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The Effect of *Escherichia coli* and *Staphylococcus aureus* Isolated from Seminal Fluid of Infertile Patients on Mouse Sperm Function Tests and *in vitro* Fertilization

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By

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بسم الله الرحمن الرحيم

لله مُلكُ السَّمواتِ والارضِ يَخلُقُ مَا يَشَاءُ يَهَبُ

لِمَن يَشاءُ انَاثاً ويَهَبُ لِمَن يَشاءُ الذُكورَ ﴿٤٩﴾

او يُزَوِّجُهُم ذُكرَاناً واناثاً ويَجعَلُ مَن يَشَاءُ عَقيماً ﴿٥٠﴾

انّهُ عَليمٌ قَديرٌ

صدق الله العظيم

الشورى

الاهداء

الى ...من حفر اول حرف في تاريخ البشرية ... اجدادي العظام

الى... كل من علمنمي حرفا واوصلني الى هنا... اساتذتي الكرام

الى ...اول من وضع في يدي القلم وغرس في قلبي حب العلم... والدتي الحنون

الى... مصدر اصراري وعزيمتي وقوتي ...والدي العزيز

الى ...من احاطوني بعطفهم ورعايتهم ...اخوتي واخواتي الاحباء

اهدي ثمرة جهدي عرفانا مني لحسن صنيعهم.

دنيا

AKNOLEDGMENT

In the name of **ALLAH**, gracious, most merciful, praise is to **ALLAH**, the lord of heavens and blessing is upon all this prophets and upon the last of prophets and messengers, Mohammed and upon his family and followers.

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Dunya

Supervisors Certification

We certify that this thesis was prepared under our supervision in Al-Nahrain University, College of Science as a partial fulfillment for the requirements to award the degree of Master of Science in Biotechnology.

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Summary

The present study was focusing on the effect of seminal fluid infection on sperm functions and fertilizing capacity of mouse spermatozoa. Two kinds of bacteria were isolated from seminal fluid of 14 infertile patients (23-51 years) during their attendance at the Institute of Embryo Research and Infertility Treatment involves *Staphylococcus aureus* (*Staph. aureus*) and *Escherichia coli* (*E. coli*), which were identified by biochemical tests and API systems. On the present project, the virulence effects of these two kinds of bacteria and their culture filtrates at viable number 3, 6, 12, 24, 36 and 48 $X10^3$ cell/ml were analyzed on the mouse sperm function tests involving sperm concentration, percentage of sperm motility, percentage of progressive motile spermatozoa, percentage of abnormal sperm morphology, percentage of sperm agglutination and percentage of *in vitro* fertilization at zero time and after 30 and 60 minutes of bacterial or culture filtrates incubation.

The results showed that *E coli* infection had highly harmful effects on most of the spermatozoa parameters studied of mouse spermatozoa than *Staph aureus*. *E. coli* was significantly ($P<0.05$) reduced the motility of mouse spermatozoa after 30 minutes of incubation with the count $6X10^3$ cell/ml, meanwhile *Staph aureus* begin its negative effect at the count $24X10^3$ cell/ml after 30 minutes of incubation. The percentages of abnormal sperm morphology and sperm agglutination were significantly ($P<0.05$) affected by *E. coli* at bacterial count $36X10^3$ cell/ml after 30 minutes of incubation and mouse spermatozoa were destroyed in average 40% after 60 minutes of incubation, meanwhile *Staph. aureus* had no effects on these two parameters. The present investigation showed that the percentage of the IVF outcomes was significantly ($P<0.05$) reduced in the presence of *E. coli* at all counts and after any time of incubation, in contrast, the spermatozoa incubated with *Staph. aureus* showed a reduced

ability for IVF till the bacterial concentration $24X10^3$ cell/ml after 30 minutes of incubation. Culture filtrates of *E. coli* and *Staph. aureus* had lower effects on mouse sperm function tests than its living cells. Culture filtrates of *E. coli* developed its negative effectiveness significantly ($P<0.05$) on the percentage of sperm motility at the count $24X10^3$ cell/ml after 30 minutes of incubation. Meanwhile, the culture filtrates of *Staph. aureus* at a count of $48X10^3$ cell/ml reduced the percentage of sperm motility significantly ($P<0.05$). The percentages of abnormal sperm morphology and sperm agglutination were significantly ($P<0.05$) affected by *E. coli* culture filtrates at count 36 and 48 X 10^3 cell/ml after 30 minutes of incubation while *Staph. aureus* had no effect on both parameters.

Spermatozoa which were incubated for 30 min. with culture filtrates of *E. coli* significantly ($P<0.05$) reduced the percentage of IVF at the counts 3 and 6 X 10^3 cell/ml to 12.50% and 27.77%; respectively, whereas, the fertilizing capability of spermatozoa incubated for 60 min with *Staph. aureus* culture filtrates were 37.5% and 17.64% at the counts 36 and 48 $X10^3$ cell/ml after 60 minutes of co-incubation**.**

In conclusion, bacterial infections of seminal fluid have serious and harmful effects on sperm functions and fertilizing capability of mouse spermatozoa.

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List of Abbreviations

Abbreviation	Key
ABP	Acute Bacterial Prostatitis
ART	Assisted Reproductive Technique
AIP	Asymptomatic Inflammatory Prostatitis
CBP	Chronic Bacterial Prostatitis
CFSAN	Center of Food Safety and Applied Nutrition
DNase	Deoxyribonucleas
ET	Embryo Transfer
FSH	Follicle Stimulating Hormone
GIFT	Gamete Intrafallopian Transfer
hCG	Human Chorionic Gonadotropin
hMG	Human Menopausal Gonadotropin
ICSI	Intracytoplasmic sperm injection
IP	Intraperitonial
IU	International Unit
IVF	In Vitro Fertilization
IVM	In Vitro Maturation
LH	Leutinizing Hormone
LPS	Lipopolysaccharid
MEM	Minimum Essential Medium
NBP	Chronic Non-Bacterial Prostatis
PPS	Pelvic Pain Syndrome
ROS	Reactive Oxygen Species

STI	Sexual Transmitted Infection
TNase	Thrmodeoxyribonuclease
UTI	Urinary Tract Infection
WBC	White Blood Cell
WHO	World Health Organization
ZP	Zona Pellucida

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Chapter One

1-1 Introduction

Infertility is a world wide public health problem that affect couples in reproductive age. However, between 15% and 20% of couples around the world have difficulty conceiving a child at some point in their lives(Int. 10), thus affecting 50 to 80 million people (Gregoriou *et al*., 1998). It is primarily a male factor is considered in 40% of infertile couples and in an additional 20% it is a combination of male and female factors (Int. 10). Male infertility factors are numerous but according to a study achieved by Khalili *et al* (2000), bacterial infections could be considered as one of the most important male infertility factors, it represents approximately 10% of all male infertility problems.

It is generally accepted that one of the potentially correctable causes of male infertility is symptomatic and asymptomatic infection of the male urogenital tract (Weidner *et al*., 1991). Male genital tract infection may lead to deterioration of spermatogenesis, impairment of sperm function and / or obstruction of the seminal tract (Keck *et al*., 1998). Pathogenic microorganisms represent a high percentage in seminal fluid *Staphylococcus aureus* and *Escherichia coli* bacteria represented approximately 58.6% of it. Therefore, pathogenic *E.coli* and *Staph.aureus* bacteria could be considered as an important pathogenic bacteria due to their widely virulence factors and their spacious to spread. *E. coli* and *Staph. aureus* could be interfere with sperm motility, viability, normality, agglutination, sperm-egg interactions and fertilization *in vivo* or *in vitro* (Liu *et al*., 2002).

Aims of the study:

1- To investigate the effect of different counts of *E. coli* (as gram negative) and *Staph. aureus* (as gram positive) and its culture filtrates on sperm function tests of mouse spermatozoa after different periods of incubation.

2- To assess the effect of co-incubation of these two bacteria and thies culture filtrates on successful rate of *in vitro* fertilization

Chapter Two

Literature Review

2.1 Infertility

Infertility is the inability to achieve pregnancy after 12 month or more of regular unprotected intercourse with the same partner (Pizzorno and Murray, 1993).

Sherins (1995) stated that infertility is considered primary when it develops in a woman who had never been pregnant and secondary when it develops in woman who has had one or more pregnancies. Murray (1997) reported that approximately 15-20% of couples attempting to achieve pregnancy are unable to do so; however, in 60% of all couples experiencing infertility, a male factor is involved in 40% of the couples and in an additional 20 represented a combination of male and female factors (Int. 10).

Kretser (2001) showed that, several important factors are known to affect fertility. They include age, nutrition, environment, oxidants, sexually transmitted diseases, smoking, alcohol , drug use, and stress. Rubenstein and Brannigan (2005) pointed out that the impact of sexually transmitted disease or the reproductive tract infections are frequently the cause of secondary infertility. In both male and female, microbial and viral infections are important causes of infertility.

Historically, infertile couples focused primarily on conditions of the female. Murray (1997) believed that in about one third of the cases of infertility, it is the man who is responsible; in another one third, both male and female are responsible. Over 50% of men have a wide causes of male factor infertility (Int. 10). These factors include varicoceles, hypogonadism, antisperm antibody, hormonal abnormalities, seminal fluid abnormalities, ductal blockage, infections, life style factors, medications, cancer, genetic disorders, low sperm count, incompatibility of the hasband semen to his wife cervical mucous and the inability of the sperm to bind probably to the egg, sexually transmitted diseases

and difficulties with erection and ejaculation i.e. retrograde ejaculation (Ankrom *et al*., 2000).

Ankrom and his coworkers (1998) concluded that infections are one of the most important male infertility factors. they found that approximately 10% of male infertility has a variety of microorganisms infect their urogenital tract system.

2.2 Microbial Infection and Male Infertility:-

Bacterial and viral infections of the genital tract are important etiological factors for male infertility (WHO, 1992). Different kinds of microbes can be isolated from seminal fluid and male reproductive system which might play an important role in male infertility involving *Staphs aureus, Staph saprophyticus, Neisseria gonorrhea, Mycobacterium tuberculoses, Salmonella, Clostridia, E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa , Chlamydia trachomatis.* And diseases Mumps, Tuberculosis, Brucellosis, Gonorrhea, Typhoid, Influenza and small pox

2.2.1 *ESCHERICHIA*

The genus *Escherichia* is originally described and named by Theodore Escherich in 1885 as the bacterium coli commune. Later, it was named *Escherichia coli*, heading the bacterial family Enterobacteriaceae (Todare, 2002). However, this family is among the most important bacterial families in the field of medicine and therapy in which a number of genera (e.g. *Salmonella, Shigilla* and *Yersinia*) are considered as human intestinal pathogens (Slayers and Whitt, 1994).

2.2.1.1 Morphological, Cultural and Biochemical Characteristics of *Escherichia coli*

Escherichia coli is a short, straight gram-negative bacillus, non-sporing, usually motile with flagella, it occurs singly, or in pairs in rapidly growing liquid cultures, it is often have capsule or microcapsule and a few strains produce a profuse polysaccharide slime (Todare, 2002). It is facultative anaerobic, capable of fermentative and respiratory metabolism. It grows on optimum temperature of 37ºC on a wide range of simple culture media and on simple synthetic media (Slayers and Whitt, 1994). Under anaerobic growth conditions, *E. coli* requires carbohydrates, it ferments glucose to pyruvate which is converted into lactic, acetic and formic acids (Macone *et al*., 1981). *E. coli* colonies are usually circular and smooth with an entire edge, some strains, particularly those isolated from patient with cyctic fibrosis, produce mucoid colonies (CFSAN, 2002).

Pathogenic *Escherichia coli* have the ability to produce α-haemolysin in erythrocyte containing media, and other strains produce a cell-associated β-haemolysin that may be released when the cells are lysed (Smith, 1963).

2.2.1.2 Pathogenicity of *Escherichia coli*

For more than half a century after its discovery, *E. coli* was thought as the major commensal in faeces and it was regarded as having only low pathogenicity. This view changed progressively with recognition of the complexity of the faecal flora and the variety of superficially similar intestinal infections due to *E. coli* (Smith, 1963).

The disease resulted from infection with *E. coli* may be classified into two groups, specific infections and non-specific infections. Specific infections are those in which colonization of mucosal surface is an essential preliminary event and in which the principle signs and symptoms of disease are related to the site for colonization, i.e. urinary tract infection. In such infection, colonization alone

is not sufficient to cause disease which results from the subsequent action of other virulence factors and mechanisms (Sussman, 2000).

Non-specific infections differ from the previous type, in which the main signs and symptoms are not related to a site of mucosal colonization. Thus, non-specific infections may result from the direct contamination of wound or the peritoneal space during surgery, or they may result from secondary spread from of a specific infection i.e. sepicaemia following a urinary tract infection (Maslow *et al*., 1993).

Todare (2002) mentioned that *E. coli* is responsible for three types of infections in human: urinary tract infection (UTI), neonatal meningitis and intestinal diseases (Gastroenteritis). A recent studies by many scientists showed that *E. coli* appear to be the bacteria that most frequently causes the genital tract infection (GTI) which has been defined by the presence of leukocytes and pathological *E. coli* in culture semen of infertile men (Sanocka-Mociejewska *et al.*, 2005).

Auroux (1988) suggested that *E.. coli* have direct effect on spermatozoa by diminished their motility, morphology and their ability fertilize and indirect affects as that *E. coli* infection could change the constituent and qualities of the seminal fluid. Marcus *et al*., (1995) pointed out that *E. coli* is an important agent which cause urinary tract and sexually transmitted infections (STI).

In a study by Galdiro *et al.* (1988), the lipopolysaccharide (LPS), porins, and peptidoglycan fragments have been reported to be toxic for human spermatozoa and may lead to reduced fertility or sterility. Sokkar *et al*. (2003) reported that *E. coli* endotoxin have a pathological effect on spermatozoa.

2.2.1.3 Virulence Factors of *Escherichia coli*

A number of virulence factors have been identified in *E. coli* and others probably remain to be discovered. Many investigators thought that the pathogenetic processes that operate in a given infection always involve more

than one virulence factor usually interact in so complex manner (Siitonen *et al*., 1993).

Todare (2002) summarized the virulence determinants of various strains of pathogenic *E. coli* as following:

a-Adhesins

CFA I / II, Type 1 fimbriae, P fimbriae, S fimbriae, intimin (non-fimbrial adhesin)

b-Invasins

Hemolysin, siderophores and siderophores uptake systems, shigella-like "invasions" for intracellular invasion and spread.

c-Motility / Chemotaxis

Flagella

d-Toxins

LT toxin, ST toxin, shigella-like toxin, cytotoxins, Endotoxin (LPS).

e-Antiphagocytic Surface Properties

Capsules, K antigens, LPS.

f-Defense against Serum Bactericidal Reactions

LPS, K antigens.

g-Defense against Immune Responses

Capsules, K antigens, LPS, Antigenic Variation.

h-Genetic Attribution

Genetic exchange by transduction and conjugation transmissible plasmids, R factors and drug resistance plasmids, toxin and other virulence plasmids.

2.2.1.3.1 Colonization of *Escherichia coli*

Bacterial pathogens must first be able to colonize an appropriate target tissue of the host (Beachey, 1981; Beachey *et al*., 1988). Colonization begins with the specific attachment of the bacterium to receptors expressed by forming lining of the mucosa (Wizemann *et al*., 1999). Attachment is often mediated by adhsin proteins: i.e. bacterial lectin and the carbohydrate recognizing protein (Geme, 1997; Jenkinson and Lamont, 1997).

2.2.1.3.2 Adhesions of *Escherichia coli*

Adhesins expressed on the microbial surface help dictate the tissue tropism of invading bacteria and can modulate host cell responses to infection. *E. coli* assemble adhesions on their surface as monomers, as simple oligomers, or as supermolecular fibers called fimbriae or pili (Hultgren *et al*., 1985). One of the best understood mechanisms of bacterial adherence is attachment mediated by cell surface structure called pili or fimbriae. Fimbriae are long, flexible structures that extend outward from the bacterial surface of *E. coli* and allow for contact between the bacterium and the host cell (Hultgren *et al*., 1993).

2.2.1.3.3 *Escherichia coli* Toxins

Toxins are mechanisms for gaining access to environment in our bodies and to the nutrients sequestered within them, for releasing these nutrients in usable form, and for moving toxins and nutrients to new hosts when they are expanded. *E. coli* bacteria have the ability to injure the host cell by two kind of toxins the endotoxin (Lipopolysaccharide) and exotoxin.

2.2.1.3.3.1 *Escherichia coli* endotoxins (Lipopolysaccharides)

Endotoxin is an integral component of the gram-negative outer membrane that plays an essential structural role in cellular survival and division. Its toxic activity is minimal during normal bacterial growth and there is no significant

toxin release into the surrounding environment. The power of this kind of toxin is seen when endotoxin is released by cell lysis, and the lipid A protin stimulates a massive immune response resulting in septic shock that is often fatal to the host (Sousa. 2003). Ørskov and his co-workers (1977) described the components of *E. coli* lipopolysaccharides as:

a- **Lipid A:** inserted into the outer membrane of the cell and this is attached to the oligosaccharide.

b- **The core oligosaccharide:** attached to the later part and exposed to the environment is the outer most part of the molecule.

c- **The polysaccharide side chains**.

Endotoxins are directly involved in the genesis of disease symptoms that range from fever of one extreme to the potentially fated endotoxic shock of septicemia at the other (Morrison and Ryan, 1992; Levine *et al*., 1993).

2.2.1.3.3.2 *Escherichia coli* Exotoxins

Exotoxin may be described as secreted proteins whose site of action is some distance from the site of infection, and which exert their specific effects at low concentrations often by enzymatic activities of *E. coli*. The major exotoxin are those that cause damage to the cell membrane and enter host cells and exert their toxic activities against intracellular targets(Sousa. 2003).

2.2.2 *Staphylococcus*

Staphylococcus is one of the most important genus in the family *Staphylococcaceae* which includes three lesser known genera, *Gamella, Micrococcus and Salinicoccus*. In 1878, Koch was the first who recognized it and then after two years Pasteur cultivated it in liquid media (Easman and Adlam, 1983). Humphrey *et al* (1997) mentioned that, in 1882 Ogsteen was introduced the name *Staphylococcus* (Greek staphyle means bunch of grapes,

coccus means berry) now used as the genus name for a group of gram positive cocci. Todare (2005) stated that, "Although the genus *Staphylococcus* comprises 30 species, only *Staphylococcus aureus* and *Staphs epidermides* are significant in their interaction with humans".

2.2.2.1 Morphological, Cultural and Biochemical Characteristic of *Staphylococcus aureus*

Staphylococci are gram-positive bacteria that found in microscopic pairs, cubic and clusters resembling groups due to its manner of dividing in two planes. It is perfectly spherical cells about 1 µm in diameter. These microorganisms are non-motile, non-spore forming (Cruikshank *et al*., 1975), they can grow on solid media at 30-37ºC and forming circular colonies, two to three millimeter in diameter with a smooth shiny surface after 24 hours of incubation (Easman and Adlam, 1983). Archer (1996) described that *Staphs aureus* forms a fairly large yellow colony on a rich medium due to the presence of triterpenoid carotenoids or its derivatives which were located in the cell membrane.

Staph aureus is often heamoloytic on blood agar, facultative anaerobes that grow by aerobic respiration or by fermentation yielding principally lactic acid. It is catalase-positive but oxidase negative, can grow at a temperature of 15-45ºC and in NaCl concentration as high as 15% (Weatherall *et al.*, 1996). It is mannitol fermenting and α-hemolytic on blood agar, producing coagulase enzyme (Todar, 2005).

2.2.2.2 Pathogenicity of *Staphylococcus aureus*:

Since the beginning of twentieth century, *Staphylococcus aureus* has been recognized as one of the most important human pathogens (Archer, 1996). Milish (1992) concluded that *Staph aureus* is an opportunistic bacterial pathogen responsible for a diverse supportive (pus-forming) infections and toxinoses in human. It causes superficial skin lesions such as boils, styes and furunculosis, as

well as more serious infections (pneumonia, mastitis, phlebitis, meningitis) and deep-seated infections (osteomyelitis and endocarditis).

Lowy (1998) reported that *Staph aureus* is a major cause of hospital acquired (nosocomial) infections of surgical wounds and infections associated with indwelling medical devices. It causes food poisoning by releasing enterotoxins into the blood stream. It also causes bronchopneumonia, post-influenzal secondary infection, lymphangitis, lymphadentis, bacteremia, and invading the kidney, liver and lung as secondary complications of infection. Bone-joint infections some times occur as a result of *Staph aureus* infection.

Day *et al*. (2002) summarized that *Staph aureus* is a major cause of severe infection in humans and yet is carried without symptoms by a large, proposition of the population occurring to *Staphylococcus aureus* responsible of urinary tract infection, acute and chronic bacterial prostatitis infection.

Meyer *et al*. (1980) discovered that the metabolic products of *Staphylococcus aureus* have pathogenic effects on bull semen.

2.2.2.3 Virulence Factors of *Staph aureus*:

Staph aureus expresses many cell surface-associated extracellular factors. Some of which contribute to the ability of the organism to overcome the body's defenses and to invade, survive in and colonize the tissue (Koneman *et al*., 1992).

Several bacterial components and secreted products are suggested to affect the pathogenesis of *Staph aureus* infection, which are:

2.2.2.3.1 Surface Adherence Proteins:

Staph aureus cells express on their surface proteins that promote attachment to host proteins such as laminin and fibronectin (Aly and Levit, 1987; Todare, 2005).

2.2.2.3.2 Invasins Proteins and Toxins:

The invasion of host tissues by *Staphylococci* apparently involves the production of a huge array of extracellular proteins and toxins. These are:

a) **α-toxin** (**α-hemolysin**) is the best characterized and most potent membrane-damaging toxin of *Staphylococcus aureus*
b) **β-toxin** is a sphingomyelinase which damages membranes rich in this lipid.
c) **σ-toxin** is a very small peptic toxin produced by most strains of *Staph. aureus*.
d) **γ-toxin and leukocidin** is a multicomponent protein toxins produced as separate components which act together to damage membranes.
e) **Membrane-Damaging Toxins** That lyses eukaryotic cell membranes i.e. Leukocidin (Todar, 2005).
f) **Exotoxins** that damage host tissues or otherwise provoke symptoms of disease. i.e. enterotoxins (Todare, 2005).

2.2.2.3.3 Surface Factors and Enzymes

a-Capsule

Expresses a surface polysaccharide of either serotype 5 or 8. *Staph aureus* strains isolated from infections express high levels of the polysaccharide capsules which enhance microbial virulence by rendering the bacterium resistant to phagocytosis in the absence of complement resulting in bacterial persistence of infected host blood stream (O'Riordan and Lee, 2004).

b-Protein A:

Protein A is a surface protein of *Staph. aureus* which binds IgG molecules by their Fc region. In serum, *Staph. aureus* disrupts opsonization and phagocytosis by the wrong binding orientation of protein A to IgG molecules (Deisenhofer, 1981).

c-Catalase

This enzyme plays a major role in protection of the bacterial cells from the poisonous action of hydrogen peroxide by breaking it to hydrogen and oxygen (Koneman *et al*., 1992).

d- Coagulase

Coagulase is an extracellular protein which binds to prothrumbin in the host to form a complex called staphylothrombin. However, *Staph. aureus* could protect themselves from phagocytic and immune defenses by causing localized clotting (Harley and Prescott, 1993).

2.3 Seminal Tract Infections

Acute and chronic infections of the genital tract may play a contributing role in male factor infertility. Various infectious mechanisms may impair fertility such as deterioration of spermatogenesis, impairment of sperm function and obstruction of the seminal tract (Bar-Chama and Fesch, 1993; Purvis and Christiansen, 1996).

According to the World Health Organization (WHO, 1992) definition of seminal tract infections, the parameters that considering the seminal fluid is infected are ; significant bacteriospermia $\geq 10^3$ (bacteria /ml ejaculate); detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*; significant leukocytospermia $\geq 10^6$ (peroxidase positive leukocytes /ml ejaculate).

2.4 Geneto urinary Tract Infections

It is generally known that urine and semen have partially the same pathway to the outside of body. Because the urinary system and reproductive system are closely related to each others, any infection in the urinary system can inter to the reproductive system and causing problems with fertility (Ohi *et al.*, 1992).

Tollon *et al* (1997) noticed that semen infections or bacteriospermia is frequently associated with urinary tract infections; he believed that symptomatic urinary tract infection (UTI) is the main cause of infertility in men. As reported by WHO (1993), the urogenital tract infection is the infection source of testes, epididymis, prostate gland, seminal vesicles, bladder and urethra.

2.4.1 Testicular Infection

Testes are a pair of oval-shaped glands which produce of spermatozoa and the male hormones which are responsible for the secondary sex characteristics of the male. Testes are lied in a cap-like structure called scrotum which keep the testes of a temperature about 2-3ºC below the temperature of the internal organs (Int. 9).

Testes could be infected and produce many complications. The infection and inflammation of testis, usually affects the adjacent epididymis. Acute infection of the testis is called orchitis which is severe infection and inflammation of the testis(Richard, 1999). Orchitis may leads to infertility or endocrine disturbance or both. The two major distinguishing etiologies of orchitis are blood-borne bacterial infection and viral infection. The most bacterial pathogens are *E. coli, Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Brucella*, *Niseria gonorrhea and* diseases Typhoid, Influenza, Mumps and small pox as a viral infections can cause testicular atrophy (Hargreave *et al*., 1998).

Because the testis possesses a relatively high infection resistance, orchitis rare without an initial epididymitis, therefore bacterial orchitis is usually secondary to bacterial involvement of the epididymitis. Testes infection could be occurred as a complicated epididymitis resulting in severe entire testes infection and pus forming, thus testes may need to be surgically drained (Osegbe. 1992).

If the infectious process causes deterioration in the blood- testis barrier, this may led to significant formation of sperm antibodies which can be detected

in serum and seminal plasma (Jarow *et al*., 1990). In many cases, testicular infections are due to bacterial spreads from urinary system. In men over 50 years of age, infection may have spread from infected urine while in younger men urethral infection may be the cause. If the bladder has infection, the testicular infection can certainly occur. Sexually transmitted infections such as *chlamydia* are common causes of urethral infection which may also cause orchitis. Hargreave *et al*. (1998) reported that infections from other parts of the body can, rarely, travel in the blood to the testes e.g. tuberculosis symptoms were usually develop quickly after 50 days from the infection. The testis swelling, scrotum enlarged painful, fever, headache, and nausea. Dangerous complications developed without testicles treatment, destruction and destroying of seminiferous tubules could be occurring.

Patients with epididymo-orchitis have chronic inflammatory changes in the seminiferous tubule which expected to disrupt the normal process of spermatogenesis and cause hypospermatogenesis and spermatogenic arrest(Joly-Guillo and Lasry. 1999; Int 8). According to a study in 1999 by Ludwing and his co-workers, genital tract infection affect spermatogenesis and sperm function resulting in reducing fertility. Ureaplasma urealyticum which was isolated from testicular biopsy specimens have a critical ability to interfere with spermatogenesis. It has been shown that in up to 60% of the patient with acute orchitis is at least the spermatogenesis temporarily impaired (Berger . 1984).

2.4.2 Epididymis Infection

Epididymis is the organ of ejaculation and maturation of the spermatozoa it runs down the middle of the back of the testis from top to bottom. Each epididymis is a long tube with 6 meters in length that are tightly coiled to form a comma- shaped organ located along the superior and posterior margins of the testis (Steren and Lowe, 1997). Its function is to carry the sperm from the testes

to the vas deferens, which is the muscular tube that carries the sperm (Scott and Giblbert. 2004).

The epididymis its more susceptible to infection than other structures in the scrotum and this infection is called epididymitis (Int 5). Infection can subsequently lead to scare tissue which can block the sperm from moving through the testis (Makferlane, 2005). Most cases of epididymitis are from sexually transmitted diseases in men who are less than 40 years. Other causes include especially in patients with sarcoidosis, brucellosis and leprosy which can result in epididymal blockage (Dohle, 2003). In men who are older than forty, the most common organisms to cause epididymitis are *E. coli* (Makfarlane, 2005).

Intestinal bacteria such as *E. coli* most often cause epididymitis, where they travel from an infected site to the bladder or some other site in the urinary tract (Vikram and Dogar. 2004). Joly-Guillo and Lasry (1999) mentioned that in older men with larger prostates, there is often more urine left in the bladder at the end of urination. This allows the bacteria to grow, and then the bacteria can move back words through the genital tract and cause the infection of the epididymis. Recently, Scott and Giblbert,(2004) discovered that epididymitis may develop after surgery or a diagnostic test that involves the bladder or urethra. In these cases, the infection is usually caused by intestinal bacteria that have entered the urethra or bladder, either during the procedure or through the catheter. Rarely, epididymitis can occur when a systemic infection spreads through the blood stream and moves to the epididymis (Internet 8).

Scott and Giblbert. (2004) showed that the complication of epididymis are as follows:-

I- Chronic epididymitis which lead to ejaculatory duct obstraction and failure of sperm aspiration from the epididymitis.

II- Infraction, abscesses and atrophy

It was certified that ejaculatory duct obstruction is a common cause of male infertility and infection are present in at least 22-50% of those men (Dohle, 2003). Fever, burning with the urination, urgency urination, urination frequency small amount, intense pain in the scrotum and epididymis swollen are the symptoms of epididymitis (Int 5). Patients with chronic epididymitis may be having a chronic discomfort of varying degrees in the epididymis and scrotum. It may be very hard to treat, through with time it may distroy itself. In sever cases where everything else has been tried, and where fertility is no longer an issue the epididymis may need to be surgically removed (Internet 5).

2.4.3 Male Accessory Glands Infection

Male reproductive system consists of important organs called male accessory genital glands which are complex of three different structures: seminal vesicles; prostate gland; and the bulb urethral glands. These glands secrete fluids that enter the urethra(Richard, 2003). In general, it was reported that male accessory glands may invaded and infected, and the main gland exposed to infection is the prostate. Prostatitis is an infection or inflammation of the prostate gland that represents as several syndromes with varying clinical features (Krause, 1992).

In meeting of National Institute of Diabetes and Digestive and Kidney disease (NIDDK) it has been classified prostatitis to a new classification system focuses on the clinical and diagnostic problems (Nieckel. 1998) as follows :

2.4.3.1 Acute Bacterial Prostatitis (ABP)

The acute bacterial prostatitis occurs from ascending urethral infection or from reflex of infected urine into the prostate or both (Berger *et al.,* 1997). Lipsky (1999) reported that *E. coli* is the most common cause of acute bacterial prostatitis, patient with acute bacterial prostatitis have a lower back pain, fever, urinary urgency, warm tender and enlarged prostate (Berger *et al.,* 1997).

