatic sexual-health clinic attendees have been reported [Bacterial Vaginosis].

5.8.2 *M. hominis* findings among infertility types and infertility factors

M. hominis positive case was obtained from a secondary (1/43, 2%, P-value=0.430) unexplained infertility (1/21, 5%) (tables 4.15, 4.16, 4.19).

The correlation between positive *Mycoplasmal* secondary infertility and unexplained factor may suggest a link between *M. hominis* infections during pregnancy and delivery complications and consequent development of unexplained factor infertility. Similar findings in a Turkish population, where the prevalence of *M. hominis* in the cervical canal in unexplained infertile women were compared to healthy controls and prospectively investigated, *Mycoplasmic* infection was detected in one case each in infertile and control patients [Guven *et al.*, **2007**].

5.8.3 *M. hominis* screening program

Culture was the most commonly employed method for detection of genital *Mycoplasma* and was considered the "gold standard" for the detection of genital *Mycoplasmas* from clinical samples [Cheah *et al.*, **2005**], but culture is slow, lacks sensitivity, is prone to contamination with other micro-organisms, special handling, complex media, and cultivation. Furthermore, positive samples need further testing to determine the species and requires repeated observations with light microscopy [Petrikkos *et al.*, **2007**, Cassell and Waites, **1995**].

Comparison between culture method and PCR has been performed and showed that a PCR assay was as sensitive as culture for detection of *M. hominis* from clinical samples. In addition it was very specific [Luki *et al.*, **1998**]. An advantage of using PCR is that the system can detect the presence of both live and dead microorganisms in the sample.

When comparing the original PCR protocols with the developed real-time assay targeting *M. hominis gap* gene which we choose to isolate *M. hominis* from endocervical specimens, it offered interesting advantages such as rapidity, closed system, which eliminates the risk of carry-overs, real-time monitoring of PCR activity, quantification of amplification product [Baczynska *et al*., **2004**].

The real-time technology where measurement of the fluorescence emitted during amplicon production is performed during each PCR cycle is considered as a breakthrough in PCR.

5.8.3.1 Qualitative real-time Light Cycler PCR with hybridization probes for detection of *M. hominis.*

A qualitative real-time Light Cycler PCR assay performed used for the detection of *M. hominis* in endocervical specimens from female patients.

The specimens were tested for *M. hominis* by a commercially available kit (Tib-Molbiol Light Mix Kit and Light Cycler Fast Start DNA Master HybProbe) for detection of DNA from *M. hominis* (table 3.1).

We selected the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*gap*) as target for development of a qualitative and quantitative real-time PCR for the detection of *M. hominis*.

A 129 bp long fragment of the glyceraldehyde-3-phosphate dehydrogenase (gap) gene from *M. hominis* was amplified with specific primers and detected with probes labelled with Light Cycler Red 640 and detected in channel 640 (table 3.3).

An additional PCR product of 349 bp was formed from the IC and detected in channel 705, IC was included in each sample during real time PCR run to control the presence of inhibitors.

Few real-time PCR assays and associated studies have been described for *M. hominis.* A real-time PCR assay targeting *M. hominis gap* gene have been evaluated for infertility [Baczynska *et al*., **2004**; F´erandon *et al*., **2011**].

In the present study, a melting curve analysis was used to determine the presence of specific amplification products.

The Light Cycler PCR reproducibility was able to detect 4 positive samples, (sample number 7, 36 from infertility group, samples number 43, 61 control groups) all our positive samples had melting peaks with T_m values from 61.2^o to 62^o (Mean T_m=61.65, table 4.25), no differences were noticed in the T_m between *M. hominis* isolates which determines how well the sequence of probes matches the sequence of template DNA.

5.8.3.2 Absolute quantitative real time PCR for *M. hominis* positive results

In the present study, the light cycler fast start DNA master hyprobe was able to specifically quantify concentrations of the *M. hominis* gab gene. Quantification of DNA concentrations performed by Light Cycler PCR Software and was based on standard dilution series with known concentrations of genomic *M. hominis* DNA.

The concentrations of standard dilution series were: 10^6, 10^5 10^4, 10^3, 10^2, 10^1 copy/reaction. Using the assay, we were able to quantify *M. hominis* in endocervical specimens' from infertile and control group.

The results were interpreted with Light Cycler 2.0 (Roche Diagnostics). Samples with high DNA load had low C_p values, while low DNA load had high C_p values.

In our study (sample number 43), a 22 year old women from control group complaining from low abdominal pain and irregular menstruation showed the

highest DNA copy number in this study, the observed DNA concentration was 9140000 ($9.14x10^5$) DNA copies/reaction (1,828,000,000 DNA copies/ml).

High loads of *M. hominis* in the endocervix specimens would be associated with inflammation responses in the pelvic, resulting in manifestation of clinical symptoms and signs of PID. Such a possible association of higher *M. hominis* loads with symptoms as pain throughout the lower abdomen and irregular menstruation cycles strengthens the proposition that *M. hominis* is a pathogen of PID [Health Grades Incorporated, **2013**].

Sample number 36(a mix infection with *M. genitalium*, infertility group) had a DNA concentration of 5.50 x 102 DNA copies/ reaction (1,100,000 copies/ml). What was interesting about this mix infection case in particular is that it was a male factor infertility (table 4.15) These observations agree with several studies that have associated between these two pathogens as a mixed infections and male infertility (Al-sweih *et al*., **2012**).

Among infertility group, sample number 7, secondary unexplained infertility case, asymptomatic, had the least template count: 117 DNA copies/reaction (23,400 DNA copies/ml). Such infections might not give rise to symptoms until illness, infertility or reproductive loss was to occur. In this connection, the clinical manifestations might be either minimal or even absent [Badami and Salari, ***2001****]*.

Conclusion

- *C. trachomatis and Mycpolasama* are Infectious agents that can damage biological functions of the female reproductive tract with devastating consequences like infertility and preterm delivery, but they are usually not detected by routine microbiological diagnosis.
- This case control study is considered the first in the city of Riyadh, Saudi, to screen infertile women for *C. trachomatis, M. genitalium, and, M. hominis* infections as a possible causative factor for their infertility. We hope that the present study could demonstrate the incidence and the importance of these pathogens in our society in order to establish a national standardized treatment protocol for our patients.
- *C. trachomatis* was the most frequent pathogen detected in present study. Statistical analysis confirmed that there was significant deference in the presence of *C. trachomatis* between infertile and control group. This finding calls for a national screening program for the early detection of *C. trachomatis* infection among infertile couples in Saudi Arabia.
- There is a high incidence of *Chlamydia* infection among Saudi infertile women and a high relationship between *Chlamydia* infection and unexplained infertility.
- The results obtained in this study showed that *M. genitalium* was less frequently detected from infertilility group, although it was not statistically significant ,but indicating an association between this infection and infertility. Therefore, screening of women for *M. genitalium* infection is recommended as part of investigations for infertility.
- This study showed low detection rate of *M. hominis* among infertility and control group. Further studies for detecting genital *Mycoplasma* are needed to determine the possible implication of those organisms in various genital infections.

