**Summary**

Toxoplasmosis is a zoonotic disease caused by the opportunistic protozoan *Toxoplasma gondii.* It has been well documented that toxoplasmosis is of crucial importance especially for pregnant women and immunocompromised patients. In addition to the risks of gestation complications and congenital infections, it has been suggested that toxoplasmosis has some unfavorable effects on reproductive capacity in both men and women.

The present study was carried out at Kamal AL-Samaraee Hospital in Baghdad province during the period from the first of October 2011 till the end of January 2012. In this cross sectional study, ELISA technique was used for detecting IgM and IgG in one hundred and ten infertile couples as a selective sample aged from 19 to 59 years, then FSH, LH levels, the size with structural architecture of ovaries were investigated in the positive females, testosterone hormone levels were tested and seminal fluid was examined in the positive males.

The results showed that out of 110 couples, 46 (41.81%) males and 85 (77.27%) females were positive for toxoplasmosis, and from the same sample it was found that 39 (35.45%) males sharing their females in *Toxoplasma* infection.

The results of VIDAS showed that 43 (93.47%) of males had decreasing in testosterone levels, while only one (2.18%) of them had increasing level, 22 (25.89%) females had decreasing in FSH levels versus 10 (11.72%) females with increasing levels, 19 (22.35%) females had decreasing in LH levels and 14 (16.17%) females were with increasing in these levels.

The results of seminal fluid analysis demonstrated that out of 46 positive infertile males, 26 (56.52%) males were with abnormal sperms morphology, 31 (67.39%) males were had decreased in sperms count, while males with abnormal sperms motility were 34 (73.91%).

According to ultrasound images, it was observed that 25 (29.41%) females -out of 85 positive infertile females- were with PCOS, 4 (4.71%) females had one cyst in their ovaries, 28 (32.94%) females were with enlarged ovaries and 4 (4.71%) females had small size ovaries.

The results showed that positive males and females were higher in the age group (35-39) years and they were higher in marriage duration group (5-9) years.

The present study found that out of 131 (males and females) patients afflicted with toxoplasmosis, there were 77 (58.77%) of them had history of contact with cats in their houses, 10 (7.64%) had history of infertility in their families.

Also, the results showed that out of 85 positive infertile females, 37 (43.53%) females had irregular cycle, 8 (9.42%) females had previously one successful pregnancy, 2 (2.35%) had frequent (twice or more) previous pregnancies, 15 (17.64%) females had one past abortion and the females with past frequent abortions were 16 (18.83%).

We concluded that *T. gondii* transmit from men to women and vice versa through the sexual intercourse between couples or partners. It causes disorder in hypothalamic-pituitary gonadal axis including testosterone in males and FSH, LH in females, and there was influence of decreased testosterone levels on semen parameters (morphology, motility and count) in males who were positive for toxoplasmosis.

Abnormal changes of ovaries (cystic, small or large size) as well as irregularity in menstrual cycles belong to the undesired variations in FSH and LH of females who afflicted with toxoplasmosis. Finally, Toxoplasmosiscould be the responsible of primary or secondary infertility in men and women.

**الخلاصة**

داء مقوسة كوندي هو أحد الامراض المشتركة بين الانسان والحيوان ، والذي تسببه مقوسة كوندي الاحادية الخلية الانتهازية المعيشة . ظهر انه من المؤكد ان هذا المرض يعتبر مؤثراً في النساء الحوامل والمرضى الذين يعانون من ضعف المناعة , اضافة الى مخاطر مضاعفات الحمل والخمج الولادي الخلقي ، فان داء مقوسة كوندي يؤدي الى تأثيرات سلبية على القابلية التناسلية في كل من الرجال والنساء .

أُجريتْ الدراسة الحالية في مستشفى كمال السامرائي في بغداد للفترة من بداية تشرين الاول 2011 وحتى نهاية كانون الثاني 2012 , في هذه الدراسة المقطعية تم استخدام تقنية الاليزا لتحديد الكلوبيولين المناعي (IgM) و (IgG) لمائة وعشرة ازواج (زوج وزوجة) يعانون من العقم كعينة مختارة تراوحتْ اعمارهم ما بين (19-59) سنة , وكذلك تم فحص الهرمون المنبه للجريب(FSH) والهرمون الملوتن ((LH والصفة الشكلية لمبايض الاناث المصابات , والهرمون الذكوري ((Testosterone وفحص السائل المنوي للذكور المصابين .

اظهرت النتائج ان من بين (110) زوج هناك 46 (41.81%) من الذكور و85 (77.27%) من الاناث كانوا مصابين بداء مقوسة كوندي , ومن العينة نفسها وجد ان هناك 39 (35.45%) من الذكور يشاركون زوجاتهم الاصابة بهذا الداء ، كما بينت نتائج الفايدس ان 43 (93.47%) من الذكور كان مستوى الهرمون الذكوري لهم منخفض في حين ان واحداً فقط (2.18%) كان مستوى الهرمون له مرتفع , و22 (25.89%) من الاناث كان مستوى الهرمون المنبه للجريب لهن منخفض مقابل 10 (11.72%) من الاناث كان مستوى الهرمون لهن مرتفع , و(22.35%) 19 من الاناث كان مستوى الهرمون الملوتن لهن منخفض بينما 14 (16.17%) من الاناث كان مستوى هذا الهرمون لهن مرتفع .

بينت نتائج تحليل السائل المنوي للذكور العقيمين المصابين (46 ذكر) ان 26 (56.52%) منهم كان شكل النطف لهم غير طبيعي ، و31 (67.39%) منهم كان عدد النطف لهم منخفض ، و 34 (73.91%) من الذكور كانت حركة النطف لهم غير طبيعية .

اعتماداً على صور السونار ، لوحظ ان هناك 25 (29.41%) من الاناث من بين 85 من الاناث العقيمات المصابات كان عندهن تكيس المبيض المتعدد (PCOS) , و4 (4.71%) منهن كان عندهن كيسة واحدة بالمبيض , و28 (32.94%) من الاناث كانت مبايضهن كبيرة الحجم و4 (4.71%) من الاناث كانت مبايضهن صغيرة الحجم .

اظهرت النتائج ان الذكور والاناث المصابين كانوا اكثر ضمن الفئة العمرية (35-39) سنة وكذلك كانوا اكثر ضمن الفئة الخاصة بمدة الزواج (5-9) سنة .

في الدراسة الحالية ظهر انه من بين 131 مريض (ذكور واناث) مبتلين بداء مقوسة كوندي كان منهم 77 (58.77%) على تلامس مع القطط في منازلهم ، و10 (7.64%) منهم كانوا يملكون تأريخاً للعقم في عوائلهم .

كذلك اوضحت النتائج ان من بين 85 انثى عقيمة مصابة كان هناك 37 (43.53%) من الاناث كانت الدورة الشهرية لهن غير منتظمة ، و8 (9.42%)منهن كن يمتلكن حمل ناجح في السابق و 2 (2.35%) منهن كان الحمل لهن متكرر في السابق (مرتين او اكثر) ، وهناك 15 (17.64%) من الاناث عانين من اجهاض واحد في الماضي و16 (18.83%) منهن عانين في الماضي اجهاض متكرر.

استناداً لنتائج الدراسة الحالية يمكن القول ان هناك امكانية في انتقال مقوسة كوندي من الرجل الى المرأة وبالعكس عن طريق الجماع الجنسي بين الازواج او الشريكين , كماانها تسبب اظطراب المحور تحت المهادي النخامي القندي متضمناً الهرمون الذكوري في الذكور والهرمون المنبه للجريب والهرمون الملوتن في الاناث وان هناك تأثير للمستويات المنخفضة للهرمون الذكوري على معالم السائل المنوي (الشكل والحركة والعدد) في الذكور المصابين بداء القطط ، وان التغيرات التي تطرأ على المبايض (التكيس وصغر او كبر الحجم) بالاضافة الى عدم انتظام الدورات الشهرية تعود الى الاختلافات غير المرغوبة في الهرمون المنبه للجريب والهرمون الملوتن في الاناث اللاتي ابتلين بهذا الداء . واخيراً فان داء مقوسة كوندي قد يكون مسؤولاً عن العقم الاولي او الثانوي لدى الرجال والنساء .

* 1. **Introduction**

Toxoplasmosis is a [parasitic disease](http://en.wikipedia.org/wiki/Parasitic_disease) caused by the [protozoan](http://en.wikipedia.org/wiki/Protozoan) [*Toxoplasma gondii*](http://en.wikipedia.org/wiki/Toxoplasma_gondii)]1[. This protozoan was first discovered by Nicolle & Manceaux, who in 1908 isolated it from the African rodent [*Ctenodactylus gundi*](http://en.wikipedia.org/wiki/Gundi), then in 1909 [differentiated](http://en.wiktionary.org/wiki/differentiate) the disease from [*Leishmania*](http://en.wikipedia.org/wiki/Leishmania) and named it *Toxoplasma gondii* ]2[.The name *Toxoplasma* is derived from the Greek word (Toxon) meaning arc or bow referring to the curved shape of the trophozoite and plasm meaning body] 3[.

Toxoplasmosis is an important parasitic disease worldwide and is related to certain psychiatric disorders and sterility ]4[. It has been suggested that Toxoplasmosis has some unfavorable effects on reproductive capacity in both men and women ]5[.

Congenital infection caused by transplacental transmission can lead to a wide variety of manifestations in the fetus and infant including spontaneous abortion, still-birth, a newborn with classic signs of congenital Toxoplasmosis such as hydrocephalus or microcephalus, cerebral calcifications and retinochoroiditis]6[ ]7[.

*Toxoplasma* infection in infertile human couples was higher than that in fertile couples, possibly related to the antisperm antibodies which were higher in *Toxoplasma* infected couples ]8[.

An investigation of *T. gondii* infection in men with sterility showed that among 100 cases of man’s sterility, 36% of them were serologically *Toxoplasma*-IgG and IgM positive. It was concluded that *T. gondii* infection may affect men’s fertility and cause sterility ]9[.

Latent toxoplasmosis is known to influence the morphology of infected persons and also increase the probability of the birth of male offspring in both humans and mice, all these traits can be related to the observed differences in the concentration of testosterone between *Toxoplasma*-infected and *Toxoplasma*  free subjects]10[.

It is clear that, any disorder of the hypothalamic–pituitary–gonadal (HPG) axis due to a direct or indirect reason may cause alterations in normal functions of male reproductive parameters, it is interesting to note that *Toxoplasma* and *Toxocara* infections have been identified asrisk factors affecting male infertility due to malfunctionsin spermatogenesis ]11[.

Repeated infections in women lead to fertility problems and one of the reasons why the endometrium does not allow the fertilized egg to adhere is chronic inflammation of various aetiologies]12[. Chronic inflammation leads to functional intrauterine abnormalities and to reduction in endometrial receptivity, which negatively affects the process of embryo implantation and its early development ]13[, it is in particular the case of chronic infections such as toxoplasmosis, listeriosis, brucellosis, rubella, cytomegalovirus, herpes virus ]14[.

It was hypothesized that cytokines released peripherally in response to the parasite could reach the hypothalamus and initiate a sequence of events that inhibit the pulsatile release of gonadotropin-releasing hormones (GnRH), leading to the subsequent impairment of the pituitary-ovarian axis function ]15[.

Although in several studies *T. gondii* has been isolated from caprine ]16[, ovine]17[, swine ]18[, and human semen ]19[, there is practically no risk of venereal transmission ]20[.

It was recently reported that *T. gondii* oocysts can be transmitted from dog to dog in semen ]21[. There is also an unpublished report of *T. gondii* oocysts being found in the epididymis of rats, from which it could get into the rats’ semen. This raises the theoretical possibility of sexual transmission of *T. gondii* in human, although this has never been studied ]22[.

**1.2 Aims of the Study**

1. To detect the affliction with toxoplasmosis in the infertile couples who had been visiting Kamal AL.Samaraee Hospital in Baghdad province and to determine the:

A: Disturbance in Testosterone levels and abnormalities of sperms (motility, morphology, and count) in the infected men.

B: Variation in FSH, LH levels and changes in the structural architecture of the ovaries in the infected women.

2. To investigate the toxoplasmosis in the infertile women with past abortions and successful pregnancies.

3. To recognize the possibility of sexual transmissionof *T. gondii* infectionbetween couples.

**2-1 History**

Nicolle and Manceaux (1908) found a protozoan in tissues of a hamster-like rodent, the gundi, *Ctenodactylus gundi*, which was being used for leishmaniasis research in the laboratory of Charles Nicolle at the Pasteur Institute in Tunis ]23[. Nicolle initially believed the parasite to be a piroplasm]24[, then Leishmania, but soon realized that he had discovered a new organism and named it *T. gondii* ]25[.

In retrospect the correct name for the parasite should have been *T. gundii*; Nicolle and Manceaux (1908) had incorrectly identified the host as *Ctenodactylus gondi* ]26[. Splendore (1908) discovered the same parasite in a rabbit in Brazil, also erroneously identifying it as *Leishmania*, but he did not name it ]27[.

For the next 30 years, *T. gondii*-like organisms were found in several other hosts, especially avian species]28[.Three pathologists, Wolf, Cowen, and Paige from New York, USA first conclusively identified *T. gondii* in an infant girl who was delivered full term by Caesarean section on May 23, 1938 at Babies Hospital, New York]29[.

In 1948, the introduction of the methylene blue dye test by Sabin and Feldman enabled seroepidemiological studies in humans as well as a broad range of animal species which provided evidence for a wide distribution and high prevalence of T. gondii in many areas of the world. ]30[.

In the next 50 years protective immunity was found to be mediated largely by immune lymphoid cells ]31, 32, 33[.

Mapping of *T. gondii* genes was achieved recently, and undoubtedly will help in search for better antigens for diagnosis and protection, and mechanism of disease ]34[.

**2.2 Taxonomy**

Toxoplasma belongs to the phylum Apicomplexa, which consists of intracellular parasites that have a characteristically polarized cell structure and a complex cytoskeletal and organellar arrangement at their apical end ]35[.

Other members of this phylum include the human pathogens Plasmodium (the cause of malaria) and Cryptosporidium as well as the animal pathogens Eimeria (the cause of chicken coccidiosis) and Sarcocystis] 36[.

Kingdom: ***Animalia***

Sub kingdom: ***Protozoa***

Phylum: ***Apicomplexa***

Class**: *Sporozoea***

Subclass**: *Coccidia***

Order: ***Eucoccidiida***

Suborder: ***Eimeriina***

Family: ***Sarcocystidae***

Genus: ***Toxoplasma***

Species**: *T. gondii***

Ferguson (2002) ]37[.

**2.3** **Morphology**

The parasite possesses the different morphological stages as follows:

**2.3.1** **Tachyzoites**

The term “tachyzoite” (tachos = speed in Greek) was coined by Frenkel ][38](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC106833/" \l "B73)[ to describe the stage that rapidly multiplied in any cell of the intermediate host and in nonintestinal epithelial cells of the definitive host. The term “tachyzoite” replaces the previously used term “trophozoite” (trophicos = feeding in Greek). The tachyzoite is often crescent shaped, approximately 2 by 6 μm, with a pointed anterior (conoidal) end and a rounded posterior end ]35[.

**2.3.2** **Bradyzoites**

The term “bradyzoite” (brady = slow in Greek) was also coined by Frenkel to describe the organism multiplying slowly within a tissue cyst ][38](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC106833/" \l "B73)[. The tissue cyst wall is elastic and thin (<0.5 μm thick), and it encloses hundreds of crescent-shaped bradyzoites, each approximately 7 by 1.5 μm in size ]39[.

**2.3.3 Oocysts**

Unsporulated oocysts are subspherical to spherical and are 10 by 12 μm in diameter ]40[. Under light microscopy, the oocyst wall consists of two colorless layers. Each oocyst contains two ellipsoidal sporocysts, and each sporocyst contains four sporozoites. Sporulation occurs outside the cat within 1 to 5 days of excretion depending upon aeration and temperature ]41[.



 **Figure(2.1) ( a) Asporulated oocyst of *Toxoplasma gondii*, (b) Tachyzoite of *T.gondii*]42[**



**Figure(2.2).** **The bradyzoite in mouse brain (tissue cyst), *and the tachyzoite in leukocyte* ]43[**

**2.4 Life Cycle**

The life cycle of T. gondii is facultatively heteroxenous. Intermediate hosts are probably all warm-blooded animals including most livestock, and humans. Definitive hosts are members of the family Felidae, for example domestic cats ]44[.

*Toxoplasma gondii* life cycle is divided into two parts: an asexual phase, which takes place in nucleated cells; and a sexual phase within the gastrointestinal tract of cats. Fertilized gametes are generated from sexual replication within the feline's small intestine, and excreted oocysts can last in the environment for18 months]45[.  
 Once ingested, oocysts and/ or tissue cysts rupture and invade cells in intestinal lining, and the sporozoites (released from the oocysts) or the bradyzoites (released from the tissue cysts) differentiate into tachyzoites, the fast-replicating form of the parasite ]46[. These tachyzoites may be detected in host leukocytes or may be circulating freely within the bloodstream ]47[ ]48[.

In intermediate hosts, *T. gondii* undergoes two phases of asexual development. In the first phase, tachyzoites (or endozoites) multiply rapidly by repeated endodyogeny in many different types of host cells. Tachyzoites of the last generation initiate the second phase of development which results in the formation of tissue cysts. Within the tissue cyst, bradyzoites (or cystozoites) multiply slowly by endodyogeny]39[.

Tissue cysts have a high affinity for neural and muscular tissues. They are located predominantly in the central nervous system (CNS), the eye as well as skeletal and cardiac muscles. However, to a lesser extent they may also be found in visceral organs, such as lungs, liver, and kidneys ]49[.

Tissue cysts are the terminal life-cycle stage in the intermediate host and are immediately infectious. In some intermediate host species, they may

persist for the life of the host. The mechanism of this persistence is unknown. However, many investigators believe that tissue cysts break down periodically, with bradyzoites transforming to tachyzoites that reinvade host cells and again transform to bradyzoites within new tissue cysts ]44[. If ingested by a definitive host, the bradyzoites initiate another asexual phase of proliferation which consists of initial multiplication by endodyogeny followed by repeated endopolygeny in epithelial cells of the small intestine. The terminal stages of this asexual multiplication initiate the sexual phase of the life cycle. Gamogony and oocyst formation also take place in the epithelium of the small intestine. Unsporulated oocysts are released into the intestinal lumen and passed into the environment with the faeces. Sporogony occurs outside the host and leads to the development of infectious oocysts which contain two sporocysts, each containing four sporozoites]50[.



Figure (2.3) *Toxoplasma gondii* - Complete Life Cycle ]51[.

**2.5 Transmission**

**2.5.1 Horizontal Transmission (Acquired Toxoplasmosis)**

The parasite can be transmitted horizontally through different ways:

1. Orally by:

A. Ingestion of raw or partly cooked meat, especially pork, lamb, or venison containing *Toxoplasma* cysts, it may also be ingested during hand-to-mouth contact after handling undercooked meat, or from using knives, utensils, or cutting boards contaminated by raw meat]52[ ]53[.

B. Eating food or drinking water both contaminated with oocysts]54[.

C. Consumption of unpasteurized milk or milk product, whether from goats, sheep, or cows ]55[.

D. Breast Feeding: There has also been a case of acute toxoplasmosis in a breast fed infant whose mother acquired a primary infection with T. gondii ]56[.

2. Organ Transplantation:

Toxoplasma gondii can also be transmitted by organ transplantation from an infected donor to an uninfected recipient, and this is an important cause of the disease in recipients of heart, heart-lung, kidney, liver and liver-pancreas transplants ]57[.

3.Blood Transfusion:

Parasitaemia usually occurs for only a short period of time after primary infection. Therefore, it has been suggested that there is only a low risk of acquiring an infection with T. gondii via ordinary blood transfusion ]50[.

4. Accidental Injection

Tachyzoites of T. gondii have also been transmitted via blood products, in particular those containing the white cell fraction, and by accidental injection in the laboratory ]58[. Infections can be acquired by accidental inoculation, splashing, inhalation or ingestion, and the source of infection may be semen, feces or tissues ]59[.

5.Mechanical Transmission:

Oocysts in soil do not always stay there, as invertebrates such as flies, cockroaches, dung beetles and earthworms can mechanically spread these oocysts and even carry them onto food ]60[.

**2.5.2 Vertical Transmission (Congenital Toxoplasmosis)**

The parasite can be transmitted vertically as well, mainly when women acquire primary disease during pregnancy ]61[.

Congenital toxoplasmosis can lead to a [miscarriage](http://wiki.medpedia.com/Miscarriage), a stillborn child, or a child born with signs of toxoplasmosis (an abnormally large or small head, [seizures](http://wiki.medpedia.com/Seizure), blindness, and developmental difficulties) ]62[.