2.4.3.2 Chronic Bacterial Prostatitis (CBP)

The chronic bacterial prostatitis is recurrent infection of the prostate having the same causative organisms of acute bacterial prostatitis, usually resulted from partial blockage of the male urinary tract, such blockage promote the harboring of bacteria from natural immune mechanisms and medications from getting to the site (Zermann *et al*, 1999). Patient with CBP showed mild symptoms compared with these of (ABP), including prineal and low abdominal pain, and dysuria (Tanner *et al.,* 1999).

2.4.3.3 Chronic Non-Bacterial Prostatitis (CNBP)

Chronic non-bacterial prostatitis or pelvic pain syndrome (PPS), is the most common form of prostatitis with symptom similar to those of chronic bacterial prostatitis, usually cased by infection agents such as fungi, *Mycoplasma* or viruses (Tanner *et al*., 1999).

With a new molecular biology methods (DNA analysis), Jarvi (2000) isolated unusual bacterial strains from all semen and urine specimens of type III prostatitis (NBP) including *Paeribacillus* species, *Proteobacterium* species, *Flavobacterium,* uncultured *Eubacterium* and *Bradyrhizobium* species which have no symptoms .

2.4.3.4 Asymptomatic Inflammatory Prostatitis (AIP)

The Asymptomatic Inflammatory Prostatitis is a prostatic infection with no subjective symptoms and it detected either by prostate biopsy, the presence of WBCs in expressed prostatic secretions or semen during evaluation for other disorders (Nickel, 1998).

Prostate problems can contribute to infertility in two possible causes:-

1- Any condition that prevent seminal fluid from reaching the unfertilized egg within the body of female partner
2- some defects in the quality of the seminal fluid and sperm it contain (Weidner *et al*., 1999).

Lipsky (1999) believed that infections of the prostate can cause swelling and block of the ejaculatory duct preventing sperm cells from being ejaculate and causing male oligospermia. Dohle (2003) has been proved that chronic bacterial prostatitis scarring of the prostatic and ejaculatory ducts resulting in low volume ejaculate with low fructose. The same author pointed out that the ejaculatory duct obstruction is a common cause of male infertility and infections are present in at least in 22-50% of these men. Furthermore, David (2004) stated " since the ejaculatory duct passes through the prostate gland, infections of the prostate can cause inflammatory cells to pass into the ejaculate, which may damage the sperm". However, the most common etiological causes of bacterial prostatitis are gram negative pathogens, predominantly strains of *E. coli* which have been identified in 65-80% of acute and chronic bacterial prostatic infection that affected both sperm structure and motility (Weidner *et al*., 1991), the same author mentioned that *Pseudomonas aeruginosa, Serretia, Klebsiella* and *Enterobacter aerogenes* have been isolated in 10-15% of infertile prostatitis patients. Regarding to the role of gram positive (G +ve) pathogens, *Enterococci* may cause bacterial prostatitis and associated with recurrent urinary tract infections (UTI), other G +ve bacteria as *Staph aureus, Staph saprophyticus, Neisseria gonorrhea, Mycobacterium tuberculoses, Salmonella, Clostridia* have been isolated from seminal fluid (Berger, 1997; Weidner, 1999). However, Ludwing and his colleagues (1999) discovered that the Human Immunodeficiency Virus (HIV) infection and Acquired Immunodeficiency Disease (AIDs) may lead to an increase in bacterial form of prostatitis and to an increasing in uncommon pathogens. Currently, the involvement of the seminal vesicles in bacterial prostatitis is probably very frequent (Hermabessiere, 1989). Seminal vesicles may obstruction as a complicating of infection (Hafez, 1977).

Hermabessiere (1989) showed that because of the prostate and seminal vesicles contribute the majority of seminal fluid (about 70%) , blockage at this level will reduce the volume of ejaculate. Donovan and Lipshultz (1988)

suggested that prostate and seminal vesicles may alter the composition of their fluids and consequently impair sperm fertilizing capacity.

2.4.4 Urethral Infection:

Urethra is part of urinary system. Since urethra is a unique reciprocal structure between the urinary and genital systems, its infection considered to be an important matter for both urinary and reproductive systems health (Schiefer, 1998). Urithritis means an inflammation of urethra caused by microbial infection (Schiefer, 1986). Infectious causes of urethritis are mainly sexually transmitted as gonococcal urithritis (GU), i.e. due to infections with *Neisseria gonorrhea* or non gonococcal urithritis (NGU) i.e. due to infections with *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis* (Ness *et al*., 1997; Trum *et al*., 1998).

Ness *et al*. (1997) summarized that urithrits may be associated with other infectious syndromes such as epididymitis, orchitis prostatitis or urinary tract infection (UTI). Trum *et al.* (1998) noticed that the presence of leukocyto spermia in patients with urethritis is caused by infection of *Neisseria gonorrhoeae.* It was mentioned that the lesion in the posterior urethra in the area of the vermonotanum can lead to ejaculatory disturbance (WHO, 1993; Purvis and Christiansen, 1995). According to a study by Terris (2004), urithritis has been claimed to impair male fertility.

2.5 Seminal Fluid Infection

Seminal fluid or semen is a slightly alkaline viscous mixture of sperm cells and fluids secretions from each organ in the male reproductive tract. It is usually white or grey but can occasionally appear yellowish (Dean, 2000). The sperm cells account for only 1-2 percent of the semen volume, while the other percentages are: 3% produced from epididymis, 40-80% produced by the

seminal vesicles, 13-33% produced from prostate gland and from bulburethral and urethral gland 2-5% (William, 2003).

The purpose of semen is purely for reproduction, its function as a vehicle to carry the spermatozoa into the female reproduction tract, so male fertility could be reduces by infection of the seminal fluid (Dean, 2000). Hafez, (1977) suggested that seminal fluid which passes through the urethra is routinely contaminated with bacteria that colonize this area, therefore, the urogenital infection could be reduce male fertility. Hillier and Rabe (1990) isolated 113 aerobes and 74 anaerobes bacteria, and one yeast from 36 of 37 semen samples, this includes: *Staphylococci*; *Viridans streptococci*; *Diphtheriods*; *Peptostreptococcus* species; *Bacteriods* species; *Gardnerella vaginaliz*; *Lactobacillus species*; *Actinomyces* species; *Enterococcus*; *Veillonella*; *B. streptococcus*; *Heamophilus*; *Mobiluncu*; *Escherichia coli* and *Clostridium.* In 1999 Saeed isolated pathogenic bacteria from more than 50% of seminal fluid samples obtained from non symptomatic infertile men. Stainislavov *et al* (2000) observed that although genital infection is often silent, it could severely impair male fertility. Burkharin *et al*. (2000) found that bacteria in the ejaculate have a direct effect on the seminal fluid due to the decrease in number of spermatozoa, suppression in their motility, change in their morphology and fertilizing capacity, and indirect influence due to the inhibition of spermatogenesis resulting from testicular damages. Gonzales *et al*. (1992) and Purvis and Christiansen (1996) believed that infection of seminal tract may impair accessory gland function and leading to change in the composition of seminal plasma.

Since semen contains component such as white blood cells (WBCs) and according to WHO 1992, individuals with specific seminal WBCs concentrations $\geq 10^3$/ ml is under a critical detection of male infertility . Many scientists found that it is a good indicators of the reproductive system infection and infertility even with non symptomatic reproductive system infection (Keck,

1998). Wolff *et al* (1990) suggested that the identification and quantification of leukocytes in the semen should be an integral part of every male infertility workup. But Hillier and Rabe (1990) discovered that the presence of WBCs in the semen was not associated with the isolation of many kind of bacteria i.e. *Staphylococcus, Viridons and Streptococci* therefore its a good idea to culture the semen as well as leukocytospermia because in many cases of infections immune system couldn't recognize the bacterial sign as a foreign body and this could produce more complication without symptoms.

2.6 Sperm Functions Parameters

Spermatozoa were first discovered by Leeuwenhoek in the 17th century but it was not until 1928 that the sperm count was found to be associated with fertility potential. Since that time a variety of sperm tests and semen parameters have been developed with the hope of clarifying whether or not a man could impregnate his partner. Scientists play a major role in the assessment of the hasband by undertaking a wide range of laboratory investigations (Seible and Zilberstain, 1995). However, semen analysis is the most fundamental procedure in the investigation of the male and is a description of the semen in terms of volume, appearance, viscosity, sperm concentration, sperm motility and morphology (WHO. 1992).

In 1993, Purvis and Christiansen pointed out that there are more sensitive tests available, including the post-coital test, which measures the ability of sperm to penetrate cervical mucus, and the hamster-egg penetration teste, which measures the *in vitro* ability of sperm to penetrate hamster egg. This test predicts fertility in an estimated 66% of cases in comparison to 30% with conventional sperm analysis. It is used by clinicians to estimate probability of conception, advice on appropriate treatment, and monitor treatment of the male partner.

Naessens *et al.* (1986) discovered the relationships between the presence of microorganisms and abnormal seminal fluid analysis. According to a study by Dohle (2003), urogenital inflammation which occurs in 5-12% of men attending infertility clinics have a detrimental effect on sperm quality by reducing concentration and motility, and a possible affect on the number of morphologically normal spermatozoa. The world health organization (1992) provides a definition of a normal sperm which is generally used as reference values:-

- The concentration of spermatozoa should be at least 20 million /ml.
- At least 75% of the spermatozoa should be a live (it is normal for up to 25% to be dead).
- At least 30% of the spermatozoa should be of normal shape and form.
- At least 25% of the spermatozoa should be swimming with rapid forward movement.
- At least 50% of the spermatozoa should be swimming forward, even if only slightly (Dean, 2000).

2.6.1 Sperm Concentration

The number of spermatozoa in the ejaculate has been related to male fertility (Sandler and Faragher, 1984). Although it takes only one sperm to fertilize an egg, in an average ejaculate a man may eject nearly 200 million sperm. However, because of the natural barriers in the female reproductive tract, only about 40 sperm will ever reach the vicinity of an egg (Carlson *et al.*, 1992). Sandler and Faragher (1984) observed that there is a strong correlation between the number of spermatozoa in an ejaculate and fertility. However, Pizzorno and Murray (1993) showed that in about 90% of low sperm count cases, is the deficient in sperm production, and in about 90% of these cases the cause of decrease sperm cells cannot be identified , this condition is known as "idiopathic oligospermia or azoospermia". Oligozoospermia means a low sperm count,

while azoospermia is defined as a complete absence of spermatozoa in the seminal fluid (Wyngaarden *et al*., 1992).

Infections in the male genito-urinary tract including epididymis, seminal vesicles, prostate, urinary bladder and urethra are thought to play a major role in many cases of infertility and low sperm count (purvis and Christiansen, 1993). Popadimas *et al.* (1999) discovered that there is a correlation between genital tract infection and low sperm count. According to studies by Popadimas *et al.* (1999), there is an increase in number of leukocytes in semen of male genital tract infection. Yanushpolsky *et al* (1996) found that there was a positive relationship between increasing leukospermia and impairment of seminal fluid especially sperm concentration. Furthermore, Wolff *et al* (1990) reported that treatment of seminal fluid infection resulted in significant reduction in the concentration of leukocytes and phagocytes and significant improvement in sperm concentration. Bacterial infection can reside in the epididymis and vas deferens affecting sperm development and fertility, but if the patients treated with appropriate antibiotics, an improvement was noticed in sperm counts and quality (Keck, 1998).

Obstruction or blockage of sperm transport from the testis can reduce sperm count to zero. Obstruction may occur at any point from the epididymis up to the ejaculatory ducts resulted from a variety of causes i.e. infection. Infection of the reproductive tract may cause swelling and block off the ejaculate duct preventing sperm to be ejaculated and because the prostate and seminal vesicles contribute the majority of fluid to the ejaculate (Int 6).

In another way bacteria that infect the prostate and seminal vesicles producing inflammatory cells which can destroy spermatozoa (ICSI, 2004). Lowering sperm count doesn't always means that the male is infertile, especially when retuned back and know that men are now supplying about 40% of the number of sperm per ejaculate compared to 1940 levels. Therefore 52% of couples whose sperm count were below 10 million /ml have achieved pregnancy

(Purvis and Christiansen, 1993). In 1993, Pizzorno and Murray summarized that whenever the majority of sperm are abnormal shaped or are entirely relatively non-motile; a man can be infertile despite having normal sperm concentration.

2.6.2 Sperm Motility

Sperm motility was considered as one of the most important parameters in evaluating the fertility potential of spermatozoa, to pass the cervical mucus through the genital tract to the uterus and reach fallopian tube of the female, ending penetration of the zona pellucida and fertilizing the ovum (Ijaz *et al*., 1994).

The term sperm motility usually refers to two different variables; percentage of sperm exhibiting any kind of movement and the quality of this movement which is called the forward progression. However, the normal velocity movement of sperm cells is 75 µ/sec (Blasco, 1984).

The ejaculate may be considered abnormal if more than 50% of spermatozoa within one hour after ejaculate shows a decrease in motility, this will be called asthenozoospermic semen sample (WHO, 1992). The capacity of sperm motility develops in the caput of epididymis while the forward direction motility develops in the distal region of the epididymis (Haas and Beer, 1986). Zaneveld and Polakoski (1977) indicated that the adinosin triphosphate (ATP) is necessary for contraction of spermatozoal flagellum (which develops the propel force for swimming) and generated by metabolic reaction that take place in the cytoplasm and mitochondria of the midpiece.

In general, sperm motility negatively affected by infection of the genital tract (Huwe *et al*., 1998). The effect of certain uropathogenic microorganisms *N. gonorrhoeae, Staph. aureus, Staph. epidermides* , *Mycopacterium tubercoloses* , *E. coli* and *P. aeruginosa* inhibite the sperm motility (Hong *et al*., 2003). Researchers from Hungary have shown that bacteria can prevent sperm from swimming well enough to reach an egg and fertilize it (Molner *et al*, 2003).

They stated that there are several possible reasons whey the bacteria may impede the movement of sperm". Bacteria might compete with the sperm for supplies of fructose that sperm need for energy; they might produce toxins that poisons the sperm; or they might physically interfere with the lashing of sperm tails. Bartoov *et al*., (1990) discovered that *E. coli* of O6 antigen serotype is a gential microorganism in regard to male infertility and considered the principal microorganism causing prostatitis and epididymitis. Wolff *et al*., (1993) and Diemer *et al* (1996) found that the effect of *E. coli* on spermatozoa *in vitro* is readily adhering to sperm as well as agglutinated to it. Many scientists believed and proved that *E. coli* have the ability to reduce human sperm motility (Paulson and Polakoski , 1977; El-Mulla *et al*., 1996; Huwe *et al*., 1998; Kohen *et al*., 1998; Diemer *et al*., 2000; Liu *et al*(2002)., Villegas *et al*., 2005). S*taphylococcus* has the ability to impair the spermatozoa motility due to their attachment to spermatozoa and this could be explained by their cluster like growth (Nicholson *et al*., 2000; Liu *et al*., 2002). Onemu and Iben (2001) and Liu *et al*. (2002) showed that *Staphs aureus* significantly decreased the sperm motility and viability.

Alexander (1998) certified that the antisperm antibody causes infertility through impairing sperm motility and migration inside the female reproductive tract, in addition to reducing the number of spermatozoa at the site of fertilization there is also evidence of direct effect of leukocytes in the ejaculate on sperm motility and other functions (Talbert *et al*., 1987; Yanushpolsky *et al*., 1996). The leukocytes are capable to produce cytokines and reactive oxygen species (ROS). These ROS which involve H_2O_2, free oxygen radicals and reactive nitrogen intermediate are produced by activated macrophages and granulocytes and are highly toxic to spermatozoa (Anderson and Hill, 1988; Eisermann *et al*., 1989; Aitken and West, 1990; Anderson and Politch, 1998).

2.6.3 Sperm Morphology:

Sperm morphology gives information for the function of the reproductive tract and is a predict of man's fertility potential. A normal-looking sperm has an oval head and a tail seven to fifteen times longer than the head, the length to width ratio should be 1-50 to 1-75, there should be a well defined acrosmal region comprising 40-70% of the head area. There must be no neck, midpeice or tail defects and no cytoplasm droplet more than one-third the size of a normal sperm head (Burr *et al*., 1996). Sperm cells represent a unique population in which up to 50% (up to 70% according to WHO 1992 and up to 86% according to strict criteria) of the cells can have morphological defects in normal fertile individuals (Ayala *et al*., 1996). The following categories of defects should be scored:

- Head shape / size defects, including large, small, tapering, pyriform, amorphous (>20% of the head area occupied by unstained vacuolar areas), or double heads, or any combination of these.
- Neck and midpiece defects, including absent tail, non inserted or bent tail (the tail forms an angel of about 90º to the long axis of the head), distended / irregular / bent midpiece, abnormally thin midpiece or any combination of these.
- Tail defects, including short, multiple, hairpin, broken, irregular width, or coiled tails, tails with terminal droplets, or any combination of these (Ombelet *et al*., 1997).

Defects in sperm morphology may impair the swimming strength or the ability of the sperm to penetrate and fertilize an egg (Seibel and Zilberstain, 1995). Diemer (2000) indicated that pathogenic microorganism could damage the spermatozoa and cause infertility. Auroux (1988) showed that *E. coli* is the bacteria that are most frequently met in cultures from the seminal fluid of infertile male, and it effects the sperm morphology and its ability to fertilize.

Wolff and Anderson (1988) examined under the microscope a multiple adherence of *E. coli* to spermatozoa causing various ultrastructure damages. Electron microscopic evaluation revealed multiple and profound alterations in the ultrastructure of spermatozoa such as membrane defects and cytoplasmic vacuoles exclusively in human spermatozoa of *E. coli* infected samples (Diemer *et al*., 2000).

2.6.4 Sperm Agglutination

Agglutination of spermatozoa means that motile spermatozoa stick to each other, head to head, midpiece to midpiece, tail to tail or mixed e.g. midpiece to tail (Urry, 1985). The adherence of either immotile or motile spermatozoa to mucus threads, to cells other than spermatozoa or to debris is not considered agglutination and should not be recorded as such (Comhair *et al*., 1980).

The presence of agglutination is suggestive of existence of an immunological factor of fertility and may be due to bacterial cells or antisperm antibody. Fakhrildin (2000) suggested that spermatozoal agglutination may be due to the cytotoxinc materials secreted from the inflammatory cells which cause sperm clumping and agglutination. Burkharin *et al*, (2000) reported that bacteria-spermia have a direct effect on the spermatozoa by decreasing sperm motility. *E. coli* have the ability of agglutination the spermatozoa and thus inhibit sperm motility (Monga and Roberts, 1994). Wolff *et al*. (1993) discovered that adherence of *E. coli* to sperm is mediated by mannose and mannose-binding structures present on both cell types which result in agglutination between bacteria and the spermatozoa cells.

2.6.5 Fertilization and Embryonic Development

The presence of bacteria in the original semen have been reported to have an effect on fertilization capacity of spermatozoa and embrynic development

either *in vivo* or *in vitro* (Bolton *et al*., 1986). Many scientist thought that fertilization, cleavage or pregnancy rates in *in vitro* could be impair at the presence of bacteria in semen (Riedel *et al*., 1984; Forman *et al*., 1987; Stoval *et al*., 1993; Liversedge *et al*., 1996). Hewitt *et al* (1985) concluded that bacterial infected semen sample could degenerate the hamster oocytes *in vitro* and reduced the fertilization and cleavage rates of human oocytes.

According to Troy and Herron (1986) and Wolff (1992), bacterial and viral agents are the most common cause of endometritis, fetal deformation and abortion. El-Mulla *et al*. (1996) noticed that *E. coli* have a negative effect on the inducibility of the acrosome reaction *in vitro* which may impair the fertilizing capacity on human spermatozoa. Shalika *et al*. (1996) reported that *E. coli* and *Staph aureus* have a negative effect on *in vitro* fertilization and should be treated. In according to the increasing WBC in seminal fluid due to infections, Plante *et al*., (1994) showed that WBC affect the fertilization potential of the spermatozoa possibly through the action of the molecules that they secrete mainly reactive oxygen species (ROS) and cytokines. Potts *et al*., (1999) suggested that the capacity for sperm oocyte penetration could be reduced through the damaging spermatozoa by ROS mediators. The ROS will initiate the peroxidation of unsaturated fatty acids of the sperm plasma membrane and this alters markedly the fluidity of the plasma membrane fusion in the normal process of fertilization (Mancini *et al*., 2000).

Many researchers indicated that the microbial infection of the seminal fluid may induce antisperm antibodies which interfere with the fertilization process and sperm-egg fusion (Cooper *et al.,* 1990). Nicholson *et al,* (2000) suggested that it is necessary to use semen preparation methods in IVF technology to minimize or rather remove the microbes originating from the semen sample. In this respect the virulence of these microbes *in vivo* is less importance than in culture condition in which microbes can divide rapidly.

2.7 *In vitro* Fertilization Technology:

In vitro fertilization (IVF) is an assisted reproductive technology (ART) in which one or more eggs are fertilized outside a female's body. IVF is an elective medical treatment, it provides a couple who has been otherwise unable to conceive with a chance to establish a pregnancy (Saith *et al*., 1998).

IVF has come a very long way since the first IVF pregnancy was reported in 1973 by team in Melbourne, Australia, and since the first IVF birth in 1978 by Dr. Edward and Dr. Steph in England (Int 2). Since then, the technology has been further refined and developed by physicians and embryologists, with over million children have been wide conceived and nearly 50.000 babies born with the aid at this technology (Internet 3; Internet 4). The possibility of a pregnancy being achieved for any one patient cannot be predicted, it depends on many variables such as the reproductive health of both the wife and the husband (Internet 3). This technique was devised for use in cases of infertility when the woman's fallopian tubes are damaged; man's sperm count is low. It is also now used to enable prospective parents with more complication reproductive problems e.g. inability to produce eggs, poor sperm quality to bear a child (Internet 3; Internet 1).

2.7.1 Requirements for IVF Technology

IVF simply is the uniting of an egg and a sperm *in vitro* out side the woman's body. Once the egg is fertilized, the embryo is put back into the woman's uterus to complete full term development (Saith *et al*., 1998). The paramount importance in a program of IVF is a deliberate attempt to stimulate as closely as possible, the events that occur naturally in oocyte maturation and spermatozoa capacitating (Richard and Tureck, 1995).

2.7.1.1 Ovulation Induction

Once the couple is deemed acceptable for the IVF technology, the wife is scheduled well in advance for follicular stimulation in a program uses hormonal therapy to stimulate multiple follicular development (superovulation)rather than the usual single ovum as in a natural cycle (Hamilton and Armstrong, 1991). Historically, the use of agents to induce ovulation were designed to induce ovulation in women, who themselves, did not ovulate. The second use of ovulation induction was to increase the number of eggs reaching maturity in a single cycle to increase chances for conception (Passmore *et al*., 2003). Human and mammalian ovaries could be stimulated by different types of hormones like the clomiphene citrate, Human Menaposal Gonadotropin (HMG), Follicle Stimulating Hormon (FSH), Human Chorionic Gonadotropin (HCG), and pregnant Mare's Serum Gonadotropin (PMSG) (Int 2).

HMG and HCG were used in the superovulation program to increase the number of follicles in laboratory and farm animals (Shaher, 1994). The HMG which is commercially called pergonal, humegon, repronex or menotropins are a mixture of follicle-stimulationg hormone (FSH) and lutinizing hormone (LH) that are naturally produced by the pituitary gland. HMG are used to cause the ovaries to produce several follicles, which can then be harvested for use in IVF (Int 2).

HCG or profasi (as commercially called) is a natural hormone, that helps with the final maturation of the eggs and triggers the ovaries to release the mature eggs. It also stimulates the corpus luteum to secrete progesterone to prepare the lining of the uterus for implantation of the fertilized egg (Internet I). Ovulation usually occurs about 36 hour after the HMG injection and 13-16 hours after HCG injection in mice (Whitingham, 1979). Betteridge (1977) and Lauria *et al*. (1982) reported that the success of superovulation program in animals depends on various factors such as physiological status of the animal at

the time of administration, animal response, age, breeding, nutrition and the hormonal preparation used.

2.7.1.2 *In vitro* Maturation (IVM)

After collection of oocytes, cumulus oocyte complexes were cultured within medium for maturation. Oocytes were considered mature only if they extruded a polar body of metaphase II during culture (Lenz *et al*., 1983). The whole maturation process requires not less than 4 hours. The maturation involves nuclear, meiotic, cytoplasmic events which include changes in the organization of the plasma membranes as a part of cytoplasmic maturation (Bellve and O'Brien, 1983). The oocyte is protected by the zona pellucida which is a network of various proteins that encloses the oocyte. Depending on the species, the zona pellucida consists of different glycoproteins that are proposed to function as receptors for spermatozoa .Therefore, during the fertilization process, sperm must first bind to and then penetrate the zona pellucida in order to reach and fuse with oocyte plasma membrane (Hinsch *et al*., 1999).

2.7.1.3 Sperm Capacitation

Capacitation is the final step in the maturation of mammalian spermatozoa and is required to render them competent to fertilize an oocyte (Yanagimachi, 1994). Although the term capacitation was coined by Austin in 1952, this concept was first described simultaneously by both Austin and Chang in 1951 (Austin, 1951; Chang, 1951). Chang *et al*. (1977) showed that the physiological capacitation is not only takes place within the female reproductive tract but also *in vitro* by using a certain incubation media.

In vivo, capacitation occurs in female reproductive ducts under hormonal control. Estrogen and minimal amount of gonadotropins may be required for capacitation to occur (Dale and Elder, 1997). *In vitro* capacitation occurs spontaneously in a defined medium. It appears that the media in which the

sperm are incubated play an integral role in many sperm processes, $CaCl_2$, bovine serum albumin and $NaHCO_3$ are needed to induce capacitation in mouse spermatozoa (Go and Wolff, 1985; Visconti *et al*., 1999; Gadella and Harrison. 2000). During capacitation, various biochemical and physical changes occur in the spermatozoa and spermatozoal membrane (Brucker and Lipford, 1995).

Certain materials coating the surface of ejaculated sperm are removed or altered during capacitation and these include the so-called decapacitating factors and other proteins and glycoprotiens, while the cholesterol content of the membrane are reduced during this process (Oliphant *et al*., 1995; Yanagimachi, 1994; Fraser, 1998; Kopf *et al*., 1999).

Capacitation also involves changes in sperm motility, which has become known collectively as hyperactivation and is characterized by vigorous flagellum movements (Suarez *et al*., 1983; Yanagimachi; 1994). The definitive indication that a sperm has undergone capacitation is of course the successful fusion of a spermatozoon with the egg certainly after the acrosome reaction in response to physiological inducers by a zona pellucid or progesterone (Cross and Razy-Faulker . 1997; Kopf *et al*., 1999).

2.7.1.4 Sperm Acrosome Reaction

The oocyte of all eutherian mammals is surrounded by a thick glycoprotein coat, the zona pellucida, which at the time of ovulation itself is surrounded by the granulose of the freshly expanded cumulus oophorus (Yanagimachi, 1994). Before it can fertilize an egg, a spermatozoon must pass through the zona pellucida and for this to happen the spermatozoon undergoes the acrosome reaction soon after binding to the zona. The mammalian acrosome is a cap-like membrane limited a secretary granule which covers the anterior part of the nucleus on the sperm head (Friend *et al*., 1977). Acrosome reaction is an irreversible exocytotic event characterized by fusion of the outer acrosomal membrane, with overlying plasma membrane allowing the release of acrosomal

content which include numerous hydrolytic enzymes, that lyses part of the zona pellucida and the sperm (usually one per oocyte under physiological conditions), with its nucleus covered only by the inner acrosomal membrane, passes through the zona pellucida and across the perivitelline space to fuse with the oolemma of the egg (Meizel, 1978; Meizel, 1984).

Acrosome reactions occur spontaneously due to a gradual increase in intracellular Ca^{2+}, pH and due to the self aggregation of sperm receptor for zona pellucida (Sailing, 1989; Yanagimachi, 1994). Progesterone is a major component of the fluid within the preovulatory follicle which is thought to contribute to physiological induction of the acrosomal reaction (Cheng *et al*., 1998a). Cheng *et al*., (1998b) suggested that, because not all the spermatozoa in ejaculate have exposed to progesterone-receptor, only a limited number of spermatozoa undergo the acrosome reaction in response to progesterone.

In vitro, the sperm acrosome reaction can be induced in capacitated spermatozoa by incubation it with calcium-ionophore A23187, heparin (Varner *et al*., 1993), epidermal growth factor (Breitbort *et al*., 1997), atrial natriuretic peptide (Ronit *et al*., 1998), solubilized zona pellucida (Kopf *et al*., 1999) and platelet activating factor (Wu *et al*., 2001).

2.7.2 *In vitro* Fertilization:

Following egg retrieval, the husband is asked to do a sperm collection. The sperm is processed by a technique known as washing to remove any additional cellular material, chemical substances or bacterial cells within the ejaculate. A pure suspension of sperm is then added to the eggs. Each egg is inseminated with 50000-100000 motile spermatozoa (Mastroianni and Biggers, 1981; Renou *et al*., 1983). Before many years ago, men whose semen parameters were considered too poor to fertilize their wife's oocytes, even *in vitro*, had to be excluded from assisted reproductive technique (ART), but after that a procedure termed intracytoplasmic sperm injection (ICSI) resolved this problem. By this

technique, scientists now able to treat cases with poor semen parameters, even collected directly from the testes and use these for ICSI (Richared and Tureck, 1995). The day following the retrieval, oocytes can be examined for evidence of fertilization. If there are an excess number of embryos, a decision can be made regarding cryopreservation or embryo freezing.

Generally, one cannot transfer all of embryos during one session. Therefore, for woman under the age of 35, it would be considered appropriate to transfer up to 2-3 embryos and store other embryos for further use (IFRH, 2002). Following embryo transfer, the patient will usually rest quietly for 30 minutes prior to her departure for home.

The success rate of the *in vitro* fertilization is highly variable among programs. Successful animal and human *in vitro* fertilization (IVF) is dependent on maintenance of suitable conditions for gametes and early embryos. A number of different culture conditions have been used with success for IVF (Sokoloski and Wollf, 1984). Culture media for IVF are usually supplemented with an exogenous protein source such as serum, bovine serum albumin or fetal calf serum which has the ability to enhance *in vitro* development of human and mouse embryo (Padilla *et al*., 1988; Robert *et al*., 1991). Control of temperature, humidity and the concentration of gases such as carbon dioxide (5%) which regulates the pH of the medium, are also very important considerations which affected the frequency of sperm ovum fusion and rates of post fusion events (Rivera and Hansen, 2001).