- We observed a significantly maximum number of infertile females in the ages of 26 - 39 years indicating a shift in the fertility potential age, this gradual loss of fertility in 20s and 30s as observed in our study opens the door for further studies on age and its effect on fertility in women in our region.
- Signs of cervicitis were presented only among infertility group with a statistically significant difference, which revealed a correlation between cervicitis and infertility.
- The finding of a significantly raised rate of hormonal factor infertility, indicating that women with hormonal factor have a higher risk for infertility than women with other factors.
- We found that *C. trachomatis* detection was statistically higher among infertile women with low-seated abdominal pain, indicating an association with this symptom.
- We have performed a qualitative, specific Light Cycler protocol using specific hybridization probes, which may prove to be a rapid alternative to the traditional cultivation method.
- Information on bacterial load in genital swabs was also obtained by a rapid quantitative real-time PCR. The final amplification and quantification was performed in closed tubes, which reduces the risk of contamination by amplicon carry-over contamination.
- Future studies on *C. trachomatis*, *M. genitalium*, and *M .hominis* should consider larger sample sizes to allow for identification of the predictors of infection, this will add key data to our knowledge of STIs in this part of the world.

Chapter (6)

References

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Chapter (7)

Appendix

Table 7.1 Shows 100 endocervical specimen results from infertility group.

Patient information							Infertility factors				
S.No	Population Group	Hosp. No	Age	Age Range	Nat	Type of Infertility	Hormonal	Ovarian	Tubal	Male factor	Unexplained
1	Infertility	618570	37	33-39	Saudi	Secondary	Yes	No	No	No	No
2	Infertility	1106365	22	19-25	Saudi	Primary	Yes	No	No	No	No
3	Infertility	533442	46	40-46	Saudi	Secondary	No	No	No	Yes	No
4	Infertility	533140	26	26-32	Saudi	Primary	No	No	No	Yes	No
5	Infertility	1107349	23	19-25	Saudi	Primary	No	No	No	Yes	No
6	Infertility	118389	36	33-39	Saudi	Primary	Yes	No	No	No	No
7	Infertility	494413	45	40-46	Saudi	Secondary	No	No	No	No	Yes
8	Infertility	28842	28	26-32	Saudi	Primary	Yes	No	No	No	No
9	Infertility	1029456	32	26-32	Saudi	Primary	No	Yes	No	No	No
10	Infertility	1082562	24	19-25	Saudi	Primary	Yes	No	No	No	No
11	Infertility	830988	31	26-32	Saudi	Primary	No	No	Yes	No	No
12	Infertility	1036189	26	26-32	Saudi	Primary	No	No	No	No	Yes
13	Infertility	5005033	34	33-39	Saudi	Primary	No	No	Yes	No	No
14	Infertility	1094031	32	26-32	Saudi	Secondary	Yes	No	No	No	No
15	Infertility	577813	29	26-32	Saudi	Primary	No	Yes	No	No	No
16	Infertility	1057806	35	33-39	Saudi	Secondary	No	Yes	No	No	No
17	Infertility	951700	35	33-39	Saudi	Secondary	Yes	No	No	No	No
18	Infertility	1080181	29	26-32	Saudi	Primary	No	No	No	No	Yes
19	Infertility	300889	34	33-39	Saudi	Primary	No	No	Yes	No	No
20	Infertility	536310	33	33-39	Saudi	Secondary	Yes	No	No	No	No
21	Infertility	24436	36	33-39	Saudi	Primary	No	No	Yes	No	No

S.No	Population Group	Hosp. No	Age	Age Range	Nat	Type of Infertility	Hormonal	Ovarian	Tubal	Male factor	Unexplained
22	Infertility	573336	24	19-25	Saudi	Secondary	No	No	No	No	Yes
23	Infertility	774736	27	26-32	Saudi	Primary	No	Yes	No	No	No
24	Infertility	949295	35	33-39	Saudi	Secondary	Yes	No	No	No	No
25	Infertility	89287	32	26-32	Saudi	Secondary	No	No	No	Yes	No
26	Infertility	846167	42	40-46	Saudi	Secondary	No	Yes	No	No	No
27	Infertility	1006083	31	26-32	Saudi	Primary	No	No	No	No	Yes
28	Infertility	1103188	27	26-32	Saudi	Secondary	Yes	No	No	No	No
29	Infertility	1006207	41	40-46	Saudi	Secondary	No	Yes	No	No	No
30	Infertility	1104860	29	26-32	Saudi	Primary	No	No	No	No	Yes
31	Infertility	612885	30	26-32	Saudi	Primary	Yes	No	No	No	No
32	Infertility	859848	40	40-46	Saudi	Secondary	No	No	No	No	Yes
33	Infertility	947046	28	26-32	Saudi	Primary	No	Yes	No	No	No
34	Infertility	972499	36	33-39	Saudi	Primary	No	Yes	No	No	No
35	Infertility	1043067	37	33-39	Saudi	Secondary	No	No	Yes	No	No
36	Infertility	954939	40	40-46	Saudi	Secondary	No	No	No	Yes	No
37	Infertility	1093915	46	40-46	Saudi	Secondary	Yes	No	No	No	No
38	Infertility	990063	28	26-32	Saudi	Primary	No	Yes	No	No	No
39	Infertility	220640	24	19-25	Saudi	Primary	No	No	No	No	Yes
40	Infertility	1014250	38	33-39	Saudi	Primary	No	No	No	No	Yes
41	Infertility	997290	32	26-32	Indian	Primary	No	Yes	No	No	No
42	Infertility	1028081	26	26-32	Saudi	Primary	No	Yes	No	No	No
43	Infertility	957767	35	33-39	Saudi	Secondary	No	Yes	No	No	No
44	Infertility	995903	32	26-32	Saudi	Primary	No	No	No	Yes	No
45	Infertility	802736	27	26-32	Saudi	Primary	No	No	Yes	No	No
46	Infertility	1036360	25	19-25	Saudi	Primary	Yes	No	No	No	No
47	Infertility	371422	39	33-39	Saudi	Secondary	No	Yes	No	No	No
48	Infertility	1044717	40	40-46	Saudi	Secondary	No	No	No	No	Yes