However, it is during the third trimester that the highest level of transmission occurs. This is thought to be related to the much larger size of the uterus. The transmission rate from a maternal infection is about 45%. Of these 60% are sub-clinical infections, 9% result in death of the foetus and 30% have severe damage such as hydrocephalus, intracerebral calcification, retinochoroiditis and mental retardation ]63[.

Also, if a women foetal loss is proven due to *Toxoplasma gondii* infection, her subsequent pregnancies are safe as far as this parasitic infection is concerned, until she becomes immunocopromised during subsequent pregnancies. However, occasional reports of congenital toxoplasmosis transmitted, by an immunocompetent woman infected before conception, to her fetus are on record ]64[.

**2.6 Epidemiology**

The prevalence of *T. gondii* infection may vary greatly from country to country, in different regions in the same country, and among different population groups of the same region ]65[.

These differences are related to the characteristics of the parasite, such as the level of infection potency and peaceful coexistence with the host, the capacity to infect thousands of animal species, including aquatic, land and winged animals, and the capacity to inhabit various regions of the globe (in lower proportion in very cold areas, arid regions and at high altitudes). Other factors that interfere with the epidemiology of *T. gondii* are feeding habits and cultural characteristics ]66[.

**2.6.1 Toxoplasmosis in Iraq**

Toxoplasmosis was first recorded in animals by Matchattie (1938) where he found the *T. gondii* in smear from spleen and lungs of two stray dogs in Baghdad ]67[. In 1963, Najim and Al- Saffar found that the rate of infection was 40.5 % among females with history of abortion. The rate of positive reaction among normal children from Baghdad with age between 7- 12 years was 4.9 % using skin test, while it was 11.4 % in a group of mentally defective children ]68[. By using skin test in 1968 Najim *et al*, observed that the rate of infection was 22.8 % among afflicted women with abortion, stillbirth and threatened abortions while 16.6 % among women with normal pregnancy ]69[.

Tawifiq in 1983 found that the rate of toxoplasmosis in Baghdad among pregnant women was 30 % ]70[, while in 1988 Naizi and Omar , they found that it was 39 % of 726 cases of pregnant women in Baghdad by using Enzyme Linked Immuno Sorbet Assay ( ELISA ) Technique ]71[ . Flaih in 1993 found that the rate of toxoplasmosis in Baghdad among pregnant women was 18 % by using indirect fluorescent antibody assay (IFAT) ]72[. Al- Dujaily in 1998 recorded that the rate of toxoplasmosis in Baghdad among aborted women was 34.7% through three serological methods: agglutination test (DAT) ELISA and IFAT ]73[.

AL-Timimi (2004) observed, by using latex and IFAT methods among 168 women with history of abortion in Baghdad the rates were 44% and 29 % respectively ]74[.

AL – Dalawi (2007) found that the rate of infection was 31.6 % among 227 aborted women (suddenly and previously) in Baghdad by using ELISA Technique ]75[. AL- Saadi (2008) found that the rate of infection was 32.1% from total aborted women in Baghdad by using ELISA Technique ]76[.

Sabah & Mahfoth (2009) showed that the seropositivity rate for antitoxoplasma IgG antibodies among schizophrenic patients was (53%) significantly higher than in healthy individuals (23%) ]77[.

In Al-Najaf Province, Dargham(2011) showed that 33.6% of the sudden aborted women were positive for toxoplasmosis by using Latex test. This is followed by examining specific IgM and IgG antibodies which were 2.5% and 16.4% respectively, by using ELISA technique ]78[.

In Kirkuk, Abdul–Razzaq (2005) found that the rate of infection among pregnant women was 61. 2 %, and among non married woman was 33.33 % ]79[. In Basra province, Yacoob *et.al* (2006) found that the rate of infection was 59.1% among aborted women ]80[. In Tikrit city, Al-Doori (2010) showed that the rate of infection in women by using latex test, ELISA (IgG) and ELISA (IgM) was 95%, 86% and 49% respectively, and in their husbands 82%, 66% and 28% respectively ]81[.

**2.6.2 Toxoplasmosis in the Arabian Countries**

In Qatar, Abu-Madi *et.al* (2010) found thatAmong 823 women of childbearing age (15–45 yr), 289 (35.1%) had IgG antibodies and 43 (5.2%) had IgM antibodies against *T. gondii*, and with testing of patients who were deemed to be at high risk for TORCH pathogens, e.g., pregnant women, their fetuses, neonates, and acquired immunodeficiency syndrome (AIDS) patients by using ELIZA technique, *Toxoplasma* *gondii* IgG seroprevalence increased with advancing age in both males and females, with a combined prevalence of 8% in 2 to 10 years of age compared with 54% in patients over 45 years of age ]82[.

Other researches indicated that the prevalence of toxoplasmosis among aborted women in Jordan was 43 %] 83[, 95.5% in Kuwait ]84[, in Bahrain 28% ]85[, in Sudan 41.7% ]86[, 34% in united Arab Emirates ]87[ , 81.4% in Egypt ]88[ and in Saudi Arabia 29% ]89[ .

In Syria, Abou - Daoud and Schwab (1960), by using skin test method, found that the rate of infection was 26 % ]90[. In Libya, Mousa *et.al* (2011) showed that out of 143 serum samples, 64 (44.8%) were positive for toxoplasma by using ELISA technique ]91[.

**2.6.3. Toxoplasmosis in the world**

Up to one third of the world's human population is estimated to carry a *Toxoplasma* infection]92[. The [Centers for Disease Control and Prevention](http://en.wikipedia.org/wiki/Centers_for_Disease_Control_and_Prevention) notes that overall [seroprevalence](http://en.wikipedia.org/wiki/Seroprevalence) in the United States as determined with specimens collected by the National Health and Nutritional Examination Survey ([NHANES](http://en.wikipedia.org/wiki/National_Health_and_Nutrition_Examination_Survey)) between 1999 and 2004 was found to be 10.8%, with seroprevalence among women of childbearing age (15 to 44 years) 11% ]93[.

Worldwide, over 6 billion people have been infected with *T. gondii*]94[. Seroprevalence, measured by IgG against *T. gondii,* varies worldwide, being reported to be 6.7% in Korea]95[, 12.3% in China]96[, and 47% in France (rural area) ]97[.In Germany, Netherlands and Brazil also have high prevalence of around 80%, over 80% and 67% respectively]98[.

Tender *et.al* (2000) found that the rates of positive sero-prevalence, were 58% in Central European countries, 51–72% in several Latin-American countries and 54–77% in West African countries. Low seroprevalence, 4–39%, was reported in southwest Asia, China and Korea as well as in cold climate areas such as Scandinavian countries (11–28%) ]50[.

In Iran, Abdi *et.al* (2008) showed that 247 of the 553 pregnant women were found to be positive for IgG *T. gondii* antibodies and the rate of seropositivity of latent *T.gondii* infection was 44.8 % ]99[. In Turkey, between 2004 and 2010 *T. gondii* IgM and IgG seropositivity rates were found detected as 1.34 and 24.6, respectively. These rates are relatively low when compared with the *Toxoplasma* endemic regions of Turkey where raw meat consumption is high ]100[.

**2.7 Pathogenesis**

*Toxoplasma gondii* has been shown to migrate over long distances in the host’s body, crossing biological barriers, actively enter the blood stream, invade cells and cross substrates and non-permissive biological sites such as the blood-brain-barrier, the placenta and the intestinal wall. At the same time, the parasite minimizes exposure to the host’s immune response, by rapidly entering and exiting cells. These two functions share common mechanisms which depend on Ca++ regulation ]101[.

Unlike many bacteria and viruses, *T. gondii* actively enters the cell, in a mechanism which is mediated by the parasites’ cytoskeleton and regulated by a parasite-specific calcium depended secretion pathway ]102[.

Although conventional medicine considers latent toxoplasmosis asymptomatic, recent research has linked toxoplasmosis with a number of neurological pathologies ]103[. In the latent phase, the cysts provoke the immune system (e.g. lymphocytes, plasma cells and macrophages), stimulating mild inflammation ]104[.

Infection may be congenitally or postnatally acquired, congenital infections acquired during the first trimester are more severe than those acquired in the second and third trimester, in which focal lesions develop in the placenta and the fetus may become infected. At first there is generalized infection in the fetus. Later, infection is cleared from the visceral tissues and may localize in the central nervous system ]105[.

Mild disease may consist of slightly diminished vision, whereas severely diseased children may have the full tetrad of signs: retinochoroiditis, hydrocephalus, convulsions and intracerebral calcification. Of these, hydrocephalus is the least common, but most dramatic, lesion of toxoplasmosis ]64[.

**2.8 Clinical Manifestations**

Toxoplasmosis can be categorized into four groups: ]106[.

a- Acquired toxoplasmosis (immunocompetent patients - mild disease)

|  |  |  |
| --- | --- | --- |
| Fever | Malaise | sore throat |
| lymphadenopathy | night sweats | maculopapular rash |

b- Toxoplasmosis (immunocompromised patients)

|  |  |  |
| --- | --- | --- |
| Fever | malaise | sore throat |
| lymphadenopathy | night sweats | maculopapular rash |
| pneumonitis | hepatomegaly | splenomegaly |
| chorioretinitis | myocarditis |  |

c- Ocular toxoplasmosis

|  |  |  |
| --- | --- | --- |
| eye pain | photophobia | blurred vision |
| scotoma ("blind spot") | chorioretinitis | retinitis |
| Blindness |  |  |

d- Congenital toxoplasmosis

|  |  |  |
| --- | --- | --- |
| abortion | Low Apgar scores | lethargy |
| anemia | jaundice | rash |
| Diarrhea | lymphadenopathy | thrombocytopcnia |
| CNS damage | encephalopathy | convulsions |
| cerebral calcification | hydrocephalus | mental retardation |
| Chorioretinitis | blindness | Hepatomegaly |
| Splenomegaly |  |  |

**2.9 Immune Response against Toxoplasmosis**

Infection with *T.gondii* cause innate , humoral (H.I) and cell mediated (C.M.I) immune responses , H.I and C.M.I components of the adaptive responses are both needed because the parasite is intracellular pathogen , but it does also move through the extracellular space in order to find new host cell]107[.

Infection is correlated with H.I response where the parasite stimulates the immune system to produce specific Abs IgG and IgM in serum ]108[,IgM isotype appears early one week after infection and their rate increases during approximately 1-2 months and are detectable in maximum rate during one year ]109[, IgG appears 2-3 weeks after the appearance of IgM and reach peak level with in 6 months and remained high for long time, IgA have an elevation paralled with that of 1gM but are detectable in its maximum level for 6 months ]110[.

Parasite replication in the intestine eventually leads to host cell lysis and parasite egress and tachyzoites disseminate throughout the host ]111[. This process is poorly understood, but recent work suggests that CD11c + dendritic cells (DC) may act as Trojan horses to spread the infection ]112[. In immunodeficient hosts or during primary infection with highly virulent strains, the immune system cannot control parasite replication and clinical disease results ]113[.

The most important type of defense mechanisms during toxoplasmosis is a C.M.I that mean a variety of effector cells and different mediators are implicated in protective immunity T. lymphocytes Natural killer cells and activated macrophages]31[.

By the late 1980s, IFN-γ produced by CD4+ and CD8+ T cells was identified as the major mediator of protection against *T. gondii* ]33[, and there were suggestions that accessory cell products were responsible for inducing IFN-γ production from T lymphocytes and natural killer (NK) cells ]114[.

Sher, Kasper and Remington laboratories established that IFN-γ production in response to *T. gondii* was largely IL-12-dependent in both immunodeficient and immunocompetent mice, with important roles for IL-2 and tumor necrosis factor-α (TNF-α) as co-factors ]115[. Macrophages ]116[, DC ]117[ and neutrophils ]118[ were all identified as sources of IL-12 during toxoplasmosis, although DC, including conventional CD8+ and plasmacytoid DC ]119[, now appear to be the major contributors ]117[.

The studies of IFN-γ-dependent effector mechanisms that limit parasite replication have underlined the importance of the IL-12/IFN-γ axis. IFN-γ signals through signal transducer and activator of transcription 1 (STAT1) to activate a variety of antimicrobial effector mechanisms, including the upregulation of inducible nitric oxide synthase (iNOS), ROI produced by iNOS were initially thought to be the host’s primary means of controlling parasite replication ]120[.

In mothers previously exposed to *T. gondii*, the fetus is very rarely infected, suggesting that natural maternal immunity to *T. gondii* is sufficient to protect the fetus from verticaltransmission ]121[. In principle, this protection could be mimickedusing a vaccine, and numerous strategies have beenemployed to elicit protective immunity in mouse models of congenital toxoplasmosis, including recombinant protein ]122[ and DNA-based vaccine strategies ]123[, but to date, a human vaccine has not been developed ]124[.

**2.10 Diagnosis**

**2. 10.1 History of Case**

**2. 10.2 Clinical Signs**

Clinical signs of toxoplasmosis are non-specific and are not sufficiently characteristic for a definite diagnosis. Toxoplasmosis in fact mimics several other infectious diseases ]60[.

**2.10.3 Laboratory Diagnosis**

**2.10.3.1 Direct Methods**

**2.10.3.1.1 Microscopic Examination**

A rapid diagnosis may be made by microscopic examination of *T. gondii* tachyzoites in fluid specimens such as blood or CSF ]125[. Also smear is made from biopsy or autopsy of skeletal muscle, lung, brain and eye to reflect the presence of tissue cyst ]126[.

**2.10.3.1.2 Histological Diagnosis**

This can made by finding *T . gondii* in host tissue , removed by biopsy or at necropsy . This procedure is particularly useful in immune – suppressed patients or patients with ( AIDS ) in whom Abs synthesis may by delayed and low .This method can by made by standard . Staining of histological section (Wright – Giemsa), but tachyzoites are difficult to detect and when recognized it is not possible to identify the parasite as a species]127[. Immunofluorescent antibody histochemical staining technique is successful for demonstration of the parasite in tissue section because it is difficult to see with ordinary stains ]128[.

**2.10.3.1.3 Isolation**

Inoculation of blood or body fluids CSF, amniotic fluid brain and retinal fluid into tissue culture, inoculation into mice, after 8-10 days, exudates examined for tachyzoites ]129[. In neonate isolation of organism from the placenta is usually diagnostic tool for congenital toxoplasmosis ]130[. A definite laboratory confirmation of active toxoplasmosis infection (especially in immunocompromised patients and pregnant women) can be established by inoculation of body fluids or tissue into mice or cell culture ]58[.

**2.10.3.2 Indirect Methods**

**2.10.3.2.1 Skin Test**

This test is described by Freckle, JK. (1968), the existence of specific immunity was assessed by intradermal delayed hypersensitivity test (IDHT). The test was performed using *Toxoplasma gondii* as the Ag and injection 0.1 ml of pure solute as the negative control. An area of 5cm erythematic after 48 hr. considered as positive results. ]131[.

The test does not give any false positive results it is useful for surveys, false negative results can be seen in the first 2-3 weeks of infection and in first nine months of age infancy. Therefore it is not useful in congenital and acute toxoplasmosis ]132[.

**2.10.3.2.2 Serological Tests**

**2.10.3.2.2 .1 Sabin Feldman Dye Test**

The dye test was described in 1948 by Sabin and Feldman ]133[. This is the first test developed for the laboratory diagnosis of *T. gondii* infection; it is still considered the “gold standard” ]50[.

Live *Toxoplasma* tachyzoites are incubated with a complement-like accessory factor and the test serum at 37°C for 1 hour beforemethylene blue is added. Specific antibody induces membrane permeability in the parasite so that the cytoplasmis able to leak out and the tachyzoite does not incorporate the dye and so appears colourless. Tachyzoites notexposed to specific antibody (i.e. a negative serum sample) take up the dye and appear blue. The DT is bothspecific and sensitive in humans, but may be unreliable in other species. In addition, it is potentially hazardous aslive parasite is used. It is expensive and requires a high degree of technical expertise ]133[.

**2.10.3.2.2.2 Direct Agglutination Test (DAT)**

The principle of this test is based on the appearance of clear visible agglutination reaction between Ag and Ab present in sera. The test was firstly used by Fulten Turk 1959 ]134[.

The test is sensitive, easy, simple and rapid to perform. The test results are compatible with the result of Sabin-Feldman dye test–on cross reaction identified with sera contains another ]135[.

**2.10.3.2.2.3 IgG – Avidity Test**

In 1989, Hedman, introduced a new method which was based on the affinity of immunoglobulins bound to *Toxoplasma gondii* polyvalent antigens, and benefited from the high density of urea to differentiate the high affinity of immunoglobulin which was later called avidity test. This test is recently used for the detection of *Toxoplasma* IgG avidity ]136[. In the initial steps of toxoplasmosis infection IgG avidity is low but the avidity of IgG due to the previous infections is very high. According to recent reports, when the avidity is lower than 40%, it is indicative of an initial infection or active steps and when the avidity is higher than 60%, it indicates an old infection ]137[.

In a pregnant women whose sample is taken in second or third trimester rather than ideally in first trimester, and she is found IgG positive but IgM negative, it is more advisable to perform IgG avidity test. High avidity IgG tests indicate that she acquired the infection more than 4 months ago. But the low avidity is not a confirmatory test for recent infection ]138[.

**2.10.3.2.2.4 Indirect Fluorescent Antibody Technique** **(IFAT)**

The IFAT was widely used to demonstrate *T. gondii*-specific antibodies: serially diluted serum samples are incubated with live, inactivated *Toxoplasma* fixed to a glass slide. *T. gondii* specific antibodies present in the serum would bind to the inactivated parasite, and the complex is then detected using fluorescein isothiocyanate-labeled anti-human Ig (or anti-IgG or anti-IgM) ]36[.

IFAT is safer to perform and more economical than the DT. It appears to measure the same antibodies as the dye test, and its titers tend to parallel dye test titers ]56[. However, the IFA interpretation is subjective and time consuming. False positive results may occur with sera containing antinuclear antibodies and rheumatoid factor, and false negative results of IFA for IgM may occur due to blockage by *T. gondii*-specific IgG ]139[.

**2.10.3.2.2.5. Fast Dipstick Dye Immuno Assay (DDIA)**

Fast Dipstick Dye Immuno Assay is a recent test to detect IgG or IgM antibodies of human toxoplasmosis. The assays employ a blue colloidal dye particles conjugated to sheep anti-human IgG and rabbit anti-human IgM as the visualizing agents and soluble antigen of tachyzoites of *Toxoplasma* *gondii* (TSA) as the detective antigen. The mixture of dye-labeled antihuman antibody – special human antibody was captured by TSA onto nitrocellulose membrane dipstick by means of immunochromatography ]140[.

The assays are rapid (the whole test can be completed within 15 minutes), simple, cheap, and they do not require any equipment, they are sensitive and specific for the detection of anti**-***Toxoplasma gondii* IgG or IgM antibodies and generally agree closely with the results of EIA ]65[.

**2.10.3.2.2.6 Enzyme Linked ImmunoSorbent Assay (ELISA)**

It is very sensitive and specific test. ELISA test is usually done on a sample of blood to detect the presence of either antigen or antibody in the blood. There are many types of ELISA, such as Double Sandwich ELISA, Micro ELISA, Macro ELISA and Dot ELISA. All types used enzyme labeled anti IgG and IgM antibody in the procedure ]141[.

In patient with recently acquired infection IgM, *T.gondii* Ab are detected initially, and in most cases these titers become negative within a few months .In some patients , positive lgM *T. gondii*specific titer can be observed during chronic stage of the infection . lgM Abs have been reported to persist as long as12 months after the acute infection ]142[. New born infants suspected to have congenital toxoplasmosis should be tested by both an IgM–and an IgA–capture EIA. The detection of *Toxoplasma gondii*–specific IgA antibodies is more sensitive than IgM detection in congenitally infected babies ]143[.

**2.10.3.2.2.7 Enzyme Linked Fluorescent Assay (ELFA)**

It is a simple, rapid and accurate technique for measurement of anti- toxoplasma IgM or IgG in patients with acute or chronic toxoplasmosis. All of the steps are performed automatically by the VIDAS instrument ]144[.

The assay principle combines an enzyme immunoassay methods by immunocapture with a final fluorescent detection, ELFA method having high specificity for detection of Ig (e.g.: IgM and IgG) ]145[.

**2.10.3.3 Nucleic Acid Recognition Methods**

**2.10.3.3.1 Polymerase Chain Reaction (PCR)**

Several polymerase chain reaction (PCR)-based assays have been developed for the detection of DNA from *T. gondii* ]146[, the sensitivity of the PCR is dependent on the copy number of the target sequence (P30: 1 copy; B1: 35 copies; rRNA: 110 repeat units). Recently, the method for amplification of the B1 repetitive sequence has been used to analyze the lens aspirates of congenitally infected human cataract patients ]147[.