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3.1 ~~Equipments,~~ Materials ~~and Animals~~

3.1.1 ~~Equipments~~Apparatus and Equipments~~:~~

~~The following equipment were used through the study:~~

~~Apparatus or Equipments~~	Company / Country
Autoclave	Karl kolb /~~West Germsny~~Germany
Oven	Gall~~ae~~n-kamp / England
Balance	~~Mettler/ USA~~Ohaus / France~~خط بين الاثنين~~
Water bath	Gallenkamp / England
Sensitive balance	~~Mettler/ USA~~Delta Range / Switzerland
Distillator	G~~allan kam~~FL ~~p~~/ ~~England~~Germany
Incubator	Gallenkamp / England~~Memmert/west Germany~~
~~Laminar air flow~~	~~Memmert/west Germany~~
~~Light~~ Compound light ~~Microscope~~microscope	Olympus / ~~Japane~~Japan
~~Millipore filter urit~~	~~Millipore filter corp/ USA~~
~~Micropipettes~~	~~Brand/ West Germany~~
~~Shaker~~ Shaking wat~~ek~~r bath	Kotterman /~~ USA~~ Germany
Sperctrophotometer	Miltonroy / USA
Power supply	LKB / Sweden
pH- ~~mexer~~meter	~~Griffen/ Sweden~~Metter Gmbh-Teledo / England
Inverted microscope	Leitz-wetzlar / Germany
Sterio micr~~e~~oscope	Will DM_3 / Switzerland

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Co2 CO2 incubator	Lec, limited, private, cowlick / NettinghamNottingham, England
Referigerator	UK National / Japan بينهم الجهازين خط في اسفل الخلية خطان
Pasteur pipettes	John Poutten Ltd./ / England
Micropipettes	–Brand / Germany بينهم خطوط
Millipore filter unit	Millipore Filter Corp / USA

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~~3.1.2 Materials~~

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3.1.2.~~1~~ Chemicals

~~The following chemicals were used in the study:~~

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Chemical~~s~~	Company / Country
~~Ethanol~~Ethyl alcohol	BDH / ~~UK~~England
Crystal violet	BDH / England~~UK~~
Iodine	BDH / England~~UK~~
Safranin	BDH / England~~UK~~
NaCl	Fluka / Switzerland
HCl	BDH / England~~UK~~
NaOH	~~MERCK~~ Merck /~~(West~~ Germany~~)~~
Sulfanilic acid	BDH / England~~/ UK~~
Acetic acid	BDH / England~~UK~~
Dimethyl~~—~~α-~~raphthylamin~~naphthylamin	BDH / England~~UK~~
Mineral oil	Bio merieux / France
Toluidine ~~Blue~~blue	Fluka / Switzerland
~~تاغي~~Gelatin	~~تاغي~~Difco~~/ USA~~
Potassium nitrate	Difco / USA
Glycerol~~Beef extract~~	BDH / England
Xylol ~~Ethyl alcohol~~	BDH / England

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Glycerol	BDH/ England UK
Xylol	BDH/ England تفصيل بخطوط
N,N,N,N-Tetramethyl-p-phenylene diamine dihydrochloride	BDH / England

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~~3.1.2.2~~

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~~3.1.2.2~~3.1.3 Media and ~~e~~Components

~~The following media were used~~

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Medium~~Media~~	Company/ Country
Ready to use medium~~Brain heart infusion agar~~ بدون هذا الفراغ~~Bifco /USA~~	
Brain heart infusion broth~~Dnase agar~~	Bio-life/ Italy~~Oxaid / Engaland~~
Mannitol-salt agar	Bio-life/ Italy
Nutrient agar	Oxoaid / England~~England~~
Nutrient broth	BDH / England~~UK~~
Staph 110 agar~~Api staph media~~	Difco / USA~~Bio-life~~
Peptone broth	Oxoaid / England~~England~~
MaConky agar	Difco / USA
Beef extract~~Blood agar~~	Biolife / Italy
Pepton water~~Cimon citrate agar~~	Oxoid / England
Eosin methyline blue agar~~Api 20 E media~~	Oxoid / England ~~Bio merieux / France~~
Trypton	Oxoaid / England ~~England~~
Minimum essential base(TSI)	~~Sigma / USA~~Sigma/ USA
Bovine-serum albumin	Sigma/ USA
Cimon-citrate agar	BDH / England
Triple sugar iron agar~~Rabbit plasma~~	BDH / England
~~Minimum essential media (MEM)~~	~~Sigma / USA~~
~~Bovine serum Albumin~~	~~Sigma / USA~~
Laboratory prepared medium	

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Blood agar	Bio-life/ Italy
Gelatin medium	Bio-life/ Italy
Indol medium	Oxoid / England
Minimum essential medium	Sigma/ USA
TNase agar	Difco/ USA

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Laboratory prepared medium	
تلقى هذه الفراغات	تلغى هذه الفراغات
Blood agar	Bio-life/ Italy
Gelatin medium	Bio-life/ Italy
Indol medium	Oxoid / England
Minimum essential medium	Sigma/ USA
TNase agar	Difco/ USA

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3.1.4 Reagents and ~~s~~Solutions:

Ready to use (Biomerieux / Franc)	Laboratory - prepared
VP1	Kovacs reagent
VP2	Dye reagent
NIT1	Catalase reagent test
NIT2	Oxidase reagent test
ZYMA	Normal saline solution
TDA	Staining solution
NID	
VP	
OX	

3.1.5 Hormones:

Two types of hormones were obtained commercially from Serrano (Italy). They are:

3.1.5.1 Human menaposal gonadotropins(HMG) : commercial name is pergonal-500 (contains 75 IU FSH + 75 IU of LH; Serono, Italy).

3.1.5.2 Human chorionic gonadotropin(HCG): ~~commertiall~~commercial ~~nam~~name is Profasi (contains 500 IU of LH; Serono, Italy).

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3.1.6 Api System Kits

Two types of Api- system kits obtained commercially from Bio-Merieux (France).

They are:

3.1.6.1 Api 20 E (for *EschirichiaEscherichia coli* identification).

3.1.6.2 Api Staph (for *Staph. aureus* identification).

3.1.2.3 Reagents

Reagent	Company/ Country
VP1	Biomerieux / France
VP2	
ZYMA	
ZYMB	
NIT1	
NIT2	

3.1.7 Experimental Animals:

Healthy adult mice of 72 males and 122 females were obtained from the animal house of Institute of Embryo Research and Infertility Treatment at AL-Nahrain university aging (10-12) weeks were used in this study. The animals

were housed in a small plastic cages measuring 29 x 12.5 x 115 cm, three females or three males each. Floors of cages were covered with soft crushed wood shaving, washed once a week with soap and tap water and sterilized with 70% ethyl alcohol throughout the period of the study. Mice were kept under suitable environmental conditions such as room temperature at about 24 ± 2ºC and exposed to 14 hour day light program , daily food in the form of pellet were supplied by Center for Agricultural Researches were accessible freely to the animal with tap water (Petter and Pearson, 1971)

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~~3.1.2.4 Animals and Animal Care~~

~~Healthy adult mice of Swiss albino strain were obtained from the animal house of institute for embryo research and infertility treatment, from the animal house of Biology Department in Baghdad University and from the animal house of Al-Nahrain Research Center for Biotechnology. One hundred male and 180 female of mice with a range of age (8-9) weeks were used in this study.~~

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~~The animal were housed in small plastic cages measuring 29 x 12.5 x 115 cm, each cage contains three females or three males. Floors of cages were covered with soft crushed wood shaving, washed once a week with soap and tap water and sterilized with 70% ethyl alcohol throughout the period of the study (Petter and Pearson, 1971; Williams, 1976).~~

~~The animals were kept under suitable environmental conditions such as the temperature of room was maintained at about 24 ± 2ºC and exposed to 14 hour~~

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~~day light program daily food in the form of pellet were supplied by center for agricultural researches were accessible freely to the animal with tab water (Williams, 1976).~~

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~~3.1.2.5 Hormones~~

~~Reagent~~	~~Company/ Country~~
~~Human menaposal gonadotropins HMG~~	~~LG life science / Korea~~
~~Human Chorionic gonadoropin HCG~~	~~Boxmeer / Holand~~

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3.2 Methods

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~~3.3 Methods~~

3.2~~3~~.1 Collection of Seminal Fluid Samples

Semen samples w~~as~~ere collected from 14 infertile patients in ~~a~~ a clear, dry and ~~d~~ ~~sterile~~ sterile disposable Petri-dishes, after 3 to 7 days of sexual ~~obstinence~~abstinence period. ~~The~~Semen collection ~~The~~ ~~coll~~rrection ~~done were~~ ~~were~~was performed in a special room ~~in~~ at the I~~i~~nstitute of Embryo Research and Infertility Treatment. ~~The sample~~Samples then ~~should reach~~were transferred to the microbiology ~~microbiologic~~ laboratory at biotechnology department; college of science of Al-~~Nahrain~~Nahrain University within 2 hour for culturing ~~(WHO, 1999)~~.

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Table (3-1) Clinical data of the infertile patients involved in this study

Parameters of infertile patients*	Rang	Mean
Duration of infertility	2-8	4.357
Age	23-51	32.071

Percentage of sperm function tests	Concentration x10^6	15-72	40.714
	(%) motility	10-65	33.428
	Progressive motility (%)	0-25	12.142
	Agglutination (%)	0-18	5.928
	Morphology (%)	12-48	34.142

* no. 14

All infertile patients were primary infertile.

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3.2.2 Sterilization

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3.2.2.1- Moist ~~h~~Heat ~~s~~Sterilization:

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—Autoclave was used to sterilize media, buffers and solutions, at 121°-C for (15Ib/in~~2~~^{2}) for 15min.

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3.2.2.2- Dry Heat Sterilization

—Electric oven was used to sterilize glasswares and others by heating at 180°-C for 2 hours.

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3.2.2.3- Filtration (Membrane Sterilization):

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Millipore filter unit was used to sterilize the bovine serum

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~~A~~albumin by dissolved it in –a few of ~~M~~minimum essential medium. Millipore also used to obtains culture filtrates from bacterial cell growth in

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broth. Two types of filter pores (0.4 um and 0.22 um) were used.

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3.2.3 Preparation of ~~m~~Media:

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3.2.3.1 Ready to Use Media:

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They were prepared and sterilized according to the instructions fixed on their containers.

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3.2.3.2 ~~Laboratory Prepared~~Laboratory Prepared Media:

3.2.3.2.1~~-~~ Blood agar Media (Harley and Prescott~~,~~, 1996~~) :~~):

~~~~~~~~This was prepared by autoclaving blood agar base after adjusting pH to 7.0

~~P~~ ~~p~~then cooled to 45°~~-~~C and 5% of human blood was added, mixed well and

poured into Petri~~-~~dishes (15 ml each), it was used for isolation of *E.~~-~~ coli* isolates.

~~-~~3.2.3.2.2~~-~~ Gelatin Medium (Harley and Prescott~~,~~ ~~(~~1996~~):~~

~~~~~~~~Brain-Heart infusion broth was used after adding 12% of dissolved

~~gelatine~~gelatin to it, the pH was adjusted to 6 and autoclaved, this medium was used

for identification of *E.~~-~~ coli* isolates.

3.2.3.2.3 ~~-~~Indole Medium (Atlas *et al.*, (1995)~~:~~

This was prepared by dissolving 15 g trypton in 1 liter of distilled water and

autoclaved, after adjusting pH to 7.0 the medium was used for identification

~~of~~of ~~*Eschirichia*~~*Escherichia*~~.~~ *coli* isolates.

3.2.3.2.4~~-~~ TNase A~~a~~gar Medium~~:~~

Toluidine blue-DNA agar was prepared by adding 0.1 g of toluidine blue to

1 L of autoclaved DNase agar.

3.2.3.2.5~~-~~ Minimum e~~E~~ssential ~~m~~Medium:

This medium was prepared by dissolving 600 mg of ~~bovin~~bovine serum albumin in 500 ml of minimum essential base, and adjusting pH to 7.3-7.4, then sterilized by membrane filtration by using 0.4 and 0.22 ~~M~~millipore filters ~~papers~~, and then stored under 4°C. This medium was used in all tests of seminal fluid parameters and *in vitro* fertilization techniques

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~~minimum essential base, and adjusting pH to 7.3-7.4 ,then sterilized by~~

~~membrane filtration by using 0.4 and 0.22 Millipore filters papers, then stored~~

~~under 4 C. This medium was used in all tests of seminal fluid parameters and *in*~~

~~*vitro* fertilization techniques~~.

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3.2.4 Preparation of Reagents and Solutions:

3.2.4.1- Ready to Use Reagents:

All ready to use reagents were obtained From Bio-merieux. (France).

3.2.4.2- Laboratory Prepared Reagents:

3.2.4.2.1- Kovac's Reagent (Atlas *et al*., 1995)

This was prepared by dissolving 0.1 gram of lactic acid to distilled/deionized water and brings complete the volume to 250 ml, then it was mixed thoroughly and kept in refrigerator. This reagent was used in indole test.

~~water and brings volume to 250 ml, then it was mixed thoroughly and kept in refrigerator. This reagent was used in indole test.~~

3.2.4.2.2 Catalase ~~r~~Reagent ~~;~~ (Harley and Prescott~~.~~, (1996)~~:~~

-A concentration of 3 % H_2O_2 was prepared-.

ال٢ تكتب صغيرة

3.2.4.2.3 Oxidase ~~r~~Reagent (Baron *et al.*, 1994)~~:~~

A concentration of 1% N,N,N,N-Tetramethyl-p-phenylenediamine dihydrochloride was prepared in sterile distilled water when needed

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3.2.4.2.4 Normal Saline Solution (Atlas *et al.*, 1995)

This was prepared by dissolving 0.85 g of NaCl in 100 ml of distilled water then autoclaved after ~~pH was~~ adjuste~~d~~ing the pH to 7.0**.**

~~then autoclaved after pH was adjusted to 7.0.~~

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3.2.4.2.5 Staining S~~s~~olution

This solution was prepared by dissolving 0.3 of methylene blue powder in 30 ml of 95 % ethanol.

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~~95 % ethanol.~~

3.2~~3~~.2 ~~5~~ Bacterial ~~Isolation~~Culturing

Each sample ~~is~~ was divided into ~~2~~ two parts~~,~~parts; one ~~of them~~was cultured in a ~~positive~~ staphylococci medium staph 110 medium and the other on

Enterobacteracea medium macConkey agar, and incubated at 37ºC aerobically overnight.

~~bacterial media medium staph 110 medium and the other on negative~~Enterobacteracea ~~media~~medium maConkey agar~~, and incubated at 37ºC aerobically over night., all media were prepared according to the manufacturer and sterilized by autoclave.~~

3.~~23~~.~~25~~.1 *Staphylococcus aureus* ~~Isolation culturing~~Culturing

~~Mannitol Salt Agar~~ Staph 110 agar ~~It~~ was used for ~~the selective isolation~~, cultivation and enumeration of ~~*Staphylococci*~~ *Staph aureus* from ~~clinical and nonclinical specimens~~samples. ~~It is usually used for differentiating *Staphylococcus aureus* (mannitol fermenting). Mannitol fermenting *Staphylococci* produce a yellow zone surrounding their growth~~After incubation at 37°C for 24-48 hrs colonies were ~~described (~~described (Harley and Prescott., 1996).

~~i.~~ 3.~~23~~.~~25~~.2 *Escherichia coli* ~~Isolation c~~Culturing:

~~i.~~ **~~MaConkey Agar~~** ~~(Ronald, 1995)~~

MacConkey agar ~~is~~ was a differential medium for the ~~selection and~~ recovery of *E. coli*. After incubation at 37 C for 24 h, colonies were described (Atlas *et al*., 1995).

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of Enterobacteriaceae and related enteric grem negative rods. Lactos is

of the sole carbohydrate. *E. coli* is lactose fermenting bacteria, produce.After incubation at 37 C for 24 h, colonies that are were varying shads of rod because of the conversion of the neutral red described (Atlas et al., 1995).

indicator dye.

3.2.6 Bacterial Identification

3.3.3 Identification of *Staphylococcus aureus* and *E. coli*

3.23.63.1 Morphological Tests Microscopical Examination (Koneman *et al.*, 1992; Harley and Prescott., 1996):

Smears of bacterial isolates (*Staphylococcus aureus* and *E. coli*)were) were stained by Gram staining method then observed under the oil immersion objective of the compound light microscopmicroscope.

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(Koneman *et al*., 1992; Prescott, 1996)

On direct Gram stained smears of *Staphylococcus aureus* which cultured on Brain Heart Infusion agar with 7-5% appeared as gram-positive cocci in grape like clusters where as *E. coli* which cultured on MaConkey agar gram-negative single or paired bacilli.

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3.23.63.2 Biochemical Tests

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3.2.6.2.1 3.3.3.2.1 *Staphylococcus* Biochemical Tests

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I-A- Catalase Test (Atlas *et al*., 1995Taylor and Chazar, 1972)

This test was performed by placing putting part of a suspected material from a colony where cultured grown on nutrient agar onto a glass slide, then a drop of 3% H_2O_2 was added. Appearance of bubbles indicated positive results.

on nutrient agar onto a glass slide, and addingthen a drop of 3% hydrogen peroxideH2O2 was added. to the inoculums. The aAppearance of bubbles indicated the positive results,

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B-Oxidase Test (Atlas *et al*., 1995)

This test was done by using moisten filter paper with a few drops of a freshly prepared solution of N,N,N,N-Tetramethyl-p-phenylene diamine Dihydro-chloride. Aseptically, a clump of cells was picked up from the slant growth with a sterile wooden stick and smeared on the moisten paper. The development of purple color within 5-10 second indicated a positive test, result is indicative of negative result.

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Dihydrochloride. Aseptically, a clump of cells was picked up from the slant growth with a sterile wooden stick and smeared on the moisten paper. The

development of purple color within 5-10 second indicated a positive test, result is indicative of negative result.

ICI- Tube Coagulase Test (Jawetz *et al.*, 1987)

D A dilution of plasma 1:10 plasma toin saline (0.85% NaCl) was prepared and placed in

smalltest tube. The strain under test was inoculated into the tube, by adding 0.1A portion ml of 0.1 ml of 18-24 hrs of each isolate culture was added then incubated at 37°-C of 18-24 hrs. Broth culture (about 10^8 cocci). The tube was incubated at 37ºC and examined for coagulation after 1, 3 and 6 hours. Tubes that showed nNegative results were left -at room temperature overnight and re-examined. The conversion of the plasma into a soft or stiff gelsinto stiff ,gels was observed by tilting the tube to a horizontal position.

IDH- TNase Test (Koneman *et al.*, 1992)

Toluidine blue-DNA agar was prepared by adding 0.1 g of toluidine blue to 1L of DNase agar. After solidification of the toluidine blue-DNA agar plates, a 33- mm wells waswere made. Tubes, each contains of 10 -ml of -Brain-Heart Infusion broth waswere inoculated with cultures theof the bacterial isolates strainsthen incubated for 18 hrs at 37ºC. The bacterial cells were precipitated by centrigugationcentrifugation at 5000 rpm for 10 min. The precipitatesupernatant was heated at 100ºC for 15 min, then 0.05 ml of the heated sample was added to the each well. Plates were incubated at 37ºC for 24 hrs. Positive results was indicated by the appearance of a pink zone around the well.

E- IV- DNase Test (Harley and Prescott, 1993)

DNase agar plates were prepared according to the manufacturer. The bacterial strains were streaked on solidified medium and incubated at 37ºC overnight. Ten ml of HCl was added to each plate. The appearance of a clear zone around the colonies indicated positive results. Mannitol Fermentation Test (Atlase *et al.*, 1995):

It was used to differentiate *Staphylococcus aureus* (mannitol-fermenting) from *Staph**Staphylococcus epidermidis* (nonmannitol-fermenting). The suspected isolates were cultured on Manitol-salt agar, Mannitol-fermenting staphylococci forming yellow zone surrounding their growth after incubation at 37°-C for 24 hrs.

V

- Identification of *Staphylococcus aureus* by API Staph system (Biomerieux)

ApI-Stph system was used to identify the different species of *Staphylococci* (Herber *et al.*, 1988). This system was designed originally to identify *Staphylococci*. The ApI-Stph system includes twenty different fast biochemical reactions, as present in table ( ) in which their results were converted to seven digit numeral profile according to the reactions patterns, whether are positive or negative (Etienne *et al.*, 1988).

Instruction for use (bioMerieux):

- The organism was subcultured onto blood agar 18-24 hrs at 35-37ºC.
- Purity of microorganism was checked.
- The ampoule of ApI medium was opened in sterile conditions.
- A homogenized bacterial suspension was prepared.

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Using a sterile pipette, the microtubes were filled with the inoculation ApI Staph medium.

Anaerobic conditions for ADH and URE tests were performed by addition of mineral oil.

Strips were incubated at 35-37ºC.

Reading the Strips

Adding 1 drop of each the following reagents development the following reactions:

VP test: VP1 and VP2 reagents:

After 10 min, a violet-pink color indicates a positive reaction. Pale pink or light pink color indicates negative reaction.

NIT test: NIT1 and NIT 2 reagents:

After 10 min a red color indicates a positive reaction.

PAL test: ZYM A and ZYM B reagents:

After 10 min, a violet color indicates a positive reaction.

3.3.3.2.2. 3.2.6.2.2 *Escherichia coli* Biochemical Tests

A

I- Tryptophanase (Indole production from tryptophan hydrolysis) Test (Atlas Ronald *et al*., 1995).

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PInoculateep tryptone brothwater was inoculated by the isolate and incubated at 37ºC for 24-48 hrs. A quantity of 0.05 ml of Kovac's reagent was added and mixed gently. Positive result was recorded by the appearance of ring on the surface.

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~~hrs. tubes with the bacterial cultures; incubate the tubes at 37ºC for 24-48 hrs. A quantity of 0.05 ml After incubation 10 drops of Kovac's reagent was added directly to the culture and mixed gently.tube. If~~

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~~the culture produces tryptophanase, the presence of indole will be detected by the immediate formation of a red layer at the top of the broth.Positive result was recorded by the appearance of ring on the surface.~~

~~IBI~~- Gelatinase ~~t~~Test (~~Ronald~~Atlas *et al.*, 1995):

Gelatin agar was used to detect gelatin liquefaction by inoculating tubes containing the medium and incubating at 37°C for 48 hr. After ~~keeping into~~keeping into the refrigerator (4°C) for 30 minutes, positive results were recorded by observing gelatin liquefaction.

C- Citrate utilization Test (Colle *et al.*,1996):

—Cimon citrate agar was streaked by the isolate, then incubated at 37°C for 24-48 hrs. *E. coli* ~~was~~ revealed no color change to blue as negative result.

~~48 hrs. *E.coli* was revealed no color change to blue as negative result .~~

D- Triple Sugar Iron Test (TSI) (Atlas *et al.*, 1995)

——Isolates were cultured on the TSI agar slants by stabbing and streaking on surface. After incubating for 24-48 hr at 37°C, the color ~~changed~~ of the

medium changed from red to yellow was observed as an indicator of acid formation, pushing the agar to the top was an indication of CO_2 production.

~~Surface. After incubating for 24-48 hr at 37C, color changed of medium from red to yellow was observed as an indicator of acid formation, pushing the agar to the top was an indication of CO2 production.~~

~~3.2.7 Nutrient broth gelatin tube (12% gelatin) was inoculated and incubated at 37ºC for 24-48 hrs. After incubation, the tube placed in a refrigerator for 30-60 min. Positive result indicated by the remaining of the gelatin in liquid manner.~~

~~III- Nitratase (Ronald et al., 1995)~~

~~Beef extract peptone broth supplemented with 0.5% potassium nitrate that inoculated with the bacterial culture were incubated at 37ºC for 24-48 hrs. After incubation, 2-3 drops of reagent A and 2-3 drops of reagent B were added to the tube positive result indicated by the immediate formation of a red color.~~

~~IV- Citrate Utilization (Ronald et al., 1995)~~

~~A slant of Simmon's Citrate agar medium was inoculated and incubated at 37ºC for 24-48 hrs. After incubation a royal blue color was the positive test.~~

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~~V- Identification of E. coli with the ApIi 20 E Ssystem~~ Identification

~~3.2.7.1 APi-Staph sSystem (-~~Atlas *et al.*, 1995)~~:~~

~~——~~Api-Staph kit was used to identify different species of *Staphylococci*. This system was designed originally to identify *Staphylococci*. It consists of 20 microtubes each one containing dehydrated substrates. After the organism was cultured onto blood agar plate for 18-24 hrs at 35-37ºC and the purify of microorganism was checked, a homogenized bacterial suspension in saline solution was prepared, the ~~G~~gallery were inoculated with a bacterial suspension Anaerobic conditions for ADH, URE were performed by addition of mineral oil. Then the gallery incubated at 37°C for 18-24 hrs. During incubation, metabolism products produce color changes that were either spontaneous or revealed by the addition of reagents.

~~This system was designed originally to identify *Staphylococci*. It consist of 20 microtubes each one containing dehydrated substrates. After the organism was cultured onto blood agar plate for 18-24 hrs at 35-37ºC and the purify of microorganism was checked, a homogenized bacterial suspension in saline solution was prepared, the Gallery were inoculated with a bacterial suspension Anaerobic conditions for ADH, URE were performed by addition of mineral oil. Then the gallery incubated at 37 C for 18 –24 hrs. During incubation, metabolism products produces color changes that were either spontaneous or revealed by the addition of reagents.~~

3.2.7.2 ~~(Ronald et al., 1995)~~Api 20 E (Harley and Prescott~~.~~, 1996)~~:~~

~~——~~The Api 20 E ~~system~~kit ~~is a standardized, miniaturized version of conventional procedure~~was used ~~s~~for the identification of Enterobacteriaceae

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and other gram negative bacteria. After the isolate was cultured and purified onto MacConkey agar plate for 18-24 hrs at 35-37ºC, a homogenized bacterial suspension was prepared in saline solution. The Galleries were inoculated with a bacterial suspension, anaerobic conditions for ADH, LDH, ODC and URE tests were performed by addition of mineral oil. Then the kit was incubated at 37°-C for 18 -24 hrs.

The system uses 20 miniature microtube, reaction chamber (each microtube consist of a tube and cupules section)

Instruction for use:

The organism was cultured onto MaConkey agar plate 18-24 hrs at 35-37ºC.

Purify of microorganism was checked.

The ampoule of ApI 20E was opened in sterile conditions.

A homogenized bacterial suspension in saline solution was prepared.

By using a sterile pipette, the microtubes were filled with the inoculation ApI 20E medium.

Anaerobic conditions for ADH, LDH, ODC and URE tests were performed by addition of mineral oil.

After inoculation, incubate the test strip and agar slant at 37ºC for 18-24 hrs.

Add the test reagents to the TDA, IND and VP.

After reading the carbohydrate reaction add 1 drop of 1.5% H_2O_2 to the MAN tube and observe for evolution of bubbles.

3.3.4 Maintenance of Bacterial Strains

Maintenance of bacterial strains were performed according to Maniatis *et al*., (1982) as follow:

I- Short Period

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~~Colonies of bacteria were maintained for few weeks on nutrient agar media, plate were tightly warpped in parafilm and stored in the refrigerator at 4ºC. For longer storage, strains of bacteria were maintained in slant containing nutrient agar. These slant were prepared in 10 ml screw-capped bottles containing 3-4 ml of nutrient agar. Bottles were inoculated with the bacterial strains and incubated at 37ºC overnight then stored in the refrigerator for one month.~~

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~~II- Long Period~~

~~Bacterial strains could be stored for a year by adding 15% of glycerol to bacterial growth on nutrient broth as 1:1 and stored in deep freezing.~~

3.23.5.8 Preparation of BBacteria and BBacterial Culture FiltratesSupernatant CConcentrations (Atlas *et al*., 1995)

——After inoculation of *Staphylococcus aureus* and *E. coli* separately in nutrient broth and incubated at 37°C overnight**,** viable plate count technique was used for the enumeration of bacterial cells after prepar~~ation~~ing serial dilutions by using normal saline solution to ~~prepar~~prepare~~ing~~ the ~~concentration of~~count of bacterial cells $3x10^3$, $6x10^3$, $12x10^3$, $24x10^3$, $36x10^3$, $48x10^3$ and filtrating it with 0.22 um ~~M~~millipore filter paper for preparing bacterial culture filtrates.

~~broth and incubated at 37°C overnight, viable plate count technique was used for the enumeration of bacterial cells after preparation serial dilutions by using~~

~~normal saline solution to preparing the concentration of bacterial cells $3x10^3$, $6x10^3$, $12x10^3$, $24x10^3$, $36x10^3$, $48x10^3$ and filtrating it with 0.22 Millipore filter paper for preparing bacterial culture filtrates.~~

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3.2.9 Maintenance of *Escherichia. coli* and *Staph.ylococcus aureus* :

3.2.9.1 Daily Working Cultures (Baron and Fingold, 1994):

After inoculation of nutrient broth by the bacterial isolates, tubs were incubated at 37°C for 24 hr then kept in refrigerator.

~~incubated at 37 C for 24 hr then kept in refrigerator.~~

3.2.9.2 Short- tETerm sStorage (Maniniatis *et al*., 1982) :

Isolates of *E. coli* were maintained on MacConky agar and *Staphylococcu.s aureus* on Staph 110 plates for few weeks. The plates were tightly wrapped with Para-film, and then stored at 4°C.

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Staph.aureus on Staph 110 plates for few weeks. The plates were tightly wrapped with Para film, and then stored at 4 C.

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3.2.9.3 Medium-tTerm sStorage (Maniniatis *et al*., 1982):

Isolates of bacteria were maintained as stab cultures for few months. Such cultures were prepared in small screw-capped bottles containing 2-3 ml of nutrient agar medium and stored at 4°C.

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Such cultures were prepared in small screw-capped bottles containing 2-3 ml of nutrient agar medium and stored at 4 C.

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3.2.9.4 Stock Cultures (Contreras *et al*., 1991):

Ten ml of 15-20% glycerol were added to the screw tubes containing nutrient broth. After autoclaving, inoculate with bacteria and incubate at 37°C for 24 hr aerobically, then kept in freezer.

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broth. After autoclaving, inoculate with bacteria and incubate at 37 C for 24 hr aerobically, then kept in freezer.

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After inculcation of *Staphylococcus aureus* and *E. coli* separately in nutrient broth and incubated it under 37°C over night, the bacterial growth were measured in spectrophotometer under were length 600. The reading value should be reach 0.5 because at this value the number of *E. coli* and *Staphylococcus aureus* cells in one milliliter is approximately 1x10^{8}. Half of bacterial growth was filtrated with 0.22 millipore filter paper to gain the bacterial supernatant.

The different concentrations of bacterial cells and supernatant 3x10^{3}, 6x10^{3}, 12x10^{3}, 24x10^{3}, 36x10^{3}, 48x10^{3} were prepared independent on this growth value.

3.3.6 *In Vitro* Maturation of Oocytes (IVM)

Immature oocytes were chosen for IVM which that surrounded by multilayers of compact cumulus cells with homogenous cytoplasm.

Each 5-6 immature oocytes were grouped within culture media (MEM; Denmark) in a new petridishes under CO_2 incubator at 5% CO_2 and 37°C with satisfies humidity for 6 hrs (Floka and Okolski, 1981).