S.No	Population Group	Hosp. No	Age	Age Range	Nat	Type of Infertility	Hormonal	Ovarian	Tubal	Male factor	Unexplained
49	Infertility	948362	37	33-39	Saudi	Secondary	No	No	Yes	No	No
50	Infertility	1045593	36	33-39	Saudi	Secondary	Yes	No	No	No	No
51	Infertility	1093646	29	26-32	Saudi	Primary	No	Yes	No	No	No
52	Infertility	954484	30	26-32	Saudi	Primary	Yes	No	No	No	No
53	Infertility	924778	26	26-32	Saudi	Secondary	Yes	No	No	No	No
54	Infertility	909315	24	19-25	Saudi	Secondary	No	No	Yes	No	No
55	Infertility	354520	39	33-39	Saudi	Secondary	No	No	Yes	No	No
56	Infertility	753958	29	26-32	Saudi	Primary	No	Yes	No	No	No
57	Infertility	811517	23	19-25	Saudi	Primary	Yes	No	No	No	No
58	Infertility	950715	23	19-25	Saudi	Primary	No	No	Yes	No	No
59	Infertility	443481	36	33-39	Saudi	Secondary	No	No	Yes	No	No
60	Infertility	681710	23	19-25	Saudi	Primary	Yes	No	No	No	No
61	Infertility	928755	25	19-25	Saudi	Primary	No	Yes	No	No	No
62	Infertility	444923	38	33-39	Saudi	Primary	No	No	No	Yes	No
63	Infertility	1054733	24	19-25	Saudi	Secondary	No	No	No	No	Yes
64	Infertility	684077	35	33-39	Saudi	Primary	Yes	No	No	No	No
65	Infertility	1049622	26	26-32	Saudi	Primary	No	Yes	No	No	No
66	Infertility	748992	30	26-32	Saudi	Secondary	No	No	No	No	Yes
67	Infertility	1049346	41	40-46	Saudi	Secondary	No	No	No	Yes	No
68	Infertility	763548	29	26-32	Saudi	Secondary	No	No	No	No	Yes
69	Infertility	687583	35	33-39	Saudi	Secondary	No	No	Yes	No	No
70	Infertility	844690	27	26-32	Saudi	Primary	Yes	No	No	No	No
71	Infertility	778702	33	33-39	Saudi	Primary	Yes	No	No	No	No
72	Infertility	1073540	19	19-25	Saudi	Primary	Yes	No	No	No	No
73	Infertility	1055234	42	40-46	Etopian	Primary	No	No	No	No	Yes
74	Infertility	846267	36	33-39	Saudi	Secondary	No	Yes	No	No	No
75	Infertility	1076051	38	33-39	Saudi	Primary	No	No	No	No	Yes

S.No	Population Group	Hosp. No	Age	Age Range	Nat	Type of Infertility	Hormonal	Ovarian	Tubal	Male factor	Unexplained
76	Infertility	830564	36	33-39	Saudi	Secondary	No	No	Yes	No	No
77	Infertility	721575	33	33-39	Saudi	Primary	No	Yes	No	No	No
78	Infertility	1092709	36	33-39	Saudi	Primary	No	No	No	Yes	No
79	Infertility	1085803	28	26-32	Saudi	Primary	Yes	No	No	No	No
80	Infertility	885842	39	33-39	Morocan	Secondary	No	Yes	No	No	No
81	Infertility	1053502	25	19-25	Saudi	Primary	Yes	No	No	No	No
82	Infertility	920503	28	26-32	Saudi	Primary	No	No	No	No	Yes
83	Infertility	1095502	43	40-46	Saudi	Primary	No	No	No	No	Yes
84	Infertility	325838	37	33-39	Saudi	Secondary	No	No	Yes	No	No
85	Infertility	643436	34	33-39	Saudi	Secondary	Yes	No	No	No	No
86	Infertility	1055921	34	33-39	Saudi	Secondary	No	Yes	No	No	No
87	Infertility	481736	36	33-39	Saudi	Secondary	No	Yes	No	No	No
88	Infertility	651201	32	26-32	Saudi	Primary	Yes	No	No	No	No
89	Infertility	824778	34	33-39	Saudi	Secondary	No	No	No	Yes	No
90	Infertility	1067897	21	19-25	Saudi	Primary	Yes	No	No	No	No
91	Infertility	641332	32	26-32	Saudi	Secondary	No	No	Yes	No	No
92	Infertility	1060112	46	40-46	Pakistan	Secondary	No	No	No	Yes	No
93	Infertility	11095942	42	40-46	Saudi	Primary	No	No	No	No	Yes
94	Infertility	596140	38	33-39	Saudi	Secondary	No	No	No	No	Yes
95	Infertility	1069321	40	40-46	Saudi	Primary	No	Yes	No	No	No
96	Infertility	457305	42	40-46	Saudi	Secondary	No	No	No	Yes	No
97	Infertility	1092618	37	33-39	Saudi	Primary	No	No	No	No	Yes
98	Infertility	1020378	21	19-25	Yemeni	Primary	No	No	No	No	Yes
99	Infertility	409172	30	26-32	Saudi	Primary	Yes	No	No	No	No
100	Infertility	1096797	29	26-32	Saudi	Primary	Yes	No	No	No	No

S.No	Positive on	Have you had any of the following problems?					
		History in STI	Burning sensation during urination	Genital Bleeding	Irregular menstruation	Abnormal Discharge?	Low-seated abdominal pain?
1	Neg	No	No	Yes	No	No	Yes
2	Neg	No	No	No	No	Yes	No
3	Neg	No	No	No	No	No	Yes
4	Neg	No	No	No	No	No	No
5	Neg	No	Yes	No	No	No	No
6	Neg	No	No	No	No	Yes	Yes
7	*M. hominis*	No	No	No	No	No	No
8	Neg	No	No	No	No	No	No
9	Neg	No	No	No	No	No	No
10	Neg	No	No	No	No	No	No
11	Neg	No	No	No	No	No	No
12	Neg	No	No	No	No	No	Yes
13	*C. trachomatis*	No	No	No	No	No	Yes
14	Neg	No	Yes	Yes	No	No	Yes
15	Neg	No	No	No	No	No	No
16	Neg	No	Yes	No	No	No	Yes
17	Neg	No	No	No	No	Yes	No
18	Neg	No	No	No	No	No	No
19	Neg	No	Yes	Yes	No	No	No
20	Neg	No	No	No	No	Yes	No
21	Neg	No	No	No	No	No	No
22	*C. trachomatis*	No	No	No	No	Yes	No

S.No	Positive on	Have you had any of the following problems?					
		History in STI	Burning sensation during urination	Genital Bleeding	Irregular menstruation	Abnormal Discharge?	Low-seated abdominal pain?
23	Neg	No	No	No	No	No	No
24	Neg	No	No	No	No	No	No
25	Neg	No	No	No	No	No	No
26	Neg	No	Yes	No	No	No	No
27	Neg	No	No	No	No	No	No
28	Neg	No	No	No	No	Yes	Yes
29	Neg	No	No	No	No	Yes	No
30	*M. genitalium*	No	No	No	No	No	No
31	Neg	No	No	No	No	No	No
32	Neg	No	No	No	No	No	No
33	Neg	No	No	No	No	No	No
34	Neg	No	No	No	Yes	Yes	No
35	Neg	No	No	No	Yes	No	Yes
36	*M. hominis & M. genitalium*	No	No	No	No	Yes	No
37	Neg	No	No	No	No	No	No
38	Neg	No	No	No	Yes	Yes	No
39	*C. trachomatis*	No	No	No	Yes	No	Yes
40	Neg	No	No	No	No	No	No
41	Neg	No	No	No	No	No	No
42	Neg	No	Yes	No	No	No	No
43	Neg	No	No	No	No	No	No
44	Neg	No	Yes	No	No	No	Yes
45	*C. trachomatis*	No	No	No	No	No	Yes
46	Neg	No	Yes	No	No	No	Yes