**2.10.3.3.2. Real-Time PCR**

Real-Time PCR is a sensitive and specific technique, which enables rapid detection of amplification products as well as hybridization of amplicon-specific probes, similar to PCR followed by Southern blot analysis. The method, which will ultimately replace traditional PCR, enables an overall time for amplification and detection of less than two hours ]36[. This quantification of parasite DNA can be used to determine the number of parasites in tissues and fluids, such as the amniotic fluid of patients suspected of being congenitally infected with *T. gondii* ]148[.

**2.11 Infertility**

Infertility primarily refers to the biological inability of a person to contribute to [conception](http://en.wikipedia.org/wiki/Fertilization). Infertility may also refer to the state of a woman who is unable to carry a [pregnancy](http://en.wikipedia.org/wiki/Pregnancy) to [full term](http://en.wikipedia.org/wiki/Full_term). There are many biological causes of infertility, some which may be bypassed with medical intervention ]149[.

Reproductive endocrinologists, the doctors specializing in infertility, consider a couple to be infertile if:

* The couple has not conceived after 12 months of contraceptive-free intercourse if the female is under the age of 34. (12 months is the lower [reference limit](http://en.wikipedia.org/wiki/Reference_limit) for *Time to Pregnancy* (TTP) by the World Health Organization).
* The couple has not conceived after 6 months of contraceptive-free intercourse if the female is over the age of 35 ]150[.

Alternatively, the NICE guidelines(NICE: National Institute For Clinical Excellence) define infertility as failure to conceive after regular unprotected sexual intercourse for 2 years in the absence of known reproductive pathology ]151[.

**2.11.1 Primary and secondary infertility**

Primary infertility is affecting individuals who have had no previous successful pregnancies while secondary infertility is infertility affecting individuals who have previously had a successful pregnancy, but are currently unable to conceive. Technically, secondary infertility is not present if there has been a change of partners ]152[.

**2.11.2 Prevalence**

The World Health Organization (WHO) estimates that 60 to 80 million couples worldwide currently suffer from infertility ]153[. Infertility varies across regions of the world and was estimated to affect 8 to 12 per cent of couples worldwide ]154[. Underlying these numbers exists a core group of couples, estimated to be (3-5%), who are infertile due to unknown or unpreventable conditions. A prevalence of infertility above this level suggests preventable or treatable causes ]155[. Infertility tends to be highest in countries with high fertility rates, an occurrence termed “barrenness amid plenty” ]156[. Many studies have been conducted in the Africa, where the reported prevalence of infertility ranges from 9% in Gambia to 30% in Nigeria ]157[.

In Great Britain, male factor infertility accounts for 25% of infertile couples, while 25% remain unexplained. 50% are female causes with 25% being due to [anovulation](http://en.wikipedia.org/wiki/Anovulation) and 25% tubal problems ]158[

**2.11.3 Unexplained Infertility**

In the US, up to 20% of infertile couples have unexplained infertility, in these cases abnormalities are likely to be present but not detected by current methods. Possible problems could be that the egg is not released at the optimum time for fertilization that it may not enter the fallopian tube, sperm may not be able to reach the egg, fertilization may fail to occur, transport of the zygote may be disturbed, or implantation fails. It is increasingly recognized that egg quality is of critical importance and women of advanced maternal age have eggs of reduced capacity for normal and successful fertilization. Also, polymorphisms in [folate pathway](http://en.wikipedia.org/wiki/Folate_pathway) genes could be one reason for fertility complications in some women with unexplained infertility ]159[.

**2.11.4Common Causes**

**2.11.4.1 Causes in Males**

**2.11.4.1.1 Sperm Disorders**

The main cause of male infertility is low [semen quality](http://en.wikipedia.org/wiki/Semen_quality). A large proportion of infertile men fail to impregnate their female counterpart because of lack of sperm (azoospermia) or too little sperm (oligozoospermia); infertility may also be due to abnormal sperm morphology (tetratozoospermia) and insufficient sperm motility (asthenozoospermia) ]160[.

Ejaculate analysis has been standardised by the WHO and disseminated by publication of the WHOLaboratory Manual for Human Semen and Sperm-Cervical Mucus Interaction(5th edition) 2010 ]161[ as the following characteristics in the below table:

**Table 2.1: Lower reference limits (5th centiles and their 95% confidence intervals) for semen characteristics ]161[.**

|  |  |
| --- | --- |
| **Parameter** | **Lower reference limit** |
| Semen volume (mL) | 1.5 (1.4–1.7) |
| Total sperm number (106 per ejaculate) | 39 (33–46) |
| Sperm concentration (106 per mL) | 15 (12–16) |
| Total motility (PR+NP) | 40 (38–42) % |
| Progressive motility (PR) | 32 (31–34) % |
| Vitality (live spermatozoa) | 58 (55–63) % |
| Sperm morphology (normal forms) | 4 (3.0–4.0) % |

**2.11.4.1.2 Other Causes**

The diagnosis of male fertility must be focus on a number of prevalent disorders as the following ]162[:

* Congenital factors (cryptorchidism, testicular dysgenesis, and congenital absence of the vas deferens)
* Acquired urogenital abnormalities (obstructions, testicular torsion, and testicular tumour)
* Urogenital tract infections
* Increased scrotal temperature (e.g. as a consequence of varicocele)
* Endocrine disturbances
* Genetic abnormalities
* Immunological factors
* Systemic diseases
* Exogenous factors (medications, toxins, irradiation, lifestyle factors)
* Idiopathic (40-50% of cases)

**2.11.4.2 Causes in Females**

**2.11.4.2.1 Ovulation Disorders**

The most common cause of female infertility is abnormalities in menstrual function. These disorders include ovulatory dysfunction and abnormalities of the uterus or outflow tract ]163[.

In women, failure to ovulate normally can be caused by genetic or environmental factors, including toxic exposures. Endocrine problems such as thyroid disease can also interfere with normal ovulation ]164[.

Ovulation disorders generally arise from disruption of the hypothalamic-pituitary ovarian axis. Causes of ovulation disorders include ]165[:

* Polycystic ovarian disease (POD) –Leventhal syndrome characterized by enlarged ovaries with follicular cysts, ammenorrhoea, abnormal hair growth and obesity.
* Diminished ovarian reserve.
* Gonadal dysgenesis (Turner syndrome).
* Ovarian tumour.
* Premature ovarian failure (POF).
* Hypothalamic dysfunction due to environmental, physical, and emotional stress.
* Pituitary adenoma with or without hyperprolactinaemia.
* Pituitary hypofunction.
* Hypogonadism and or corpus luteum insufficiency with luteal phase deficiency.
* Premature menopause.
* Anovulation.
* Oral or injectable contraceptives.

**2.11.4.2.2 Other Causes**

**2.11.4.2.2.1** [**Acquired**](http://en.wikipedia.org/wiki/Female_infertility#Acquired) **Factors**

According to the [American Society for Reproductive Medicine](http://en.wikipedia.org/wiki/American_Society_for_Reproductive_Medicine) (ASRM), Age, Smoking, Sexually Transmitted Infections, and Being Overweight or Underweight can all affect fertility ]166[.

* [**Age**](http://en.wikipedia.org/wiki/Female_infertility#Age)

Female age is the single most important determinant of spontaneous as well as treatment-related conception, with a gradual decline in fertility especially after the age of 35 years ]167[.

* [**Tobacco Smoking**](http://en.wikipedia.org/wiki/Female_infertility#Tobacco_smoking)

Nicotine and other harmful chemicals in cigarettes interfere with the body’s ability to create [estrogen](http://en.wikipedia.org/wiki/Estrogen), a hormone that regulates [folliculogenesis](http://en.wikipedia.org/wiki/Folliculogenesis) and [ovulation](http://en.wikipedia.org/wiki/Ovulation). Also, cigarette smoking interferes with folliculogenesis, embryo transport, endometrial receptivity, endometrial angiogenesis, uterine blood flow and the uterine myometrium ]168[.

* [**Sexually Transmitted Disease**](http://en.wikipedia.org/wiki/Female_infertility#Sexually_transmitted_disease)

In many cases in the developing countries, infertility in women results from untreated pelvic inflammatory disease (PID), a sequel of sexually transmitted diseases (STDs) or other reproductive tract infections ]169[.

* [**Body Weight and Nutritional Disorders**](http://en.wikipedia.org/wiki/Female_infertility#Body_weight_and_eating_disorders)

Too much body fat causes production of too much estrogen and the body begins to react as if it is on birth control, limiting the odds of getting pregnant, and too little body fat causes insufficient production of estrogen and disruption of the [menstrual cycle](http://en.wikipedia.org/wiki/Menstrual_cycle) ]170[. Proper nutrition in early life is also a major factor for later fertility ]171[.

* [**Chemotherapy**](http://en.wikipedia.org/wiki/Female_infertility#Chemotherapy)

[Chemotherapy](http://en.wikipedia.org/wiki/Chemotherapy) poses a high risk of infertility. [Antral follicle count](http://en.wikipedia.org/wiki/Antral_follicle_count) decreases after three series of chemotherapy, whereas [follicle stimulating hormone](http://en.wikipedia.org/wiki/Follicle_stimulating_hormone) (FSH) reaches menopausal levels after four series. Other hormonal changes in chemotherapy include decrease in [inhibin B](http://en.wikipedia.org/wiki/Inhibin_B) and [anti-Müllerian hormone](http://en.wikipedia.org/wiki/Anti-M%C3%BCllerian_hormone) levels ]172[.

**2.11.4.2.2.2** [**Genetic Factors**](http://en.wikipedia.org/wiki/Female_infertility#Genetic_factors)

There are many [genes](http://en.wikipedia.org/wiki/Gene) wherein [mutation](http://en.wikipedia.org/wiki/Mutation) causes female infertility ]171[. Genetic factors diagnoses to consider are Turner syndrome (45, X or variant), congenital adrenal hyperplasia (autosomal recessive), congenital absence of uterus and vagina (most cases are 46, XX, normal female development and isolated Müllerian aplasia), complete androgen insensitivity syndrome (X-linked androgen mutation in 46, XY female phenotype), gonadal agenesis/dysgenesis, hypogonadotrophic hypogonadism, and Kallmann syndrome (variable etiology dominant, recessive, X-linked) ]173[.

**2.11.4.2.2.3** [**Anatomic Location**](http://en.wikipedia.org/wiki/Female_infertility#By_anatomic_location)

* [**Hypothalamic-pituitary Factors**](http://en.wikipedia.org/wiki/Female_infertility#Hypothalamic-pituitary_factors) e.g. [Hyperprolactinemia](http://en.wikipedia.org/wiki/Hyperprolactinemia)]174[.
* **Ovarian Factors**: as mentioned in ovulation disorders ]165[.
* [**Tubal (ectopic)/peritoneal Factors**](http://en.wikipedia.org/wiki/Female_infertility#Tubal_.28ectopic.29.2Fperitoneal_factors) e.g.:

1. [Endometriosis](http://en.wikipedia.org/wiki/Endometriosis) ]175[
2. [Pelvic inflammatory disease](http://en.wikipedia.org/wiki/Pelvic_inflammatory_disease) (PID, usually due to [chlamydia](http://en.wikipedia.org/wiki/Chlamydia_infection)) ]176[.
3. [Tubal occlusion](http://en.wikipedia.org/wiki/Tubal_occlusion) ]177[

* [**Uterine Factors**](http://en.wikipedia.org/wiki/Female_infertility#Uterine_factors) e.g.: [Uterine malformations](http://en.wikipedia.org/wiki/Uterine_malformation) ]178[
* [**Cervical Factors**](http://en.wikipedia.org/wiki/Female_infertility#Cervical_factors) e.g.:

1. [Cervical stenosis](http://en.wikipedia.org/wiki/Stenosis_of_uterine_cervix) ]179[
2. [Antisperm antibodies](http://en.wikipedia.org/w/index.php?title=Antisperm_antibodies&action=edit&redlink=1) ]180[

* [**Vaginal Factors**](http://en.wikipedia.org/wiki/Female_infertility#Vaginal_factors) e.g.: Vaginal obstruction ]181[

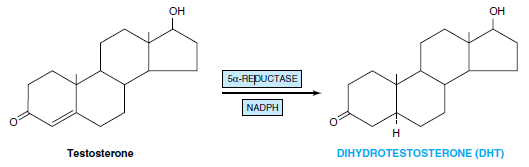
**2.12 Some Important Hormones in Couples Fertility**

**2.12.1 Testosterone in the Male**

Testosterone is a powerful anabolic hormone. It is essential both to the development of secondary sexual characteristic in the male and for spermatogenesis. It is secreted by the Leydig cell of the testes under the influence of luteinizing hormone (LH). In circulation, approximately 97% of testosterone is protein bound, principally to sex hormone binding globulin (SHBG) and a lesser extent to albumin ]182[.

**2.12.1.1 Testosterone Synthesis**

In the human testicle, the predominant pathway leading to testosterone synthesis is through pregnenolone to 17- - hydroxypregnenolone to DHEA (the Δ5 pathway), and then from DHEA to androstenedione, and from androstenedione to testosterone. As for all steroids, the rate-limiting step in testosterone production is the conversion of cholesterol to pregnenolone. LH controls the rate of side-chain cleavage from cholesterol at carbon 21 to form pregnenolone, and thus regulates the rate of testosterone synthesis. In its target cells, the double bond in ring A of testosterone is reduced through the action of 5- reductase, forming the active hormone dihydrotestosterone (DHT) ]183[.



**Figure2.4**Dihydrotestosterone is formed from testosterone through action of the

enzyme 5-reductase]184[

**2.12.1.2 Functions of Testosterone**

In utero, testosterone is necessary for the development of male genitalia in 46, XY fetuses ]185[. After birth, the serum concentration in boys remains approximately twice that of girls until puberty. In boys, a more than 10-fold increase during puberty leads to the development of secondary sexual characteristics, whereas in girls, a 2-fold increase leads to the development of pubic and axillary hair ]186[.

In men, testosterone is necessary for the maintenance of spermatogenesis, secondary sexual characteristics, bone density, muscle mass, and libido and it is thought to play a role in memory recall ]187[.

Testosterone regulates the population of [thromboxane A2](http://en.wikipedia.org/wiki/Thromboxane_A2) receptors on [megakaryocytes](http://en.wikipedia.org/wiki/Megakaryocytes) and [platelets](http://en.wikipedia.org/wiki/Platelets) and hence platelet aggregation in humans ]188[.

Recent studies have shown conflicting results concerning the importance of testosterone in maintaining cardiovascular health ]189[.

**2.12.1.3 Regulation of Testosterone**

The amount of testosterone synthesized is regulated by the [hypothalamic-pituitary-testicular axis](http://en.wikipedia.org/wiki/Hypothalamic-pituitary-gonadal_axis).When testosterone levels are low, gonadotropin-releasing hormone ([GnRH](http://en.wikipedia.org/wiki/Gonadotropin-releasing_hormone)) is released by the [hypothalamus](http://en.wikipedia.org/wiki/Hypothalamus) which in turn stimulates the [pituitary gland](http://en.wikipedia.org/wiki/Pituitary_gland) to release FSH and LH. These later two hormones stimulate the testis to synthesize testosterone. Finally increasing levels of testosterone through a negative [feedback](http://en.wikipedia.org/wiki/Feedback) loop act on the hypothalamus and pituitary to inhibit the release of GnRH and FSH/LH respectively ]190[.

**2.12.2 FSH and LH in the Female**

Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) originate from basophil-staining cells, whose secretory granules are about 200 nm in diameter. These cells constitute 10–15% of anterior pituitary cells, and they are located throughout the entire anterior lobe ]191[.

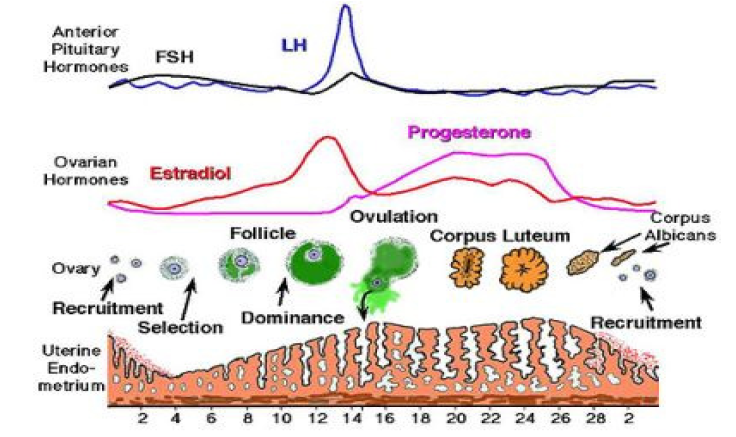
Follicle-Stimulating Hormone and Luteinizing Hormone are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by these sex hormones through a negative feedback relationship. Gonadotropin releasing hormone (GnRH), produced in the hypothalamus, controls the release of FSH from the anterior pituitary. Like other glycoproteins, such as LH, TSH, and HCG, FSH consists of subunits designated as alpha and beta. Hormones of this type have alpha subunits that are very similar in structure; therefore the biological and immunological properties of each hormone are dependent on the unique beta subunit ]192[.

**2.12.2.1 Syntheses of FSH and LH**

The differential gene expression that leads to the production and release of gonadotropins by cells in the pituitary is influenced by GnRH and ovarian hormones through feedback loops. Slower GnRH pulse frequency enhances FSH beta subunit expression and increases LH amplitude. In turn, increased GnRH pulses stimulate LH beta subunit expression while promoting FSH release. As a result, LH amplitude decreases while the mean concentration rises. Thus, ovarian steroid modification of hypothalamic GnRH pulsatility controls pituitary gonadotrophin production ]191[.

**2.12.2.2 The Role of FSH and LH in the Menstrual Cycle**

FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells; follicular steroidogenesis is promoted and LH production is stimulated. The LH produced then binds to the theca cells and stimulates steroidogenesis. Increased intraovarian estradiol production occurs as follicular maturation advances, thereupon stimulating increased FSH receptor activity and FSH follicular binding ]193[.High levels of FSH in women could cause a loss of or poor ovarian function, polycystic ovary syndrome or can indicate that menopause has set in. All of these conditions will have a negative impact on fertility. Low levels of the hormone can indicate that ova are not being produced, for the pituitary gland is not functioning correctly, which means that there are significant levels of stress present or that the person is severely underweight which is causing problems to occur ]194[.

**Figure 2.5: FSH & LH levels throughout the menstrual cycle ]195[**

**2.13 Semen Analysis**

During ejaculation, semen is produced from a concentrated suspension of spermatozoa, stored in the paired epididymides, mixed with, and diluted by, fluid secretions from the accessory sex organs. It is emitted in several boluses. Comparison of pre- and post-vasectomy semen volumes reveals that about 90% of semen volume is made up of secretions from the accessory organs, mainly the prostate and seminal vesicles, with minor contributions from the bulbourethral (Cowper’s) glands and epididymides ]196[.

Andrological examination is indicated if semen analysis shows abnormalities compared with reference values. Important treatment decisions are based on the results of semen analysis and standardisation of the complete laboratory work-up is essential ]161[. If the results of semen analysis are normal according to WHO criteria, one test should be sufficient. If the results are abnormal in at least two tests, further andrological investigation is indicated. It is important to distinguish between the following:

• Oligozoospermia: < 15 million spermatozoa/mL.

• Asthenozoospermia: < 32% motile spermatozoa.

• Teratozoospermia: < 4% normal forms ]197[.

**2.14 Ultrasound for Detecting the Changes in Ovary**

The transvaginal ultrasound provides a more accurate view of the internal structure of the ovaries, avoiding apparently homogeneous ovaries as described with transabdominal scans, particularly in obese patients. With the transvaginal route, high-frequency probes (>6 MHz), which have a better spatial resolution but less examination depth, can be used because the ovaries are close to the

vagina and/or the uterus and because the presence of fatty tissue is usually less disruptive (except when very abundant) ]198[.

**2.14.1 Polycystic Ovary Syndrome (PCOS)**

Polycystic Ovary Syndrome is a heterogeneous syndrome characterized by persistent anovulation, oligo- or amenorrhea, and hyperandrogenism in the absence of thyroid, pituitary, and/or adrenal disease. Many, but not all women with PCOS haverelatively high circulating levels of LH, compared with FSH, believed to be due to insensitivity to steroid hormone feedback. This complex disorder likely has its origins both within and outside the hypothalamic-pituitary- ovarian axis, and metabolic, neuroendocrine, and other endocrine regulators likely contribute to its manifestation ]199[. The characteristic features are accepted as being an increase in the size (volume) of the ovary due to a greater number of follicles and volume of stroma as compared with normal ovaries ]198[.