3.23.710 Mouse and Mouse sSSpermatozoa

house of institute for Embryo Research and Infertility Treatment at university of Baghdad with a range of age (10-12) weeks were used in this study. The animals were housed in small plastic cages measuring 29 x 12.5 x 115 cm, each cage contains three females or three males. Floors of cages were

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~~covered with soft crushed wood shaving, washed once a week with soap and tap water and sterilized with 70% ethyl alcohol throughout the period of the study (Petter and Pearson, 1971; Williams, 1976).~~

~~Mice were kept under suitable environmental conditions such as the temperature of room was maintained at about 24 ± 2ºC and exposed to 14 hour day light program daily food in the form of pellet were supplied by Center for agricultural researches were accessible freely to the animal with tab water (Williams, 1976).~~

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~~house of institute for Embryo Research and Infertility Treatment at university of~~

~~Baghdad with a range of age (10-12) weeks~~

~~were used in this study. The animals were housed in small plastic cages~~

~~measuring 29 x 12.5 x 115 cm, each cage contains three females or three males.~~

~~Floors of cages were Covered with soft crushed wood shaving, washed once a~~

~~week with soap and tap water and sterilized with 70% ethyl alcohol throughout~~

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~~the period of the study(Petter and Pearson, 1971; Williams, 1976).~~

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~~temperature of room was maintained at about 24 ± 2ºC and exposed to 14 hour~~

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~~agricultural researches were accessible freely to the animal with tab water~~

~~(Williams, 1976).~~

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3.23.710.. 11 ~~Seminal Fluid Collection~~Collections of Mouse Spermatozoa

~~Seminal fluid was collected from the mouse~~ Aafter ~~slaughter~~male mouse was killed by cervical dislocation, ~~them. Bothe~~both ~~vas deference~~ coda were excised and washed by culture medium MEM then every coda was ~~putted~~plased in a sterile small Petri dish contains 0.5 ml of culture medium MEM and crushed with ~~microscissore~~micro scissor ~~until~~ to be homogenized.

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~~and washed by culture medium (MEM ; Denmark), then sperm were collected by flushing of vas deference with 1 ml every coda was putted in a sterile~~

~~small Petri dish syringe (G 28) contains 0.51 ml of culture medium MEM and crushed with~~

~~microscissore until to be homogenized.~~

~~(Fakhrildin *et al*., 2001).~~

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3.23.710.22 ~~Co~~Incub~~tamin~~ation of the m~~M~~ouse spermatozoa and bacteria ~~Seminal Fluid~~

In order to ~~be acquainted~~investigate ~~with~~ the effect of *Staph~~ylococcus~~ aureus* and *~~Escherichia~~. coli* which were isolated and identified from seminal fluid of infertile patients on mouse spermatozoa, these two kinds of bacteria with ~~ther~~their supernatant were added to the mice spermatozoa *in vitro* with different bacterial co~~ncentration~~unts (3~~x~~X10^3, 6X~~x~~10^3, 12X~~x~~10^3, 24X~~x~~10^3, 36X~~x~~10^3 and 48X~~x~~10^3 cell/ml) or its supernatant. Then the outcomes of sperm function tests and *in vitro* fertilization were assessed.

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~~*Escherichia coli* bacteria which isolated and identificated from seminal fluid infertileof infertile~~

~~humanpatientss on mouse spermatozoa, these two kinds of bacteria with there~~

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~~supernatant were added to the mice spermatozoa *in vitro* with different bacterial~~

~~concentrations (3 X 10^3, 6 X 10^3, 12 X 10^3, 24 X 10^3, 36 X10^3 and 48 X 10^3~~

~~cell/ml) or its supernatant .Then the outcomes of sperm function tests and *in vitro* fertilization were assessed.~~

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~~seminal fluid, these two kinds of bacteria with there supernatant were analyzed *in vitro* by adding it to the mice seminal fluid and contaminated it with different bacterial or supernatant concentrations (3, 6, 12, 24, 36 and 48) x 10^3 cell / ml and experiments sperm function tests and *in vitro* fertilization under their influences.~~

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3.2~~3~~.~~7~~10.~~3~~3 Sperm Function Tests(SFTs) (NAFA and ESHER~~.~~, 2002):

Typical measurements of SFTs were— done after 0, 30, and 60 ~~minutesof~~minutes of co- incubat~~ed~~ion with bacteria or its supernatants. The spermatozoa tests ~~are~~were ~~S~~sperm concentration, percentage of sperm motility,

percentage of progressive sperm ~~grade activity~~motility, percentage of sperm agglutination, percentage of abnormal sperm morphology and percentage of *in vitro* fertilization. For each sample,~~–~~ a drop of culture medium containing spermatozoas was mounted between slides and covered with standard cover slip. The preparations were ~~scared~~examined under magnification of 40X~~x~~ objective. These sperm function tests are:

~~incubated with bacteria or its supernatants. in microscopic. Semen analysis include~~~~The spermatozoa tests are:~~

~~Sperm~~

~~concentration, percentage of sperm motility, percentage of sperm grade activity,~~

~~percentage of sperm agglutination and, percentage of abnormal sperm morphology~~

~~and percentage of *in vitro* fertilization. For each sample a drop of culture~~

~~medium containing sperms was mounted between slides and covered with~~

~~standard cover slip. The preparations were scared under magnification of 40x objective.~~

~~These sperm function tests are:~~

~~(WHO, 1992).~~

~~These sperm function tests were assessed in pre and post semens bacterial polluted at zero time and after 30 and 60 min. For each sample a drop of culture media containing sperm was mounted between slide and covered with standard cover slip.~~

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~~The preparations were scared under magnification of 40x objective. These sperm function tests are:~~

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~~3.3.7.3.1~~3.2.10.3.1 ~~-~~Sperm Concentration (Hinting.,1989);

~~——~~Sperm concentration per ~~millileter~~milliliter was ~~reported from~~ calculated by multiplying the mean number of spermatozoa in 10 random microscopic fields ~~and multiplying the mean number of sperm with~~by a factor 10^6

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~~spermatozoa in 10 random micorscopicmicroscopica fields and multiplying the mean number by multiplying sperm concentration with of spermsemen with a factor 106volume.~~

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~~Total sperm count~~ Sperm ~~concentration~~(concentration (sperm/ml) = mean number of sperm ~~X~~XX 10^6

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sperm concentration x volume

3.3.7.3.3.2.10.3.22 Sperm Motility and gGGrade ActivityaActivity , (Pale and Elder, 1997; WHO, 1999)(NAFA and ESHRE, 2002):

The prepared slide examined for determination of sperm motiliy wereslide examined for determination of sperm motiliymotility was deterexamined under room temperature. Ten randomly fields were selected and counted at least one hundred spermatozoa. The percentage of motile spermatozoa was calculated as follow:

$$\text{Percentage of Sperm Motility} = \frac{\text{No. of motile spermatozoa}}{\text{Total no. of spermatozoa}} x100$$

under room temperature. Ten randomly fields were selected and counted at least

one hundred spermatozoa. The percentage of motile and immotile spermatozoa was calculated as follow:

Percentage of sperm motility = X100 يكتب بين المنة واليساري

(no. of motile spermatozoa البسط) Total no. of spermatozoa مقام خط كسر في المقام يكتب

counted in sSperm grade activity, which wereactivity was classified undergraded in four grades:scales:

A: Rapid regular progressive forward movement.

B: -Moderate progressive forward movement.

C: ~~Slow~~Non--progressive forward movement.

D: Immotile spermatozoa.

~~D Immotile Sperm.~~

$$\text{Sperm grade activity \%} = \frac{\text{no. of spermatozo a (a or b or c or d)}}{\text{Total no. of spermatozo a}} \text{ x 100}$$

The ~~percentage of progressive motile spermatozoa were~~percentage of progressive motile spermatozoa was also counted by taking the mean number of forward progressive m~~m~~otile spermatozoa (a + b~~), it should be more than 50% total sperm count.~~):

$$\text{Normal sperm motility\%} = \frac{\text{no. of progressive motile spermatozoa (a + b)}}{\text{Total no. of spermatozoa}} \text{ x 100}$$

~~Both should be taken within 60 min of collection.~~

3.2.10.3.3 Sperm Morphology (WHO, 1999)

The examination of morphologically abnormal sperms was performed using the same prepared slides for sperm motility. At least 100 sperm were counted and percentage of morphologically abnormal sperms was calculated from the following formula:

$$\text{Morphological abnormal sperms \%} = \frac{\text{no. of abnormal sperms}}{\text{Total no. of sperms}} \text{ x 100}$$

~~3.3.7.3.3 Sperm Morphology (WHO, 1999)~~

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The examination of morphologically abnormal sperms was performed using the same prepared slides for sperm motility. At least 100 sperm were counted and percentage of morphologically abnormal sperms was calculated from the following formula:

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$$\text{Morphological abnormal sperms \%} = \frac{\text{no. of abnormal sperms}}{\text{Total no. of sperms}} \text{ x } 100$$

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Normal value should be equal to more than 50% normal morphology.

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3.3.7.3.3.2.10.3.4 4 Sperm Agglutination

After the counteding of at least one hundred sperm, the calculation of agglutinated spermatozoa it as the following formula:was calculated using the following formula

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$$\text{Percentage of agglutination sperm} = \frac{\text{no. of agglutinated sperms}}{\text{Total no. of spermatozoa count}} \text{ X } 100$$

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3.23.810.4 *In ~~vitro~~ Fertilization*vitro Fertilization Technologyique IVF

3.23.810.4.1 SuperovulationSuperovulation PProgram

Female mouse was injected intraperitonialy (IP) with 7.5 IU of human menapousal gonadotropin (HMG). 24 hrs later, another IP injection (7.5 IU) of hMG. Human chorionic gonadotropin (hCG) was IP injected (15 IU) after 24 hrs from the last HMG injection.

~~The mice females were superovulated with 2 dosage of human menaposal gonadotropins HMG during 48 hrs, 10 IU in each 24 hrs. and one dosage of 15 IU human chorionic gonadotropins HCG injected after 72 hrs from the first injection were administrated intraperitonially.~~

3.2.10.4.~~3.3.8.~~2 Oocytes C~~C~~ollection and C~~C~~lassification

After~~At~~ 15-16 hrs ~~hrs after~~from ~~H~~HCG injection, ~~the mice~~ female mouse was killed by cervical dislocation, ~~s were sacrificed and their~~their reproductive organs were excised in sterile conditions and washed by minimum essential medium (MEM). Under the stereo microscope, the adipose tissue and the ovaries were removed. Oocytes were collected from the oviducts by inserting a sterile syringe (28 gauge needle) and flashing 1 ml of MEM medium. Oocytes were classified into mature, immature and atretic according to the presence of first polar body and other morphological features.

~~**reproduction organs were excised in sterile conditions, washed by media minimum essential media MEM, and put in a sterile petridish with media MEM. Then under the stereo microscope the adipose tissue and the ovary**~~

~~were removed and after that, the oocytes were collected form the oviducts by flashing it with inserting a sterile syringe (24 gauge needle) containing media MEM inside the uterine born and taking care to push the media to get the oocytes from the open end of he oviducts. The oocytes were about 10-20 ova from each oviduct were classified into mature, immature and atretic.~~

3.2.10.4.~~3.3.8.~~3 *In vitro* O~~O~~ocytes ~~*In vitro*~~ M~~M~~aturation IVM (Fakhrilden *et al.*, 2005):

—For *in vitro* oocyte, imaturation oocytes were grouped (2-3 oocytes) and incubated in 5% CO_2 incubator at 37 C for 4-6 hrs, while mature oocytes were grouped (5-6 oocytes) and incubated with mouse spermatozoa for *in vitro* fertilization. However, atretic oocytes were discarded.

~~For *in vitro* oocytes maturation after collection, it were putted in a new sterile petridish with MEM media and incubated in a CO_2 incubator at 37ºC with a 5% CO_2 in humidified air for at least four hrs.~~

3.2.10.5.~~3.3.8.~~4 ~~*In*~~*In* ~~*vitro*~~ F~~F~~ertilization ~~T~~Technique ~~and~~ E~~E~~mbryonic D~~D~~evelopment (Fakhrildin *et al.*, 2001)

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~~After collected and incubated the sperms for at least 30 min and contaminated with bacteria or its supernatant, it were added to maturation oocytes as 1 x 10^6 sperm per ova, covered with a drop of paraffin oil and incubated together in CO_2 incubator at 37ºC with a 5% CO_2 in humidified CO_2 incubator. Twenty~~ Mature oocytes were grouped (5-6 oocytes) within 0.5 ml of MEM medium and ~~coverd~~covered with mineral oil, 0.2 ml of mouse spermatozoa-containing medium was introduced ~~incide~~inside mature oocytes-containing medium, and then incubated in a humidified 5% CO_2 incubator at 37 C for 16-18 hrs. ~~four hrs after insemination, p~~Percentages of fertilization and embryonic development were assessed under inverted microscope by examin~~ation~~ing the presence of first polar body and / or ~~the~~early embryonic ~~division~~cleavage.

3.2.11 Statistics (SPSS~~,~~ 1993)~~:~~

~~—~~Depending on the nature of the crude data, statistical tests were used to determine the mean and standard error of mean (S.E.M). Multi factorial analysis of varians was applied to report the level of statistical significance among mean of groups. Statistically, the level of significance 0.05 was considered to distinguish between means of groups.

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Chapter Four

Results and Discussion

4-1 Sperm Function Tests

Two kinds of pathogenic bacteria, Gram-negative *E. coli* and Gram-positive *Staph. aureus* were isolated from semen samples of 14 infertile patients (23-51 year) attended the Institute of Embryo Research and Infertility Treatment at Al-Nahrain university.

The percentage of *Staph aureus* isolates were 64% and 14% for *E. coli*. Rehewy *et al.* (1979) isolated *E. coli* and *Staph aureus* from 73% semen samples of infertile patients. Moreover, Sewenson (1980) isolated aerobic bacteria from 63.3% of semen samples of 109 infertile patients, While, Eggert-Kruse *et al.* (1995) isolated *E. coli* isolates from 13% of semen samples of infertile patients. Therefore, the results of the present study concerning in the isolates of both pathogens are agreement with the results stated by the above mentioned authors.

The effect of different count of *E. coli* (3, 6, 12, 24, 36 and 48 $X10^3$ cell/ml) were incubated with mouse spermatozoa on sperm function tests (sperm concentration, percentage of motile spermatozoa, percentage of abnormal sperm morphology, and percentage of sperm agglutination) at zero time and after 30 and 60 minutes of incubation is shown in tables 4-1, 4-2 and 4-3. *E. coli* growth has non significant ($P>0.05$) effect on all mouse sperm function tests at zero time of incubation when assessed with their control groups (table 4-1). However, after 30 minutes of mouse spermatozoa co-incubation with all concentrations of *E. coli* (table 4-2), there is non significant ($P>0.05$) differences in the sperm concentration as compared to the control groups. After the same time(30 min.), the percentage of sperm motility was decreased significantly ($P<0.05$) as compared to their control groups under the effect of all bacterial counts except the group of bacterial count $3X10^3$ cell/ml. In agreement with this result, Diemer *et al*, (2000) found that *E. coli* had a direct inhibitory effect on sperm motility depend upon its count.

Table 4-1: Effects of *E. coli* on mice sperm function tests at zero time of incubation.

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		56.800 ± 2.357	52.872 ± 1.929	53.040 ± 2.463	57.6000 ± 2.798	53.140 ± 2.384	54.76 ± 1.808	54.18 ± 2.977	53.41 ± 2.220	53.660 ± 2.051
Percentage of sperm motility		76.585 ± 2.462	71.470 ± 2.179	69.217 ± 2.342	71.919 ± 2.009	70.900 ± 1.332	68.980 ± 1.332	80.625 ± 2.985	79.012 ± 2.520	80.288 ± 2.737
Sperm grade activity(%)	**A**	23.126 ± 0.947	21.420 ± 0.970	19.590 ± 0.981	22.220 ± 0.683	20.641 ± 0.956	21.318 ± 0.733	28.259 ± 1.392	29.883 ± 1.881	26.008 ± 2.059
	B	22.600 ± 0.850	20.880 ± 0.760	19.214 ± 0.959	32.380 ± 1.159	34.123 ± 0.571	30.476 ± 0.827	29.658 ± 2.437	29.731 ± 1.303	29.600 ± 1.029
	C	30.859 ± 1.723	29.170 ± 1431	30.312 ± 1.194	17.318 ± 0.809	16.136 ± 0.263	17.186 ± 0.824	22.708 ± 0.228	19.498 ± 0.154	24.68 ± 2.310
	D	23.415 ± 1.123	28.520 ± 0.540	30.883 ± 0.752	28.081 ± 0.970	29.100 ± 1.480	31.020 ± 0.900	19.375 ± 0.109	20.768 ± 0.322	19.712 ± 0.601
Abnormal sperm morphology (%)		13.732 ± 1.663	12.885 ± 1.400	13.243 ± 1.670	13.341 ± 1.678	12.290 ± 0.974	11.322 ± 1.189	14.465 ± 1.316	13.748 ± 0.817	12.261 ± 0.522
Agglutination (%)		16.818 ± 1.216	15.924 ± 1.000	15.211 ± 1.271	12.943 ± 0.583	12.257 ± 0.316	12.591 ± 0.927	14.712 ± 1.245	14.227 ± 1.078	13.190 ± 0.944

Table 4-2: Effects of *E. coli* on mice sperm function tests after 30 min. of incubation.

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		55.61 ± 2.181	51.36 ± 1.785	50.14 ± 2.090	55.70 ± 2.562	52.11 ± 2.296	50.23 ± 2.103	52.66 ± 2.308	47.18 ± 2.044	44.28 ± 1.991
Percentage of sperm motility		74.392 ± 2.224	66.433 ± 1.839	60.250* ± 1.377	69.739 ± 1.923	47.386* ± 0.916	35.625* ± 0.826*	78.232 ± 1.500	7.630* ± 0.402	2.108* ± 0.232
Sperm grade activity (%)	**A**	21.105 ± 0.932	16.735 ± 0.636	14.678* ± 0.387	22.095 ± 0.614	1.950* ± 0.242	0.390 ± 0.051*	26.585 ± 0.893	0.000*	0.000*
	B	21.155 ± 0.770	18.828 ± 0.649	17.152 ± 0.727	31.210 ± 1.081	8.212* ± 0.492*	1.722 ± 0.460	28.864 ± 2.258	0.000*	0.000*
	C	32.132 ± 1.558	30.904 ± 1.019	27.420 ± 0.186	16.434 ± 0.785	37.224* ± 1.537*	33.513* ± 1.626	22.783 ± 0.329	7.630* ± 0.099	2.108* ± 0.184
	D	23.608 ± 1.303	33.567 ± 0.861	39.750* ± 1.157	30.261 ± 1.052	52.614 ± 2.610	64.375* ± 1.903	21.768 ± 0.411	92.370* ± 3.392	97.892* ± 3.748
Abnormal sperm morphology (%)		13.247 ± 1.461	13.239 ± 1.505	14.797 ± 2.392	13.199 ± 1.463	14.252 ± 1.495	15.723* ± 1.792	14.191 ± 1.051	20.372* ± 1.612	24.521* ± 1.320
Agglutination (%)		15.624 ± 0.969	16.770 ± 1.416	17.437 ± 1.833	12.810 ± 0.512	15.287 ± 0.827	16.919 ± 1.236	15.191 ± 1.378	20.706* ± 2.163	25.391* ± 2.426

* less than 0.05 level of significance

Also, it was reported that the *E. coli* at concentration 10^4 cell / ml decreased sperm motility *in vitro* (Monga and Robert., 1994).

The results of percentages of abnormal sperm morphology and sperm agglutination were significantly ($P<0.05$) reduced when mouse spermatozoa incubated with *E. coli* at counts 36 and 48 $X10^3$ cell/ml as compared to the control groups (table 4-2). In agreement with many scientists which noticed that *E. coli* concentration of 10^4 caused sperm agglutination about 40-70% of motile spermatozoa, P-fimbria caused tail-tail agglutination, type I fimbriae caused head-head agglutination (Monga and Robert, 1994). In according with Greskovich *et al* (1993), irreversible morphological damage to the sperm could occur after microbial infections of the semen and resulting in an increasing abnormal sperm morphology. In table (4-3) mouse spermatozoa incubated with *E. coli* for 60 minutes, the sperm concentration showd non significant ($P>0.05$) decreased at the *E. coli* counts 3, 6, 12 and 24 $X10^3$ cell/ml as compared to the control groups, but at *E. coli* counts 36 and 48 $x10^3$ cell/ml a significant ($P<0.05$) reduction in mouse spermatozoa concentration as compared to their control groups. Paulson and Polokoski (1977) reported that the incubation of sperm with live *E. coli* causes irreversible necrospermia and damaging spermatozoa for 80% of sperm cells. Moreover, Dimer *et al*. (1996) demonstrated a multiple adhesion of *E. coli* to spermatozoa causing various ultrastructural damages. The percentage of motile spermatozoa was significantly ($P<0.05$) reduced at bacterial counts 6, 12, and 24 $X10^3$ cell/ml while bacterial counts of 36 and 48 $X10^3$ cell/ml cause total suppression for sperm motility as compared to their control groups. Wolff *et al* (1993) and Diemer *et al* (1996) concluded that *E. coli in vitro* readily adhere to the spermatozoa and inhibited their motility at a microorganism/sperm ratio of more than 1 under field of electronmicroscopy.

Table 4-3: Effects of *E. coli* on mice sperm function tests after 60 min. of incubation

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		54.18 ± 2.005	50.28 ± 1.424	48.14 ± 1.731	54.06 ± 2.031	48.04 ± 1.846	47.16 ± 1.967	50.43 ± 2.117	33.32* ± 1.795	28.94* ± 1.469
Percentage of sperm motility		72.708 ± 1.902	54.420 ± 1.455	45.316* ± 1.022	68.416 ± 1.905	31.869* ± 1.083	18.797* ± 0.800	77.061 ± 1.630	0.000*	0.000*
Sperm grade activity (%)	**A**	19.240 ± 0.705	12.778 ± 0.320	6.647* ± 0.197	21.677 ± 0.531	0.315* ± 0.044	0.000*	25.483 ± 0.751	0.000*	0.000*
	B	20.881 ± 0.949	14.045 ± 0.456	9.140* ± 0.200	29.625 ± 0.932	5.412* ± 0.322	0.000*	27.836 ± 2.187	0.000*	0.000*
	C	33.587 ± 1.393	27.597 ± 1.416	29.529 ± 1.051	17.114 ± 0.792	26.143 ± 0.614	18.797 ± 0.961	23.742 ± 0.605	0.000*	0.000*
	D	27.293 ± 1.507	45.580 ± 1.113	54.684* ± 1.693	31.584 ± 1.090	68.131* ± 3.666	81.203* ± 2.659	22.939 ± 0.8333	89.80* ± 2.870	78.772* ± 2.801
Abnormal sperm morphology (%)		13.384 ± 1.095	14.892 ± 2.138	15.424 ± 2.840	13.607 ± 1.829	17.104 ± 2.204	18.306 ± 2.585	14.276 ± 0.926	13.562 ± 0.935	12.100* ± 0.511
Agglutination (%)		15.839 ± 1.217	17.345 ± 1.755	18.989 ± 1.997	13.233 ± 0.604	19.371* ± 1.300	20.445* ± 1.946	15.412 ± 1.250	15.323 ± 0.974	16.331 ± 1.100

* less than 0.05 level of significance

Therefore, more time of incubation result in more living bacterial growth and its metabolic products which in turn produce great impact on spermatozoa. Percentages of abnormal sperm morphology and sperm agglutination were significantly ($P<0.05$) increased at the *E. coli* concentrations (24 $X10^3$ cell/ml), except groups of *E. coli* with counts 36 and 48 $X10^3$ cell/ml which appeared non significant ($P>0.05$) increased as compared to its control groups. Explanation of this result may be through damaging of spermatozoa and then reducing sperm concentration due to active destroying effect of *E. coli* to the host cells after 60 minutes of incubation. In 2003, Donek reported that the *E. coli* endotoxin could increase the abnormal forms of spermatozoa as distal cytoplasmic droplet and distilling position of the tail.

Table (4-4, 4-5 and 4-6) represented the effect of different concentrations of *E. coli* culture filtrates involving (3, 6, 12, 24, 36 and 48 $X10^3$ cell / ml) on mouse sperm function tests at zero time and after 30 and 60 minutes of co-incubation. In general, non significant ($P>0.05$) differences were noticed for mouse sperm function tests when co-incubated with all counts of E. coli filtrates at zero time (table 4-4). After 30 minutes of incubation of *E. coli* culture filtrates with mouse spermatozoa (table 4-5), non significant ($P>0.05$) differences were assessed for mouse sperm concentration and percentage of sperm agglutination as compared with the control groups. While the percentage of sperm motility was significantly ($P<0.05$) reduced at bacterial concentrations (24, 36 and 48 $X10^3$ cell/ml), and a significant ($P<0.05$) increase in abnormal sperm morphology at (36 and 48 $X10^3$ cell/ml) as compared to the control groups. Mouse sperm concentration was non significantly ($P>0.05$) reduced after 60 minutes of incubation with different counts of *E. coli* culture filtrates (table4-6).

Table 4-4: Effects of *E. coli* culture filtrates on mice sperm function tests at zero time of incubation.

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		57.68 3.700	56.68 3.926	54.98 2.696	56.18 2.500	55.64 3.000	54.68 2.143	58.68 1.577	55.32 2.636	56.66 2.387
Percentage of sperm motility		78.515 1.872	76.456 3.506	76.835 21.627	75.042 1.910	75.240 2.378	73.376 2.551	76.395 1.312	76.862 1.811	77.992 2.482
Sperm grade activity (%)	**A**	22.638 1.872	23.048 1.329	21.627 1.698	23.791 1.548	23.575 1.077	23.137 1.672	23.833 1.200	24.054 1.378	23.928 1.161
	B	28.332 1.332	27.093 1.603	29.102 1.249	26.344 1.793	26.093 1.822	26.298 1.139	28.952 1.686	28.066 1.298	26.681 1.113
	C	27.545 1.238	26.315 1.519	26.106 1.526	24.907 1.400	25.572 1.571	23.941 1.700	23.810 1.011	24.742 1.358	26.383 1.600
	D	21.485 1.655	23.544 1.850	23.165 1.332	24.958 1.536	24.760 1.855	26.624 1.438	23.405 1.256	23.138 1.917	23.008 1.500
Abnormal sperm morphology (%)		15.303 0.802	14.691 0.990	15.133 1.063	15.890 0.930	15.245 0.583	4.942 0.870	13.384 0.950	13.110 0.244	12.994 0.824
Agglutination (%)		16.311 0.900	15.68 08.45	17.013 1.127	13.954 0.985	13.532 0.710	13.612 0.623	15.422 0.904	15.314 0.634	14.580 0.970

Table 4-5: Effects of *E. coli* culture filtrates on mice sperm function tests after 30 min. of incubation.

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		55.49 2.666	52.19 2.149	52.00 1.942	53.90 2.242	52.00 1.969	51.30 1.588	56.80 1.261	51.00 1.903	53.70 2.134
Percentage of sperm motility		76.393 1.121	73.940 1.407	69.522 1.233	73.308 1.239	62.936 1.826	51.856* 1.997	75.345 1.183	40.826* 1.757	29.515* 1.310
Sperm grade activity (%)	**A**	22.091 1.120	21.287 1.022	18.710 1.270	22.629 1.515	18.036 0.992	15.075* 1.032	23.406 1.085	10.068* 0.870	7.442* 0.447
	B	27.373 1.053	26.650 1.281	23.925 1.008	25.978 1.572	20.064 1.365	16.296* 0.939	27.187 1.510	11.517* 0.800	9.645* 1.017
	C	26.929 1.110	26.003 1.352	26.887 1.344	24.701 1.174	24.836 1.349	20.485 1.613	24.752 1.833	19241 1.000	14.428* 0.802
	D	23.607 1.303	26.060 1.734	30.478 1.200	26.692 1.300	37.064* 2.800	48.144* 2.285	24.655 1.340	59.174* 2.852	68.485* 2.780
Abnormal sperm morphology (%)		15.627 0.955	14.951 1.241	15.820 0.889	15.135 0.790	17.315 0.801	17.9 0.943	13.099 0.870	18.942* 0.700	19.836* 1.003
Agglutination (%)		16.202 0.825	16.556 0.935	17.004 0.988	13.514 0.758	14.954 0.934	15.326 0.777	15.72 0.683	17.600 0.823	18.349 1.002

* less than 0.05 level of significance

Table 4-6: Effects of *E. coli* culture filtrates on mice sperm function tests after 60 min. of incubation.

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		52.67 ± 1.759	51.00 ± 1.516	50.30 ± 1.111	52.20 ± 1.265	50.90 ± 1.345	49.50 ± 0.808	54.75 ± 1.095	٥٠,١١ ± 1.245	٥١,٩٧ ± 1.150
Percentage of sperm motility		75.875 ± 1.942	68.829 ± 1.133	60.963 ± 1.045	70.285 ± 1.147	51.947* ± 1.555	40.221* ± 1.604	74.147 ± 1.090	30.863* ± 1.245	18.792* ± 1.150
Sperm grade activity (%)	**A**	21.903 ± 1.003	18.805 ± 0.902	16.777* ± 0.879	21.150 ± 1.067	15.827* ± 0.812	10.194* ± 0.589	22.173 ± 0.735	7.457* ± 0.402	2.428* ± 0.211
	B	28.073 ± 1.530	25.785 ± 1.171	22.152 ± 0.980	24.852 ± 1.292	17.362* ± 0.947	12.764* ± 0.900	26.599 ± 1.267	8.796* ± 0.525	5.507* ± 0.387
	C	25.899 ± 0.923	24.239 ± 1.276	22.034 ± 0.755	24.283 ± 0.937	18.758* ± 1.015	17.263* ± 1.152	25.275 ± 2.043	14.610* ± 0.933	10.857* ± 0.400
	D	24.125 ± 1.102	31.171 ± 1.056	39.037* ± 1.134	28.715 ± 1.180	48.053* ± 3.154	59.779* ± 2.537	25.853 ± 1.638	69.137* ± 3.153	81.208* ± 3.305
Abnormal sperm morphology (%)		15.854 ± 0.842	16.481 ± 1.319	17.242 ± 0.736	16.850 ± 0.707	18.021 ± 1.125	18.736* ± 1.270	14.564 ± 1.044	18.991* ± 1.060	20.780* ± 1.933
Agglutination (%)		17.020 ± 0.724	17.758 ± 1.223	18.291 ± 0.930	13.173 ± 0.650	15.009 ± 1.230	16.862 ± 1.145	15.041 ± 0.560	20.143* ± 1.700	22.062* ± 1.519

* less than 0.05 level of significance

At the same time the percentage of sperm motility showed a significant ($P<0.05$) reduction at bacterial countss (12, 24, 36 and 48 $X10^3$ cell/ml)and a significant ($P<0.05$) increase in abnormal sperm morphology and sperm agglutination was found at counts 24, 36 and 48X10 cell/ml in comparison with the control group (table 4-6). Donek (2003) certified a significant decrease in the motility of spermatozoa by endotoxin of *E. coli* administrated intravenously.