S.No	Positive on	Have you had any of the following problems?					
		History in STI	Burning sensation during urination	Genital Bleeding	Irregular menstruation	Abnormal Discharge?	Low-seated abdominal pain?
47	Neg	No	No	No	No	No	No
48	Neg	No	No	No	No	Yes	No
49	Neg	No	Yes	Yes	No	No	Yes
50	Neg	No	Yes	No	No	No	No
51	Neg	No	No	No	No	No	No
52	Neg	No	No	No	No	No	No
53	Neg	No	Yes	No	No	No	Yes
54	Neg	No	No	Yes	No	Yes	Yes
55	Neg	No	No	No	No	No	No
56	Neg	No	No	No	No	No	No
57	Neg	No	No	No	No	No	No
58	*M. genitalium*	No	No	No	Yes	No	No
59	Neg	No	No	No	No	No	Yes
60	Neg	No	No	No	No	No	No
61	Neg	No	No	No	No	No	No
62	Neg	No	No	No	Yes	Yes	No
63	*C. trachomatis*	No	No	No	No	No	Yes
64	Neg	No	No	No	No	No	No
65	Neg	No	No	No	No	No	No
66	Neg	No	No	No	No	No	No
67	Neg	No	No	No	No	No	Yes
68	Neg	No	No	Yes	No	Yes	No
69	Neg	No	No	Yes	No	No	No
70	Neg	No	No	No	No	No	No

S.No	Positive on	Have you had any of the following problems?					
		History in STI	Burning sensation during urination	Genital Bleeding	Irregular menstruation	Abnormal Discharge?	Low-seated abdominal pain?
71	Neg	No	No	No	No	No	No
72	Neg	No	No	No	No	No	No
73	Neg	No	No	No	No	No	No
74	Neg	No	No	No	No	No	No
75	Neg	No	Yes	No	No	No	No
76	Neg	No	No	No	No	Yes	Yes
77	Neg	No	No	No	No	Yes	No
78	Neg	No	No	No	No	No	No
79	Neg	No	No	No	No	No	No
80	Neg	No	No	No	No	No	Yes
81	Neg	No	No	No	No	No	No
82	Neg	No	No	No	No	No	No
83	*C. trachomatis*	No	No	No	No	No	No
84	Neg	No	No	No	No	No	No
85	Neg	No	No	No	No	No	No
86	Neg	No	No	No	No	No	Yes
87	Neg	No	Yes	Yes	No	Yes	No
88	Neg	No	No	Yes	No	No	No
89	*C. trachomatis*	No	Yes	No	No	No	Yes
90	Neg	No	Yes	No	No	No	No
91	Neg	No	No	No	No	No	No
92	Neg	No	No	No	No	Yes	No
93	*C. trachomatis*	No	No	No	No	No	No
94	Neg	No	No	Yes	No	No	Yes

S.No	Positive on	Have you had any of the following problems?					
		History in STI	Burning sensation during urination	Genital Bleeding	Irregular menstruation	Abnormal Discharge?	Low-seated abdominal pain?
95	Neg	No	No	No	No	No	No
96	Neg	No	No	No	No	No	No
97	Neg	No	No	Yes	Yes	Yes	Yes
98	*M. genitalium*	No	Yes	No	Yes	Yes	No
99	Neg	No	No	No	No	Yes	Yes
100	Neg	No	No	No	No	No	No

S. No.	Have you had any of the following problems?				Completed by the examining doctor	
	Previous abortion	Pelvic inflammatory disease?	Etopic pregnancy?	Premature delivery and birth weight?	Signs of vaginosis?	Signs of cervicitis?
1	No	No	No	No	No	No
2	No	No	Yes	No	No	No
3	No	No	No	No	No	No
4	No	No	No	No	No	No
5	No	No	No	No	No	No
6	No	No	No	No	No	Yes
7	No	No	No	No	No	No
8	No	No	No	No	No	No
9	No	No	No	No	No	No
10	No	No	No	No	No	No
11	No	No	No	No	No	No
12	No	No	No	No	No	No
13	No	No	No	No	No	No
14	No	No	No	No	Yes	No
15	No	No	No	No	No	No
16	No	No	No	Yes	No	No
17	No	No	No	No	No	No
18	No	No	No	No	No	No
19	No	No	No	No	No	No
20	No	No	No	No	No	No
21	No	No	No	No	No	No
22	No	No	No	No	No	No
23	No	No	No	No	No	No

S. No.	Have you had any of the following problems?				Completed by the examining doctor	
	Previous abortion	Pelvic inflammatory disease?	Etopic pregnancy?	Premature delivery and birth weight?	Signs of vaginosis?	Signs of cervicitis?
24	No	No	No	No	No	No
25	No	No	No	No	No	No
26	No	No	No	No	No	No
27	No	No	No	No	No	No
28	No	No	No	Yes	No	No
29	No	No	No	No	No	No
30	No	No	No	No	No	Yes
31	No	No	No	No	No	No
32	No	No	No	No	No	No
33	No	No	No	No	No	No
34	No	No	No	No	No	Yes
35	No	No	No	No	No	No
36	No	No	No	No	No	No
37	No	No	No	No	No	No
38	No	No	No	No	No	No
39	No	No	No	No	No	No
40	No	No	No	No	No	No
41	No	No	No	No	No	No
42	No	No	No	No	No	No
43	No	No	No	No	No	No
44	No	No	No	No	No	No
45	No	No	No	No	No	No
46	No	No	No	No	No	No
47	No	No	No	No	No	No
48	No	No	No	No	No	No

S. No.	Have you had any of the following problems?				Completed by the examining doctor	
	Previous abortion	Pelvic inflammatory disease?	Etopic pregnancy?	Premature delivery and birth weight?	Signs of vaginosis?	Signs of cervicitis?
49	No	No	No	No	No	No
50	No	No	No	No	No	No
51	No	No	No	No	No	No
52	No	No	No	No	No	No
53	No	No	No	No	No	No
54	No	No	No	Yes	No	No
55	No	No	No	No	No	Yes
56	No	No	No	No	No	No
57	No	No	No	No	No	No
58	No	No	No	No	No	No
59	No	No	No	Yes	No	No
60	No	No	No	No	No	No
61	No	No	No	No	No	No
62	No	No	No	No	No	No
63	2 abortion	No	No	No	No	No
64	No	No	No	No	No	No
65	No	No	No	No	No	No
66	No	No	No	No	No	No
67	No	No	No	No	No	No
68	No	No	No	No	No	No
69	No	No	Yes	No	No	No
70	No	No	No	No	No	No
71	No	No	No	No	No	No
72	No	No	No	No	No	No
73	No	No	No	No	No	No

S. No.	Have you had any of the following problems?				Completed by the examining doctor	
	Previous abortion	Pelvic inflammatory disease?	Etopic pregnancy?	Premature delivery and birth weight?	Signs of vaginosis?	Signs of cervicitis?
74	No	No	No	No	No	No
75	No	No	No	No	No	No
76	No	No	No	No	No	No
77	No	No	No	No	No	Yes
78	No	No	No	No	No	No
79	No	No	No	No	No	No
80	No	No	No	No	No	No
81	No	No	No	No	No	No
82	No	No	No	No	No	No
83	No	No	No	No	No	No
84	No	No	No	No	No	No
85	No	No	No	Yes	No	No
86	No	No	No	No	No	No
87	No	No	No	No	No	No
88	No	No	No	No	No	No
89	No	No	No	No	No	No
90	No	No	No	No	No	No
91	No	No	No	No	No	No
92	No	No	No	No	No	No
93	No	No	No	No	No	No
94	No	No	No	No	No	No
95	No	No	No	No	No	No
96	No	No	No	No	No	No
97	No	Yes	No	No	No	No
98	No	No	No	No	No	No

S. No.	Have you had any of the following problems?				Completed by the examining doctor	
	Previous abortion	Pelvic inflammatory disease?	Etopic pregnancy?	Premature delivery and birth weight?	Signs of vaginosis?	Signs of cervicitis?
99	No	No	No	No	No	Yes
100	No	No	No	No	No	No

Table 7.2 Shows 100 endocervical specimen results from control group.