**Figure 2.6 Polcystic ovaries (B mode, transvaginal route). In both left and right ovaries, the ovarian length and width are increased as well as the ovarian area. The follicle number, with a diameter mainly between 2 and 5 mm, is more than 12. ]198[**

* 1. **Toxoplasmosis and Infertility**

The data obtained from limited studies performed in animal models as well as in infertile couples, have supported the relationship between [Toxoplasma](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url_Species=http://www.ncbi.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=5810&lvl=0) infection and [infertility](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url=http://ukpmc.ac.uk/search/?page=1&query=%22infertility%22). The hypothesis concerning [infertility](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url=http://ukpmc.ac.uk/search/?page=1&query=%22infertility%22) mechanisms due to [T.gondii](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url_Species=http://www.ncbi.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=5811&lvl=0) in females include [development](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url_go_term=http://www.ebi.ac.uk/ego/GTerm?id=GO:0007275) of [endometritis](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url=http://ukpmc.ac.uk/search/?page=1&query=%22endometritis%22) and fetal rejection due to local release of [T.gondii](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url_Species=http://www.ncbi.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=5811&lvl=0) from latently located cysts in endometrial tissue on stimulation during plansenta formation; impaired folliculogenesis in ovaries and [uterine atrophy](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url=http://ukpmc.ac.uk/search/?page=1&query=%22uterine%20atrophy%22) and reproductive failure occur due to [hypothalamic dysfunction](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url=http://ukpmc.ac.uk/search/?page=1&query=%22hypothalamic%20dysfunction%22) as a result of chronic [toxoplasmosis](http://ukpmc.ac.uk/abstract/MED/21341172/?whatizit_url=http://ukpmc.ac.uk/search/?page=1&query=%22toxoplasmosis%22) ]7[.

*Toxoplasma* infection changes the concentration of serum testosterone in mice and human rather than changed concentration of testosterone influences the probability of the Toxoplasma infection, it is possible that the decrease of testosterone is an adaptive mechanism of infected mice aimed to compensate toxoplasmosis-induced immunosuppression observed during latent *Toxoplasma* infection ]10[.

Toxoplasmosis can affect main reproductive parameters in male rats including sperm motility, concentration and morphology, which are the most predictive of their fertilizing capacity. White rats, which are considered to be the best model for human’s toxoplasmosis due to their natural resistance to *Toxoplasma* infection, may be used for further investigation on the possible effect of toxoplasmosis on man’s fertility ]11[.

Several conditions can interfere with spermatogenesis and reduce sperm quality and production. More factors such as drug treatment, chemotherapy, toxins, infections, air pollutions and insufficient vitamins intake, parasites such as *T. gondii* tachyzoites have harmful effects on spermatogenesis and sperm normal production ]200[.

Male sterility or loss of pregnancy can result from infection with some pathogens (e.g. mumps, toxoplasmosis, brucellosis, and herpes) but this seems to be a variable side effect of pathology ]201[.

Stahl *et.al* (1994) demonstrated that Nya:NYLAR female mice undergo acquired hypogonadotrophic hypogonadism secondary to hypothalamic dysfunction within a few weeks after infection with *T. gondii* ]202[.

Stahl *et.al* (1995) found that the integrity of the pituitary-ovarian axis of female mice chronically infected with *T. gondii* was evaluated by administering GnRH to stimulate the release of gonadotrophins from the pituitary, then monitoring the secondary effects on the ovary, the atrophied ovaries of the infected mice were found to be responsive to single injections of GnRH, thereby confirming the release of endogenous gonadotrophins from the pituitary. So they proposed that inadequate levels of the readily releasable pool of pituitary gonadotrophins, indicated to result from a hypothalamic inhibition of the pulsatile release of GnRH, are responsible for the weak ovarian responses ]15[.

Classically, infection with *T. gondii* triggers production of proinflammatory cytokines, including IFN-γ and IL-1β ]203[. IL-1β -induced modulation of hypothalamic GnRH release, and probably synthesis, is mediated by augmented release of the neurotransmitters, norepinephrine and dopamine from neurons in the brain stem and hypothalamus that are inhibitory to GnRH ]204[.

Opportunistic infection, such as cerebral toxoplasmosis, CMV, toxoplasmosis and TB, have rarely been reborted as a cause of hypogonadism in patients with AIDS ]205[.

Congenital toxoplasmosis is characterized by a meningoencephalitis with an intense perivascular inflammation involving particularly the basal ganglia and periventricular regions. It is likely that important hypothalamic regulatory centers are involved in this process resulting in hypothalamic-pituitary dysfunction ]206[.

Interestingly symptomatic CNS toxoplasmosis and the involvement of the pituitary are not rare during primary infection even in immunocompetent hosts

]207[, because there is an association between atypical genotypes of the parasite and severe toxoplasmosis ]208[.

The infection of *Toxoplasma gondii* is very common in infertile patients; the infection rate of female is higher than that of male. The infection of *Toxoplasma gondii* should be paid high attention to and treated in time ]209[.

**2.16 Toxoplasmosis is a sexually transmitted infection in animals**

Toxoplasma gondii-positive seminal samples were used in the artificial insemination (AI) of four female dogs free from *Toxoplasma* infection. Seven days after AI, all of the female dogs presented serologic conversion (IFAT). Fetal reabsorption occurred in two of the dogs, while the others sustained full-term gestation. Several T. gondii cysts were detected in the brains of four offspring. These results suggest that T. gondii can be sexually transmitted in domestic dogs ]21[.

Artificial insemination using semen containing experimentally added tachyzoites can establish toxoplasmosis in ewes and cause reproductive pathologies during the acute and chronic phases of the disease ]210[.

Recently, Dass *et.al* showed that Toxoplasma gondii is transmitted through sexual intercourse in brown rats. Toxoplasma gondii cysts could also be observed from vaginal lavage of females 12 hours after mating, indicating that Toxoplasma gondii was successfully ejaculated. Mating with an infected male resulted in transmission of infection to females ]211[.

**2.17 Possibility of *T. gondii* transmission through sexual intercourse in human**

De Paepe and Waxman (1989) described involvement of the testis with *Mycobacterium avium intracellularae, Toxoplasma*, CMV in 39% of patients with disseminated tuberculosis ]212[. Another studyindicated that 32% of patients with opportunistic infectionshad CMV, *Mycobacterium avium-intracellulare*, or *Toxoplasma gondii*in the testes ]19[.

De Paepe, *et.al*  (1990) showed that the testes of an autopsy sample of 56 patients with acquired immunodeficiency syndrome (AIDS) and systemic opportunistic infections were studied for the presence and type of testicular infection, then light-microscopic evidence of opportunistic organisms (cytomegalovirus, Mycobacterium avium-intracellulare, and *Toxoplasma*) was present in 22 cases (39%). Based on the prevalence and histologic features of the testicular infections and the biological characteristics of the specific organisms, the possible sexual transmission of opportunistic organisms in AIDS is discussed ]19[.

According to study of Al-Doori (2010), *Toxoplasma gondii* was isolated from seminal fluid of 5 men from 23 seminal fluid samples of natural infected men with 21.73%, this study showed the presence of tissue cysts in all groups of mice which were injected by these seminal fluid. The study proved the transmission of *T. gondii* from males to females through copulation process in mice, then to their offspring. This study agreed all studies that suggested the possibility of transmission this parasite from male to female by sexual intercourse in humans ]81[.

**3.1 Patients,** **Location and Time**

Samples were collected from a total of 110 infertile couples as a selective patients aged from 19 to 59 years in Kamal AL-Samaraee Hospital, the Fertility and *In Vitro* Fertilization (IVF) Center in Baghdad province. This cross sectional study has been done during the period from the first of October/ 2011 till the end of January/ 2012.

**3.2 Samples Collection**

Before blood collection, and after their agreement, all couples were asked about their age, marriage duration, family history, and contact with cats, as well as other questions according to questionnaire sheets (as observed in appendices 1 and 2).

The blood collected from men and their women was used to investigate the presence of *Toxoplasma* Abs (IgM, IgG) then, the levels of testosterone hormone in the infected males and levels of FSH, LH in the infected females were measured. Other observation methods were done in positive patients such as semen analysis (morphology, motility, and count of sperms) in males, ultrasound examination for ovaries in females.

**3.2.1 Blood Collection**

Five milliliters (5mL) of blood was collected by vein puncture using syringe with needle gauge 23, transported to unhepranized tube and allowed to clot at room temperature and sera were separated by centrifugation at 1500g for 5 min, then stored and frozen at (– 20oC) ]213[.

**3.2.2 Semen Collection**

Semen samples were collected according to WHO standard procedure/ 2010 as following:

Firstly, selective infertile males were asked about days of sexual abstinence. The semen sample was collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence, and then every patient was given a clean, wide mouth, sterile, dry, graduated plastic and warm disposable container.

The container was labeled with the man’s name, identification number, and the date and time of collection. The samples were obtained by masturbation in a private room near the semen analysis laboratory, in order to limit the exposure of the semen to fluctuations in temperature and to control the time between collection and analysis.

After masturbation the semen fluid samples were immediately incubated at 37°C, and waiting for complete liquefaction ]214[.

**3.2.3 Imaging for Ovaries by Ultrasound**

For allselective infertile females, the ultrasound examination was taken in AL-Samaraee Hospital/ ultrasound department and the ovaries were examined by specialist gynecologist. The majority of ultrasound examinations were performed transvaginally to optimize image quality. After the longest medial axis of theovary had been determined, the second dimension was measured,and then the vaginal probe was rotated 90 degree to obtainthe third dimension. Ovarian volume was calculated accordingto a simplified formula for an ellipsoid (0.5 x length x widthx thickness) ]198[.

**3.3 Materials**

Materials used in the work of studycan be summarized as the tables below:

**Table 3.1: Instruments, their company and origin**

|  |  |  |
| --- | --- | --- |
| **Instrument** | **Company** | **Origin** |
| **ELISA system** | **Sanofi-Pasteur** | **France** |
| **miniVIDAS** | **BioMerieux** | **France** |
| **Light microscope** | **Olumpus** | **Japan** |
| **U/S machine (AG50149)** | **Siemens** | **Germany** |
| **Transvaginal probe** | **Siemens** | **Germany** |
| **Incubator** | **Fisher scientific** | **Germany** |
| **Centrifuge** | **Hettich** | **Germany** |
| **Refrigerator** | **Philips** | **Italy** |
| **Deep freezer** | **Daewoo S.** | **Korea** |
| **Rotatory shaker** | **Kankolb** | **Germany** |
| **Automatic washer** | **Techan** | **Austria** |
| **Timer** | **Organon** | **Belgian** |
| **Water bath** | **Memmert** | **Germany** |
| **Improved Neubauer chamber** | **Superior** | **Germany** |

**Table 3.2: Laboratory kits, their company and origin**

|  |  |  |
| --- | --- | --- |
| **Reagents** | **Company** | **Origin** |
| ***Toxoplasma* Ab (IgM) Enzyme Immunoassay Test Kit.** | **BioCheck, Inc** | **U.S.A** |
| ***Toxoplasma* Ab (IgG) Enzyme Immunoassay Test Kit.** | **BioCheck, Inc** | **U.S.A** |
| **Testosterone (VIDAS) hormone Kits** | **BioMerieux** | **France** |
| **FSH (VIDAS) Kits** | **BioMerieux** | **France** |
| **LH (VIDAS) Kits** | **BioMerieux** | **France** |

**Table 3.3: Equipment, their company and origin**

|  |  |  |
| --- | --- | --- |
| **Equipment** | **Company** | **Origin** |
| **Plan tubes** | **V.K. company** | **England** |
| **Automatic micro pipettes** | **Start scientific** | **U.K** |
| **Rack** | **Diagnostic** | **England** |
| **Tips** | **Sterillin** | **England** |
| **Filter paper** | **Difico** | **U.S.A** |
| **Disposable tube** | **AFMA-Dispo** | **Jordan** |
| **Khan tube** | **AFMA-Dispo** | **Jordan** |
| **Syringes (23Gx1.5)** | **Medical Ject** | **Syria** |
| **Micropipettes (Eppendrof)** | **Eppendrof** | **Germany** |

**Table 3.4: Other tools, their company and origin**

|  |  |  |
| --- | --- | --- |
| **Others** | **Company** | **Origin** |
| **Spirit** | **local** | **Iraq** |
| **Cotton** | **local** | **Iraq** |
| **Gloves** | **Blossom** | **U.S.A** |
| **Tourniquet** | **Medical Ject** | **Syria** |

**3.4 Methods**

**3.4.1 Enzyme Immunoassay (ELISA) for the Detection of IgM Antibodies to *Toxoplasma gondii* in Human Serum.**

**3.4.1.1 Principle of the Test**

Purified *Toxoplasma gondii* antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the *Toxoplasma gondii* IgM specific antibody, if present will bind to the antigen. All unbound materials were washed away.

HRP-conjugate is added, which binds to the antibody-antigen-complex. Excess HRP-conjugate is washed off and solution of TMB reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time.

The intensity of the color generated is proportional to the amount of IgM-Specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls ]215[.

**3.4.1.2 Content of the kit**

|  |  |
| --- | --- |
| **Microtiter wells** | **purified Toxoplasma antigen coated wells (12x8) wells** |
| **Enzyme conjugate Reagent (Red color)** | **Red cap 1vail (12ml)** |
| **Sample diluent (blue color)** | **1Bottle (22ml)** |
| **Negative control** | **Range state on label. Natural cap.**  **(100L/vial)** |
| **Cut-off calibrator** | **Yellow cap. *Toxoplasma* M index = 1**  **(100L /vial)** |
| **Positive control** | **Range stated on label. Red cap. (100L/vial)** |
| **Wash Buffer concentrate (20x)** | **1 bottle (50ml)** |
| **TMB Reagent (Tetra methylbenzidine)** | **one-step: 1 vial (11ml)** |
| **Stop solution** | **1N HCL. Natural cap. 1 vial (11ml)** |

**3.4.1.3** **Assay procedure**

1. Before starting the work all reagents were allowed to reach room temperature (18-25 oC), and one volume of Wash Buffer (20x) was diluted with 19 volume of distilled water, then the desired number of coated wells were placed into the holder.
2. Preparing 1:40 dilution of test samples, negative control, positive control and calibrator by adding 5l of the samples to 200l of sample diluent.
3. 100l of diluent sera, calibrator and controls were dispensed into appropriate wells.
4. The microtiter plate was incubated at 37 oC for 30 minutes.
5. At the end of incubation period, the liquid was removed from all wells, then microtiter plate was rinsed and flicked five times with diluted wash buffer(1x)
6. 100l of enzyme conjugate was dispensed to each well, and mixed gently for 10 seconds.
7. Wells were incubated at 37 oC for 30 minutes.
8. Enzyme conjugated was removed from all wells. Then micro titer plate was rinsed, flicked five times with diluted wash buffer(1x)
9. 100l of TMB reagent was dispensed to each well, and mixed gently for 10 seconds.
10. Wells were incubated at 37 oC for 15 minutes.
11. 100l of stop solution (1N HCL) was added to stop reaction, and mixed gently for 30 seconds. Then the color in wells was changed from blue to yellow.
12. The optical density (O.D) was Read at 450 nm within 15 min. with a microwell reader.

**3.4.1.4 Calculation of Results**

*Toxoplasma* IgM index of each determination was calculated by dividing the mean value of each samples (Xs) by calibrator mean value Xc.

**3.4.1.5 Interpretation of Results**

|  |  |
| --- | --- |
| **Negative** | **Toxo M Index less than 0.90** |
| **Equivocal** | **Toxo M Index between 0.91-0.99** |
| **Positive** | **Toxo M Index of 1.00 or greater** |

**3.4.2 Enzyme Immunoassay (ELISA) for the Detection of IgG Antibodies to *Toxoplasma gondii* in Human Serum.**

**3.4.2.1 Principle of the Test**

Purified *Toxoplasma gondii* antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the *Toxoplasma gondii* IgM specific antibody, if present will bind to the antigen. All unbound materials were washed away.

HRP-conjugate is added, which binds to the antibody-antigen-complex. Excess HRP-conjugate is washed off and solution of TMB reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG-Specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls ]216[.

**3.4.2.2 Content of the kit**

|  |  |
| --- | --- |
| **Microtiter wells** | **purified Toxoplasma antigen coated wells (12x8) wells** |
| **Enzyme conjugate Reagent (Red color)** | **Red cap. 1vail (12ml)** |
| **Sample diluent (green color)** | **1Bottle (22ml)** |
| **Negative Calibrator** | **0 IU/ml. Natural cap. (100L/vial)** |
| **Cut-off calibrator** | **32 IU/ml. Yellow cap. (100L/vial)** |
| **Positive calibrator** | **100 IU/ml. Red cap. (100L/vial)** |
| **Positive calibrator** | **300 IU/ml. Green cap. (100L/vial)** |
| **Negative control** | **Range state on label. Blue cap.**  **(100L/vial)** |
| **Positive control** | **Range stated on label. Purple cap.**  **(100L/vial)** |
| **Wash Buffer concentrate (20x)** | **1 bottle (50ml)** |
| **TMB Reagent (Tetrametylbenzidne)** | **one-step: 1 vial (11ml)** |
| **Stop solution** | **1N HCL. Natural cap. 1 vial (11ml)** |

**3.4.2.3 Assay procedure**

1. Before starting the work all reagents were allowed to reach room temperature (18-25 oC), and 1 volume of Wash Buffer (20x) was diluted with 19 volume of distilled water, then the desired number of coated wells were placed into the holder.
2. Preparing 1:40 dilution of test samples, negative control, positive control and calibrators by adding 5l of the samples to 200l of sample diluent.
3. 100l of diluent sera, calibrators and controls were dispensed into appropriate wells.
4. The microtiter plate was incubated at 37 oC for 30 minutes.
5. At the end of incubation period, the liquid was removed from all wells, then microtiter plate was rinsed and flicked five times with diluted wash buffer(1x)
6. 100l of enzyme conjugate was dispensed to each well, and mixed gently for 10 seconds.
7. Wells were incubated at 37 oC for 30 minutes.
8. Enzyme conjugated was removed from all wells. Then micro titer plate was rinsed, flicked five times with diluted wash buffer(1x)
9. 100l of TMB reagent was dispensed to each well, and mixed gently for 10 seconds.
10. Wells were incubated at 37 oC for 15 minutes.
11. 100l of stop solution (1 N HCL) was added to stop reaction, and mixed gently for 30 seconds. Then the color in wells was changed from blue to yellow.
12. The optical density was Read at 450 nm within 15 min. with a microwell reader.

**3.4.2.4 Calculation of Results**

*Toxoplasma* IgG index of each determination was calculated by dividing the mean value of each sample (Xs) by calibrator mean value Xc.

**3.4.2.5 Interpretation of Results**

|  |  |
| --- | --- |
| **Negative** | **Toxo G Index less than 0.90 or (< 32 IU/ml).** |
| **Equivocal** | **Toxo G Index between 0.91-0.99** |
| **Positive** | **Toxo G Index of 1.00 or greater, or WHO IU/ml value greater than 32 IU/ml** |

**3.4.3 VIDAS Method for the Measurement of FSH Level in Woman Serum**

**3.4.3.1Principle**

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptor (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready- to- use and predisposed in sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

The sample is taken and transferred in to the well containing alkaline phosphatase-labeled anti-FSH (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to conjugate forming a (sandwich). Unbound components are eliminated during the washing step.

During the final detection step, the substrate (4-methyl-umbelliferone) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone). The fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of the antigen present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out ]217[.

**3.4.3.2** **Contents of the kit (60 Tests)**

Reconstitution of reagents:

|  |  |  |
| --- | --- | --- |
| Ready-to-use | STR | 60 FSH strips |
| Ready-to-use. Interior of SPRs coated with monoclonal anti- FSH immunoglobulins (mouse) | SPR | 60 FSH SPRs  2x30 |
| Reconstituted with 3ml of distilled water. Left to stand for 5 minutes, and then mixed. | C1 | FSH control  1x3 ml  (lyophilized) |
| Reconstituted with 2ml of distilled water. Left to stand for 5 to 10 minutes, and then mixed. | S1 | FSH calibrator 2x4 ml(lyophilized) |
| Ready-to-use  Bovine serum +1g/l sodium azide. | R1 | FSH diluent  1x3 ml (liquid) |
|  |  | |
| Specification sheet containing the factory master data required to calibrator the test. | 1MLE card | |

**3.4.3.3 The Solid Phase Receptacle (SPR)**

The interior of the SPR is coated during production with mouse monoclonal anti-FSH immunoglobulins. Each SPR is identified by the FSH code. Only remove the required number of SPRs from the pouch and reseal the pouch correctly after opening.

**3.4.3.4** **The Strips**

The strip consists of 10 wells covered with the labeled foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date.

The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the flourimetric reading is performed. The well in the center section contain the various reagents required for the assay.