Tables (4-7, 4-8 and 4-9) represented the effect of different concentrations of *Staph. aureus* on mouse sperm function tests in zero time and after 30 and 60 minutes of co-incubation. In general, non significant ($P>0.05$) effects of this bacteria at all its concentrations on the mouse sperm function tests were observed at zero time of incubation table (4-7). After 30 minutes of incubation, with all concentrations of *Staph. aureus*, mouse sperm concentration, percentage of abnormal sperm morphology and sperm agglutination were represented non significant ($P>0.05$) differences from the control groups (table 4-8). The percentage of sperm motility was significantly ($P<0.05$) reduced at bacterial concentrations (12, 24, 36 and 48 $X10^3$ cell/ml) as compared with the control groups. Recently, Hong (2002) proved that the *Staphylococcus aureus* suppress the sperm motility into 18.11% after 2 hrs, as well as decreased sperm viability to 47.51% after 4 hrs. Non significant ($P>0.05$) differences were noticed after 60 minutes of mouse spermatozoa co-incubated with all concentrations of *Staph. aureus* in the mouse sperm concentration, percentage of abnormal sperm morphology and sperm agglutination as compared to its control groups (table 4-9). From the same table, the percentage of sperm motility appeared to be significantly ($P<0.05$) reduced at bacterial concentrations (6, 12, 24, 36 and 48 $X10^3$ cell/ml) as compared to the control groups.

Table 4-7: Effect of *Staphs aureus* on mice sperm function tests at zero time of incubation.

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		54.850 ± 2.19	53.17 ± 2.18	50.48 ± 3.71	57.24 ± 2.77	55.39 ± 2.07	52.92 ± 2.35	56.38 ± 2.01	56.22 ± 3.99	54.77 ± 2.03
Percentage of sperm motility		79.957 ± 2.416	78.046 ± 2.638	78.422 ± 1.772	79.321 ± 2.073	77.877 ± 2.353	79.315 ± 2.059	76.266 ± 1.907	73.933 ± 2.347	73.735 ± 2.249
Sperm grade activity (%)	**A**	23.955 ± 1.288	22.918 ± 1.208	23.979 ± 1.122	20.591 ± 1.140	20.043 ± 1.249	20.457 ± 1.029	24.831 ± 2.258	22.056 ± 2.111	22.604 ± 1.280
	B	29.696 ± 1.624	26.588 ± 1.630	27.733 ± 1.303	27.911 ± 1.224	26.980 ± 1.224	27.537 ± 1.560	26.805 ± 1.414	26.680 ± 1.788	26.931 ± 1.319
	C	26.306 ± 1.157	21.935 ± 0.871	26.710 ± 0.836	30.739 ± 1.122	30.854 ± 1.319	31.321 ± 1.319	24.830 ± 1.581	25.257 ± 1.529	24.200 ± 1.743
	D	20.043 ± 1.581	21.936 ± 1.714	21.578 ± 1.469	20.679 ± 0.941	22.123 ± 1.029	20.685 ± 0.812	23.734 ± 1.503	27.007 ± 1.860	26.265
Abnormal sperm morphology (%)		14.757 ± 1.043	14.0.83 ± 0.931	14.412 ± 0.985	15.693 ± 2.382	15.363 ± 2.105	15.007 ± 2.000	14.032 ± 1.892	14.355 ± 1.555	13.712 ± 1.755
Agglutination (%)		16.365 ± 2.341	16.710 ± 2.500	16.101 ± 2.122	14.341 ± 1.852	13.33 ± 1.401	13.345 ± 1.766	13.614 ± 1.954	12.933 ± 1.600	12.657 ± 2.011

Table 4-8: Effect of *Staph aureus* on mice sperm function tests after 30 min. of incubation

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		53.26 ± 2.08	51.90 ± 1.540	48.81 ± 3.000	56.54 ± 2.325	52.68 ± 1.264	51.30 ± 1.763	54.80 ± 1.121	49.42 ± 3.692	50.28 ± 1.825
Percentage of sperm motility		79.347 ± 2.103	74.570 ± 1.369	71.301 ± 1.153	78.724 ± 1.443	59.732* ± 1.003	47.495* ± 1.190	74.598 ± 1.732	36.921* ± 0.849	17.945* ± 0.252
Sperm grade activity (%)	**A**	23.657 ± 1.351	20.768 ± 1.008	20.077 ± 0.969	21.337 ± 1.082	14.935* ± 0.981	07.731* ± 0.571	23.090 ± 2.166	1.425* ± 0.330	0.000*
	B	28.902 ± 1.495	26.751 ± 1.642	25.410 ± 1.116	26.649 ± 0.929	20.880 ± 1.000	14.359* ± 0.683	26.640 ± 1.249	14.564* ± 0.747	6.959* ± 0.800
	C	26.788 ± 1.206	27.141 ± 1.233	25.814 ± 0.6000	30.738 ± 0.855	23.917 ± 0.748	25.405 ± 1.240	24.866 ± 1.720	20.932 ± 1.057	10.986* ± 0.547
	D	20.653 ± 1.715	25.430 ± 2.014	28.699 ± 1.673	21.276 ± 1.007	40.268* ± 2.067	52.505* ± 2.144	25.402 ± 1.930	63.079* ± 2.521	82.055* ± 2.805
Abnormal sperm morphology (%)		15.020 ± 1.236	14.009 ± 0.900	15.154 ± 1.394	15.495 ± 2.564	15.031 ± 1.669	14.902 ± 1.739	14.765 ± 2.088	14.053 ± 1.712	13.864 ± 1.828
Agglutination (%)		16.258 ± 2.171	16.188 ± 2.113	15.397 ± 1.630	14.642 ± 2.100	14.098 ± 1.851	14.007 ± 1.991	13.169 ± 2.215	12.400 ± 1.833	13.111 ± 2.294

* less than 0.05 level of significance

Table 4-9: Effect of *Staph aureus* on mice sperm function tests after 60 min. of incubation

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		51.03 ± 1.500	49.00 ± 1.146	47.220 ± 2.772	53.10 ± 2.000	48.74 ± 0.851	48.235 ± 1.369	52.9 ± 0.920	46.3 ± 3.009	44.6 ± 1.283
Percentage of sperm motility		76.622 ± 2.050	66.897 ± 0.829	60.641* ± 0.910	77.824 ± 1.322	48.023* ± 0.805	33.162* ± 0.637	73.444 ± 1.019	21.122* ± 0.474	6.720* ± 0.326
Sperm grade activity (%)	**A**	22.976 ± 1.115	18.548 ± 0.985	15.867* ± 0.748	19.985 ± 0.938	12.156* ± 0.583	4.535* ± 0.316	22.401 ± 1.722	0.000*	0.000*
	B	27.852 ± 1.388	22.480 ± 0.979	18.277* ± 0.990	26.082 ± 0.655	15.770* ± 0.860	11.594* ± 0.489	26.942 ± 1.313	6.312* ± 0.316	1.390* ± 0.200
	C	25.794 ± 1.041	25.869 ± 0.927	26.497 ± 0.826	31.757 ± 0.764	20.097 ± 0.549	17.033* ± 0.840	24.101 ± 1.275	14.810* ± 0.743	5.330* ± 0.400
	D	23.378 ± 1.365	33.103 ± 1.777	39.359* ± 1.971	22.776 ± 1.342	51.977* ± 1.140	74.838* ± 1.643	26.556 ± 2.224	78.878* ± 2.293	93.280* ± 1.923
Abnormal sperm morphology (%)		14.900 ± 1.038	14.514 ± 1.395	14.301 ± 1.009	15.193 ± 2.257	15.038 ± 1.928	15.000 ± 2.008	14.920 ± 2.367	14.361 ± 1.99	14.509 ± 2.610
Agglutination (%)		16.622 ± 2.445	16.416 ± 2.352	16.724 ± 1.826	14.221 ± 1.700	13.913 ± 1.892	13.406 ± 1.8412	13.330 ± 1.800	12.900 ± 1.705	13.850 ± 1.626

* less than 0.05 level of significance

Table (4-10, 4-11 and 4-12) demonstrating the effect of different concentrations (3, 6, 12, 24, 36, 48 $X10^3$ cell/ml) of *Staph aureus* culture filtrates on mouse sperm function tests at zero time and after 30 and 60 minutes of incubation. At zero time of incubation no significant ($P>0.05$) differences in all sperm function tests were examined. From table (4-11) mouse sperm concentration, percentages of abnormal sperm morphology and sperm agglutination have non significant ($P>0.05$) differences as compared to the control groups after 30 minutes of incubation with different counts of *Staph. aureus* culture filtrates. At the same time the percentage of sperm motility observed a significant ($P<0.05$) reduction at culture filtrates of bacterial concentration 48 $x10^3$ cell/ml only when compared to its control groups. Table4-12 shows the effect of different concentrations of *Staph. aureus* culture filtrates incubated for 60 minutes. No significant ($P>0.05$) differences were assessed for sperm concentration, as compared to its control groups. Percentage of sperm motility was significantly ($P<0.05$) decreased at bacterial concentrations (24, 36 and 48 $X10^3$ cell/ml) as compared to its control groups. After incubation for the same period *Staph. aureus* has no effect on percentages of abnormal sperm morphology and sperm agglutination. These results in agreement with result were obtained by Hong *et al* (2002) who observed that *Staph. aureus* has no activity for adherence to the spermatozoa or stimulate any morphological change. From previous results of bacterial growth cells have harmful effects on mouse sperm function tests more than the effects of its culture filtrates and may be explained by the fact that bacterial cells continue division, reproduction, producing more metabolic toxic products and enzymes and have antigenic activity as well as could impair spermatozoal motility by the motility of bacteria. Huwe *et al* (1998) suggested that toxic effects of *E. coli* were associated with its growth.

Table 4-10: Effect of *Staph aureus* culture filtrates on mice sperm function tests at zero time. of incubation

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	3	6	Control	12	24	Control	36	48
Sperm concentration (no. x 10^6)		54.604 ± 2.987	53.410 ± 2.031	53.690 ± 2.151	57.280 ± 2.251	54.630 ± 1.288	55.080 ± 1.626	57.048 ± 1.903	56.620 ± 0.875	56.080 ± 2.367
Percentage of sperm motility		67.295 ± 2.481	75.802 ± 2.712	74.660 ± 1.581	78.244 ± 2.300	76.607 ± 1.870	77.677 ± 1.764	76.706 ± 1.520	75.473 ± 2.323	76.682 ± 2.395
Sperm grade activity (%)	A	22.592 ± 1.340	22.264 ± 1.223	22.092 ± 0.979	28.070 ± 1.760	27.857 ± 1.600	26.639 ± 1.802	26.315 ± 1.742	25.081 ± 1.536	25.925 ± 1.667
	B	28.518 ± 0.927	28.403 ± 1.580	27.863 ± 1.330	26.315 ± 2.788	25.692 ± 2.301	26.025 ± 2.521	25.034 ± 1.561	24.392 ± 1.700	25.000 ± 1.551
	C	25.185 ± 1.400	25.135 ± 1.384	24.705 ± 1.371	23.859 ± 2.014	23.058 ± 2.009	25.013 ± 2.217	25.357 ± 1.655	26.200 ± 2.055	25.757 ± 2.030
	D	23.705 ± 1.269	24.198 ± 1.296	25.340 ± 1.472	21.756 ± 1.583	23.393 ± 0.994	22.323 ± 1.864	23.294 ± 1.950	24.327 ± 1.729	23.318 ± 1.135
Abnormal sperm morphology (%)		15.778 ± 0.955	15.946 ± 0.800	14.703 ± 1.152	14.757 ± 1.044	13.685 ± 0.933	14.095 ± 1.036	13.963 ± 1.270	12.842 ± 0.952	13.783 ± 1.281
Agglutination (%)		14.6 ± 0.851	15.012 ± 1.122	14.460 ± 0.775	15.473 ± 1.323	15.051 ± 0.903	14.424 ± 0.834	16.872 ± 1.200	16.259 ± 1.066	16.418 ± 0.858

Table 4-11: Effect of *Staph aureus* culture filtrates on mice sperm function tests after 30 min. of incubation

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		25.980 ± 2.212	51.1645 ± 1.503	51.659 ± 1.872	55.180 ± 1.199	50.400 ± 1.393	52.917 ± 1.426	56.161 ± 1.585	54.245 ± 0.966	54.909 ± 1.059
Percentage of sperm motility		75.391 ± 1.922	74.606 ± 1.815	73.149 ± 1.415	76.732 ± 1.270	74.511 ± 0.995	72.160 ± 1.130	76.179 ± 0.809	69.568 ± 1.752	61.720* ± 1.721
Sperm grade activity (%)	**A**	21.529 ± 1.254	20.693 ± 1.013	21.090 ± 0.959	27.845 ± 1.350	25.826 ± 1.327	24.532 ± 1.221	25.964 ± 1.516	23.524 ± 1.333	20.204 ± 1.156
	B	27.196 ± 0.825	27.513 ± 1.281	26.524 ± 1.310	25.748 ± 2.000	24.875 ± 2.131	25.245 ± 2.221	24.410 ± 1.392	23.782 ± 1.621	21.267 ± 1.250
	C	26.666 ± 1.846	26.400 ± 1.791	25.625 ± 1.762	23.139 ± 1.752	23.800 ± 2.500	22.383 ± 2.090	25.805 ± 1.696	22.162 ± 1.655	20.249 ± 1.346
	D	24.609 ± 1.853	25.394 ± 1.782	26.851 ± 1.547	23.268 ± 1.934	25.489 ± 1.180	27.840 ± 2.299	23.821 ± 1.927	30.432 ± 2.550	38.280 ± 1.800
Abnormal sperm morphology (%)		16.030 ± 1.136	15.365 ± 0.755	15.732 ± 1.386	15.38 ± 1.310	14.400 ± 0.798	13.355 ± 0.919	13.800 ± 1.158	13.540 ± 1.085	12.852 ± 1.113
Agglutination (%)		15.162 ± 1.030	15.227 ± 1.036	15.360 ± 0.6285	14.692 ± 1.100	14.141 ± 0.881	14.780 ± 0.707	16.342 ± 1.006	15.777 ± 1.036	15.902 ± 0.700

* less than 0.05 level of significance

Table 4-12: Effect of *Staph aureus* culture filtrates on mice sperm function tests after 60 min. of incubation

Sperm function tests		Bacterial Concentration (no. x 10^3) cells / ml								
		Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
Sperm concentration (no. x 10^6)		50.660 ± 2.059	48.300 ± 1.347	45.290 ± 2.182	53.820 ± 1.547	49.600 ± 2.023	50.55 ± 1.873	54.36 ± 2.300	51.00 ± 1.759	53.00 ± 1.660
Percentage of sperm motility		73.278 ± 1.267	72.779 ± 1.606	70.024 ± 1.123	75.595 ± 0.911	66.700 ± 0.513	60.220* ± 1.797	74.284 ± 0.834	52.620* ± 1.536	38.042* ± 1.188
Sperm grade activity (%)	**A**	20.763 ± 1.14	20.745 ± 0.713	19.787 ± 0.940	26.700 ± 1.170	22.256 ± 1.267	19.445 ± 0.633	24.865 ± 1.067	16.562* ± 1.360	12.463* ± 0.582
	B	26.675 ± 0.727	25.710 ± 1.089	24.874 ± 1.200	25.944 ± 1.328	22.087 ± 2.131	20.244 ± 1.800	23.450 ± 1.022	16.342* ± 1.644	13.214* ± 1.327
	C	25.840 ± 1.092	26.324 ± 1.300	25.363 ± 1.260	22.951 ± 1.011	22.357 ± 1.973	20.531 ± 1.707	25.969 ± 2.014	19.716* ± 1.166	12.365* ± 0.927
	D	26.722 ± 2.168	27.221 ± 1.972	29.976 ± 1.763	24.405 ± 2.343	33.300 ± 2.344	39.780* ± 2.737	25.716 ± 2.050	49.380* ± 1.633	61.958* ± 2.933
Abnormal sperm morphology (%)		16.388 ± 1.182	14.634 ± 0.562	15.292 ± 1.252	14.840 ± 1.126	13.368 ± 0.851	13.815 ± 0.835	14.800 ± 1.573	13.725 ± 1.316	12.757 ± 1.099
Agglutination (%)		15.309 ± 0.600	15.625 ± 1.510	15.350 ± 0.497	15.281 ± 0.863	15.600 ± 0.937	14.000 ± 0.638	16.000 ± 0.836	15.105 ± 0.822	14.669 ± 0.671

* less than 0.05 level of significance

On the other hand, Diemer *et al*. (1996) demonstrated that the culture filtrate of *E. coli* has no inhibitory effect on the sperm cells. Nicholson *et al* (2000) reported that *Staph. aureus* cells have the ability to impair the spermatozoal motility due to their attachment ability and cluster like growth. The same authors proved that the inhibitory effect of *Staph. aureus* on sperm motility is due to various toxins and enzymes that produce from it through its metabolism.

Figures (4-1, 4-2 and 4-3) showed the effect of all concentrations of *E. coli* and *Staph. aureus* on mouse sperm concentrations after 0, 30 and 60 minutes. Non significant ($P>0.05$) differences were observed, except a significant ($P<0.05$) reduction in the sperm concentration of mouse spermatozoa co-incubated for 60 minutes with $36X10^3$ cell/ml and $48X10^3$ cell/ml of *E. coli* as compared to *Staph. aureus*. This result may be attributed to the fact that *E. coli* has damage activity for host cells during its growth by producing exotoxins, involving *E. coli* LT toxin and the *E. coli* ST toxin (Todar, 2005). On the other hand, *Staph. aureus* has a less activity and virulence factors than *E. coli*. Other investigators found that *Staph. aureus* may produce various toxins and enzymes that may be exert damaging effects on human sperm, but its mechanism is unclear (Hong *et al*., 2002).

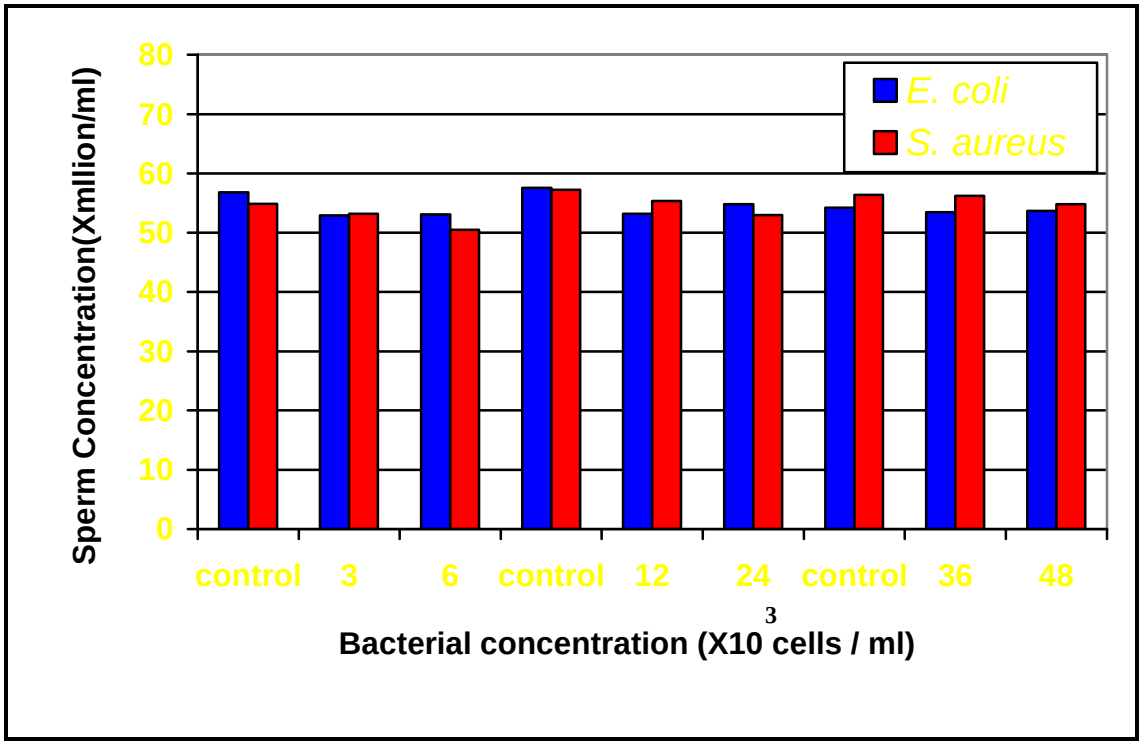

Figure (4-1): The effect of *E. coli* and *Staph aureus* on mice sperm concentration at zero time of incubation.

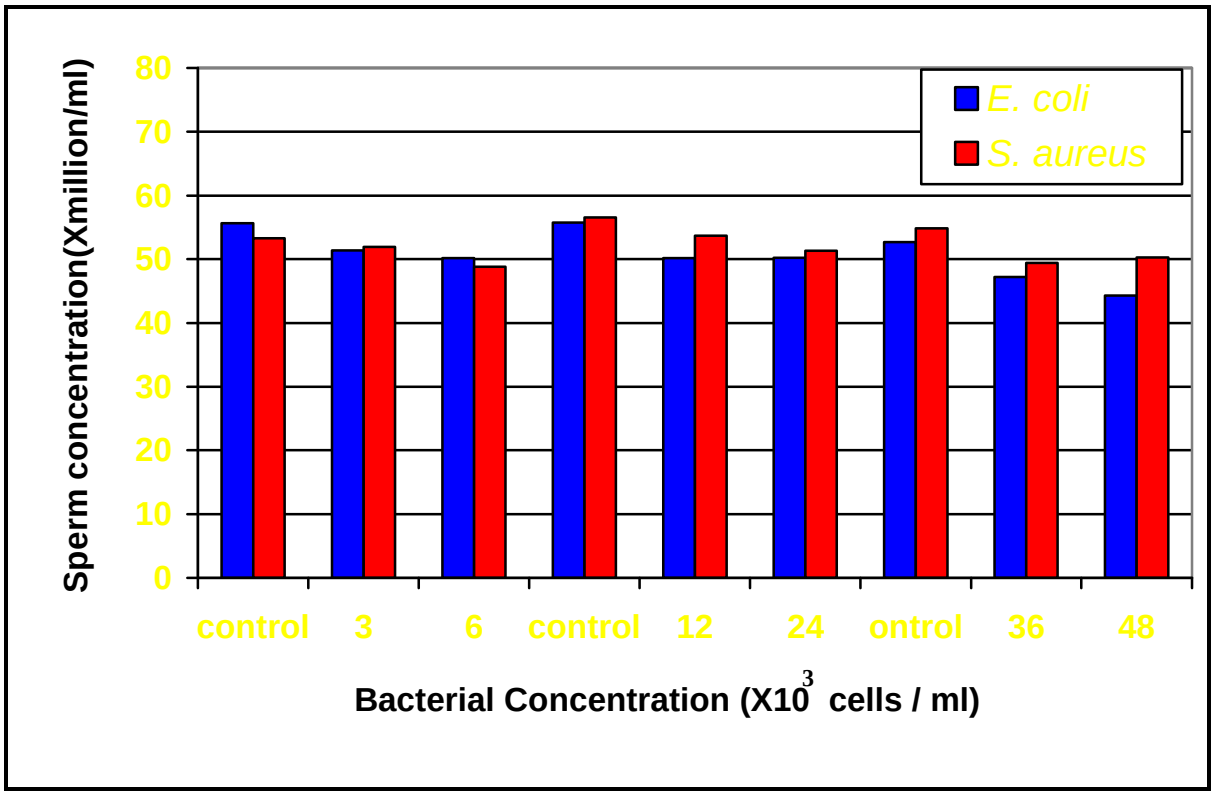

Figure (4-2): The effect of *E. coli* and *Staphs aureus* on mice sperm concentration after 30 min of incubation.

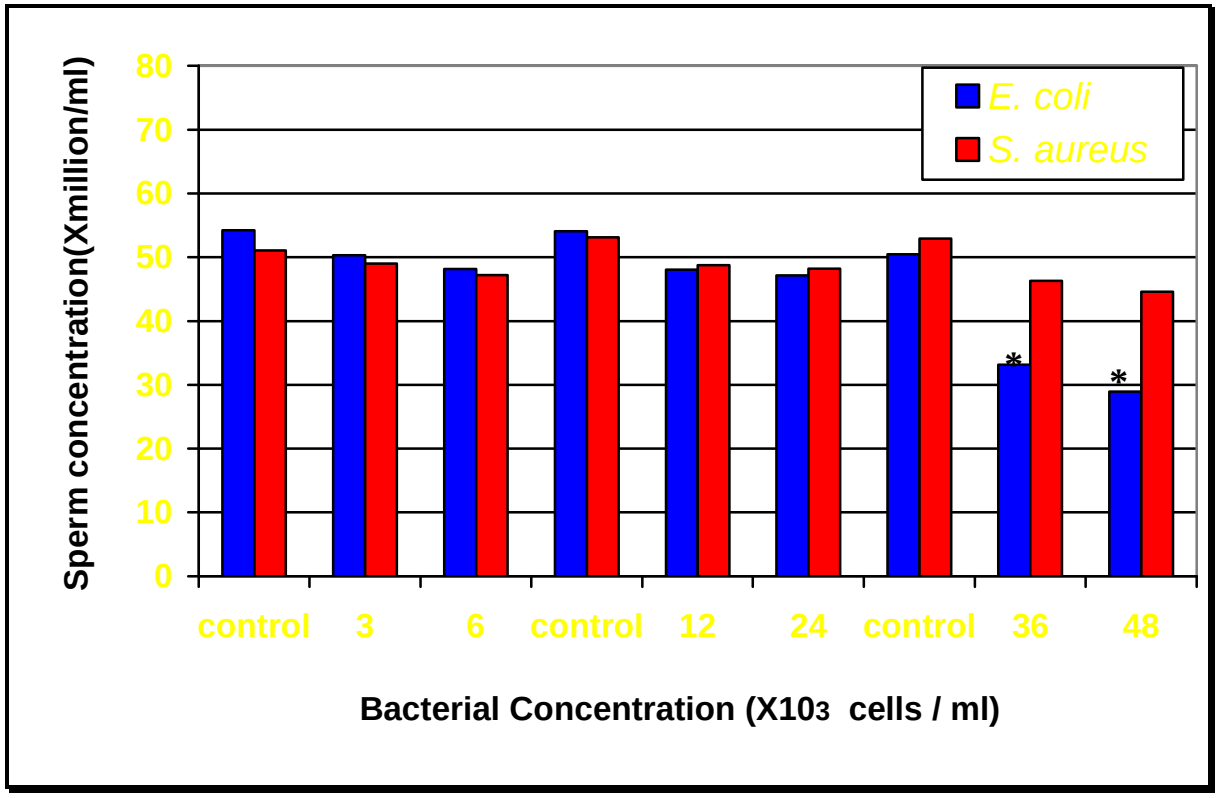

Figure (4-3): The effect of *E. coli* and *Staph aureus* on mice sperm concentration after 60 min of incubation.

***** less than 0.05 level of significance

Effects of all counts of *E. coli* and *Staph. aureus* on the percentage of mouse sperm motility after 0, 30 and 60 minutes of incubation were shown in figures (4-4, 4-5 and 4-6). The present results revealed significant ($P<0.05$) differences between the two kinds of bacterial counts 6, 12, 24, 36 and $48X10^3$ cell/ml after 30 minutes of incubation (figure 4-5) and at bacterial conunts 3, 6, 12, 24, 36, and $48X10^3$ cell/ml after 60 minutes of incubation (Figure 4-6). This comparison demonstrated that *E .coli* has a great effect to induce suppression on mouse sperm motility than *Staph. aureus*. Similar result was reported by other workers who found that *Staph. aureus* have a lower activity than *E. coli* on motility of spermatozoa (Shalika *et al*., 1996).

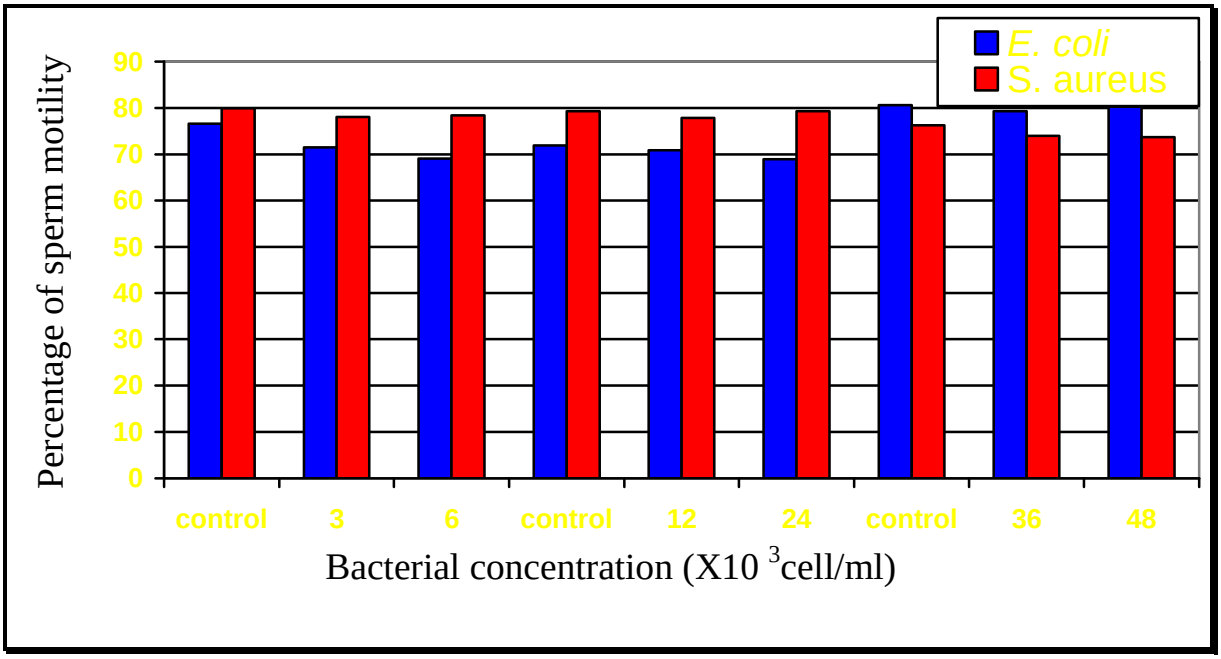

Figure (4-4): The effect of *E. coli* and *Staphs aureus* on percentage of mouse sperm motility at zero time of incubation.