Patient information						Positive on	Have you had any of the following problems?		
S.No	Population Group	Hosp. No	Age	Age Range	Nat		History in STI	Burning sensation during urination	Genital Bleeding
1	Control	458282	24	19-25	Saudi	Neg	No	No	No
2	Control	452949	25	19-25	Saudi	Neg	No	No	No
3	Control	56267	43	40-46	Saudi	Neg	No	No	No
4	Control	382328	31	26-32	Saudi	Neg	No	No	No
5	Control	407032	28	26-32	Saudi	Neg	No	No	No
6	Control	227994	23	19-25	Saudi	Neg	No	No	No
7	Control	554777	39	33-39	Saudi	Neg	No	No	No
8	Control	186948	45	40-46	Saudi	Neg	No	No	No
9	Control	176131	35	33-39	Saudi	Neg	No	No	No
10	Control	133704	38	33-39	Saudi	Neg	No	No	No
11	Control	31480	44	40-46	Saudi	Neg	No	No	No
12	Control	174679	45	40-46	Saudi	Neg	No	No	No
13	Control	552147	23	19-25	Saudi	Neg	No	Yes	No
14	Control	450246	29	26-32	Saudi	Neg	No	Yes	Yes
15	Control	259911	38	33-39	Saudi	Neg	No	Yes	No
16	Control	466159	32	26-32	Saudi	Neg	No	Yes	No
17	Control	310959	41	40-46	Saudi	Neg	No	No	No
18	Control	21383	44	40-46	Saudi	Neg	No	No	No
19	Control	280376	19	19-25	Saudi	Neg	No	No	No
20	Control	151093	43	40-46	Saudi	Neg	No	No	No
21	Control	390009	23	19-25	Saudi	Neg	No	No	No
22	Control	425906	33	33-39	Saudi	Neg	No	No	No
23	Control	295863	24	19-25	Saudi	Neg	No	Yes	No
24	Control	383018	44	40-46	Saudi	Neg	No	No	Yes

Patient information						Positive on	Have you had any of the following problems?		
S.No	Population Group	Hosp. No	Age	Age Range	Nat		History in STI	Burning sensation during urination	Genital Bleeding
25	Control	559804	41	40-46	Saudi	Neg	No	No	No
26	Control	92719	45	40-46	Saudi	Neg	No	No	No
27	Control	164404	43	40-46	Saudi	Neg	No	No	Yes
28	Control	265239	33	33-39	Saudi	Neg	No	No	No
29	Control	550612	45	40-46	Saudi	Neg	No	No	No
30	Control	486885	22	19-25	Saudi	Neg	No	No	No
31	Control	197221	35	33-39	Saudi	Neg	No	No	No
32	Control	173198	44	40-46	Saudi	Neg	No	No	No
33	Control	218926	41	40-46	Saudi	Neg	No	Yes	No
34	Control	208926	43	40-46	Saudi	Neg	No	Yes	No
35	Control	262918	45	40-46	Saudi	Neg	No	Yes	No
36	Control	221014	44	40-46	Saudi	Neg	No	No	Yes
37	Control	221114	37	33-39	Saudi	Neg	No	No	No
38	Control	551789	30	26-32	Saudi	Neg	No	No	No
39	Control	427768	33	33-39	Filipino	Neg	No	No	No
40	Control	291690	40	40-46	Saudi	Neg	No	Yes	Yes
41	Control	542843	34	33-39	Saudi	Neg	No	No	No
42	Control	351239	44	40-46	Saudi	Neg	No	Yes	No
43	Control	435180	22	19-25	Saudi	*M. hominis*	No	No	No
44	Control	91828	40	40-46	Saudi	Neg	No	No	No
45	Control	261690	22	19-25	Saudi	Neg	No	Yes	No
46	Control	51187	45	40-46	Saudi	Neg	No	Yes	Yes
47	Control	462337	44	40-46	Saudi	Neg	No	No	No
48	Control	138191	42	40-46	Saudi	Neg	No	Yes	No
49	Control	235279	45	40-46	Saudi	Neg	No	No	No
50	Control	555145	25	19-25	Saudi	Neg	No	No	No

S.No	Patient information					Positive on	Have you had any of the following problems?		
	Population Group	Hosp. No	Age	Age Range	Nat		History in STI	Burning sensation during urination	Genital Bleeding
51	Control	557854	43	40-46	Saudi	Neg	No	No	No
52	Control	107563	26	26-32	Saudi	Neg	No	No	Yes
53	Control	410377	44	40-46	Filipino	Neg	No	No	No
54	Control	499414	38	33-39	Saudi	Neg	No	No	No
55	Control	34478	40	40-46	Saudi	Neg	No	Yes	No
56	Control	541600	40	40-46	Saudi	Neg	No	Yes	No
57	Control	441945	21	19-25	Saudi	Neg	No	No	Yes
58	Control	4573	42	40-46	Saudi	Neg	No	No	No
59	Control	364414	43	40-46	Saudi	Neg	No	No	Yes
60	Control	119323	44	40-46	Saudi	Neg	No	No	No
61	Control	448778	37	33-39	Saudi	*M. hominis*	No	Yes	No
62	Control	197330	45	40-46	Saudi	Neg	No	Yes	No
63	Control	311742	37	33-39	Saudi	Neg	No	No	No
64	Control	300879	31	26-32	Saudi	Neg	No	No	No
65	Control	47654	43	40-46	Saudi	Neg	No	No	No
66	Control	545120	38	33-39	Saudi	Neg	No	No	No
67	Control	138662	41	40-46	Saudi	Neg	No	No	No
68	Control	406414	45	40-46	Saudi	Neg	No	No	No
69	Control	174150	34	33-39	Saudi	Neg	No	No	No
70	Control	210376	45	40-46	Saudi	Neg	No	Yes	No
71	Control	384679	45	40-46	Filipino	Neg	No	No	No
72	Control	13190	45	40-46	Saudi	Neg	No	No	No
73	Control	16988	44	40-46	Saudi	Neg	No	No	No
74	Control	274585	42	40-46	Filipino	Neg	No	Yes	No
75	Control	106968	46	40-46	Indian	Neg	No	No	No
76	Control	409595	23	19-25	Saudi	Neg	No	No	No