**3.4.3.5 Description of the** **VIDAS FSH Strip**

|  |  |
| --- | --- |
| **Reagents** | **Wells** |
| **Sample well** | **1** |
| **Empty well** | **2-3-4-5** |
| **Conjugate: alkaline phosphatase labeled monoclonal anti-FSH immunoglobulins (mouse) + 1 g/l sodium azide (400µl).** | **6** |
| **Wash buffer: sodium phosphate(0.1 mol/l) pH 7.5 + 1g/l sodium azide (600µl).** | **7-8** |
| **Wash buffer: diethanolamine (1.1 mol/l, or 11.5%) pH 9.8 + 1 g/l sodium azide (600µl).** | **9** |
| **Reading Cuvette with substrate: 4-methyl-umbellifeerly phosphate (0.6 mmol/l) + diethanolamine (DEA) (0.62mol/l) +6.6% , pH 9.2) + 1g/l sodium azide (300µl).** | **10** |

**3.4.3.6 Procedure**

1- The required reagents were removed from the refrigerator and allowed to come to room temperature for at least 30 minutes before used.

2- One FSH strip was used and one FSH SPR from the kit for each sample, control or calibrator to be tested.

3- "FSH" was selected on the instrument to enter the test code. The calibrator identified by "S1", and tested in duplicate. If the control is to be tested, it should be identified by "C1".

4- The calibrator, control and samples were mixed by using vortex type mixer.

5- 200µl of sample, calibrator or control was pipetted into the sample well.

6-The SPRs and strips were inserted into the instrument.

7- The assay was initiated as directed in the operator's manual.

8- After 40 minutes, the assay was completed.

9-Then SPRs and strips were removed from the instrument.

10- The used SPRs and strips were disposed into an appropriate recipient.

**3.4.3.7 Range of expected values**

|  |  |
| --- | --- |
| 1.7 - 12.0 mIU/ml | **Men** |
| **Women** | |
| 6.3 - 24.0 mIU/ml | -Ovulation peak(D0) |
| 3.9 - 12.0 mIU/ml | -Follicular phase:  1st half(D-15 to D-9) |
| 2.9 - 9.0 mIU/ml | 2nd half(D-8 to D-2) |
| 1.5 - 7.0 mIU/ml | -Luteal phase)D+3 to D+15) |
| 17.0 - 95.0 mIU/ml | Menopause: |

**3.4.4 VIDAS Method for the Measurement of LH level in Woman Serum**

**3.4.4.1Principle**

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptor (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready- to- use and predisposed in sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

The sample is taken and transferred in to the well containing alkaline phosphatase-labeled anti-LH antibodies (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to conjugate forming a (sandwich). Unbound components are eliminated during the washing step.

During the final detection step, the substrate (4-methyl-umbelliferone) is cycled in and out of the SPR. the conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of the antigen present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out ]218[.

**3.4.4.2** **Contents of the kit (60 Tests)**

Reconstitution of reagents:

|  |  |  |
| --- | --- | --- |
| Ready-to-use | STR | 60 LH strips |
| Ready-to-use. Interior of SPRs coated with monoclonal anti- LH immunoglobulins (mouse) | SPR | 60 LH SPRs  2x30 |
| Reconstituted with 3ml of distilled water. Left to stand for 5 minutes, and then mixed. | C1 | LH control  1x3 ml  (lyophilized) |
| Reconstituted with 2ml of distilled water. Left to stand for 5 to 10 minutes, and then mixed. | S1 | LH calibrator 2x3 ml(lyophilized) |
| Ready-to-use  Phosphate buffer (0.05 mol/l, pH 7.5) + protein and chemical stabilizers + 1g/l sodium azide. | R1 | LH diluent  1x3 ml (liquid) |
| Specification sheet containing the factory master data required to calibrator the test. | | 1MLE card |
|  | |  |

**3.4.4.3 The Solid Phase Receptacle (SPR)**

The interior of the SPR is coated during production with mouse monoclonal anti- LH immunoglobulins. Each SPR is identified by the LH code. Only remove the required number of SPRs from the pouch and reseal the pouch correctly after opening.

**3.4.4.4** **The Strips**

The strip consists of 10 wells covered with the labeled foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date.

The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the flourimetric reading is performed. The well in the center section contains the various reagents required for the assay.

**3.4.4.5 Description of the** **LH Reagent Strip**

|  |  |
| --- | --- |
| **Reagents** | **Wells** |
| **Sample well** | **1** |
| **Empty well** | **2-3-4-5** |
| **Conjugate: alkaline phosphatase labeled monoclonal anti**-**LH immunoglobulins (mouse) + 1 g/l sodium azide (400µl).** | **6** |
| **Wash buffer: sodium phosphate(0.1 mol/l) pH 7.5 + 1g/l sodium azide (600µl).** | **7-8** |
| **Wash buffer: diethanolamine (1.1 mol/l, or 11.5%) pH 9.8 + 1 g/l sodium azide (600µl).** | **9** |
| **Reading Cuvette with substrate: 4-methyl-umbellifeerly phosphate (0.6 mmol/l) + diethanolamine (DEA) (0.62mol/l) +6.6% , pH 9.2) + 1g/l sodium azide (300µl).** | **10** |

**3.4.4.6 Procedure**

1- The required reagents were removed from the refrigerator and allowed to come to room temperature for at least 30 minutes before use.

2- One LH strip and one LH SPR were used from the kit for each sample, control or calibrator to be tested.

3- "LH" was selected on the instrument to enter the test code. The calibrator was identified by "S1", and tested in duplicate. If the control needs to be tested, it should be identified by "C1".

4- The calibrator, control and samples were mixed by using vortex type mixer.

5- 200µl of sample, calibrator or control was pipetted into the sample well.

6-The SPRs and strips were inserted into the instrument.

7- The assay was initiated as directed in the operator's manual.

8- After 40 minutes, the assay was completed.

9-Then SPRs and strips were removed from the instrument.

10- The used SPRs and strips were disposed into an appropriate recipient.

**3.4.4.7 Range of expected values**

|  |  |
| --- | --- |
| 1.1 - 7.0 mIU/ml | **Men** |
| **Women** | |
| 9.6 - 80.0 mIU/ml | -Ovulation peak(D0) |
| 1.5 - 8.0 mIU/ml | -Follicular phase:  1st half(D-15 to D-9) |
| 2.0 - 8.0 mIU/ml | 2nd half(D-8 to D-2) |
| 0.2 - 6.5 mIU/ml | -Luteal phase)D+3 to D+15) |
| 8.0 - 33.0 mIU/ml | Menopause: |

**3.4.5 VIDAS Method for Measurement of Testosterone level in man**

**3.4.1Principle**

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA).

The Solid Phase Receptor (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready- to- use and predisposed in sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

The sample is taken and transferred in to the well containing alkaline phosphatase-labeled testosterone derivative. The testosterone present in the serum and the testosterone derivative in the conjugate compete for the anti-testosterone specific antibody sites coated to the inner surface of the SPR. Unbound components are eliminated during the washing steps.

During the final detection step, the substrate (4-methyl-umbelliferone is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of the testosterone present in the sample.

At the end of the assay, the results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out ]219[.

**3.4.5.2 Content of the kit** (**30 tests**)

Reconstitution of reagents

|  |  |  |
| --- | --- | --- |
| Ready-to-use | STR | 30 TES. strips |
| Ready-to-use  SPRs sensitized with polyclonal anti-testosterone immunoglobulins (rabbit) | SPR | 30 TES. SPRs  1x30 |
| Reconstituted with 2 ml of distilled water. Waited for 5 to 10 minutes, and then mixed. | C1 | TES. control  1x2 ml  (lyophilized) |
| Reconstituted with 3 ml of distilled water. Waited for 5 to 10 minutes, and then mixed. | S1 | TES. calibrator  1x3 ml(lyophilized) |
| Specification for the factory master data required to calibrator the test. | | 1MLE card |

**3.4.5.3 The Solid Phase Receptacle (SPR)**

The SPR is coated during production with polyclonal anti-testosterone immunoglobulins (rabbit).

**3.4.5.4 The strips**

The strip consists of 10 wells covered with the labeled foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date.

The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the flourimetric reading is performed. The well in the center section contains the various reagents required for the assay.

**3.4.5.5 Description of Testosterone Reagent Strip**

|  |  |
| --- | --- |
| **Reagents** | **Wells** |
| **Sample** | **1** |
| **Empty wells** | **2-3-4** |
| **Conjugate: alkaline phosphatase labeled Testosterone derivative + calf serum gelatin (procine) + releasing agent + 0.9 g/l sodium azide (400µl)** | **5** |
| **Empty well** | **6** |
| **Wash buffer: Tris-NaCl (0.05 mol/l) pH 7.4 + 0.9g/l sodium azide (600µl).** | **7-8** |
| **Wash buffer: diethanolamine EDTA (1.1 mol/l, or 11.5%) pH 9.8 + 1 g/l sodium azide (600µl).** | **9** |
| **Reading Cuvette with substrate: 4-methyl-umbellifeerly phosphate (0.6 mmol/l) +DEA (0.62 mol/l or 6.6%) pH 9.2 + 1g/l sodium azide (300µl).** | **10** |

**3.4.5.6 Procedure**

1- The required reagents were removed from the refrigerator and allowed to come to room temperature for at least 30 minutes.

2- One (TES) strip and one (TES) SPR were used from the kit for each sample, control or calibrator to be tested.

3- "TES" was identified by the test code on the instrument. The calibrator was identified by "S1", and tested in duplicate. If the control needs to be tested, it should be identified by "C1".

4- The calibrator, and/or the control and samples were mixed by using vortex type mixer.

5- 200µl of sample, calibrator and control was pipetted into the sample well.

6-The SPRs and strips were inserted into the instrument.

7- The assay was initiated as directed in the operator's manual.

8- After 60 minutes, the assay was completed.

9-The SPRs and strips were removed from the instrument.

10- The used SPRs and strips were disposed into an appropriate recipient.

**3.4.5.7 Range of expected values**

|  |  |
| --- | --- |
| 0.1 - 0.9 ng/ml | Cyclic Women |
| 3.0 - 10.6 ng/ml | Men |

**3.4.6. Seminal Fluid Analysis**

**3.4.6.1 Sperms Motility**

After liquefaction of the sample, sperm motility within semen was assessed according to WHO standard procedure 2010 as follows ]214[:

* The semen sample was mixed well.
* A Standard volume of semen, 10 *µl* was placed onto a clean glass slide.
* It was covered with a coverslip, 22 mm × 22 mm.
* The slide was examined with phase-contrast optics at ×200 or × 400 magnifications.
* At least 200 spermatozoa were evaluated in a total of at least five fields in each replicate, in order to achieve an acceptably low sampling error.
* The motility of each spermatozoon was graded as follows:
* Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed.
* Non-progressive motility (NP): all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed.
* Immotility (IM): no movement.
* Reporting the average percentage for each motility grade to the nearest whole number.
* The lower reference limit for total motility (PR + NP) is 40% (5th centile, 95% CI 38–42).
* The lower reference limit for progressive motility (PR) is 32% (5th centile, 95% CI 31–34).

**3.4.6.2 Sperms Count**

Determination of sperms number comprised the following steps: ]214[

* The count of spermatozoa was determined by using the improved Neubauer haemocytometer which had two separate counting chambers, each of which had a microscopic 3 mm × 3 mm pattern of gridlines etched on the glass surface.
* 1:20 dilution was made with the aid of a white blood-cell pipette.
* Fixative for diluting semen was done through dissolving 50 g of sodium bicarbonate (NaHCO3) and 10 ml of 35% (v/v) formalin in 1000 ml of purified water. Each chamber of the haemocytometer was filled with the replicate dilutions, one replicate per chamber.
* The haemocytometer was stored horizontally for at least 4 minutes at room temperature in a humid to prevent drying out. The immobilized cells will sediment onto the grid during this time.
* The haemocytometer was examined with phase-contrast optics at ×200 or × 400 magnifications. One chamber, grid by grid, was examined and continued counting until at least 200 spermatozoa had been observed and a complete grid had been examined.
* The concentration of spermatozoa in semen is their number (*N*) divided by the volume in which they were found, i.e. the volume of the total number (*n*) of grids examined for the replicates (where the volume of a grid is 100 nl), multiplied by the dilution factor. That is, *C* = (*N/n*) × (1/100) × dilution factor.
* The lower reference limit for sperm concentration is 15 × 106 spermatozoa per ml (5th centile, 95% CI 12–16 × 106).

**3.4.6.3 Sperms Morphology**

Determination of sperm morphology comprised the following steps ]214[:

* Preparing a smear of semen on a slide.
* Air-drying, fixing and staining the slide.
* Mounting the slide with a coverslip if the slide is to be kept for a long time.
* Examining the slide with brightfield optics at ×1000 magnification with oil immersion.
* Assessing approximately 200 spermatozoa per replicate for the percentage of normal forms or of normal and abnormal forms.

The following categories of defects should been noted:

* Head defects: large or small, tapered, pyriform, round, amorphous, vacuolated (more than two vacuoles or >20% of the head area occupied by unstained vacuolar areas), vacuoles in the post-acrosomal region, small or large acrosomal areas (<40% or >70% of the head area), double heads, or any combination of these.
* Neck and midpiece defects: asymmetrical insertion of the midpiece into the head, thick or irregular, sharply bent, abnormally thin, or any combination of these.
* Principal piece defects: short, multiple, broken, smooth hairpin bends, sharply angulated bends, of irregular width, coiled, or any combination of these.
* The lower reference limit for normal forms is 4% (5th centile, 95% CI 3.0–4.0).

**Statistical analysis:**

The following statistical data analysis approaches were used in order to analyze and assess the results of the study:

1. **Descriptive data analysis**:
2. Statistical tables (Frequencies and percent).
3. Mean value.
4. Standard Deviation.
5. Contingency causes correlationship coefficient for association tables.

**2. Inferential data analysis:**

These were used to accept or reject the statistical hypotheses; they included the following:

1. Contingency coefficient test.
2. Testing the contingency coefficients of the contingency tables.
3. Odds Ratio.
4. Binomial test

**3-Computer & programs:**

All the statistical analyses were done by using Pentium-4 computer through the SPSS program (version-10) and Excel application ]220[

Note: The comparison of significant (P-value) in any test were: S= Significant difference (P<0.05).

HS= Highly Significant difference (P<0.05).

NS= Non Significant difference (P>0.05).

**4.1** **Results**

Table (4-1) shows that out of 110 infertile couples which represent the total number of samples, 46 (41.81%) males and 85 (77.27%) females were positive for toxoplasmosis.

In the same direction and out of those infertile couples, the positive couples were 39 (35.45%), (i.e. 39 husbands were sharing their wives in *Toxoplasma* infection. P-value was highly significant (HS) in couples and females (P=0.003), (P=0.000) respectively, while in male was non significant (NS) (0.105).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Patients** | | **Toxoplasmosis** | | **Total**  **Number (No.) & percent (%)** | **P-value**  **(Binomial Test)** |
| **Positive** | **Negative** |
| **Males** | **No.** | **46** | **64** | **110** | **P=0.105**  **(NS)** |
| **%** | **41.81** | **58.19** | **100** |
| **Females** | **No.** | **85** | **25** | **110** | **P=0.000**  **(HS)** |
| **%** | **77.27** | **22.73** | **100** |
| **Couples** | **No.** | **39** | **71** | **110** | **P=0.003**  **(HS)** |
| **%** | **35.45** | **64.55** | **100** |

**Table (4-1): Demonstration of the Affliction with Toxoplasmosis in the Infertile Couples with Comparison between Positive and Negative Groups**

The relationship between the age and the positive infertile patients is demonstrated in table (4-2). This table demonstrates that the number and percentage of males was zero in the age group (<20) and (20-24) years, and between (25-29) years was 2 (4.34%). In this age group, it was lower than the other age groups, while it was 14(30.35%) in age groups (35-39) years, which was higher than other age groups.

Females were recorded 1(1.17%) in the age group (45-49) years, they were lower than females in all other age groups, and were zero in age group (≥50) years, while they were 23 (27.06%) in the age group (35-39) years, they were higher than female in other age groups. The comparison significance (C.S.) in all age groups was (HS) (P-value = 0.000).

First row in each age group represents the number of patients, and second row show the presence (%) of males to females in each age group, while third row demonstrates the distribution (%) of gender (males or females) along all age groups.

The odds ratio of males to females in less than 35 years to equal and more than this age (<35:35≥years)was approximately 1:6 (1:5.963)**.** According to this ratio, every one male parallel 6 females in the age of less than 35 years, thus, females were higher in younger ages than males.

The mean value of the age in males was 40, in females it was 31.18, and in all patients (couples) was 34.28

**Table (4-2): Distribution of the Positive Infertile Couples According to the Age Groups.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Age Groups in the**  **Positive males & females (years)** | **No. & (%)** | **Gender** | | **Total** | **C.S.**  **P-value**  **C.C. Test** |
| **Male** | **Female** |
| **< 20** | **No.** | **0** | **2** | **2** | **C.C.=0.477**  **P=0.000**  **HS**  **Odds Ratio**  **(<35:35≥)**  **(M:F)**  **(1:5.963)** |
| **% Age Groups** | **0.0** | **100** | **100** |
| **% Gender** | **0.0** | **2.35** | **1.52** |
| **20 - 24** | **No.** | **0** | **16** | **16** |
| **% Age Groups** | **0.0** | **100** | **100** |
| **% Gender** | **0.0** | **18.83** | **12.22** |
| **25 - 29** | **No.** | **2** | **18** | **20** |
| **% Age Groups** | **10** | **90** | **100** |
| **% Gender** | **4.34** | **21.17** | **15.37** |
| **30 - 34** | **No.** | **8** | **17** | **25** |
| **% Age Groups** | **32** | **68** | **100** |
| **% Gender** | **17.39** | **20.00** | **19.08** |
| **35 - 39** | **No.** | **14** | **23** | **37** |
| **% Age Groups** | **37.83** | **62.17** | **100** |
| **% Gender** | **30.35** | **27.06** | **28.24** |
| **40 - 44** | **No.** | **9** | **8** | **17** |
| **% Age Groups** | **52.94** | **47.06** | **100** |
| **% Gender** | **19.56** | **9.42** | **12.97** |
| **45 - 49** | **No.** | **6** | **1** | **7** |
| **% Age Groups** | **85.71** | **14.29** | **100** |
| **% Gender** | **13.04** | **1.17** | **5.35** |
| **50 ≥** | **No.** | **7** | **0** | **7** |
| **% Age Groups** | **100** | **0.0** | **100** |
| **% Gender** | **15.22** | **0.0** | **5.35** |
| **Mean ± SD** | | **40.00**  **± 7.94** | **31.18**  **± 6.61** | **34.28**  **8.24** |

Table (4-3) demonstrates marriage durationin positive infertile patients. The mean value was (8.35) and standard deviation was (4.09).

The infertile patients (males and females) were 70 (53.44%) in marriage duration group (5-9) years, they were higher in this group, while they were 3 (2.29%) in the marriage duration group(20≥) years.

**Table (4-3): Distribution of the Positive Infertile Patients According to the Marriage Duration.**

|  |  |  |
| --- | --- | --- |
| **Marriage Duration in**  **Positive Patients**  **(years)** | **No.** | **%** |
|  |
| **< 5** | **15** | **11.45** |
| **5 - 9** | **70** | **53.44** |
| **10 - 14** | **37** | **28.24** |
| **15 - 19** | **6** | **4.58** |
| **20 ≥** | **3** | **2.29** |
| **Mean ± SD** | **8.35± 4.09** | |

History of infertility in the family, contact with cats in all patients, and the disturbance in menstrual cycle in females were showed in table (4-4).

It was found that only 10 (7.64%) of positive infertile patients were with history of infertility in the family, while other positive infertile patients 121 (92.36) were without family history.

According to this table, 77 (58.77%) infertile patients had history of contact with cats in their houses, while 54 (41.23%) of them had never been in contact. Table (4-4) mentioned that 48 (56.47%) of females were with regular cycle, while 37 (43.53%) females were with irregular cycle.

**Table (4-4): Distribution of the Observed Frequencies of Some Demographical Characteristics and Related Variables.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variables** | **Status** | **No.** | **%** |
|  |
| **History of infertility in the family** | **No** | **121** | **92.36** |
| **Yes** | **10** | **7.64** |
| **Contact with cats** | **No** | **54** | **41.23** |
| **Yes** | **77** | **58.77** |
| **Menstrual cycle** | **Regular** | **48** | **56.47** |
| **Irregular** | **37** | **43.53** |

The previous pregnancy and abortion in positive infertile women were demonstrated in table (4-5). 8 (9.42%) of these women had one successful pregnancies and 2 (2.35%) of them had frequent (twice or more) previous pregnancy, while all other 75 (88.23%) women suffered from infertility without past pregnancy.