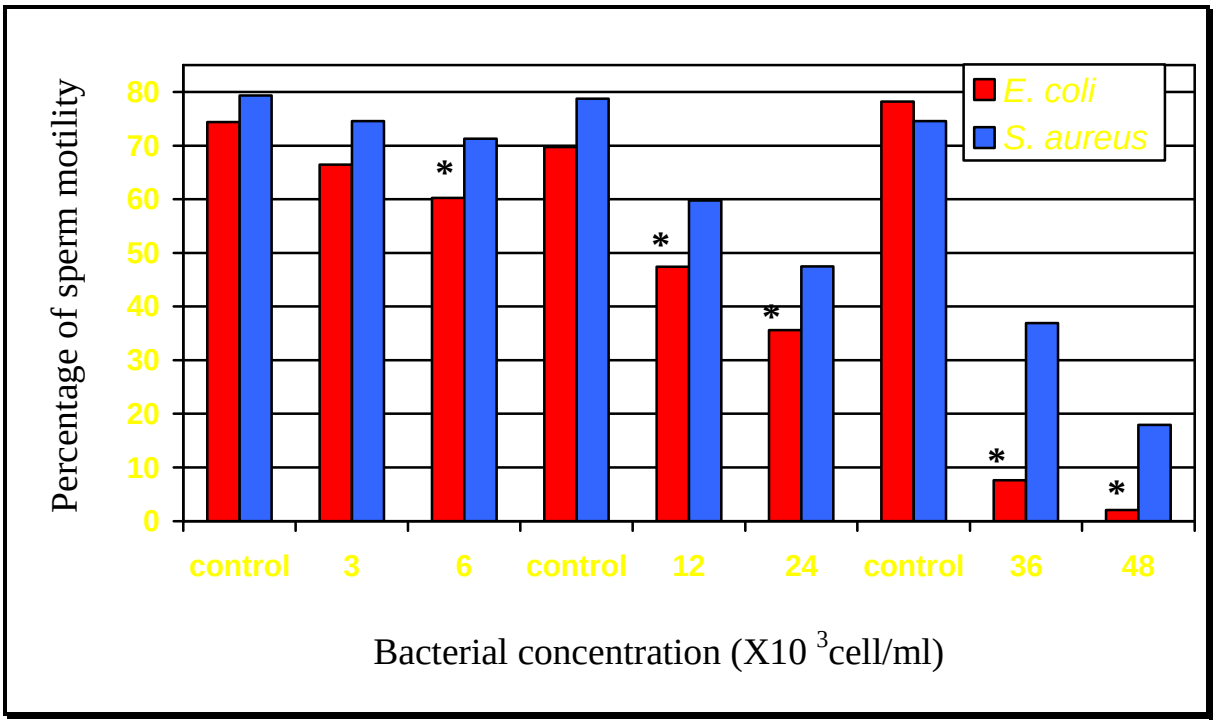

Figure (4-5): The effect of *E. coli* and *Staph aureus* on percentage of mouse sperm motility after 30 min of incubation.

* less than 0.05 level of significance

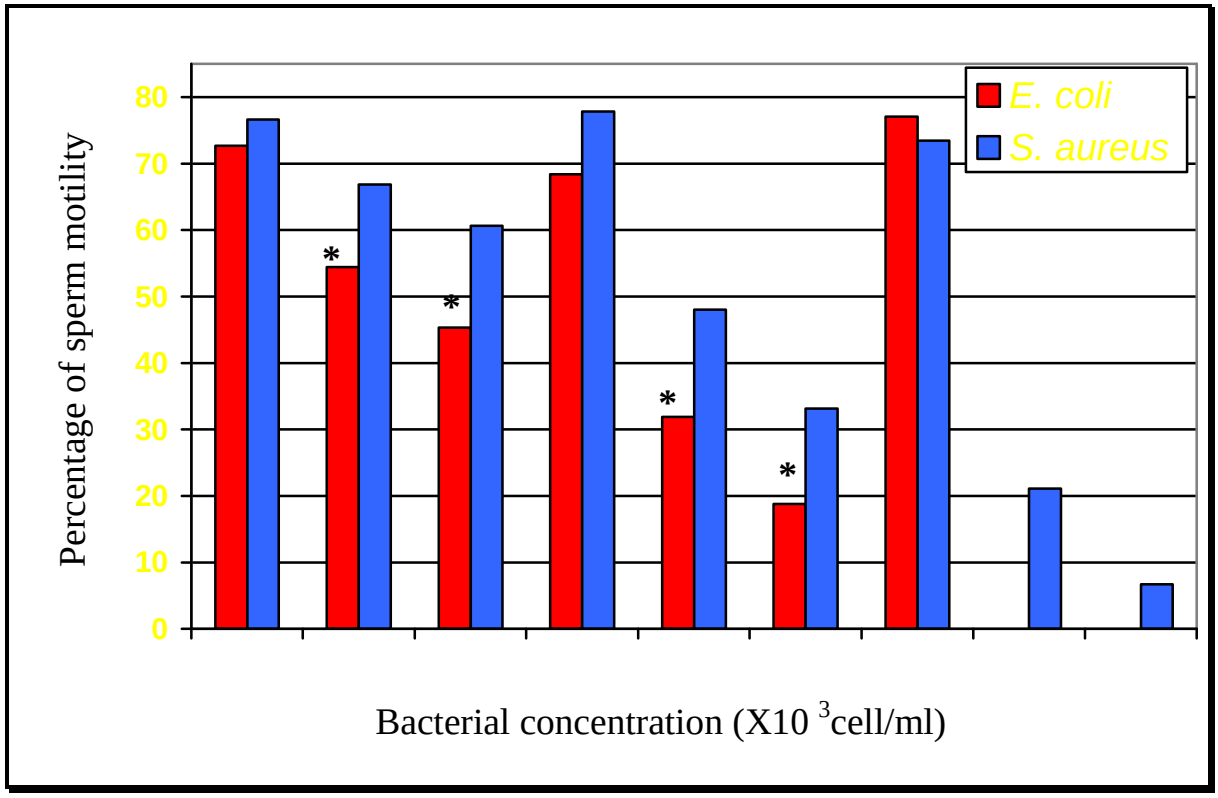

Figure (4-6): The effect of *E. coli* and *Staph aureus* on percentage of mice sperm motility after 60 min of incubation.

***** less than 0.05 level of significance

No significant (P>0.05) differences were reported between all counts of *E. coli* and *Staphs aureus* on the percentage of mouse abnormal sperm morphology at zero time of incubation were found (Figure 4-7). A significant (P<0.05) increase in the percentage of abnormal sperm morphology when incubated with 36 and 48X10^3 cell/ml of *E. coli* as compared to *Staph. aureus* after 30 min of incubation was observed (Figure 4-8). Same statistically differences were assessed for bacterial counts 12 and 24 X 10^3 cell/ml after 60 min between the two pathogens (Figur4-9).

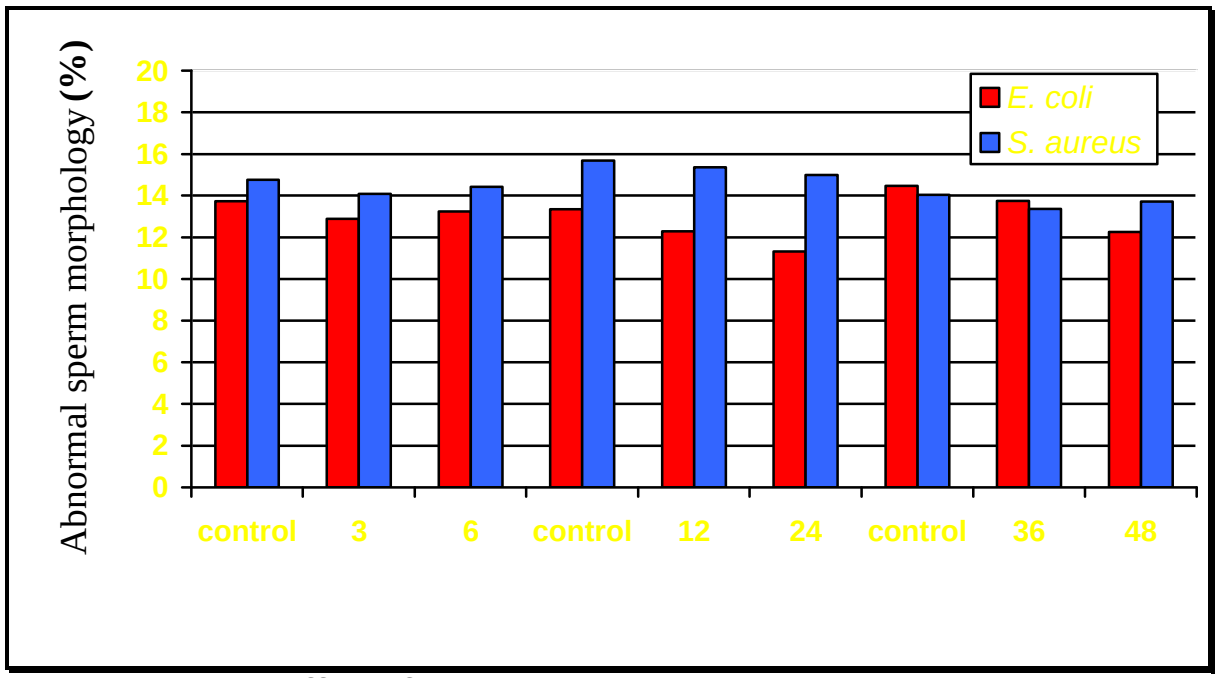

Figure (4-7): The effect of *E. coli* and *Staph aureus* on mice abnormal sperm morphology at zero time of incubation.

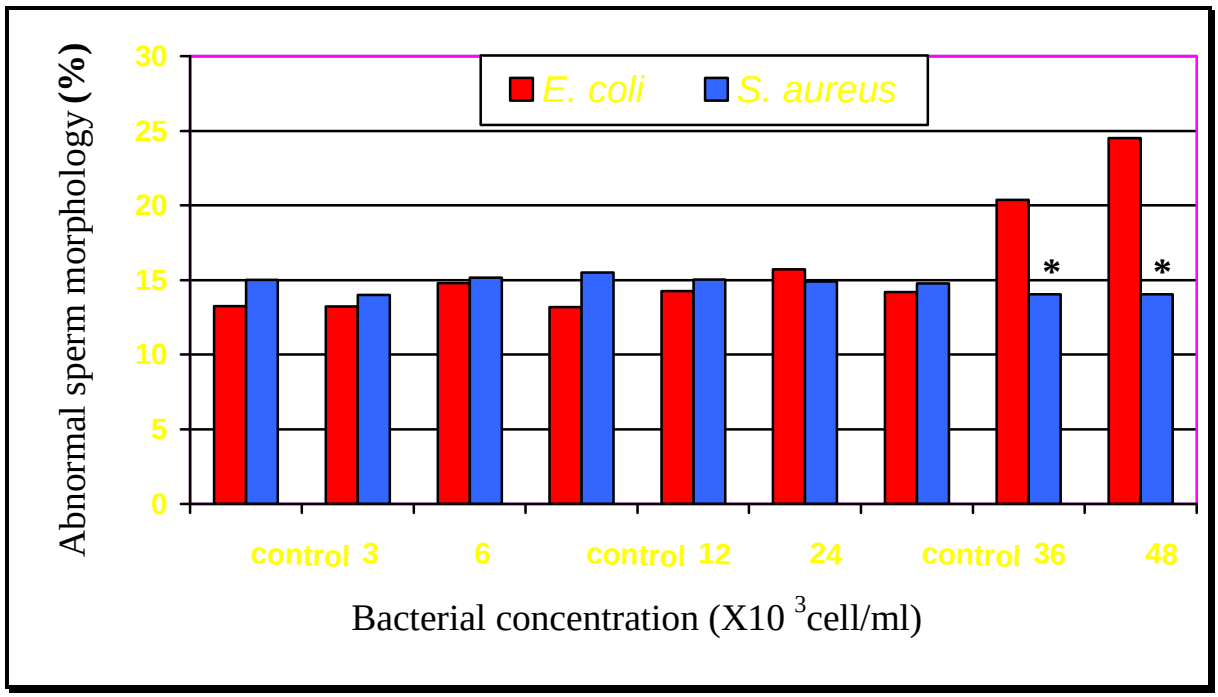

Figure (4-8): The effect of *E. coli* and *Staphs aureus* on mice abnormal sperm morphology after 30 min of incubation.

***** less than 0.05 level of significance

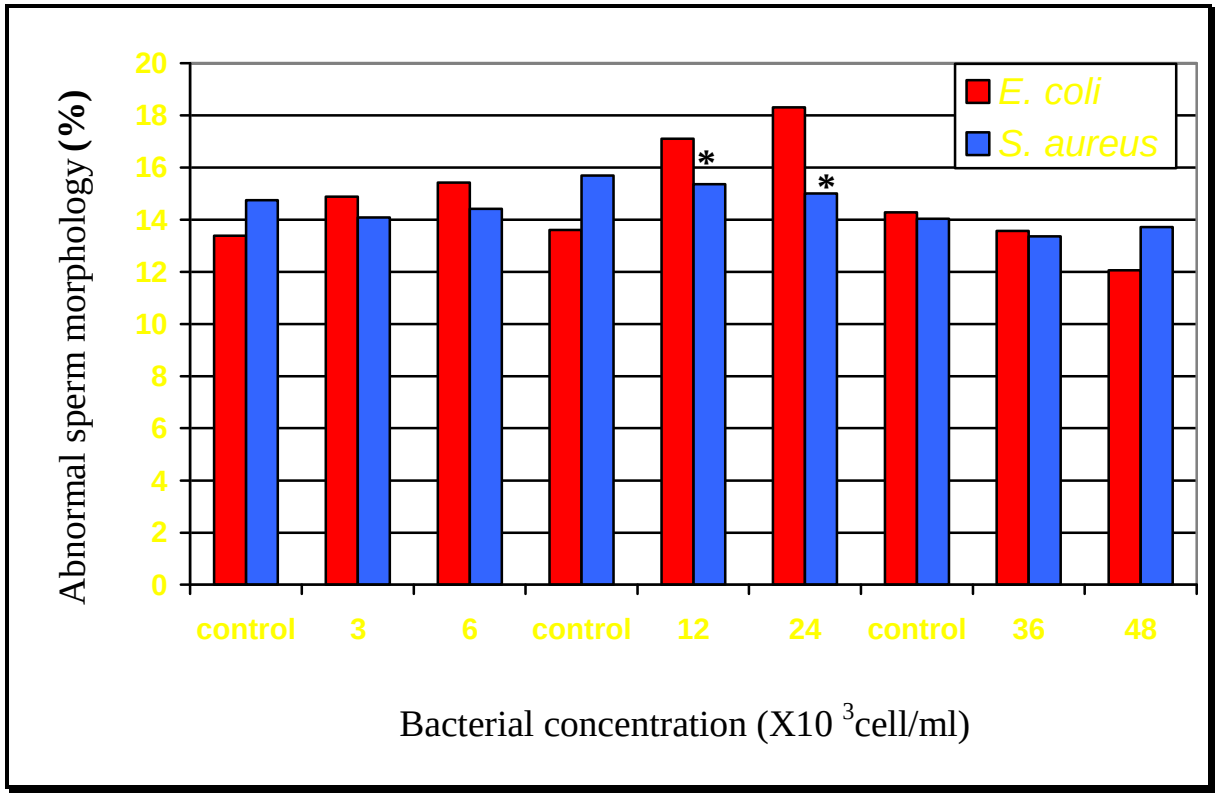

Figure (4-9): The effect of *E. coli* and *Staphs aureus* on percentage of abnormal sperm morphology after 60 min of incubation.

* less than 0.05 level of significance

Therefore, *E. coli* has impaired effect on the mouse abnormal sperm morphology more than *Staph. aureus* .This result explained by Villegas *et al.* (2005), who certified that the direct contact between *E. coli* or its products and spermatozoa could stimulate apoptosis in ejaculated human sperm. More over, Hong *et al* (2002) concluded that the *Staph. aureus* has no activity for adherence to the spermatozoa, and did not induce morphological changes on the spermatozoa. Monga and Roberts (1994) noticed that there is a receptor-ligand interaction between sperm and *E. coli* surface adhesions (p-fimbriae type 1 fimbriae and their specific receptor saccharides (alpha-gal P-1-4-beta-gal P-o-methyl [gal-gal], mannose). P-fimbriae caused tail-tail sperm agglutination, meanwhile, type 1 fimbriae causes head-head sperm agglutination therefore *E.*

coli causes about 40-70 % sperm agglutination within motile spermatozoa. On the other hand, Diemer (2000) reported that the electron microscopic evaluation revealed multiple and profound alteration in the ultrastructure of spermatozoa such as membrane defects and cytoplasmic vacuoles exclusively in spermatozoa of more than 90% infected samples.

Figures (4-10, 4-11 and 4-12) showed the effects of the different counts of *E. coli* and *Staph aureus* on the percentage of mouse sperm agglutination after 0, 30 and 60 minutes of incubation respectively. The present result appeared that the *E. coli* increased significantly ($P<0.05$) the percentage of sperm agglutination when compared to *Staphs aureus* after 30 min of co-incubation with bacterial counts 36 and $48X10^3$ cell/ml (Figure 4-11) and after 60 min of co-incubation with bacterial counts 12 and $24X10^3$ cell/ml (Figure4-12). These results may be explained by the activity of *E. coli* for adherence to spermatozoal membrane mediated by mannose and mannose-binding structures presente on both cell types resulted in clumping and agglutination between bacteria and the spermatozoa cells (Wolff *et al*., 1993). Meanwhile, *Staphs aureus* has no activity for adherence to spermatozoa and then has no effect on sperm agglutination (Hong *et al*., 2002).

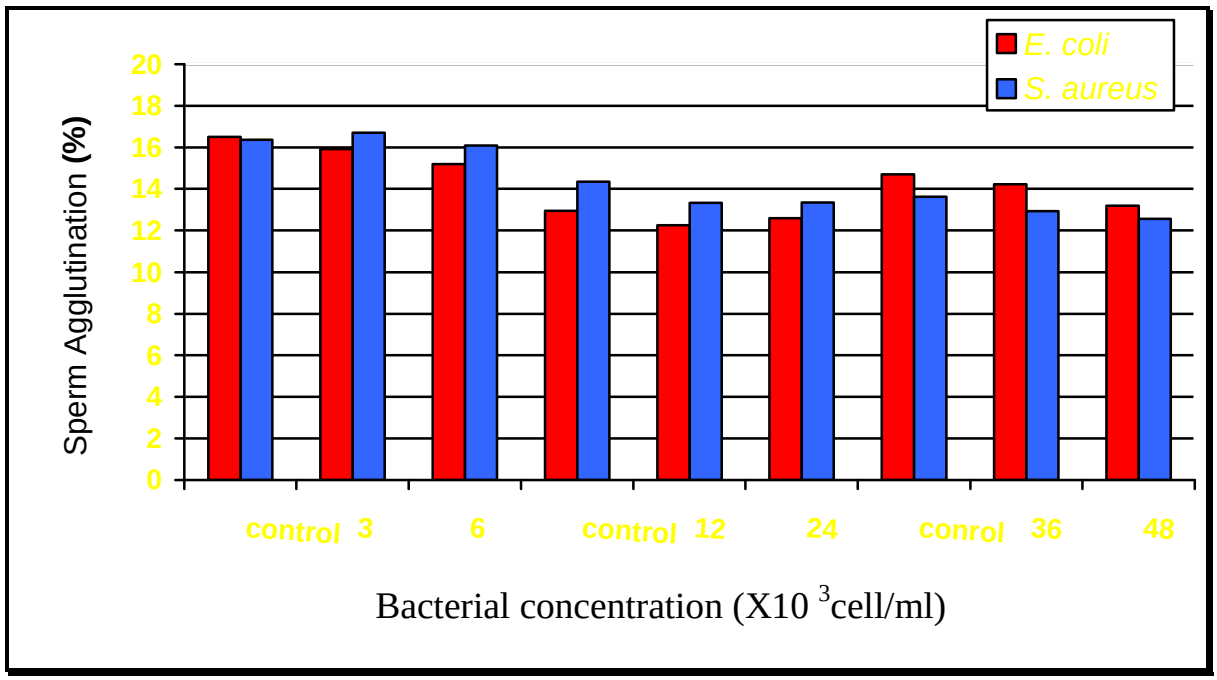

Figure (4-10): The effect of *E. coli* and *Staph aureus* on mice sperm agglutination at zero time of incubation.

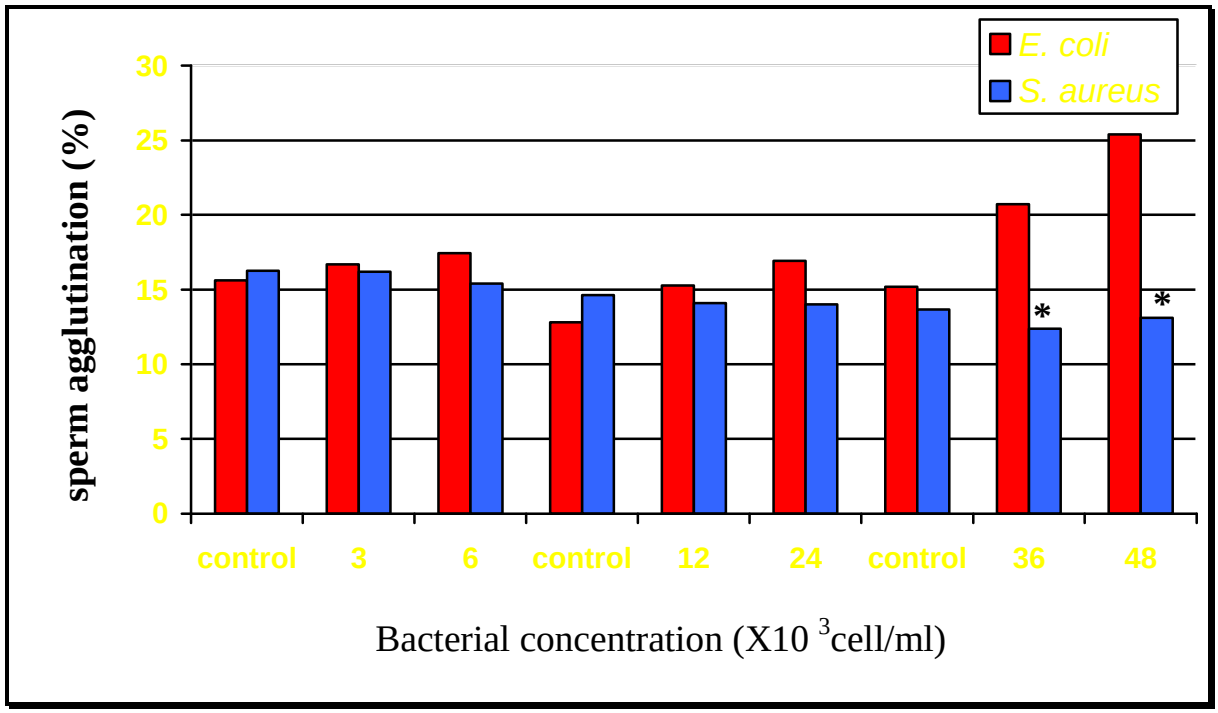

Figure (4-11): The effect of *E. coli* and *Staph aureus* on percentage of mice sperm agglutination after 30 min of incubation.

* less than 0.05 level of significance

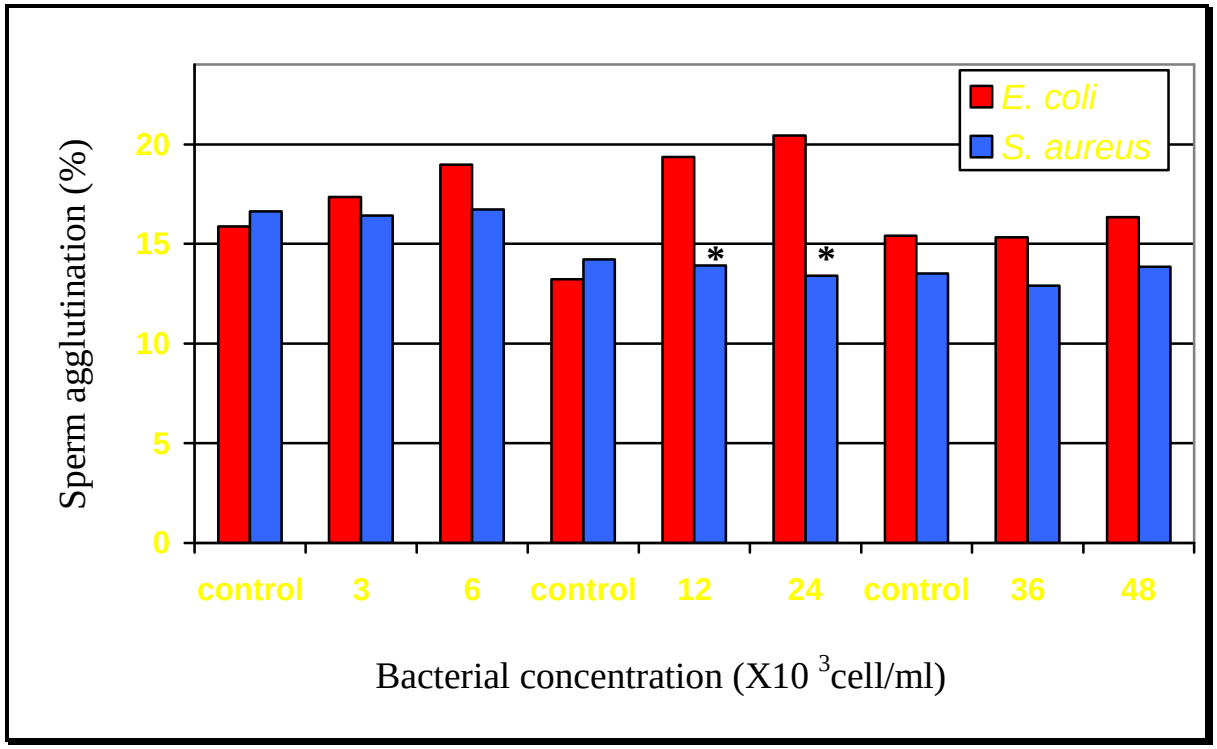

Figure (4-12): The effect of *E. coli* and *Staph aureus* on percentage of mouse sperm agglutination after 60 min of incubation.

* less than 0.05 level of significance

Figures (4-13, 4-14 and 4-15) show the effects of the different counts of *E. coli* and *Staph aureus* culture filtrate on mouse sperm concentrations after 0, 30 and 60 minutes. Non significant (P>0.05) differences were observed between *E. coli* and *Staphs aureus* at zero time and after 30 and 60 minutes of co-incubation. Therefore this result may be attributed to the weakness in the activity of both bacterial culture filtrates on mouse sperm concentration. However, Todar (2005) reported two types of *E. coli* exotoxin involving LT and ST toxins which negatively affect sperm cells. On the other hand, *Staphs aureus* has activity and virulence factors lower than *E. coli*, and then its culture filtrate may has no activity on sperm cells.

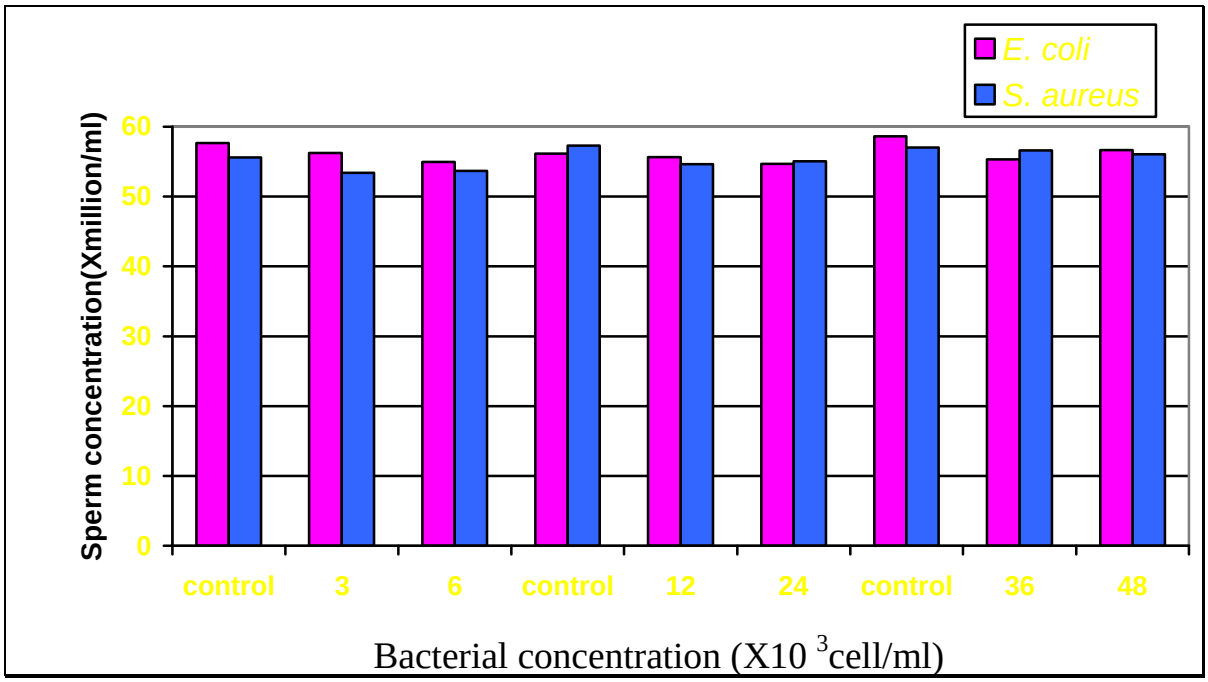

Figure (4-13): The effect of *E. coli* and *Staph aureus* culture filtrate on mice sperm concentration at zero time of incubation.