S.No	Patient information					Positive on	Have you had any of the following problems?		
	Population Group	Hosp. No	Age	Age Range	Nat		History in STI	Burning sensation during urination	Genital Bleeding
77	Control	147430	45	40-46	Saudi	Neg	No	No	No
78	Control	554915	25	19-25	Saudi	Neg	No	No	No
79	Control	394740	21	19-25	Saudi	Neg	No	No	No
80	Control	539945	42	40-46	Saudi	Neg	No	No	No
81	Control	322097	30	26-32	Saudi	Neg	No	No	No
82	Control	534790	21	19-25	Saudi	Neg	No	No	No
83	Control	52678	27	26-32	Saudi	Neg	No	No	No
84	Control	413282	42	40-46	Saudi	Neg	No	No	No
85	Control	444934	44	40-46	Saudi	Neg	No	No	No
86	Control	81473	46	40-46	Saudi	Neg	No	No	No
87	Control	303337	42	40-46	Saudi	Neg	No	No	No
88	Control	412545	43	40-46	Saudi	Neg	No	No	No
89	Control	516946	46	40-46	Egyptian	Neg	No	No	No
90	Control	331346	27	26-32	Saudi	Neg	No	No	No
91	Control	531146	30	26-32	Saudi	Neg	No	Yes	No
92	Control	281319	30	26-32	Saudi	Neg	No	No	No
93	Control	279801	45	40-46	Saudi	Neg	No	No	No
94	Control	371433	37	33-39	Saudi	Neg	No	No	No
95	Control	241499	35	33-39	Saudi	Neg	No	No	No
96	Control	189678	44	40-46	Saudi	Neg	No	Yes	No
97	Control	291106	45	40-46	Saudi	Neg	No	Yes	No
98	Control	266743	45	40-46	Saudi	Neg	No	Yes	No
99	Control	559011	21	19-25	Saudi	*C. trachomatis*	No	No	No
100	Control	474082	30	26-32	Saudi	Neg	No	Yes	No

S.No	Have you had any of the following problems?						
	Irregular menstruation	Abnormal Discharge?	Low-seated abdominal pain?	Previous abortion	Pelvic inflammatory disease?	Ectopic pregnancy?	Premature delivery and birth weight?
1	No	Yes	No	No	No	No	No
2	No	No	No	No	No	No	No
3	Yes	No	No	No	No	No	No
4	No	No	No	No	No	No	No
5	No	No	No	No	No	No	No
6	Yes	No	No	No	No	No	No
7	No	No	No	No	No	No	No
8	Yes	No	No	No	No	No	No
9	No	No	No	No	No	No	No
10	No	No	No	No	No	No	No
11	No	No	No	No	No	No	No
12	No	No	No	No	No	No	No
13	No	No	No	No	No	No	No
14	No	Yes	Yes	No	No	No	No
15	No	Yes	No	No	No	No	No
16	No	No	No	No	No	No	No
17	No	No	No	No	No	No	No
18	No	No	No	No	No	No	No
19	No	No	No	No	No	No	No
20	No	No	No	No	No	No	No
21	Yes	Yes	No	No	No	No	No
22	No	No	No	No	No	No	No
23	No	Yes	Yes	No	No	No	No
24	No	No	Yes	No	No	No	Yes
25	No	No	No	No	No	No	Yes

S.No	Have you had any of the following problems?						
	Irregular menstruation	Abnormal Discharge?	Low-seated abdominal pain?	Previous abortion	Pelvic inflammatory disease?	Ectopic pregnancy?	Premature delivery and birth weight?
26	Yes	No	No	No	No	No	No
27	No	No	No	No	No	No	No
28	No	No	No	No	No	No	No
29	No	No	No	No	No	No	No
30	No	No	No	No	No	No	No
31	No	No	Yes	No	No	No	No
32	No	No	Yes	No	No	No	No
33	No	No	No	No	No	No	No
34	No	No	No	No	No	No	No
35	No	No	No	No	No	No	No
36	No	No	No	No	No	No	No
37	Yes	No	No	No	No	No	No
38	No	No	No	No	No	No	No
39	No	Yes	No	No	No	No	No
40	No	No	Yes	No	No	No	No
41	No	No	Yes	No	No	No	No
42	No	No	Yes	No	No	No	No
43	Yes	No	Yes	No	No	No	No
44	No	No	No	No	No	No	No
45	No	Yes	Yes	No	No	No	Yes
46	No	Yes	Yes	No	No	No	No
47	No	No	Yes	No	No	No	No
48	No	No	Yes	No	No	No	No
49	No	No	No	No	No	No	No

S.No	Have you had any of the following problems?						
	Irregular menstruation	Abnormal Discharge?	Low-seated abdominal pain?	Previous abortion	Pelvic inflammatory disease?	Ectopic pregnancy?	Premature delivery and birth weight?
50	No	No	No	No	No	No	No
51	No	No	No	No	No	No	No
52	No	No	Yes	No	No	No	No
53	No	No	Yes	No	No	No	No
54	No	No	No	No	No	No	No
55	No	Yes	Yes	No	No	No	No
56	No	No	No	No	No	No	No
57	No	No	No	No	No	No	No
58	No	No	No	No	No	No	No
59	No	No	No	No	No	No	No
60	No	No	No	No	No	No	No
61	No	Yes	No	No	No	No	No
62	No	No	No	No	No	No	No
63	No	No	No	No	No	No	No
64	No	No	No	No	No	No	No
65	No	No	No	No	No	No	No
66	No	No	No	No	No	No	No
67	No	No	No	No	No	No	No
68	No	No	No	No	No	No	No
69	No	No	No	No	No	No	No
70	No	Yes	No	No	No	No	No
71	No	No	No	No	No	No	No
72	No	No	No	No	No	No	No
73	No	No	No	No	No	No	No
74	No	Yes	No	No	No	No	No

S.No	Irregular menstruation	Abnormal Discharge?	Low-seated abdominal pain?	Previous abortion	Pelvic inflammatory disease?	Ectopic pregnancy?	Premature delivery and birth weight?
75	Yes	No	No	No	No	No	No
76	No	Yes	Yes	No	No	No	Yes
77	No	No	No	No	No	No	No
78	No	No	No	No	No	No	No
79	No	No	No	No	No	No	No
80	No	No	No	No	No	No	No
81	No	No	No	No	No	No	No
82	No	No	No	No	No	No	No
83	Yes	Yes	No	No	No	No	No
84	No	No	No	No	No	No	No
85	Yes	Yes	Yes	No	No	No	No
86	No	No	No	No	No	No	No
87	No	No	No	No	No	No	No
88	No	No	No	No	No	No	No
89	Yes	No	No	No	No	No	No
90	No	No	No	No	No	No	No
91	No	No	Yes	No	No	No	Yes
92	No	No	Yes	No	No	No	No
93	Yes	No	Yes	No	No	No	No
94	No	No	Yes	No	No	No	Yes
95	No	No	Yes	No	No	No	No
96	No	No	No	No	No	No	No
97	No	No	No	No	No	No	No
98	No	Yes	Yes	No	No	No	No
99	Yes	No	Yes	No	No	No	No
100	Yes	No	No	No	No	No	No

S.No	Signs of vaginosis?	Signs of cervicitis?
1	No	No
2	No	No
3	No	No
4	No	No
5	No	No
6	No	No
7	No	No
8	No	No
9	No	No
10	No	No
11	No	No
12	No	No
13	No	No
14	No	No
15	No	No
16	No	No
17	No	No
18	No	No
19	No	No
20	No	No
21	No	No
22	No	No
23	No	No
24	No	No
25	No	No
26	No	No
27	No	No

S.No	Signs of vaginosis?	Signs of cervicitis?
28	No	No
29	No	No
30	No	No
31	No	No
32	No	No
33	No	No
34	No	No
35	No	No
36	No	No
37	No	No
38	No	No
39	No	No
40	No	No
41	No	No
42	No	No
43	No	No
44	No	No
45	No	No
46	No	No
47	No	No
48	No	No
49	No	No
50	No	No
51	No	No
52	No	No
53	No	No
54	No	No