In this study, women with history of one past abortion were 15 (17.64%), and those with past frequent abortion were 16 (18.83%), while 54(63.52%) women had no abortion.

|  |  |  |  |
| --- | --- | --- | --- |
| **Pregnancy & abortion in positive female** | **Status** | **Number** | **%** |
| **Pregnancy** | **Non** | **75** | **88.23** |
| **Once** | **8** | **9.42** |
| **frequent** | **2** | **2.35** |
| **Abortion** | **Non** | **54** | **63.52** |
| **Once** | **15** | **17.64** |
| **frequent** | **16** | **18.83** |

**Table (4-5): Demonstration the Presence of Previous Pregnancy and Abortion in Positive Infertile Females**

Hormonal medication for fertility improvement and treatment by oral antibiotic against toxoplasmosis in our patients were mentioned in the table (4-6). There were 69 (52.67%) infertile patients who had never taken any treatment, 33 (47.82%) of them were males and 36 (52.18%) were females. From all positive infertile patients, there were only 16 (12.21%) of females who had been treated with antibiotic for toxoplasmosis, 46 (35.12%) patients took hormonal treatment, 13 (28.27%) of them were males and 33 (38.83%) were females.

The percentage of male to female in patients without any treatment was (47.82%) to (52.18%), in patients with *Toxoplasma* antibiotics was (0.00%) to (100%), and those under hormonal treatment was (28.26%) to (71.74%). The comparison significance was highly significant, p-value= (0.001) in these groups.

**Table (4-6): Demonstration of the Presence and Type of Treatment in Positive Infertile Males and Females with Comparison Between Groups.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **No. & %** | **Gender** | | **Total** | **C.S.**  **P-value**  **C.C. Test** |
| **Male** | **Female** |
| **Non** | **No.** | **33** | **36** | **69** | **C.C.=0.316**  **P=0.001**  **HS** |
| **% Non** | **47.82** | **52.18** | **100** |
| **% Gender** | **71.73** | **42.35** | **52.67** |
| **Antibiotic For *Toxoplasma*** | **No.** | **0** | **16** | **16** |
| **% Antibiotic** | **0.0** | **100** | **100** |
| **% Gender** | **0.0** | **18.82** | **12.21** |
| **Hormonal treatment** | **No.** | **13** | **33** | **46** |
| **%Hormones** | **28.26** | **71.74** | **100** |
| **% Gender** | **28.27** | **38.83** | **35.12** |

According to table (4-7), out of 46 of positive infertile males in our study, males who had normal levels of testosterone concentration in their serum were 2 (4.35%), and low levels was present in 43 (93.47%) of them, while only one (2.18%) had high level.

Out of 85 positive infertile females, 53 (62.39%) of them had normal levels for follicle stimulating hormone (FSH), 22 (25.89%) females were with low, and 10 (11.72%) were seen with high levels.

Luteinizing hormone (LH) levels were normal in 52 (61.18%) females; 19 (22.35%) females had decreased levels, while in 14 (16.17%) females there were increased levels.

**Table (4-7): Demonstration of the Testosterone Levels in the Male and FSH, LH in the Females**

|  |  |  |  |
| --- | --- | --- | --- |
| **Hormones** | **Levels(concentration in serum)** | **Patients Number** | **%** |
| **Testosterone hormone in males** | **Normal** | **2** | **4.35** |
| **Abnormal (Low)** | **43** | **93.47** |
| **Abnormal (High)** | **1** | **2.18** |
| **(FSH) in females** | **Normal** | **53** | **62.39** |
| **Abnormal (Low)** | **22** | **25.89** |
| **Abnormal (High)** | **10** | **11.72** |
| **(LH) in females** | **Normal** | **52** | **61.17** |
| **Abnormal (Low)** | **19** | **22.35** |
| **Abnormal (High)** | **14** | **16.48** |

Out of total number (46) of positive infertile males, table (4-8) showed that 26 (56.52%) males were with abnormal morphology of sperms and 20 (43.48%) of them were normal in sperm morphology.

Sperms number (concentration per ml) in 31 (67.39%) of males was abnormal, while 15 (32.61%) males was recorded as normal.

Abnormal sperm motility was observed in 34 males with percent (73.91) and in 12 one of them was normal with (26.09%).

The comparison significance (C.S.) between normal and abnormal males in sperm morphology was (NS), and between males with normal and others with abnormal sperm concentration was (S), while it was (HS) in the case of sperm motility between normal and abnormal males in this semen parameter.

**Table (4-8): Demonstration of the Normal and Abnormal Semen Analysis in the Positive Infertile Males and Comparison between Normal and Abnormal.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Semen analysis in the positive males** | **No. & %** | **Abnormal** | **Normal** | **C.S.**  **P-value**  **(Binomial Test)** |
| **Sperm Morphology** | **No.** | **26** | **20** | **P= 0.461**  **(NS)** |
| **%** | **56.52** | **43.48** |
| **Sperm Concentration** | **No.** | **31** | **15** | **P= 0.026**  **(S)** |
| **%** | **67.39** | **32.61** |
| **Sperm Motility** | **No.** | **34** | **12** | **P= 0.002**  **(HS)** |
| **%** | **73.91** | **26.09** |

The relationship between abnormality in serum testosterone levels and the abnormality of sperms in positive infertile males was demonstrated in table (4-9). Males with low testosterone levels (a) when they were compared with males who had abnormal sperms morphology (b), those with abnormal sperms count (c), and males with abnormal sperms motility (d) the (C.S.) was (NS).

Males with high testosterone levels (A) when they were compared with males who had abnormal sperms shape, males with abnormal sperms count, and males with abnormal sperms motility, (C.S.) was highly significant and p-value was (0.000) in all groups ((b),(c), and (d)).

**Table (4-9): Relationship between Abnormality in the Testosterone Concentration and the Abnormality of Sperms in Positive Infertile Males.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Abnormality of**  **Testosterone levels** | **Positive Males** | | | **Abnormality of sperms** | | | **C.S.**  **P-value**  **(Binomial Test)** |
| **Shape**  **(b)** | **Count**  **( c )** | **Motility**  **(d)** |
| **Low Levels**  **a))** |  | **No.** | **43** | **26** | **31** | **34** | **(a)with (b)=0.054 (NS)**  **(a) with (c) = 0.201 (NS)**  **(a) with(d)=0.362 (NS)** |
|  | **%** | **93.47** | **56.52** | **67.39** | **73.91** |
| **High Levels**  **(A)** |  | **No.** | **1** | **26** | **31** | **34** | **(A)with (b) = 0.000 (HS)**  **(A)with (c) = 0.000 (HS)**  **(A)with (d) = 0.000 (HS)** |
|  | **%** | **2.17** | **56.52** | **67.39** | **73.91** |

Table (4-10) demonstrates the number and percentage of positive infertile females that had abnormal ovaries (in case of cystic ovary and change in size) and other positive infertile females with normal ovaries.

According to this table, females with polycystic ovaries were 25 (29.41%); females with one follicle cyst were 4 (4.71%), while females with normal ovaries were 56 (65.88%).

Ovary size was enlarged in 28 (32.94%) females, and smaller ovary in 4 (4.71%) females, while there were 53 (62.35%) of females with normal size of ovaries.

**Table (4-10): Demonstration of the Normal and Abnormal Ovaries in the Positive Infertile Females.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Appearance of the ovaries in positive females** | **Changes type** | **Number** | **%** |
| **Cystic Ovary** | **Polycystic ovary** | **25** | **29.41** |
| **One follicular cyst** | **4** | **4.71** |
| **Normal ovaries** | **56** | **65.88** |
| **Size of the ovary** | **Enlarged ovary** | **28** | **32.94** |
| **Small size ovary** | **4** | **4.71** |
| **Normal size** | **53** | **62.35** |

Low and high levels of FSH and LH were compared with abnormal ovaries (cystic ovaries) in Positive infertile females, as demonstrated in table (4-11).

Low FSH levels (f) in 22 (25.89%) females were compared with polycystic ovaries (p) in 25 (29.41%) females, and the (C.S.) in these two groups was (NS), while it was (HS) when these low levels were compared with presence of one ovary cyst (c) in 4 (4.71%) females.

High level of FSH levels (F) in 10 females was compared with polycystic ovaries (p), and the (C.S.) was (S), while it was (NS) when (F) was compared with (c).

Low levels of LH (l) and high level (L) also compared with (p) and (c) group, and the (C.S.) was according to table below:

**Table (4-11): Relationship between Abnormality in the FSH, LH Concentrations and Cystic Ovary in the Positive Infertile Females.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Abnormality of FSH and LH in positive females** | | | | | **Abnormal ovary**  **(cystic ovary)** | | **C.S.**  **P-value**  **(Binomial Test)** |
| **Polycystic**  **(p)** | **One cyst (c)** |
| **FSH** | **Low (f)** |  | **No.** | **22** | **25** | **4** | **(f)with (p) = 0.771 (NS)**  **(f)with (c) = 0.001 (HS)** |
| **%** | **25.89** | **29.41** | **4.71** |
| **High (F)** |  | **No.** | **10** | **25** | **4** | **(F)with (p) = 0.017 (S)**  **(F)with (c) = 0.180 (NS)** |
| **%** | **11.72** | **29.41** | **4.71** |
| **LH** | **Low (l)** |  | **No.** | **19** | **25** | **4** | **(l)with (p)= 0.451 (NS)**  **(l)with (c) = 0.003 (HS)** |
| **%** | **22.35** | **29.41** | **4.71** |
| **High (L)** |  | **No.** | **14** | **25** | **4** | **(L)with (p )=0.108 (NS)**  **(L)with (c )= 0.031 (S)** |
| **%** | **16.48** | **29.4%** | **4.7** |

According to the table (4-12), it was obvious that out of total number (85) of positive infertile females, 22 females with low FSH levels (f) were compared with 28 females who had enlarged ovaries (e), the (C.S.) was (NS), while it was (HS) in case of comparison between this (f) and small size ovaries (s) in 4 females.

High FSH levels in 10 females (F) were compared with enlarged ovaries (e), the p-value was (HS) with (0.001), while it was (NS) when the levels (F) compared with (s).

Low (l) and high (L) LH levels also when compared with (s) and (e), the (C.S.) was recorded as observed in the following table:

**Table (4-12): Relationship between Abnormality in the FSH, LH Concentrations and Abnormal Size of the Ovaries in the Positive Infertile Females.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Abnormality of FSH and LH Levels in Positive Females** | | | | | **Abnormal Size of Ovary** | | **C.S.**  **P-value**  **(Binomial Test)** |
| **Enlarged(e)** | **Small size (s )** |  |
| **FSH** | **Low (f)** |  | **No.** | **22** | **28** | **4** | **(f)with (e) = 0.480 (NS)**  **(f)with (s) = 0.001 (HS)** |
| **%** | **25.89** | **32.94** | **4.71** |
| **High (F)** |  | **No.** | **10** | **28** | **4** | **(F)with (e)= 0.005 (HS)**  **(F)with (s) = 0.180 (NS)** |
| **%** | **11.72** | **32.94** | **4.71** |
| **LH** | **Low (l)** |  | **No.** | **19** | **28** | **4** | **(l)with (e)=0.243**  **(NS)**  **(l)with (s)=0.003**  **(HS)** |
| **%** | **22.35** | **32.94** | **4.71** |
| **High (L)** |  | **No.** | **14** | **28** | **4** | **(L)with (e)= 0.044**  **(S)**  **(L)with (s) = 0.031**  **(S)** |
| **%** | **16.48** | **32.94** | **4.71** |

As observed in table (1-13), low FSH levels in 22 females was compared with irregular menstrual cycle in 37 females, and the (C.S.) was (NS) (p=0.067); while when high levels of FSH in10 females was compared with irregular menstrual cycles, the (C.S.) was (HS) (p=0.000).

In case of low LH levels in 19 females when compared with this disorder in cycles, the (C.S.) was (S) (p=0.022), furthermore, it was (HS) (p=0.002) when high LH levels in 14 females compared with cycle irregularity in these 37 females.

**Table (4-13): Relationship between Abnormality in the FSH, LH Concentrations and Irregular Menstrual Cycle in the Positive Infertile Females.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Abnormality of FSH and LH Levels in Positive Females** | | | | | **Irregular Menstrual Cycles** | **C.S.**  **P-value**  **(Binomial Test)** |
| **FSH** | **Low** |  | **No.** | **22** | **37** | **P= 0.067**  **( NS)** |
| **%** | **25.89** | **43.53** |
| **high** |  | **No.** | **10** | **37** | **P= 0.000**  **( HS)** |
| **%** | **11.72** | **43.53** |
| **LH** | **Low** |  | **No.** | **19** | **37** | **P= 0.022**  **(S)** |
| **%** | **22.35** | **43.53** |
| **high** |  | **No.** | **14** | **37** | **P= 0.002**  **(HS)** |
|  |  | **%** | **16.48** | **43.53** |

**4.2 Discussion**

Up to our knowledge, this is the first study- at least in Iraq and the area around- which is trying to correlate between affliction with toxoplasmosis and infertility in couples. Also, it is the first to investigate the disturbance in testosterone hormone levels and sperms disorder (motility, morphology and count) in positive males, and to study the variations in FSH and LH levels as well as observing the changes in volume and structural architecture of ovaries in positive females.

This study included 110 couples, of which 39 (35.45%) were positive for toxoplasmosis as shown in table (4-1). This result was approximately similar to the result of Zhou *et.al* ]8[ in which the positive result of *Toxoplasma* infection in the infertile couples was 34.83 %. Also, the result was in agreement with the results of Xin-cai *et.al*]221[ in which the infection rate of *Toxoplasma gondii* was 37.88% among the infertile couples.

According to table (4-1), there were 39 (35.45%) men sharing their women in seropositivity for toxoplasmosis. This result agreed with the study of Al-Doori] 81[that showed 100% agreement in the sero test between the women and their husbands for 42 couples from 100 couples.

There were many pieces of evidence about the transmission of this parasite sexually from male to female in dogs ]21[, rats ]211[, rabbits ]222[ and goats ]210[; furthermore, *T. gondii* had been isolated from caprine ]16[, ovine ]17[, bovine ]223[, swine] 18[, and human semen ]19[ ]81[.

When the socio-demographic and behavioral characteristics of the psychiatric patients were analysed by logistic regression, Cosme *et.al* ]224[ found that *T. gondii* infection was associated with sexual promiscuity (adjusted OR = 15.8; 95% CI: 3.8–64.8). They said "Interpretation of this finding should be taken with care since the CI of the OR was wide. In addition, this finding is surprising since the sexual route seems not to be effective in parasite transmission in humans and animals. Nevertheless, this route can not be absolutely excluded since *T. gondii* has been found in male genital tract" ]225[.

Table (4-1) demonstrates that out of these 110 couples, the positive males were 46 (41.81%), while positive females were 85 (77.27%). Such a difference between males and females agreed with the study of Liu and Wang ]209[ in which the infection rate of females (19.13%) is higher than that of males (6.957%).

The difference between males and females also agreed with the results of Xin-cai *et.al* ]221[ in which male and female infection rate of *Toxoplasma* *gondii* was 22.73% and 53.03% respectively.

The results also agreed with results of Al-Doori]81[ in Iraq, in which the rate of infection in women by using latex test, ELISA (IgG) and ELISA (IgM) was 95%, 86% and 49% respectively, and in their husbands 82%, 66% and 28% respectively.

Furthermore, the study was in agreement with Flegr J. and Stříž I. ]226[in Prague; they showed that the prevalence of latent toxoplasmosis was significantly higher in females (23.7%) than in males (10.9%) patients.

In present study, the infection rate of females is higher than that of males; the reason for this is likely tobe attributed to the physiological differencesbetween males and females which play an important role in determiningsusceptibility to parasitic infection. It isnow widely accepted that many hormones, including the sex-associatedhormones can directly influence the immune system and thus the susceptibilityto disease. It has been reported that female susceptibility to pathogens, especially those that infect their reproductiveorgans, varies according to the stage of the menstrual cycle ]227[.

The results disagreed with Shirbazou *et.al* ]228[ in Iran, which showed that 24(%13.33) females and 39(%20) males were positive with lgG anti-*Toxoplasma* antibody. This can be explained as follows: the number of samples in their study was not equal as to males and females, it was107 males versus 73 females from total of 180 patients, and so as the factors that interfere with the epidemiology of *T. gondii* such as feeding habits and cultural characteristics ]66[.

Table (4-2) demonstrated that the higher infection rate in males and females was recorded at age group (35-39) years with mean age (34.28± 8.24); this result was similar to the results of ]229[, in which the rates of infection were higher in age group (35-39) years than other age groups. The result disagreed with Al-Doori ]81[ results in Tikrit city; he showed that the higher rates of infection among couples lie in the age group 25-31years.

Other studies agreed with the results in the mean of ages, Aral *et.al* ]5[ in Turkey showed that the mean ages in positive infertile females were (33.0 ± 5.3).

According to table (4-2), the odds ratio of males to females in (<35:35≥)yearswas (1:5.963)**;** this result demonstrated that females were higher in younger ages than males, and it agreed with results of Suhaila ]76[ in Baghdad Province who showed that the percentages of women at (26-34) years of age range group were higher than other age ranges, and the percentage of women at (17-25) year age was high in apparently healthy non married women. Such results approximately agreed with the results in table (4-3) which showed that the mean value of marriage duration was (8.35). It concluded that the age of marriage nearly lies in 23th year after the mean of marriage duration is minused from the mean of ages in females (31.81).

This result also were in agreement with finding of Tasawar *et.al*]230[ who showed that the prevalence of *T. gondii* was higher in younger ages. Higher prevalence in young people may be due to the association with pets, poor sanitary habits and low immunity against this parasite ]231[.

According to table (4-4), there were 77 (58.77%) positive infertile patients (males and females) versus 54 (41.23%) from total of 131 positive ones who had a history of contact with cats in their houses. These results agreed with Al- Najjar results ]232[ in Mosul City; he found that the percentage of *Toxoplasma* infection in people who were in contact with cats in their houses was 53%, while it was 47% in people who did not deal with cats. Also the results are in agreement with Hossein *et.al* ]233[ in Iran whose data analysis confirmed a positive correlation between IgM reactivity in toxoplasmosis and contact with cats (P. Value =0.014).

Table (4-4) demonstrates that the history of infertility in the positive couples family was (7.64%) in positive infertile patients, while other positive infertile patients 121 (92.36) were without family history. This indicates that their infertility was a result of acquired ]166[ (include infectious and/or environmental) factors rather than genetic factors ]171[.

Menstrual cycle in 85 positive infertile females (table 4-4 and 4-13) was regular in 48 (56.47%) of them, while 37 (43.53%) females were with irregular cycles. These result agreed with limited studies in mice ]15[, and human ]206[; these studies suggested that *T. gondii* was responsible of hypogonadotrophic hypogonadism secondary to hypothalamic-pituitary dysfunction.

Table (4-5) demonstrated that the percentage of positive infertile women with history of one abortion were 17.64%, and those with past frequent abortion were 18.83%. These results approximately agreed with the study of Al-Doori ]81[ who showed that percentage of infected women who suffered from one abortion were 23.5%, two abortions were 17.6% and three / more abortions were 5.9%.

The interpretation of these results is that chronic inflammation leads to functional intrauterine abnormalities and to reduction in endometrial receptivity, which negatively affects the process of embryo implantation and its early development ]13[They are in particular the cases of chronic infections -toxoplasmosis, listeriosis, brucellosis, rubella, cytomegalovirus and herpes virus ]14[.

There were 9.42% of positive infertile women who had one successful pregnancy and 2.35% of them had frequent (twice or more) previous pregnancies, while the remaining women (88.23%) suffered from sterility without past pregnancy.

These results can be explained in the following way: if the infection occurs during thelate stages of pregnancy, the strong Th2 bias and the diminishedNK cells, macrophages, and CD8+ T-cells function may facilitate parasite survival, it is unlikely to induce abortion. Conversely, there is the likelihoodthat the Th1 response induced early during *T. gondii* infectionwill induce abortion early in pregnancy] 234[.

Table (4-6) showed that out of 131 positive infertile patients (males and females), there were only 16 (12.21%) of females who had been treated with antibiotic for toxoplasmosis, 46 (35.12%) patients took hormonal treatment, 13 (28.27%) of them were males and 33 (38.83%) were females. These results demonstrate that although those patients were under antibiotic treatment against toxoplasmosis, there was no response of this antibiotic (mostly spiramycin). The results agreed with the study of Al-Doori ]81[ who found that after one month of treatment with spiramycin, there was no improvement in the 15 infected couples by using latex agglutination test, but there was a slightly response to these antibiotics by using ELISA (IgG).

In spite of hormonal management in those 46 (35.12%) infected infertile patients, there was an obvious disturbance in testosterone in males, FSH and LH in females.