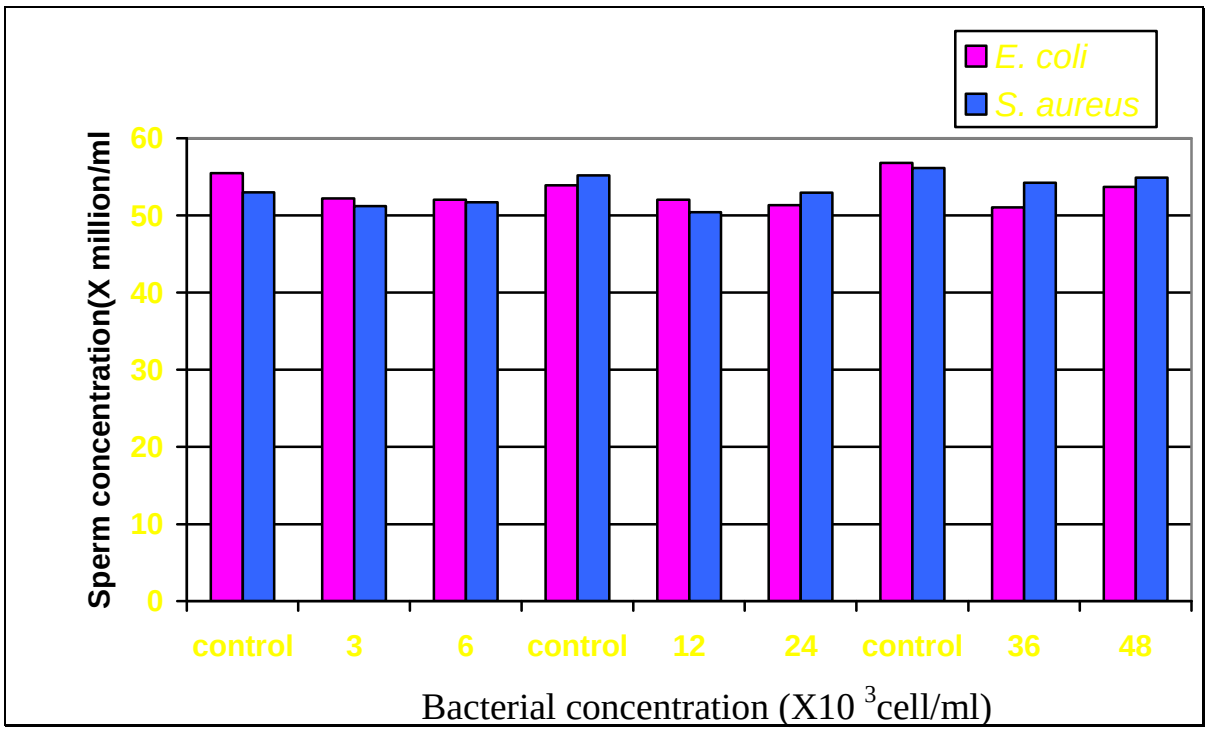

Figure (4-14): The effect of *E. coli* and *Staph aureus* culture filtrate on mice sperm concentration after 30 min of incubation.

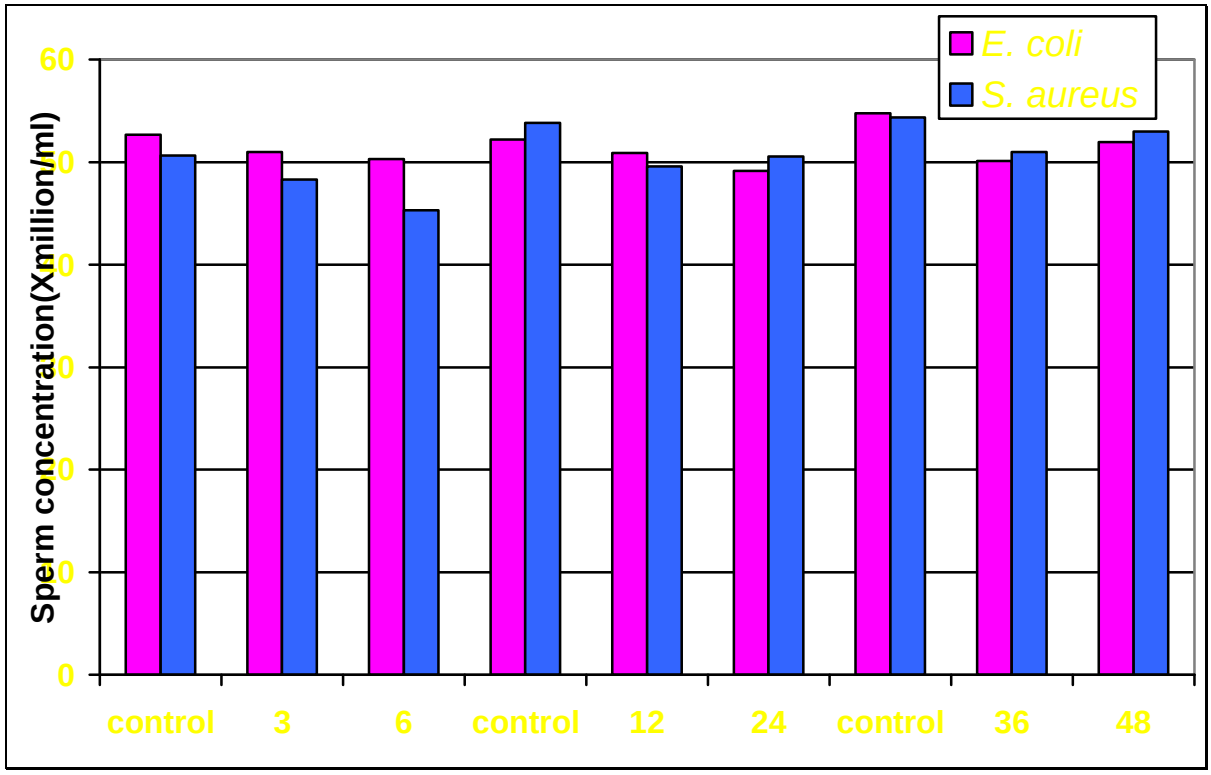

Figure (4-15): The effect of *E. coli* and *Staphs aureus* culture filtrate on mice sperm concentration after 60 min of incubation.

Non significant (P>0.05) difference were found for all concentrations of *E. coli* and *Staph aureus* culture filtrate used on the percentage of mouse sperm motility at zero time (Figure 4-16). Significant (P<0.05) reduction in the percentage of mouse sperm motility incubated for 30 minutes with culture filtrates of *E. coli* counts 24, 36 and 48 $X10^3$ cell/ml and with culture filtrates of *E. coli* counts 12, 24, 36 and 48 $X10^3$ cell/ml incubated for 60 minutes as compared to its group of *Staph aureus* culture filtrate. Sousa (2003) revealed that *E. coli* have the ability to produce microbial proteins exotoxin usually enzymes that kill host cells at very low count which may probably reduce spermatozoal motility.

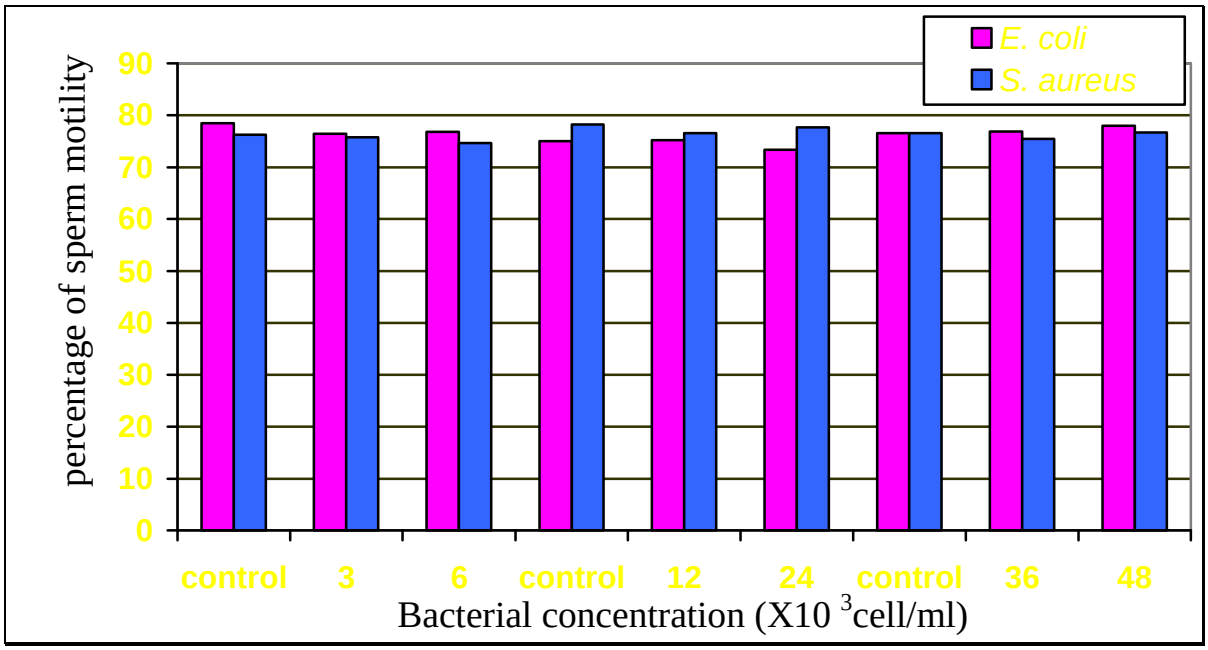

Figure (4-16): The effect of *E. coli* and *Staphs aureus* culture filtrate on mice sperm motility at zero time of incubation.

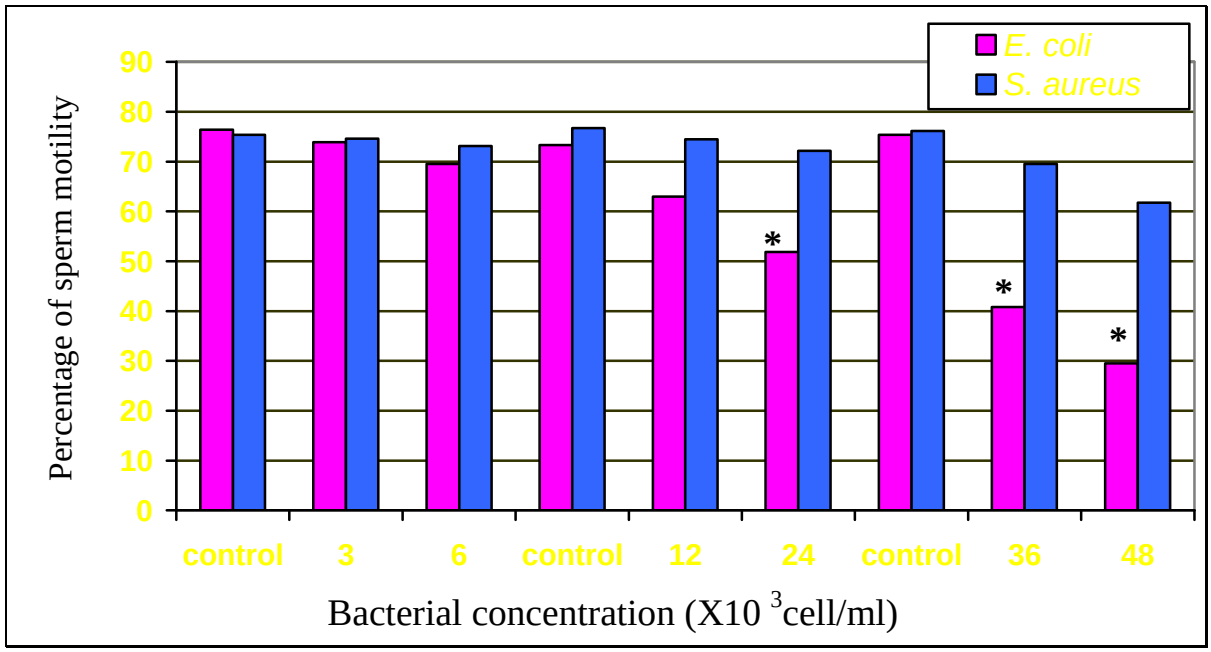

Figure (4-17): The effect of *E. coli* and *Staph aureus* culture filtrate on mice sperm motility after 30 min of incubation.

* less than 0.05 level of significance

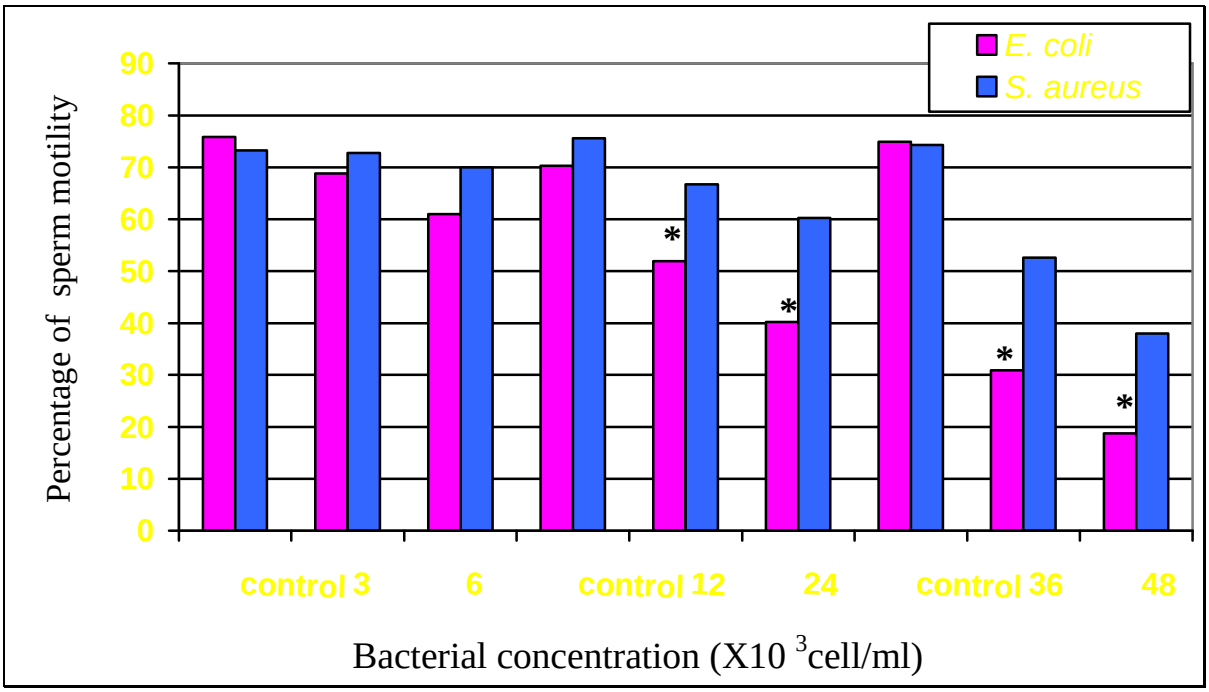

Figure (4-18): The effect of *E. coli* and *Staph aureus* culture filtrate on mice sperm motility after 60 min of incubation.

* less than 0.05 level of significance

Although *staph aureus* bacteria produce different kinds of exotoxins, its culture filtrates couldn't induce a significant reduction in spermatozoal motility as *E. coli* culture filtrates did. This could be explained by the suggestions that this strain hasn't acquired sets of all virulence genes or its concentration is very low (Sousa, 2003).

The effects of the different counts of *E. coli* and *Staphs aureus* culture filtrates on the percentage of mouse sperm morphology after 0, 30 and 60 minutes of incubation were presented in figures 4-19, 4-20 and 4-21. At zero time of incubation with all culture filtrate of *E. coli* counts, the percentage of abnormal sperm morphology was showd no significant differences (P>0.05) as compared to corresponding groups of *Staphs aureus*.

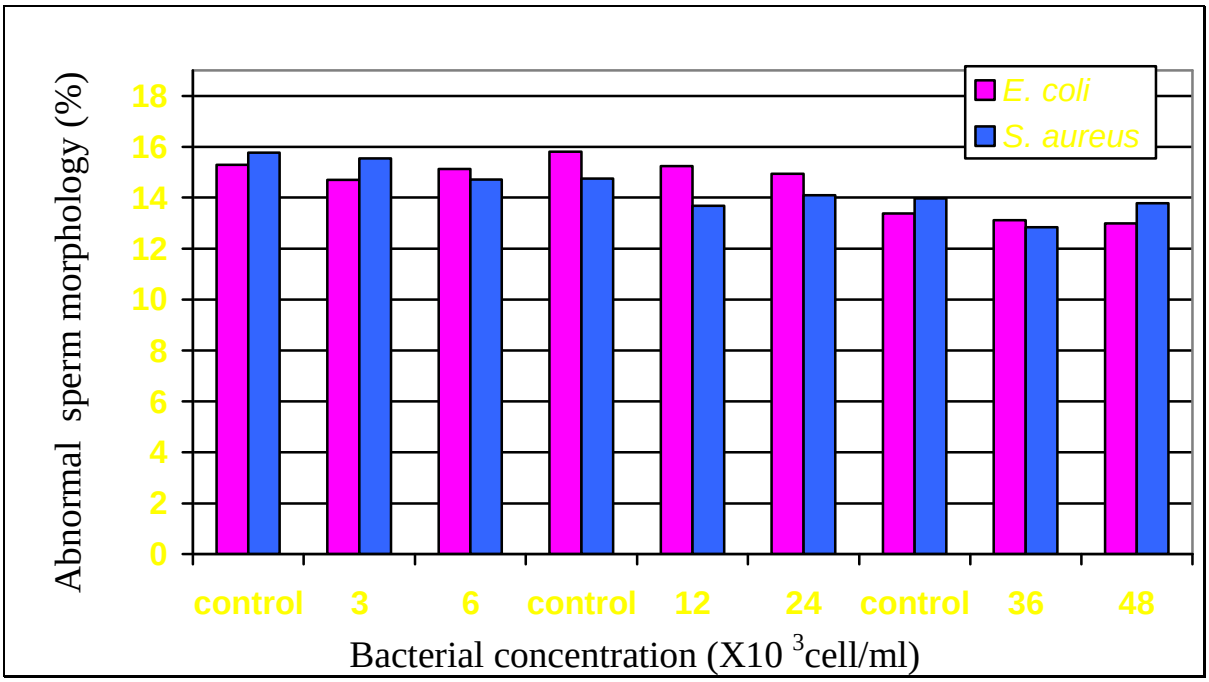

Figure (4-19): The effect of *E. coli* and *Staph aureus* culture filtrate on mice abnormal sperm morphology at zero time of incubation.

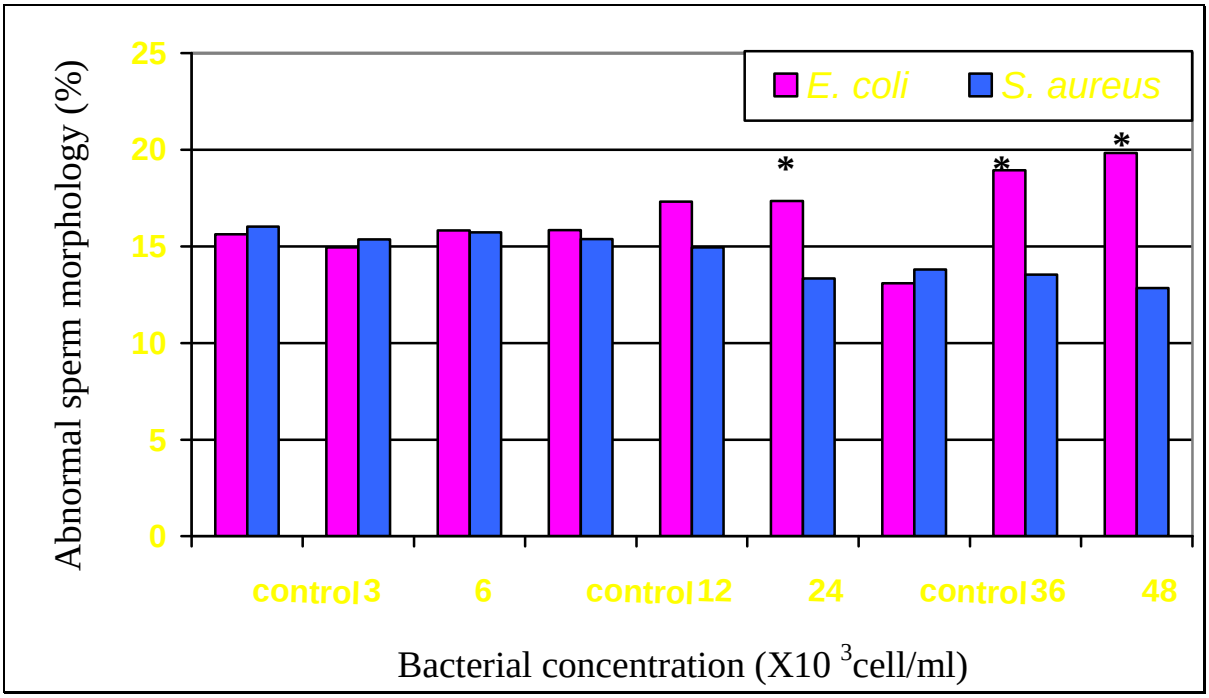

Figure (4-20) The effect of *E. coli* and *Staph aureus* culture filtrate on abnormal mouse sperm morphology after 30 min of incubation.

* less than 0.05 level of significance

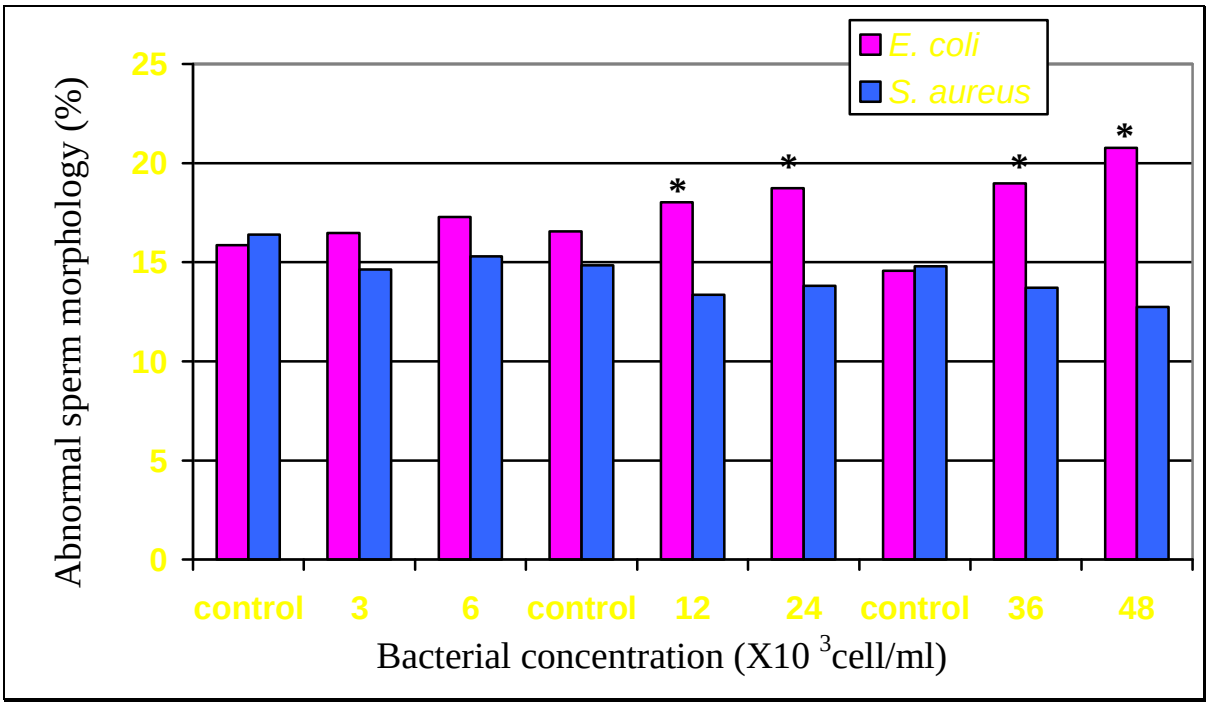

Figure (4-21): The effect of *E. coli* and *Staph aureus* culture filtrate on mice abnormal sperm morphology after 60 min of co-incubation.

* less than 0.05 level of significance

The percentage of abnormal sperm morphology was significantly (P<0.05) increased for *E. coli* culture filtrates at counts 24, 36 and 48 x10^3 cell/ml after 30 minutes incubation and at counts 12, 24, 36 and 48X10^3 cell/ml after 60 minutes of incubation as compared with groups of *Staph. aureus* culture filtrates (tables 4-20 and 4-21 Respectively), non significant (P>0.05) differences were assessed for all counts useed among the two species culture filtrates on the percentage of sperm agglutination at zero time and after 30 minutes of incubation (Figures 4-22 and 4-23). meanwhile *E. coli* culture filtrates at counts 24, 36 and 48 X10^3 cell/ml significantly (P<0.05) elevated the percentage of sperm agglutination after 60 minutes of incubation (Figure 4-24).

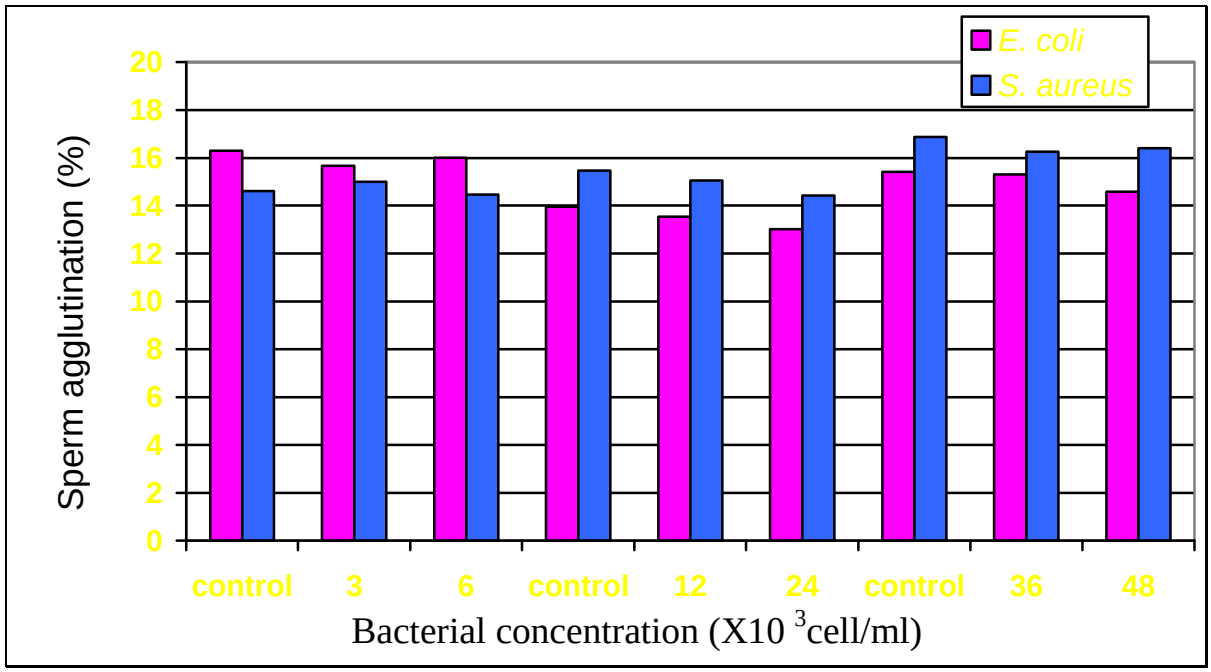

Figure (22): The effect of *E. coli* and *Staph aureus* culture filtrate on mice sperm agglutination at zero time of incubation.

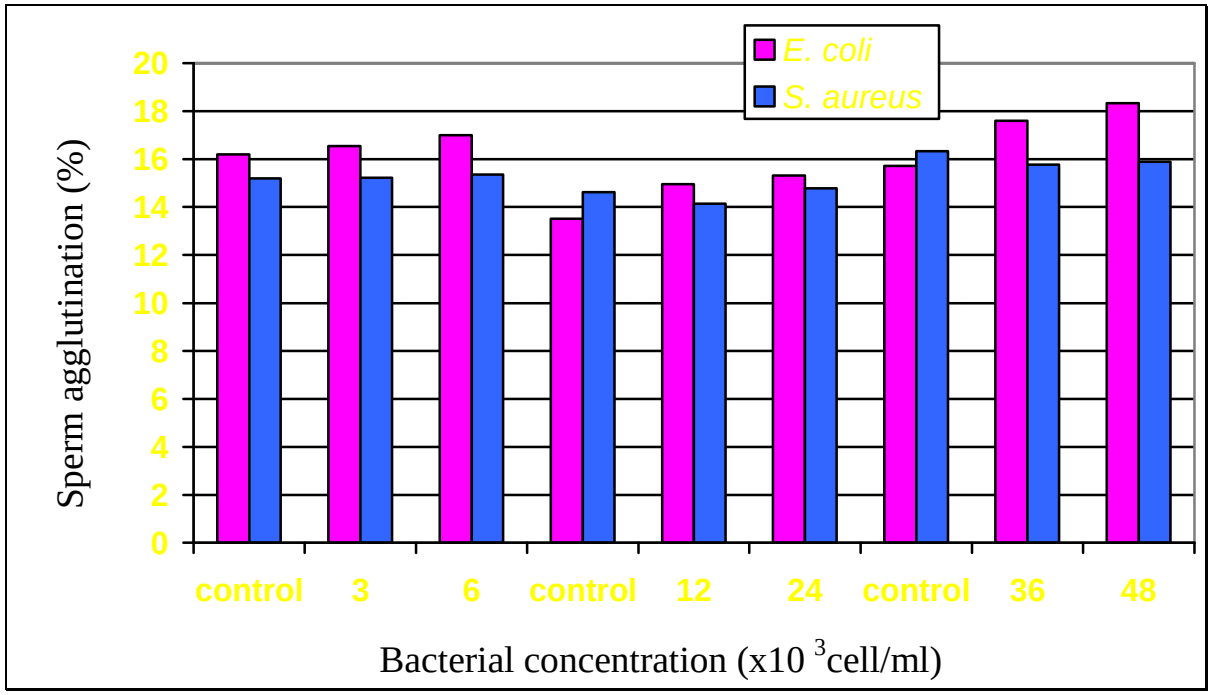

Figure (23): The effect of *E. coli* and *Staph aureus* culture filtrate on mice sperm agglutination after 30 min of incubation.