S.No	Signs of vaginosis?	Signs of cervicitis?
55	No	No
56	No	No
57	No	No
58	No	No
59	No	No
60	No	No
61	No	No
62	No	No
63	No	No
64	No	No
65	No	No
66	No	No
67	No	No
68	No	No
69	No	No
70	No	No
71	No	No
72	No	No
73	No	No
74	No	No
75	No	No
76	No	No
77	No	No
78	No	No
79	No	No
80	No	No
81	No	No

S.No	Signs of vaginosis?	Signs of cervicitis?
82	No	No
83	No	No
84	No	No
85	No	No
86	No	No
87	No	No
88	No	No
89	No	No
90	No	No
91	No	No
92	No	No
93	No	No
94	No	No
95	No	No
96	No	No
97	No	No
98	No	No
99	No	No
100	No	No

Table 7.3 List of laboratory consumables

Laboratory product	Source
Micropipette 100-1000µl	Eppendorf- Germany
Micropipette 20-200µl	Eppendorf- Germany
Micropipette 2-20µl	Eppendorf- Germany
Micropipette Tips 1000 µl	Eppendorf- Germany
Micropipette Tips 200 µl	Eppendorf- Germany
Micropipette Tips 30 µl	Eppendorf- Germany
Eppendorf tubes	Eppendorf- Germany
Cooling block32 with centrifuge adapters	Roche- Germany
Light cycle capillaries	Roche- Germany
MagNA Pure 32 sealed cartridges	Roche- Germany
MagNA Pure 32 disposable Tip Trays	Roche- Germany
MagNA Pure 35sample tubes, 2.0 ml	Roche- Germany
MagNA Pure35 Elution Tube, 2.0 ml	Roche- Germany
MagNA Pure35 Elution Tube Caps	Roche- Germany
Disposable Grooves	China

Table 7.4 List of chemicals, reagents used during the study.

Name of reagent	Source
Light Cycler Fast Start Enzyme	Roche Diagnostics-Germany
Light Cycler Fast Start Reaction Mix HybProbe	Roche Diagnostics-Germany
$MgCl_2$ Stock Solution, 25 mM	Roche Diagnostics-Germany
Water, PCR Grade	Roche Diagnostics-Germany
Vials with green caps containing premixed lyophilized primers and probes for 32 PCR reactions each of *C. trachomatis, M. genitalium, M. hominis*	TibMolbiol- Germany
Vials with white caps containing the IC of *C. trachomatis, M. genitalium, M. hominis*	TibMolbiol- Germany
Standard row with 6 lyophilized cloned plasmid standards of *C. trachomatis, M. genitalium, M. hominis* from 10^1 to10^6 target equivalents per reaction.	TibMolbiol- Germany
Sealing foil for the standard row	TibMolbiol- Germany

Table 7.5 Composition of Buffers, Solutions, and Media using during the study

Buffers, Solutions, and Media	Composition
UTM-RT medium formation	Hank's Balanced Salts, Bovine Serum Albumin, L-Cysteine, Gelatin, Sucrose, L-Glutamic Acid HEPES Buffer, Vancomycin Amphotericin B, Colistin Phenol Red, PH 7.3 +/- 0.2 @ 25°C
Tris/Borate/EDTA(TBE buffer(10X)	TBE buffer10% (pH8.3), is used for agarose gel electroghoresis. This product has been optimized for use in DNA applications. 890mMTris/Borate, 890mMboric acid, 20mM EDTA
Tris/Borate/EDTATBE buffer(1X)	200ml of the10X TBE Buffer diluted in 1800ml DW
Agarose Gel1.5%	1.5 gram of Agarose powder dissolved in 100ml of 1x electrophoresis buffer.

الملخص العربي

الملخص

مقدمة: يعتبر العقم مشكلة صحية منتشرة في جميع أنحاء العالم ، واحد من كل ستة أزواج يعانون من هذه المشكله التي تشكل عبئا اقتصاديا كبيرا على الرعاية الصحية العالمية ، تقديرات معدل العقم العالمية الحالية تشير إلى أن 15 ٪ من الأزواج يعانون من العقم . في البلدان النامية، عدد كبير من النساء يتعرضن للاصابة بالعقم بسبب أصابة بمرض التهاب الحوض (PID) ، و العدوى المنقولة جنسيا (STI) أو غيرها من عدوى الجهاز التناسلي . الأمراض المنقولة جنسيا تحديدا تعتبر مشكلة صحية عامة في جميع البلدان و مشكلة صحية رئيسية ، خاصة في أجزاء من العالم النامي.

الكلاميديا أو (*C. trachomatis*) هي بكتيريا منقوله جنسيا ، بجانب قدرتها على أحداث التهاب المسالك التناسلية ، ترتبط *C. trachomatis* مع االعديد من المضاعفات على المدى الطويل مثل التهاب الحوض ، والحمل خارج الرحم ، والعقم.

معظم المرضى المصابين لا تظهر عليهم أعراض ، و يتم التعرف على الاصابة عند إجراء الفحص الروتيني للعقم. على الرغم من أن الإصابة ب *C. trachomatis* قد تسبب العقم بسبب حدوث بعض المضاعفات ، فإن العديد من العيادات النسائية لا يؤدون الفحص الروتيني ل *C.trachomatis* هذا يجعل حجم المشكلة غير واضحة ، عندها تشخيص العديد من النساء بالاصابة بالعقم غير مفسر دون الكشف عن احتماليةعدوى الجهاز التناسلي ، الأمر الذي يؤدي إلى تشخيص غير دقيق للعقم . وعلاوة على ذلك ، العديد من النساء يخضعن لإجراءات تتعلق بفحص الجهاز التناسلي للمرأة بما في ذلك تنظير البطن التشخيصي دون الكشف عن عدوى التهاب الحوض ،مما يضاعف من حجم المشكلة.

الميكوبلازما هي أصغر بدائيات النوى المعروفه ، وقادرة على التضاعف الذاتي. هناك دليل قوي ورابط لدور الميكوبلازما (*M. genitalium*) كمسببات الإلتهاب لعنق الرحم ، التهاب بطانة الرحم ، التهاب الحوض ، وأمراض الأعضاء التناسلية ، المسالك ، و انسداد قنوات فالوب المسببه للعقم.

تم الكشف عن وجود الميكوبلازما هومينيس (*M. hominis*) عادة في المسالك التناسلية للإناث وفي حالات التهاب الحوض وفي حالات الحمى بعد الولادة .

تختلف التقارير والابحاث الخاصة بانتشار *M.hominis* عند النساء والمتزامنه مع التهاب عنق الرحم على نطاق واسع. ولقد تم الربط بين الاصابة بهذا المسبب والعقم عند النساء. يرتبط كل من *C. trachomatic* و *M. genitalium* و *M. hominis* مع أمراض مختلفة

في الجهاز البولي التناسلي، ولكن عادة لا يتم كشف عن هذه المسببات عن طريق التشخيص الروتيني الميكروبيولوجي.

كان الهدف من هذه الدراسة هو تحديد وجود *C. trachomatic* و *M. hominis* و *M.genitalium* في النساء المصابات بالعقم واللاتي يخضعن لإجراءات فحص العقم بعيادة العقم ومقارنة ذلك مع نساء طبيعيات غير عقيمات كمجموعةضبط.