As observed in table (4-7), out of 46 positive males, 43 (93.47%) of them were with low levels of testosterone, while only one (2.18%) had the high levels. The result with low testosterone levels was in agreement of Kankova *et.al* ]10[ results in Czech Republic. They showed that *Toxoplasma*-infected mice, both females and males, had lower serum testosterone concentration than controls. This can be ascribed to the fact that the decrease of testosterone concentration could be an adaptive response of infected mice to *Toxoplasma*-induced immunosuppression. By decreasing the concentration of testosterone, the infected mice could partly compensate the latent toxoplasmosis-associated down-regulated cellular immunity.

These results are also similar to the Barakat results ]235[ in Egypt, who showed that testosterone levels in the serum of infected rabbits was highly decreased in comparison with that of the controlled group from the first week till the end of experiment.

The results disagreed with study of Shirbazou *et.al* ]228[; their results showed a direct relation between the raise of *Toxoplasma* infection and cortisol, and testosterone increases in both men and women.

Male patient's results in the study agreed with Oktenli *et.al* ]236[ in their study among men. They showed that nine of 40 patients (22.5%) had lower levels of sex hormones (FSH, LH, FT and TT) than normal. A chief symptom of these patients at presentation was diminished sex drive and erection. They showed also that IL-1β levels were found to be higher in group B (patients with low sex hormone levels) than group A (patients with normal sex hormone levels). The levels of IL-1β correlated significantly in a negative manner with FSH, LH, FT and TT in all patients with acute toxoplasmosis ]236[.

Table (4-7) demonstrates that out of 85 positive infertile females, 22 (25.89%) females were with low FSH levels and 10 (11.72%) females were seen with high levels. In 19 (22.35%) females LH levels were low, while in 14 (16.17%) females the levels were upper than the normal (high). These result agreed with Stahl *et.al* ]202[ who demonstrated that Nya:NYLAR female mice undergo acquired hypogonadotrophic hypogonadism secondary to hypothalamic dysfunction within a few weeks after infection with *T. gondii*.

The our results are such because high concentrations of testosterone are known to have immunosuppressive effects ]234[, so the host (mice ]10[, rabbits ]235[ or human ]236[) lowers the levels of this hormone in serum by increasing the concentration of some cytokines like interferon-γ (IFN-γ), interleukin-1β (IL-1β) ]236[ which induce modulation of hypothalamic GnRH release and lead to hypogonadotrophic hypogonadism.

Table (4-8) shows that out of 46 positive infertile men, males with abnormal morphology of sperms were 26 (56.52%), males with abnormal sperm number (concentration per ml) were 31 (67.39%) and those with abnormal sperm motility were 34 (73.91%). These results nearly agreed withresults ofTerpsidis *et.al* ]11[ who observed in Wistar rats males (Rattus norvegicus) that motility and concentration of sperm were significantly decreased in the infected group compared to controls; and a remarkable elevation up to 30% of spermatozoa abnormalities (bent tail, lost of hook shape, head lost, double head and cytoplasmic droplet) was noticed in infected group ]11[.

The results also agreed with Barakat results ]235[which observed that pronounced histopathological testicular changes were necrosis and disappearance of epithelial lining of almost all the seminiferous tubules in mature male New Zealand rabbits after inoculation subcutaneously with 150,000 tachyzoites of *T. gondii*.

The study ofQi *et.al* ]9[ found pathological changes in the testes, epididymis, vas deferens, prostate and thalamus of male mice with experimentally-induced acute *T. gondii* infection and they concluded that acute infection can cause infertility.

According to table (4-8), the abnormality of sperm motility, count, and morphology was more than normal with highly significant in motility, significant in count, and non significant in morphology. These results indicate that *T. gondii* may cause direct or indirect defect in sperm quality which is the main cause of infertility in men.

Table (4-9) demonstrates the relation between abnormal testosterone level in the serum of infected males and the abnormality in motility, morphology and count of sperms in their seminal fluids.

Statistically, this table showed that (C.S.) was non significant (NS) between lower bound testosterone and abnormal sperms quality, but it was highly significant (HS) between upper bound testosterone and abnormality in sperms.

Medically, these results are very important; the (NS) and (HS) here indicate that low testosterone levels have an influence on sperm shape, count, and motility in infected infertile males, and there is no influence in the case of high testosterone level.

These results are supported by studies which carried out in mice, rats, and rabbits]10, 11, 235 [, but in human, up to our knowledge, there is no study correlating between abnormal sperms quality and testosterone disturbance in the infertile men who had been afflicted with Toxoplasmosis.

According to table (4-10), we observed that positive females with polycystic ovaries were 25 (29.41%), females with one follicle cyst were 4 (4.71%), females with enlarged ovaries were 28 (32.94%), and females who had small size ovaries were 4 (4.71%). This observation is in line with the previous results of experimental studies in animal (mice) only, and there is no similar study in human up to the date.

The study of Antonios *et.al*]237[ used laboratory bred female mice to study the effect of chronic toxoplasmosis on reproductive performance, and they found that -after two months post infection (p.i.)- the histopathological examination of the ovaries revealed impaired folliculogenesis and atropic degeneration, the supraoptic and paraventricular hypothalamic nuclei were deformed and showed pyknotic neurons.

Additionally, Stahl *et.al*(1994)]202[ found that female mice bred 1 month (p.i.) exhibited reproductive failure, impaired folliculogenesis, and uterine atrophy was marked and vaginal lavage showed cessation of estrus cycling and constant diestrus cytology. Coronal sections of the cerebrum disclosed widespread vasculitis, focal disruptions of the ependymal cell layer lining the lateral and third ventricles, and periventricular edema ]202[.

Studies carried out by Antonios *et.al*]237[ and Stahl *et.al* ]202[ concluded that the reproductive failure of infected mice was due to hypogonadotrophic hypogonadism secondary to hypothalamic dysfunction as a result of chronic toxoplasmosis.

The study of Stahl *et.al* (1995) ]15[ hypothesized that cytokines released peripherally in response to the parasite reached the hypothalamus and initiated a sequence of events that inhibited the pulsatile release of gonadotropin-releasing hormone (GnRH), leading to the subsequent impairment of the pituitary-ovarian axis.

Tables (4-11), (4-12) and (4-13) shows the relationship between FSH, LH disturbance and abnormal changes (size and cysts) in ovaries as well as irregularity in menstrual cycle. There was no similar study recording all these disorders in positive infertile females.

It was suggested that a variety of parasitic and host factors, as well as unrecognized cofactors, may influence disease presentations in toxoplasmosis ]238[. Patients with toxoplasmosis had significantly higher levels of IL-1β, IFN-γ and MIP-1α than those in the controlled subjects ]236[. Consequently, cytokine-induced inflammatory reactions and edema in the hypothalamic region seem to be the most factors responsible for GnRH deficiency: central disruption of the pulsatile release of hypothalamic GnRH, leading to inadequate pituitary priming, depletion of gonadotrophin reserves, and perturbation of the release of gonadotrophins from the pituitary ]236[.

**5.1Conclusions**

1. According to the results, 46 (41.81%) males and 85 (77.27%) females were positive for toxoplasmosis. Thus, we concluded that the infertile females were more susceptible to get *Toxoplasma* infection than infertile males.
2. The present study found that out of 110 infertile couples, 39 (35.45%) men were sharing their women in *Toxoplasma* infection. These results indicate another route of *T. gondii* transmission which is sexual intercourse between couples or partners.
3. The higher infection rate in males and females was in the age group (35-39) years, and the positive females were higher in younger ages than males.
4. Toxoplasmosis causes infertility after single or multiple abortions and successful pregnancies in women, depending on the period of infection in different stages of pregnancy.
5. *T. gondii* cause disorder in hypothalamic-pituitary-gonadal axis in males and females. So, the disturbance in testosterone levels will influence the sperms quality in the infected men. In the same line, the abnormal fluctuation in FSH and LH levels may cause changes in the size and structure of ovaries especially PCOS in the infected women.
6. Finally*, T. gondii* considers as one of the reasons for infertility (either primary or secondary) in males and females as a result of direct or indirect action of this parasite.

**5.2 Recommendations**

1. Investigating about *T. gondii* Abs in infertile men as a routine test in the IVF and fertility centers and treating the positive cases.
2. The semen of infertile men must be examined for this parasite before using to fertilize the eggs in case of ART (e.g. ICSI, IUI … etc.).
3. It is very important to carry out studies to confirm the transmission of *T. gondii* from male to female and vice versa during coitus in couples.
4. Making more advanced studies to develop vaccine for *T. gondii* infection in human.
5. Several immunological and molecular researches are needed to indicate the susceptibility and protectivity factors of toxoplasmosis.

**References**

1. **Ryan, K.J. and Ray C.G. (2004)**. Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 723–7.

2.**Sukthana, Y.** (**2006).** ["Toxoplasmosis: beyond animals to humans"](http://linkinghub.elsevier.com/retrieve/pii/S1471-4922(06)00023-7). Trends Parasitol. 22 (3): pp.137–42.

3. **Edwar, K. M. ;David, T. J. and Wojcech, A. K. (1999)**. The blood and Tissue Dwelling protozoa. Madical parasitology 8th ed. WB Sunders company, USA. pp. 160 – 170.

4. **Li, S.; Cui, L.; Zhao, J.; Dai, P.; Zong, S.; Zuo, W.; Chen, C.; Jin, H.; Gao, H., and Liu, Q. (2011).** Seroprevalence of *Toxoplasma gondii* Infection in Female Sterility Patients in China. Journal of Parasitology, 97 (3) pp. 529-530.

5. **Aral, A.G; Elhan, H.A.and Akarsu, C. (2011)**. Retrospective evaluation of *Toxoplasma gondii* seropositivity in fertile and infertile women. Microbiyol. Bul., 45(1): pp. 174-180.

6. **Goldenberg, R.L. and Thompson, C.** **(2003)** **.The infectious origins of stillbirth.** Am. J. Obstet. Gynecol*.,* **189:** pp. 861-873.

7. **Gibbs, RS. (2002) .The origins of stillbirth: infectious diseases.** Semin Perinatol., **26:** pp. 75-78.

8. **Zhou, Y.H. ; Lu, Y.J. ; Wang, R.B. ; Song, L.M. ; Shi F. ; Gao, Q.F. ; Luo, Y.F. ;Gu, X.F. and Wang, P. (2002).** Survey of infection of *Toxoplasma gondii* in infertile couples in Suzhou countryside (In Chinese with English abstract). Zhonghua Nan Ke Xue 8, pp. 350– 352.

9. **Qi, R.; Su, X.P.; Gao, X.L. and Liang, X.L. (2005).** *Toxoplasma* infection in males with sterility in Shenyang, China. (In Chinese with English abstract). Zhonghua Nan Ke Xue 11, pp. 503–504.

10. **Kankova, S.; Kondym, P. and Flegr, J. (2011).** Direct evidence of *Toxoplasma* induced changes in serum testosterone in mice. Expermental-parasitology 128, pp. 181-183.

11. **Terpsidis, K. I. ; Papazahariadou, M. G. ; Taitzoglou, I. A. ; Papaioannou, N. G. ; Georgiadis, M. P. and Theodoridis, I. T. (2009).** *Toxoplasma gondii*: Reproductive parameters in experimentally infected male rats. Exp. Parasitol., 121 (3): pp. 238–241.

12. **Řežábek, K. (2008).** Infertility Treatment.4th Edition, Praha, CzR: Grada Publishing, 102.

13. **Hudeček, R.; Krajčovičová, R. and Ventruba, P. (2009).** Pharmacological possibilities of embryoprotective therapy of infertile women. Klin. Farmakol. Farm., 23(2): pp. 76 – 82.

14. **Pavlinová, J.; Kinčeková, J.; Ostró, A.; Saksun, L.; Vasilková, Z. and Königová A. (2011).** Parasitic infections and pregnancy complications. Parasitological Institute of SAS, Košice, Helminthologia, 48, (1): pp. 8 – 12.

15**. Stahl ,**[**W.**](http://www.springerlink.com/content/?Author=W.+Stahl) **; Dias,** [**J. A.**](http://www.springerlink.com/content/?Author=J.+A.+Dias)  **; Turek ,**[**G.**](http://www.springerlink.com/content/?Author=G.+Turek)  **and Kaneda ,**[**Y.**](http://www.springerlink.com/content/?Author=Y.+Kaneda)  **(1995).** Etiology of ovarian dysfunction in chronic murine toxoplasmosis. [Parasitology Research](http://www.springerlink.com/content/0932-0113/), [81(2](http://www.springerlink.com/content/0932-0113/81/2/)): pp. 114-120.

16. **Dubey, J.P. and Sharma, S.P. (1980).** Prolonged excretion of *Toxoplasma gondii* in semen of goats. American Journal of Veterinary Research 41, pp. 794–795.

17. **Aganga, A.O.; Umoh, J.U.; Kyewalabye, E.K., and Ekwempu, C.C., (1988).** Comparative experimental transmission studies with Nigerian isolates and TS-I strain of *Toxoplasma gondii* in sheep. Journal of Animal Production Research 8, pp. 104–120.

18. **Moura, A.B.; Costa, A.J.; Filho, S.J. ; Paim, B.B. ; Pinto, F.R. and Di Mauro, D.C. (2007).** *Toxoplasma gondii* in semen of experimentally infected swine. Pesquisa Veterinaria Brasileira. 27, pp. 430–434.

19. **De Paepe, M.E.; Guerrieri, C., and Waxman, M. (1990).** Opportunistic infections of the testis in the acquired immunodeficiency syndrome. Mount Sinai Journal of Medicine 57, pp. 25–29

20. **Janitschke, K., and Nürnberger, F., (1975).** Studies on the significane of sexual intercourse for the transmission of *Toxoplasma gondii*. Zentralblatt fur Bakteriologie 231, pp. 323–332.

21. **Arantes TP; Lopes WD; Ferreira RM et al., (2009).** *Toxoplasma gondii*: evidence for the transmission by semen in dogs*,* Exp Parasitol. 123: pp. 190–194.

22. **Stanley Medical Research Institute (SMRI).** Toxoplasmosis–Schizophrenia Research, SMRI report. (Last updated July **2011**). Available from: http//www.stanleylab.org//.

23. **Dubey, J. P. (2008).** The History of *Toxoplasma gondii*—The First 100 Years. J. Eukaryot. Microbiol., 55:(6), pp. 467–475.

24. **Ajioka, J. W. and Soldati, D. (2007).** Preface. In: **Ajioka, J. W. and Soldati, D.** (ed.),*Toxoplasma* Molecular and Cellular Biology. Horizon. Bioscience Norfolk, UK. pp. 13–18.

25. **Nicolle, C. and Manceaux, L. (1909).** Sur un protozoaire nouveau du gondi. C. R. Seances Acad. Sci., 148: pp. 369–372.

26. **Dubey, J. P. (2007).** The history and life cycle of *Toxoplasma gondii*. In:Weiss, L. M. & Kim, K. (ed.), *Toxoplasma gondii*. The Model Apicomplexan: Perspectives and Methods. Academic Press, New York. pp. 1–17.

27. **Splendore, A. (1908).** Un nuovo protozoa parassita de’ conigli. Incontrato nelle lesioni anatomiche d’une malattia che ricorda in molti punti il Kala-azar dell’ uomo. Nota preliminare pel. Rev. Soc. Scient. Sao Paulo, 3: pp. 109–112. Cited by **Dubey, J. P. (2008).** The History of *Toxoplasma gondii*—The First 100 Years. J. Eukaryot. Microbiol., 55:(6), pp. 467–475.

28. **Dubey, J. P. (2002).** A review of toxoplasmosis in wild birds. Vet. Parasitol., 106: pp. 121–153.

29. **Wolf, A.; Cowen, D. and Paige, B. (1939).** Human toxoplasmosis: occurrence in infants as an encephalomyelitis verification by transmission to animals. Science, 89: pp. 226–227. Cited by (23).

30. **Sabin A.B. and Feldman H.A. (1948).** Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (Toxoplasma) Science. 108: pp. 660–3. Cited by (23).

31. **Frenkel, J. K. (1967).** Adoptive immunity to intracellular infection. J. Immunol., 98: pp. 1309–1319.

32. **Gazzinelli, R. T.; Hakim, F. T.; Hieny, S.; Shearer, G. M. and Sher, A. (1991).** Synergistic role of CD41 and CD81 T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. J. Immunol., 146: pp. 286–292.

33. **Suzuki, Y.; Orellana, M. A.; Schreiber, R. D. and Remington, J. S. (1988).** Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. Science, 240: pp. 516–518.

34. **Khan, A.; Taylor, S.; Su, C.; Mackey, A. J.; Boyle, J.; Glover, R. D.; Tang, K.; Paulsen, I. T.; Berriman, M.; Boothrooyd, J. C.; Pfefferkorn, E. R.; Dubey, J. P.; Ajioka, J. W.; Roos, D. S.; Wootton, J. C. and Sibley, L. D. (2005).** Composite genome map and recombination parameters derived from three archetypal lineages of *Toxoplasma gondii*. Nucleic Acids Res, 33: pp. 2980–2992.

35. **Dubey, J. P.; Lindsay, D. S. and Speer, C. A. (1998).** Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin Microbiol Rev. 11: pp. 267–299.

36. **Rorman, E.; Zamir, C.; Rilkis I., and Ben-David, H. (2006).** Congenital toxoplasmosis—prenatal aspects of *Toxoplasma gondii* infection. Reproductive Toxicology 21, pp. 458–472.

37. **Ferguson, T.R. (2002).** Apicomplexa .J.Parasitol ,18: p. 355

38**. Frenkel J. K. (1973).** Toxoplasma in and around us. BioScience. 23: pp. 343–352.

39. **Melhorn, H. and Frenkel J. K. (1980).** Ultrastructural comparison of cysts and zoites of Toxoplasma gondii, Sarcocystsis muris, and Hammondia hammondi in skeletal muscle of mice. J Parasitol. 66: pp. 59–67.

40. **Cornelissen, A. W.; Overdulve, J. P. and Hoenderboom, J. M. (1981).** Separation of Isospora (Toxoplasma) gondii cysts and cystozoites from mouse brain tissue by continuous density-gradient centrifugation. Parasitol. 83: pp. 103–108.

41. **Brown, C. R.; Hunter, C. A.; Estes, R. G.; Beckmann, E.; Forman J.; David, C.; Remington, J. S. ,and McLeod, R. (1995).** Definitive identification of a gene that confers resistance against Toxoplasma cyst burden and encephalitis. Immunology. 85: pp. 419–428.

42. **Pappas, P. W. and Wardrop , S. M. (1999) .** Toxoplasmosis in adult. New Engl. J. Med.; 298: pp. 550-553.

43. **Banos Zsuzsa, (2011).** Protozoan. Teaching pathology, Cambridge academy Report. UK. Available from: http/www.iwf.de.com.

44. **Frenkel J.K.** **(2000).** Biology of Toxoplasma gondii. In: **Ambroise-Thomas P,and Peterse E, editors.** Congenital toxoplasmosis: scientific background, clinical management and control. Paris: Springer-Verlag;. pp. 9–25.

45. **Black M.W., and Boothroyd J.C. 2000.** Lytic cycle of *Toxoplasma gondii*. Microbiol Mol Biol Rev. 64: pp. 607-23.

46. **Montoya, J.G., and Liesenfeld, O. (2004).** Toxoplasmosis. Lancet. 363: pp. 1965-76.