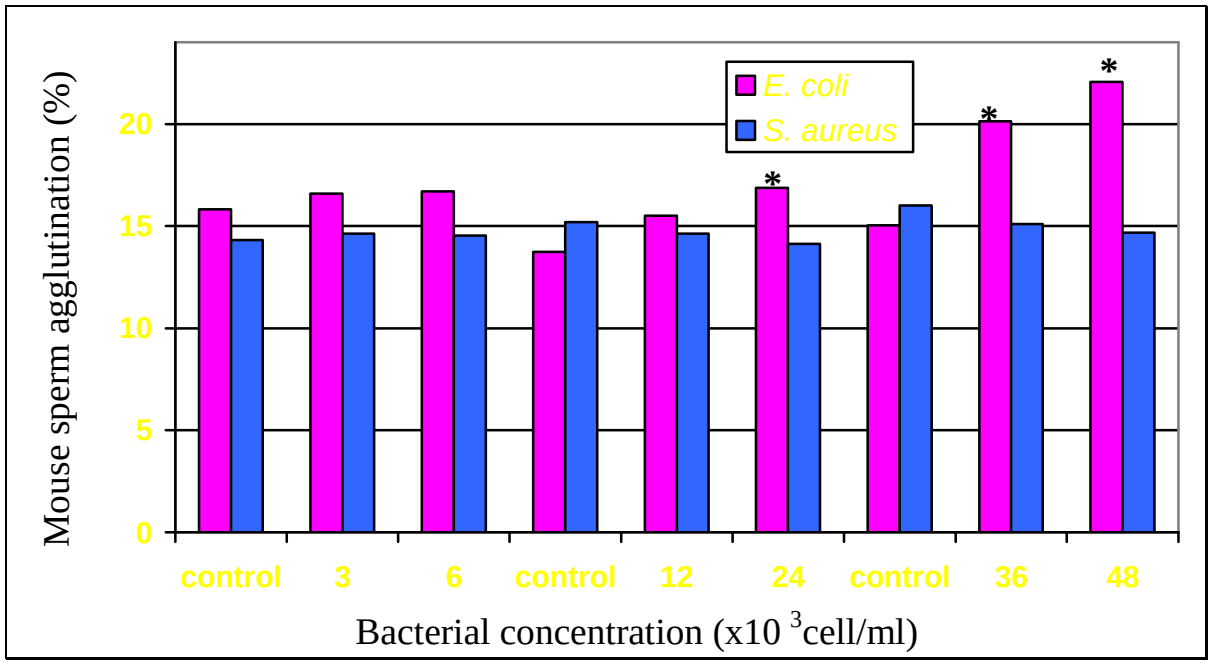

Figure (4-24): The effect of *E. coli* and *Staph aureus* culture filtrate on mice sperm agglutination after 60 min of incubation.

* less than 0.05 level of significance

In the light of the present results, culture filtrates of *E. coli* are harmful than *Staph aureus* culture filtrates on sperm agglutination. More from this result, the time of sperm-bacterial culture filtrate incubation plays a major role on the morphology and agglutination of spermatozoa which permits bacterial products like exotoxins to complete its attachment and action although Hong *et al* (2002) demonstrated that *Staphs. aureus* or its culture filtrates hasn't the ability to adherence to the spermatozoa and didn't increase sperm agglutination. Further explanation by Sousa (2003) who suggested that Gram-negative pathogens could lyses the host cells by the pore-forming toxins function by insertion into the host cell plasma membrane which causes formation of a pore or channel leading to lyses of the host cells.

Figure (4-25) shows the effect of all concentrations of *E. coli* on the percentage of progressive motile spermatozoa (grades A+B) throughout0, 30 and 60 minutes of incubation. During the first 30 minutes of incubation there was a rapid and significant ($P<0.05$) decrease in the percentage of progressive motile spermatozoa incubated with *E. coli* counts 12, 24, 36 and $48X10^3$ cell/ml as compared to the control groups (Figure 4-25). From the same figure, gradual and non significant ($P>0.05$) decrease in the percentage of progressive motile spermatozoa of control groups throughout 60 minutes of incubation .This could be explained by the fact that normal spermatozoa in natural conditions impair its motility gradually after one hr of ejaculation(William, 2003).

While figure (4-26) shows the effect of all incubated *Staphs aureus* counts on the percentage of progressive mouse motile spermatozoa (grades A+B) during zero time and after 30 and 60 minutes of incubation . Gradual and non significant ($P>0.05$) decrease in the percentage of progressive motile spermatozoa incubated with 3 and 6 X 10^3 cell/ml, as well as control groups throughout 60 minutes. The culture filtrates of E. coli counts 3 and 6 X 10^3 cell/ml is lowers counts filtrates , have a lower concentrations of pathogenic enzymes and exotoxins, thus its negative effect on spermatozoa motility (grades A+B) lower. Gradual and significant ($P<0.05$) reduction in the percentage of progressive motile spermatozoa incubated with 12 X 10^3 cell/ ml throughout 60 minutes. From the same figure, rapid and significant ($P<0.05$) decrease in the percentage of progressive motile spermatozoa incubated with 24, 36 and 48 X 10^3 cell/ ml after 30 and 60 minutes of incubation. This result could be explained by the fact that bacteria may be produce toxins (serotoxins) that poisons the sperm (Molner, 2003). Moreover, Sousa (2003) proved that *E. coli* could produce proteins (toxins) that poison the host cells. Nicholson *et al.* (2000) reported that various exotoxins and enzymes that produced from *Staph. aureus* through its metabolism could significantly reduce the spermatozoa progressive motility.

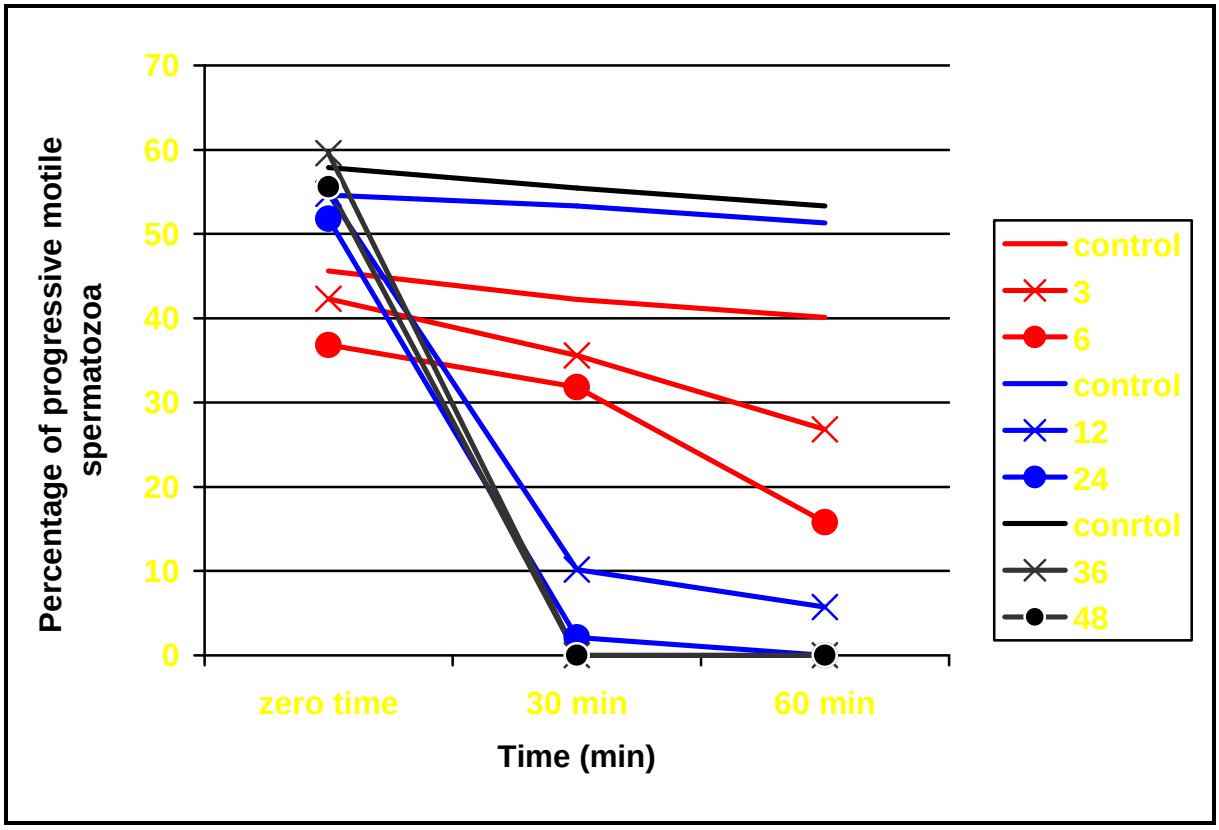

Figure (4-25): The effect of *E. coli* on percentage of progressive motility(grade A+B) of mice spermatozoa.

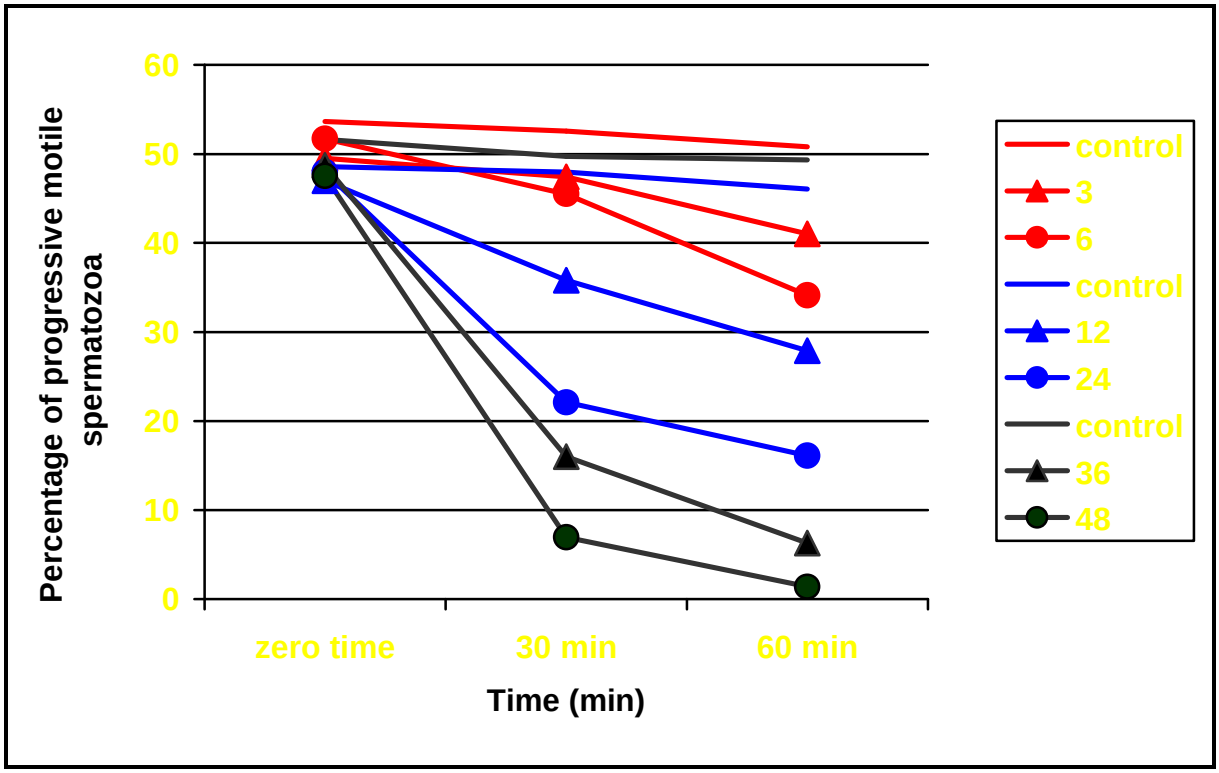

Figure (4-26): The effect of *Staph aureus* on percentage of progressive motility(grade A+B) of mice spermatozoa .

Effects of all counts of *E. coli* culture filtrates on percentage of progressive motility of mouse spermatozoa (grades A+B) at zero time and after 30 and 60 minutes of incubation were presented in figure (4-27). Non significant ($P<0.05$) reduction in the percentage of progressive motile spermatozoa at bacterial counts 3, 6 and 12 $X10^3$ cell/ml after 60 minutes of incubation. In the same figure, rapid and significant ($P<0.05$) decrease in the percentage of progressive motile spermatozoa incubated with culture filtrate of *E. coli* counts 24, 36 and 48 X 10^3 cell/ml after 30 minutes, also, same regression was registered for another 30 minutes. However, control groups have gradual and non significant ($P>0.05$) decrease in the percentage of progressive motile spermatozoa (Figure 4-27). In the figure (4-27), during 60 minutes of incubation, gradual and non significant ($P>0.05$) regression in the percentage of progressive motile spermatozoa incubated with 3, 6, 12, and 24 X 10^3 cell/ml, in additions to control groups. The percentage of progressive motile spermatozoa incubated with 36 and 48 X 10^3 cell/ml was significantly decreased after 30 minutes and 60 minutes. In agreement with Diemer *et al* (2003), spermatozoa incubated with *E. coli* bacteria revealed a significant decrease in progressive motility after 2 hr. In 1996 Diemer *et al* mentioned that *E. coli* have a direct inhibitory effect through its exotoxins in culture filtrate of *E coli* on progressive motility of spermatozoa only at sperm / bacteria ratio of approximately 1. Similarly, Liu *et al* (2002) revealed that *Staph aureus* significantly ($P<0.05$) decreased the sperm motility and viability. Prabha *et al* (2004) noticed that 72 hr old culture supernatant isolated from infertile male causing 100% immobilization of spermatozoa.

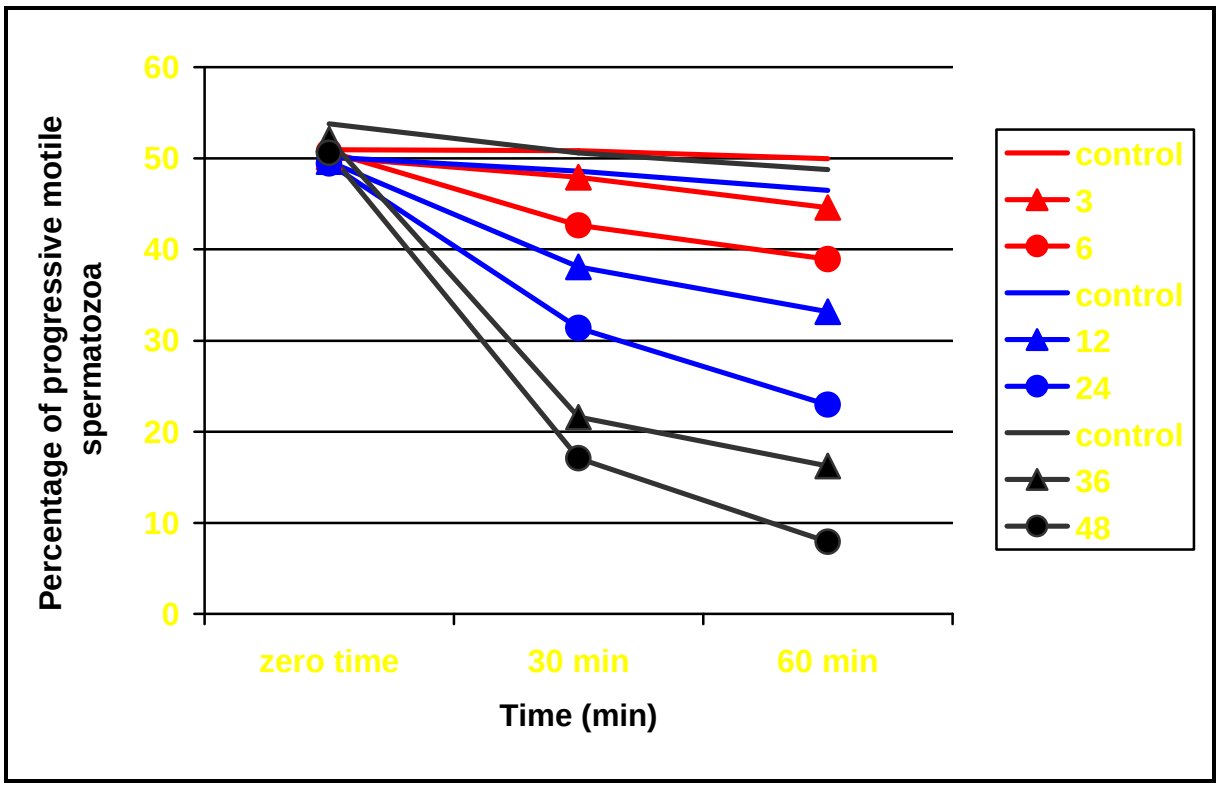

Figure (4-27): The effect of *E. coli* culture filtrates on percentage of progressive motility(gradeA+B) of mice spermatozoa.

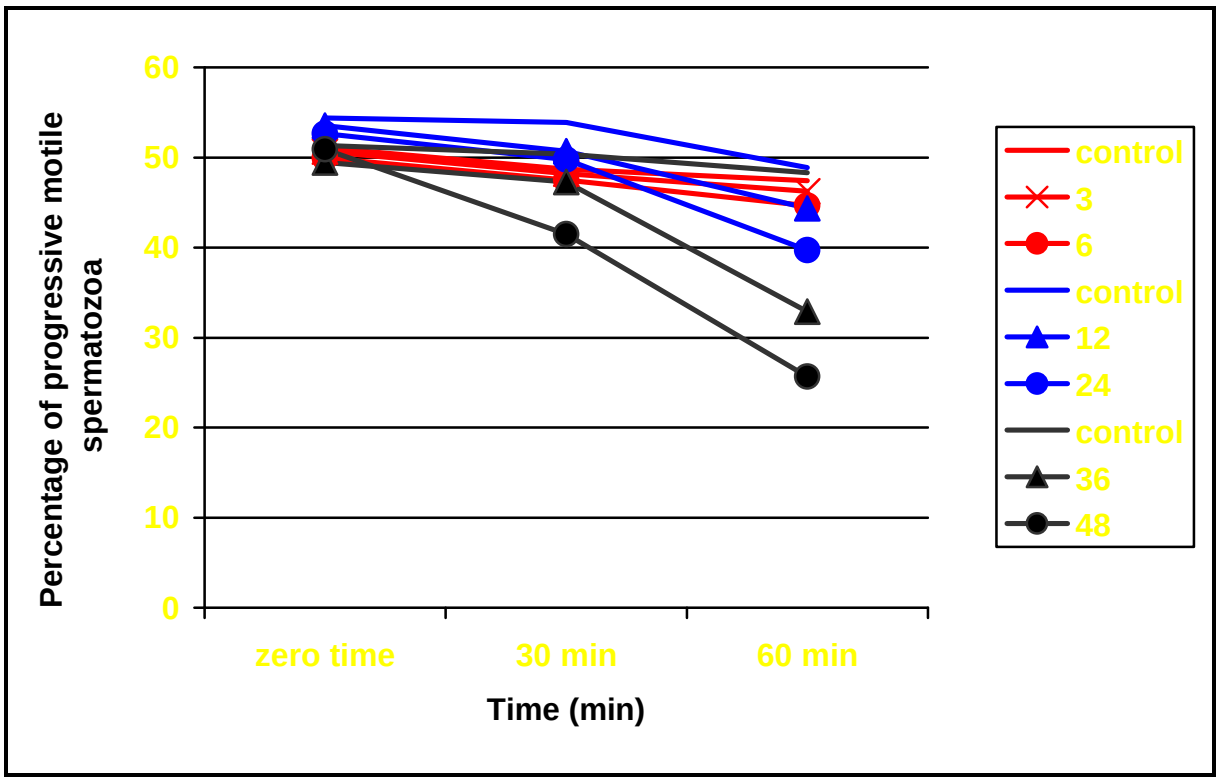

Figure (4-28): The effect of *Staph aureus* culture filtrates on percentage of progressive motility (grade A+B) of mice spermatozoa.

4.2 *In vitro* Fertilization

Results of *in vitro* fertilization (IVF) for mouse spermatozoa co-incubated with either *E. coli* or *Staph.s aureus* for different periods were presented in table (4-13). In general, outcomes of IVF have no results for spermatozoa . incubated with the different counts of *E coli* at different periods including 0, 30 and 60 minutes; meanwhile its control groups have mean percentage of IVF (55.61%). From the same table, results of IVF indicated that spermatozoa incubated with *Staphs aureus* at counts 36 and $48X10^3$ cell/ml at zero time and after 30 and 60 minutes have no results. Similarly, no IVF results were obtained for *Staph aureus* at count 24 after 30 minutes and 60 minutes, respectively. Significant ($P<0.05$) reduction in the percentage of IVF for spermatozoa incubated with *Staph aureus* at counts 3, 6, 12 and 24 X 10^3 cell/ml at zero time and at counts 3, 6 and 12 X 10^3 cell/ml after 30 minutes of incubation when compared to the control groups.

Table (4-13): The effects of different concentrations of *Escherichia coli* and *Staphylococcus aureus* on percentages of *in vitro* fertilization in mice.

Bacteria	Time								
	Zero								
	Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
E. coli	50.00*	0.00	0.00	56.25*	0.00	0.00	58.82*	0.00	0.00
Staph. aureus	56.25*	37.5	35.68	56.25*	31.25	17.64	58.88*	0.00	0.00
	30 min								
	Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
E. coli	44.44*	0.00	0.00	68.75*	0.00	0.00	52.94*	0.00	0.00
Staph. aureus	59.28*	33.52	23.33	66.66*	12.5	0.00	50.00*	0.00	0.00
	60 min								
	Control	**3**	**6**	**Control**	**12**	**24**	**Control**	**36**	**48**
E. coli	47.05*	0.00	0.00	58.82*	0.00	0.00	58.88*	0.00	0.00
Staph. aureus	66.66*	29.05	27.41	44.44*	0.00	0.00	52.94*	0.00	0.00

* Control group significantly ($p<0.05$) increased as compared to its corresponding groups.

Many scientists pointed out that the outcomes of IVF could be impaired at the presence of bacteria in semen (Stovall *et al* 1993).While Hewitt *et al* (1985) demonstrating that bacteria *in vitro* was reduced the fertilization and cleavage rates of human oocytes. Keck *et al*. (1998) reported that binding of bacteria to the sperm cell surface may impair sperm oocyte interaction. El-Mulla *et al* (1996) reported that the fertilization capacity of human spermatozoa *in vitro* could be impaired by the *E. coli* inducability effect of the acrosome reaction too early before reach ovum. Shalika *et al*. (1996) showed that *E coli* and *Staph. aureus* have reduced the fertilization *in vitro.* The effect of *E. coli* incubated with spermatozoa *in vitro* resulted in reduced sperm motility and acrosome reaction (Kohen *et al*., 1998). *E. coli* infection of ejaculates results in immobilization and impaired acrosomal function in human spermatozoa (Diemer. 2000). Furlong *et al*. (2000) explained this suggestion in according to the proacrosin protein present in the sperm acrosom which could be expressed by *E. coli* and assess zona pelucida binding activity. Therefore patients with semen culture positive for E. coli prior to the IVF procedure, and then cleared after treatment had a higher pregnancy rate (60%) compared with those patients who were positive for E. coli at the time of attempt.

Outcomes of IVF for mouse spermatozoa incubated with the different counts of *E. coli* and *Staph aureus* culture filtrates were presented in table (4-14). Culture filtrates of all counts of *E. coli* at 30 and 60 minutes have no results as compared to its control groups, except culture filtrates at counts 3 and 6 X 10^3 cell/ml have significant ($P<0.05$) reduction for IVF percentage after 30 minutes of incubation when compared to the control groups.

At zero time, no IVF results were recorded for culture filtrate at counts 36 and 48 X10^3 cell/ml, meanwhile, a significant ($P<0.05$) reduction in the percentage of IVF were noticed for culture filtrates at counts 12 and 24 X 10^3 cell/ml and non significant ($P>0.05$) differences were observed for culture filtrate at counts 3 and 6 X 10^3 cell/ml as compared to the control groups. On the

other hand, *Staphs aureus* culture filtrates of only 36 and 48X10^3 cell/ml has significant (P<0.05) reduction in the percentage of IVF after 60 minutes of incubation (Table 4-14). Patients have positive *E. coli* within semen cultures prior to the IVF procedure using washing and centrifugation have a higher pregnancy rate than those patients who were positive for *E. coli* at the time of attempt without sperm processing (Shalika *et al*., 1996). The same workers demonstrated that the *Staphs aureus* have a lower activity than *E. coli*, however the group of patients have *Staph aureus* infected semen at the time of IVF observed reduced pregnancy rate up to 17% of patients.

Table (4-14): The effects of different concentrations of *Escherichia coli* and *Staphylococcus aureus* culture filtrates on percentages of *in vitro* fertilization in mice.

Bacteria	Time								
	Zero								
	Control	3	6	Control	12	24	Control	36	48
E. coli filtrates	52.29	46.66	47.36	66.66*	17.64	6.25	53.33*	0.00	0.00
Staph. aureus filtrates	47.05	42.53	41.11	50.00	46.68	54.00	52.94	47.05	45.0
	30 min								
	Control	3	6	Control	12	24	Control	36	48
E. coli filtrates	52.29*	27.77	12.5	58.82*	0.00	0.00	60.00*	0.00	0.00
Staph. aureus filtrates	62.25	52.29	41.17	56.25	50	44.6	62.25	43.75	37.50
	60 min								
	Control	3	6	Control	12	24	Control	36	48
E. coli filtrates	68.87*	0.00	0.00	47.05	0.00	0.0	46.66*	0.0	0.00
Staph. aureus filtrates	50.00	43.75	46.66	50.00	47.05	41.17	50.0*	37.5	17.64

* Control group significantly (p<0.05) increased as compared to its corresponding groups.

Chapter Four
Results and Discussion

Chapter Five

Conclusions and Recommendations

5.1 Conclusions

1- The seminal fluid of infertile patients infected with *Staph. aureus* is four times than infected with *E. coli*.

2- Culture filtrates of *E. coli* and *Staph. aureus* have lower negative effects than its cells on the of mouse sperm function tests and the culture filtrates of *E. coli* have more side effect than *Staph. aureus* on the mouse sperm function tests.

3- Mouse *in vitro* fertilization has no results in the presence of *E. coli* infection while, reduced outcomes of mouse IVF when culture media infected with *Staph. aureus.*

5.2 Recommendations

1- Molecular and biochemical studies on the effect of *E. coli* and *Staph. aureus* on *in vitro* sperm-egg binding and plasma membrane receptors in experimental animals.

2- Immunological and histological studies on the infected male and female reproductive organs and its reproducibility in experimental animals.

3- Use of herbal medicine for treatment of infection of male reproductive system and improvement of *in vitro* and *in vivo* fertilization outcomes in experimental animals.

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الخلاصة

تضمنت الدراسة الحالية عزل وتشخيص بكتريا القولون وبكتريا العنقوديات الذهبية لاربعة عشر مريضا يعانون من العقم اثناء مراجعتهم معهد ابحاث الاجنة وعلاج العقم. تم عزل وتشخيص نوعي البكتريا باجراء الفحوصات البايوكيميائية اللازمة، ولبغية التاكد من دقة التشخيص فقد اعيد تشخيصها باستعمال نضام الابي (API) للتشخيص و الذي اعطى نتائج ايجابية تاكيدية.

تم اختبار الفعالية السمية لكلا نوعي البكتريا ورواشحهما عند العدد البكتيري ٣، ٦، ١٢، ٢٤، ٣٦ و ٤٨ X 10^3 خلية/ملليلتر على فحوصات وظائف النطف في الفئران خارج الجسم والتي تضمنت تراكيز النطف، النسبة المئوية لحركة النطف، النسبة المئوية لحركة النطف التقدمية، النسبة المئوية للشكل اللاطبيعي للنطف، النسبة المئوية لتكتل النطف والنسبة المئوية للاخصاب الخارجي عند وقت الصفر وبعد مرور ٣٠ و٦٠ دقيقة من وقت اضافة البكتريا او راشحها وحضنه.

اظهرت النتائج ان بكتريا القولون ذات تاثير سلبي اكبر من تاثير بكتريا العنقوديات الذهبية على فحوصات وظائف النطف *في الفئران* حيث بينت ان بكتريا القولون بدات باختزال النسبة المئوية لحركة النطف معنويا بعد مرور ٣٠ دقيقة من اظافة وحضن البكتريا عند العدد البكتيري 6 X 10^3خلية/مليلتر في حين ان العنقوديات الذهبية بدا تاثيرها السلبي على حركة النطف عند العدد البكتيري ٢٤ X 10^3 خلية/مليلتر و بعد مرور ٣٠ دقيقة على الاضافة والحضن. ان النسب المئوية للشكل اللاطبيعي للنطف وتكتل النطف ازداد معنويا بعد مرور نصف ساعة على اضافة وحضن العدد البكتيري ٣٦ X 10^3 خلية/مليلتر لبكتريا القولون حيث وصل تاثيرها الى تكسير ما يقارب 40 % من النطف بعد مرور ٦٠ دقيقة على الاضافة والحضن في حين ان العنقوديات الذهبية لم تسبب اي تاثير سلبي لكلا النسبتين.

في الدراسة الحالية اثرت بكتريا القولون وبكل اعدادها المضافة سلبيا على الحيامن باختزال قدرتها على التخصيب معنويا عند وقت الصفر وبعد مرور ٣٠ و ٦٠ دقيقة على الاظافة والحضن في حين ان قدرة الحيامن المحضونة مع العنقوديات الذهبية على التخصيب اختزلت عند العدد البكتيري ٢٤X ١٠^3 خلية/مليلتر وبعد مرور ٣٠ دقيقة على اضافة البكتريا وحضنها.

كان لراشحي بكتريا القولون وبكتريا العنقوديات الذهبية تاثيرسلبي اقل من خلاياها الحية على فحوصات وظائف النطف في الفئران. حيث بينت النتائج ان الراشح البكتيري لبكتريا القولون اختزل النسبة المئوية لحركة النطف معنويا عند العدد البكتيري ٢٤ X $١٠^٣$ خلية/مليلتر بعد مرور ٣٠ دقيقة على الاضافة والحضن في حين ظهر التاثير السلبي لراشح بكتريا العنقوديات الذهبية على هذه النسبة اي حركة

النطف عندالعدد البكتيري ٤٨ X 10^3 خلية/مليلترو بعد مرور ٣٠ دقيقة على الاضافة والحضن. كان لراشح بكتريا القولون تاثير سلبي على النسب المئوية للشكل اللاطبيعي للنطف و تكتل النطف عند العدد البكتيري ٣٦ و ٤٨ X 10^3 خلية/مليلتر بعد مرور ٣٠ دقيقة على الاضافة والحضن في حين لم يكن لراشح العنقوديات الذهبية اي تاثير على كلا النسبتين.

عند الاعداد البكتيرية ٣و ٦ X 10^3خلية / مليلتروبعد مرور ٣٠ دقيقة على الاضافة والحضن كان لراشح بكتريا القولون تاثير واضح على اختزال قدرة الحيامن على التخصيب حيث كانت نسبة البيوض المخصبة % 12.50 و 27.77% ، في حين كانت نسبة البيوض المخصبة تحت تاثير العنقوديات الذهبية هي% ١٧,٦٤ و% ٣٧,٥٠ عند الاعداد البكتيرية ٤٨و٣٦ X ١٠٣ خلية/مليلتر بعد مرور ٦٠ دقيقة على الاظافة والحضن.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الاحيائية

تاثير بكتريا ايشيريشيا القولون *Escherichia coli* وبكتريا العنقوديات الذهبية *Staphylococcus aureus* المعزولة من السائل المنوي للمرضى العقيمين على فحوصات وظائف النطف والاختاب الخارجي في الفئران

رسالة

مقدمة الى كلية العلوم جامعة النهرين

كجزء من متطلبات نيل درجة الماجستير في علوم التقانة الاحيائية

من قبل

دنيا قاسم محمد العزاوي

بكالوريوس تقانة احيائية 2002

ربيع الثاني ١٤٢٧

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