منهجية البحث:أجريت هذه الدراسة المقارنة خلال الفترة من أكتوبر 2012 وحتى يوليو 2013 في مستشفى الملك خالد الجامعي ومستشفى الملك عبد العزيز الجامعي و بمختبرات و مرافق مستشفى الملك خالد الجامعي.

تم جمع عينات من باطن عنق الرحم من 100 امرأة مصابة بالعقم الأولي والثانوي ومن 100 امرأة غير مصابة بالعقم كمجموعة ضبط ومقارنة. وقعت كل مشاركة بالموافقة على إقرار الموافقة وتم تعبئة استبانة تتضمن البيانات الشخصية و على الخصائص الديموغرافية، كما تم تسجيل التاريخ الطبي لكل مشاركة.

تراوحت أعمار المشاركات في هذه الدراسة من 19 - 46 وتم تقسيمهم إلى 4 فئات عمرية :19-26،25-32 ،33-39 ،40-46 تم إجراء الكشف عن المسببات السابقة عن طريق اختبار:

Qualitative and quantitative light-mix real-time PCR assay

النتائج: أثبتت النتائج أن بكتيريا *C. trachomatis* كانت الأعلى حدوثا(8% *من مجموعة العقم و1% من مجموعة الضبط*،p = 0.017)، الإصابة ببكتيريا *M. genitalium* *كانت* أقل شيوعا، فقد تم الكشف عن ثلاث حالات إصابة ضمن مجموعة العقم (3% من مجموعة *العقم* ،p =0.123)، *أما بالنسبه لبكتيريا M. hominis فقد تبين أن مريضة واحدة من مجموعة العقم كانت إيجابية(1%)مقارنة مع مريضتان إيجابيتان من مجموعة* الضبط(2%،p =0.50).

كما تم تسجيل حالة إيجابية مشتركة لكلا من: *M. genitalium* و *M. hominis* وذلك من مجموعة العقم(1%،p =0.4999).

أظهرت الدراسة أن نسب أعداد المشاركات للفئتين العمريتين 26-32 و 33-39 كانت أعلى عند مجموعة العقم(34%و35%) مقارنة بمجموعة الضبط(13%و17%) وبفروقات يعتد بها إحصائيا(P<0.0001 وP=0.004). أما بالنسبة للفئة العمرية 40-46 فقد لوحظ أن نسبة مجموعة العقم(15%) أقل من مجموعة الضبط (53%)وبفرق يعتد به إحصائيا (P<0.0001).

أظهرت الدراسة أن العدوى ببكتيريا *C. trachomatis* *كانت* واسعة الانتشار بين جميع الفئات العمرية ولم تكن مرتبطة بفئه عمرية محددة.

من بين 100 امرأه مصابة بالعقم فإن 54% منهن كن مصابات بأعراض مصاحبة و46% كن بلا أعراض مصاحبة.

بكتيربا *C. trachomatis* كانت الاكثر انتشارا بين النساء المصابات بأعراض(11%) منها بين الغير مصابات بأعراض(4%).

أكثر الأعراض المنتشرة والمصاحبة للإصابة ببكتيريا *C. trachomatis* وبفرق يعتد به إحصائيا كان عرض الام أسفل البطن(19%،p =0.044).

كل حالات M. genitalium الايجابية كانت لنساء مصابات بأعراض مصاحبة(6%) .لم يكن هناك فروقات يعتد بها إحصائي بين مجموعة العقم و الضبط بشأن العلامات والاعراض عدا *علامات التهاب عنق الرحم التي تم تسجيلها بين مجموعة* العقم فقط (6%،p =0.014

.)

نسبة المصابات بالعقم الأولي بهذه الدراسه بلغ57% والثانوي بلغ43%. كانت نسبة انتشار بكتيريا *C. trachomatis* بين المصابات بالعقم الأولي تبلغ 9% والثانوي 7% ،أما بالنسبة لبكتيريا *M. genitalium كانت جميعها تعود لمصابات بالعقم الأولي(5%)* ، الحالة الوحيدة الايجابية لبكتيريا *M. hominis* كانت لمصابة بالعقم الثانوي(2%).

الفئات العمرية 19-25و26-32 كانت أعلى وبفروقات يعتد بها إحصائيا بين المصابات بالعقم الأولي (P=0.009وP< 0.0001).

عامل العقم الهرموني كان العامل الأكثر شيوعا (29٪)، يليه عامل التبويض (23٪)، العقم الغير مفسر (21٪) عامل قنوات فالوب (15٪)، وأخيرا العامل الذكري (12٪). لوحظ أن العامل الهرموني كان أعلى من عاملي القنوات والعامل الذكري بفروقات يعتد بها إحصائيا (P=0.033 وP= 0.007).

بكتيريا *C. trachomatis* كانت الأعلى بين حالات عامل العقم الغير مفسر (24%) ، أما بكتيريا *M. genitalium فكانت نسبةحدوثها تبلغ 10% ضمن حالات عامل العقم الغير مفسر.*

ختاما : هذه الدراسة هي واحدة ضمن التقارير القليلة في المنطقة والتي تهدف لتحديد وجود *C. trachomatic* و *M. hominis* و*M. genitalium* عند النساء المصابات بالعقم.

بسبب القيود الثقافية والاجتماعية استبعدت هذه الدراسة نسبة كبيرة من النساء الذين تقل أعمارهم عن 19 سنة من العمر والنساء غير المتزوجات. النتائج التي توصلنا إليها تؤكد وجود ارتباط وعلاقة بين*C. trachomatic* وحدوث العقم عند النساء ، حيث كانت الإصابة بهذه البكتيريا بين النساء المصابات بالعقم أعلى مما كان متوقعا. وفي ضوء الميل نحو زيادة معدل الانتشار فإن إجراء المسح على النساء كإجراء روتيني عند التخطيط للحمل وكذلك النساء الماصابات بالعقم وخاصة الغير مفسر كجزء من التحقيقات الروتينية الخاصة بالعقم هو إجراء مطلوب.

لم نجد أي فروق ذات دلالة إحصائية بين النساء الغير مصابات بالعقم والعقيمات فيما يتعلق بعدوى *M.genitalium* وبكتيريا *M.hominis* وهناك حاجة إلى مزيد من الدراسات مع حجم أكبر للعينة وأكثر تركيزا على مجموعات مختلفة تتعرض لخطر الإصابةوذلك للاتجاه نحو الوقاية ومكافحة هذه الأمراض المنقولة جنسيا.

إظهرت النتائج أن تحليل RealTime PCR يمكن أن يكون بديلا سريعا وأكثر دقة وحساسية من الطرق التقليدية. ويمكن الحصول على معلومات تتعلق بالتركيز البكتيري في مسحات عنق الرحم كما يسمح الكشف عن الكلاميديا والميكوبلازما في نظام مغلق الحد من مخاطر التلوث.

المملكة العربية السعودية

جامعة الملك سعود

كلية العلوم

قسم النبات والأحياء الدقيقة

عزل الكلاميديا والميكوبلازما من عينات مأخوذة من عنق الرحم لسيدات يعانون من العقم والحمل خارج الرحم

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