47. **Courret, N.; Darche, S.; Sonigo, P.; Milon, G.; Buzoni-Gatel D.,and Tardieux I. (2006).** CD11c- and CD11b-expressing mouse leukocytes transport single *Toxoplasma gondii* tachyzoites to the brain. Blood. 107: pp. 309-16

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| 48. **Silveira, C.; Vallochi, A.L.; Rodrigues da Silva, U.; Muccioli, C.; Holland, G.N.,and Nussenblatt, R.B., *et al*. (2011).** *Toxoplasma gondii* in the peripheral blood of patients with acute and chronic toxoplasmosis. Br. J. Ophthalmol., 95: pp. 396-400.  49. **Dubey J.P. (1998).** Toxoplasmosis, sarcocystosis, isosporosis, and cyclosporosis. In: **Palmer SR, Soulsby EJL, Simpson DJH**, editors. Zoonoses. Oxford: Oxford University Press. pp. 579–97.  50. [**Tenter,A.**](http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=PubMed&term=%20Tenter%2BAM%5bauth%5d) **M. ; Heckeroth, A. R., and Weiss L. M. (2000).** Toxoplasma gondii: from animals to humans. Int J Parasitol., 30: (12-13), pp. 1217–1258.  51. **Jones, J.L.; Ogunmodede, F.; Scheftel, J.; Kirkland, E.; Lopez, A.; Schulkin, J., and Lynfield, R. 2003.** Toxoplasmosis-related knowledge and practices among pregnant women in the United States. Infect Dis Obstet Gynecol 11, pp. 139-145.  52. **Centers of Disease Control and Prevention (2004).** ["Toxoplasmosis"](http://www.dpd.cdc.gov/DPDx/HTML/Toxoplasmosis.htm) Available from: <http://www.dpd.cdc.gov/DPDx/HTML/Toxoplasmosis.htm>.  53**. Jones J.L.,and Dubey J.P. (2010).** Waterborne toxoplasmosis-recent developments. Exp. Parasitol.124: pp. 10-25.  54. **Dubey J.P. (2010).** Toxoplasmosis of animals and humans*.* Second edition. Boca Raton, Florida: CRC Press.  55. **Drake, C. L., (1998).** Toxoplasmosis . In : **Britlman,** **J. C. and Quenzer , R. W.** (eds) . Infectious disease in emergency medicine. 5th ed. pp. 650 – 653. Philadelphia, New York.  56. **Bonametti, A. M.; Passos, J. N.; Koga de Silva , E. M. and Macedo , Z. S. (1997) .** Probable transmission of acute Toxoplasmosis through breast feeding. J. Trop. Ped.; pp. 43: 116.  57. **Montoya, J.G., and Liesenfeld, O. (2004**). Toxoplasmosis. Lancet*,*363(9425): pp. 1965-76  58. **Dubey, J.P. and Beattie C.P. (1988).** Toxoplasmosis of animals and man. Boca Raton, FL: CRC Press.  59. **Canadian Laboratory Centre for Disease Control. (2001).** Material Safety Data Sheet – *Toxoplasma gondii*. Office of Laboratory Security. Available at: <http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/index>.  60. **Hill, D., and Dubey, J. P. (2002).** *Toxoplasma gondii*: transmission, diagnosis and prevention. Clin. Microbiol. Infect., 8: pp. 634–640  61. **Montoya, J.G., and Remington, J.S. (2008).** Management of *Toxoplasma gondii* infection during pregnancy. Clin. Infect. Dis. 47: pp. 554-66.  62. **Kravetz, J.D., and Federman, D.G. (2005).** Toxoplasmosis in pregnancy. Am. J. Med. 118(3): pp. 212-6. ([Abstract](http://pubmed.gov/15745715))  63. **Singh, S. 2003.** Mother-To-Child Transmission And Diagnosis Of *Toxoplasma Gondii* Infection During Pregnancy. Indian Journal of Medical Microbiology, 21 (2): pp. 69-76.  64. **Villena, I.; Chemla, C.; Quereux, C.; Dupouy, D.; Leroux, B.; Foudrinier, F., and Pinon J.M. (1998).** Prenatal diagnosis of congenital toxoplasmosis transmitted by an immunocompetent woman infected before conception. Reims Toxoplasmosis Group. Prenat. Diagn. 18(10): pp. 1079-81.  65**. Remington, S. J.; McLeod, R.; Thulliez, P., and Desmonts, G. (2001).** Toxoplasmosis. In: Remigton, J. S.; Klein, J.O(editors). Infectious diseases of the fetus and newborn infant. Philadelphia, WB Sauders Company, pp. 205-346.  66. **Vaz, R.S.; Rauli, P.; Mello, R. G., and Cardoso, M. A. (2011).** Congenital Toxoplasmosis: A Neglected Disease? – Current Brazilian public health policy. Field Actions Science Reports[Online], Special Issue 3, Online since 02 November 2011. URL: <http://factsreports.revues.org/1086>.  67. **Macattie, C. (1938).** Notes on two cases of naturally occurring Toxoplasmosis in Baghdad. Tran. Roy. Soc. Trop. Med. Hyg. 23: pp. 373-376.  68. **Najim, A.T., and AL-Saffer, G. (1963).** Sensitivity of Iraqi's to the *Toxoplasma* intradermal test. Z. Trop. Parasitol. 14: pp. 399- 401.  69. **Najim , A.T. ; AL-Saffer ,G., and Chali ,H. (1968).** Some aspects of Toxoplasmosis in gynecology in Iraq, fol. Parasite (praha ) 15 : pp. 283- 286.  70. **Tawifiq, H.S. (1983).** Preliminary study for Toxoplasmosis in pregnancy, Bull. End. Dis. 22: pp. 63-86.  71. **Niazi, A.D.; Omar, A.R.; AL- Hadithi, T. S., and Aswad, A. (1988).** Prevalence of Toxoplasmosis in pregnant women in Iraq J. Fac. Med. Baghdad 30: pp. 323- 333.  72. **Flaih, G.H. (1993).** Study of Toxoplasmosis among Iraqi woman with history of abortion. Diploma thesis, Saddam college of Medicine (now, college of Medicine, Al-Nahrain University).  73. **Khitam, Y.O.AL-Dujaily (1998).** A seroepidemology study of Toxoplasmosis among aborted woman in Baghdad M.Sc. thesis. Veterinary Medicine, University of Baghdad.  74. **Al-Timimi, R.L., (2004)**. Detection of Toxoplasmosis among different groups of aborted women during gestational age of pregnancy, Diploma, thesis, College of Health and Medical Technology, Foundation of Technical Education.  75. **Al-Dalawi, N.kh, (2007).** Hormonal Disturbances in suddenly and previously aborted women afflicted with Toxoplasmosis in Baghdad Province. M.Sc. thesis College of Health and Medical Technology, Foundation of Technical Education  76. **Suhaila, S.K. (2008)**. Prevalence, serodiagonosis and some immunological aspects of toxoplasmosis among women in Baghdad province. M.Sc. thesis, College of Health and Medical Technology, Foundation of Technical Education.  77. **Sabah S.M. and Mahfoth S. H. (2009)**. Seroprevalence of toxoplasmosis among Schizophrenic patients. Yemeni Journal for Medical Sciences, 1(3): pp. 1-6.  78. **Dargham, B.M. (2011)**. Prevalence of Toxoplasmosis, and Laboratory Serological Diagnosis and some Haematological and Biochemical Tests in infected women in Al-Najaf Province. M.Sc. thesis, College of Health and Medical Technology.  79. **Abdul-Razzaq, M. AL-Jubori (2005).**Serological diagnosis of Toxoplasmosis in Kirkuk province. M.Sc thesis, College of Health and Medical Technology.  80. **Yacoob, A.A.H. ; Bakr, S. ; Hameed , A.M.; AL-Thamery, A.A., and Fartoci, M.J. (2006) .**Seroepidemilogy of selected zoonotic infection in Basrah region of Iraq , Eastren Mediterranean Health J. 12(1): p.82.  81. **Al-Doori, M. A. M. (2010).** Epidemiology study of *Toxoplasma gondii* between couples in Tikrit city, and experimental trial about possibility of sexual transmission of infection in mice type *Mus musculus*. M.Sc thesis, College of Education, University of Tikrit.  82. **Abu-Madi M. A.; Behnke, J. M., and Dabritz, H. A. (2010).***Toxoplasma gondii* Seropositivity and Co-Infection with TORCH Pathogens in High-Risk Patients from Qatar.Am. J. Trop. Med. Hyg.82(4): pp. 626–633.  83. **Qublan, H.S.; Jumaian, N.; Abu-Salem, A.; Hamadelil, Y.; Mashagbeh, M., and Abdel-Ghani F. (2004).** Toxoplasmosis and habitual abortion. J. Obstet. Gynaecol. 22(3): pp. 296-8.  84. **Behbehani, K., and Al-Karmi, T. (1980).** Epidemology of Toxoplasmosis in Kuwait. Transactions of the Royal Society of Tropical Medicine and Hygiene; 74: pp. 209 – 212.  85. **Yousif , A. A. ; Wallace , M. R., and Baig , B. H. (1990).** Prenatal serologic screening in Bahrain. Scand. J. Infec. Dis. 23: pp. 781 – 783.  86. **Abdel – Hammed, A. A. (1991).** Seroepidemology of Toxoplasmosis in Sudan. J. Trop. Med. Hyg. 5: pp. 329 – 232.  87. **Mohammed, H. M.; Bener, A.; Uduman, S. A., and Al-Karme, M. D. (1998).** Toxoplasmosis antibody prevalence among healthy adults in United Arab Emirates. Saudi Med. J. 19 (3): pp. 8-10.  88. **Soliman, M.; Nour-Eldin, M. S.; Elnaggar, H.M.; El-Chareb, M. E., and Ramadan, N. E. (2001).** *Toxoplasma* antibodies in normal and complicated pregnancy. J. Egypt. Soc. parasitol. 31: pp. 37-46.  89. **Al-Harthi, S.A.; Jamjoom, M.B., and Ghazi, H.O. (2006)**. Seroprevalence of *Toxoplasma gondii* among pregnant women in Makkah, Saudi Arabia. Umm Al-QuraUniversity. Journal of Science and Medical Engineering18:pp. 217 – 227.  90**. Abou-Daoud, K. and Schwab, C.W. (1960).** Sensitivity of Lebanon and Syrian's to *Toxoplasma gondii* and to a similar antigen prepared from L. tropica, Am.J.Trop. Med.Hyg. 9:158.  91. **Mousa, D.A.; Mohammad, M.A.,and Toboli, A.B.(2011).** *Toxoplasma gondii* infection in pregnant women with previous adverse pregnancy outcome. Medical Journal of Islamic World Academy of Sciences 19(2): pp. 95-102.  92. **Montoya J., and Liesenfeld O. (2004).** "Toxoplasmosis". Lancet 363(9425): pp. 1965–76.  93. **Jones, J.L.; Kruszon-Moran, D.; Sanders-Lewis, K., and Wilson, M. (2007).** "*Toxoplasma gondii* infection in the United States, 1999-2004, decline from the prior decade". Am. J. Trop. Med. Hyg. **77** (3): pp. 405–10.  94. **Klaren, V.N., and Kijlstra A. (2002).** Toxoplasmosis, an overview with emphasis on ocular involvement. Ocul. Immunol. Inflamm. 10: pp. 1-26.  95. **Shin, D.W.; Cha, D.Y.; Hua, Q.J.; Cha, G.H., and Lee YH. (2009).** Seroprevalence of *Toxoplasma gondii* infection and characteristics of seropositive patients in general hospitals in Daejeon, Korea. Korean J. Parasitol. 47: pp. 125-30.  96. **Xiao, Y.; Yin, J.; Jiang, N.; Xiang, M.; Hao, L., and Lu H, *et al*. (2010).** Seroepidemiology of human *Toxoplasma gondii* infection in China. BMC. Infect. Dis., 10:4.  97. **Fromont, E.G.; Riche, B., and Rabilloud, M. (2009).** *Toxoplasma* seroprevalence in a rural population in France: Detection of a household effect. BMC Infect Dis. 9:76.  98. **Cantos, G.A.; Prand, M.D.; Siqeira, M.V. and Ehavior, R.M. (2000)**. Toxoplasmosis: occurrence of antibodies anti *Toxoplasma gondii* and diagnosis. Rev. Assoc. Med. Bras.; 46: pp. 335-41.  99. **Abdi, J.; Shojaee, S.; Mirzaee, A. and Keshavarz, H. (2008).** Seroprevalence of toxoplasmosis in pregnant women in Ilam Province, Iran. Iranian J. Parasitol. 3(2): pp. 34-7.  100. **Akyar**, **I.** **(2011).** Seroprevalence and Coinfections of *Toxoplasma gondii* in Childbearing Age Women in Turkey.Iranian J. Publ. Health. 40(1): pp.63-67.  101. **Lekutis, C.; Ferguson, D.J., and Boothroyd J.C. (2000).** *Toxoplasma gondii*: identification of a developmentally regulated family of genes related to SAG2. Exp. Parasitol. 96: pp. 89–96.  102. **Barragan, A., and Sibley, L.D. (2003).** Migration of *Toxoplasma gondii* across biological barriers. Trends Microbiol. 11: pp. 426–30.  103. **Fekadu, A.; Shibre, T. and Cleare, A. J. (2010).** Toxoplasmosis as a cause for behaviour disorders—overview of evidence and mechanisms. Folia Parasitol. 57: pp. 105–113.   * 104. **Hermes, G.;** [**Ajioka, J.**](http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=PubMed&term=%20Ajioka%2BJW%5bauth%5d)**;** [**Kelly, K.**](http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=PubMed&term=%20Kelly%2BKA%5bauth%5d)**, and** [**Mcleod, R.**](http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=PubMed&term=%20McLeod%2BR%5bauth%5d) **(2008).** Neurological and behavioral abnormalities, ventricular dilatation, altered cellular functions, inflammation, and neuronal injury in brains of mice due to common, persistent, parasitic infection. J. Neuroinflammation 5, 48.   105. **Remington, J.S.; McLeod, R., and Desmonts, G. (1995).** Toxoplasmosis. In: Remington JS, Klein JO, eds. Infectious Disease of the Fetus and Newborn Infant. Philadelphia: W.B. Saunders Company. pp. 140–267.  106. **Luft, B. J., and Remington, J. S. (1992).** *Toxoplasmosis*. Clin. Infec. Dis. 15: pp. 211 – 222.  107. **LiesensFeld, O. (1999).** Immune response to *Toxoplasma gondii* in the gut. Immunology. 201 (2): pp. 229-239.  108. **Charles, T.; Bourguin, 1. ; Mevelec, M.N.; Dubrement J.F., and Bouth[, D. ; (1990).** Ab response to *T.gondii* in sera intestinal characterization of target Ags. Immune. 58(5): pp. 1240 -1244.  109. **Duriez, T.; Dujardin .L., and Afchain, D. (2002).** Toxoplasmosis laboratory of parasitology, Faculty of Lille pharmacy, pp. 8.  110. **Hussein, A.H.; Nagaty, I.M., and fouad, M.A. (2002).** Evalution of IgM ELISA versus PCR in diagnosis recent *T.gondii* infection . Jor. Egypt, Soc. Parasitol. 32(2): pp. 639-646.  111. **Petersen, E., and Dubey, J.P. (2001).** Biology of toxoplasmosis. In DHM Joynson, TG Wreghitt (eds.), *Toxoplasmosis*, Cambridge University Press, Cambridge. pp. 1-42.  112. **Lambert, H.; Hitziger, N.; Dellacasa, I.; Svensson, M., and Barragan, A. (2006).** Induction of dendritic cell migration upon *Toxoplasma gondii* infection potentiates parasite dissemination. Cell Microbiol. 8: pp. 1611-1623.  113. **Fuentes, I.; Rubio, J.M.; Ramírez, C., and Alvar, J. (2001).** Genotypic characterization of *Toxoplasma gondii* strains associated with human toxoplasmosis in Spain: direct analysis from clinical samples. J Clin Microbiol. 39: pp. 1566-1570.  114. **Kelly, C.D.; Russo, C.M.; Rubin, B.Y., and Murray, H.W. (1989).** Antigen stimulated human interferon-γ generation: role of accessory cells and their expressed or secreted products. Clin. Exp. Immunol. 77: pp. 397-402.  115. **Khan, I.A.; Matsuura, T., and Kasper, L.H. (1994).** Interleukin-12 enhances murine survival against acute toxoplasmosis. Infect. Immun. 62: pp. 1639-1642.  116. **Gazzinelli, R.T.; Heiny, S.; Wynn, T.A.; Wolf, S., and Sher, A. (1993).** Interleukin 12 is required for the T-lymphocyte-independent induction of interferon γ by an intracellular parasite and induces resistance in T-cell-deficient hosts. Proc. Natl. Acad. Sci. USA. 90: pp. 6115-6119.  117. **Liu CH, Fan YT, Dias A, Esper L, Corn RA, Bafica A, Machado FS, Aliberti J (2006).** Cutting edge: dendritic cells are essential for *in vivo* IL 12 production and development of resistance against *Toxoplasma gondii* infection in mice. J. Immunol. 177: pp. 31-35.  118. **Bliss SK, Marshall AJ, Zhang Y, Denkers EY (1999).** Human polymorphonuclear leukocytes produce IL-12, TNF-α and the chemokines macrophage-inflammatory protein-1 α and -1 β in response to *Toxoplasma gondii* antigens. J. Immunol. 162: pp. 7369-7375.  119. **Pepper M, Dzierszinski F, Wilson E, Tait E, Fang Q, Yarovinsky F, Laufer TM, Roos D, Hunter CA (2008).** Plasmacytoid dendritic cells are activated by *Toxoplasma gondii* to present antigen and produce cytokines. J. Immunol. 180: pp. 6229-6236.  120. **Scharton-Kersten TM, Yap G, Magram J, Sher A (1997).** Inducible nitric oxide is essential for host control of persistent but not acute infection with the intracellular pathogen *Toxoplasma gondii*. J. Exp. Med. 185: pp. 1261-1273.  121. **Remington JS, McLeod R, Thulliez P, Desmonts G (2000).** Toxoplasmosis. In JS Remington, JO Klein (eds.), Diseases of the fetus and newborn infant, W H Saunders Company, Philadelphia. pp. 205-346.  122. **Letscher-Bru V, Pfaff AW, Abou-Bacar A, Filisetti D, Antoni E, Villard O, Klein JP, Candolfi E (2003).** Vaccination with *Toxoplasma* *gondii* SAG-1 protein is protective against congenital toxoplasmosis in BALB/c mice but not in CBA/J mice. Infect. Immun. 71: pp.6615-6619.  123. **Mévélec MN, Bout D, Desolme B, Marchand H, Magné R, Bruneel**  **O, Buzoni-Gatel D (2005).** Evaluation of protective effect of DNA vaccination with genes encoding antigens GRA4 and SAG1 associated with GM-CSF plasmid, against acute, chronic and congenital toxoplasmosis in mice. Vaccine. 23: pp. 4489-4499.  124. **Tait, E. D., Hunter, C. A. (2009).** Advances in understanding immunity to *Toxoplasma gondii.* Mem. Inst. Oswaldo Cruz, Rio de Janeiro. 104(2): pp. 201-210.  125. **Scotts and Bailey (1993).** Parasitology. In: Ellen, J. B.; Lancer, R.;  Peterson. Phlidalephia. pp. 784-804.  126. **Aubert, D.; Foudrinier, F.; Villena, I.; Pinon, J. M. (1996).** PCR for diagnosis and follow up of two cases disseminated toxoplasmosis after kidney grafting. J. Clin. Microbiol. 34:pp. 134-137.  127. **Gustafsson K.; (1997).** Studies of Toxoplasmosis in hares and Capercaillic , Acta universitatis Agricultura suecia veterinary. 16: pp.114.  128. **Conley, F. K. and Remington, J. S. (1998).** *T. gondii* infection of the central nervous system: use of peroxidase anti-peroxidase method to demonstrate *Toxoplasma* in formalin fixed paraffin embedded tissue sections. Human Pathology. 12: pp. 877-82.  129. **Jayaram, CK. And Paniker (2002).** Miscellaneous sporozoa and Nlicrosrora. Text book of medical parasitology, 5th ed. Jaypee brothers Medical Publisher .pp.84.  130. **Remington, J.; McLeod, R. and Desmonts, G. (1995).** Toxoplasmosis: Infectious diseases of the fetus and newborn infant, 4th ed. pp. 140-266.  131. **Frenkel, JK. (1968).** Dermal hypersensitivity to *Toxoplasma* Ag (Toxoplasmin) proc. Soc. Exp. Biol. Med. 681: pp. 634-639.  132. **Prakash, O. and Chewdhrv, P. (1969).** Acomparison of Toxoplasmosis and apparently healthy Controls Indian. J. Med. Res. 57(1).  133. **Sabin, A.B. and Feldman, H.A. (1948).** Drugs as microscochemical indicator of immunity phenomenon affecting a protozoan parasite. Science 108: pp. 660-663.  134. **Fulton, J.O. and Turk, J.L. (1959).** Indirect agglutination test for *T.gondii* latent infection. 2: pp. 10600-10609.  135. **Feldman, H. A; (1996).** Laboratory methods in current use the Study of Toxoplasmosis. Advances in ophthalmology. 3: pp. 139.  136. **Hedman, K.; Lappalainen, M.; Seppala, I. and Makela, O. (1989).** Recent primary *Toxoplasma* infection indicated by a low avidity of specific IgG. J. Infect. Dis. 159: pp. 736-40.  137. **Candolfi, E.; Pastor, R.; Huber, R.; Filisetti, D. and Villard, O. (2007).** IgG avidity assay firms up the diagnosis of acute toxoplasmosis on the first serum sample in immunocompetent pregnant women. Diagn Microbiol. Infect. Dis. 58: pp. 83-8.  138. **Singh, S. (2003).** Mother-to-child transmission and diagnosis of *Toxoplasma gondii* infection during pregnancy. Indian Journal of Medical Microbiology. 21 (2):pp. 69-76.  139. **Wilson M.; Jones J.L. and McAuley J.B**. **(2003).** *Toxoplasma*. In: **Murray P.R.; Baron EJ; Jorgensen JH; Pfaller M.A. and Yolken R.H**. editors. Manuel of clinical microbiology, 8th ed. ASM Press. 2: pp. 1970–80.  140. **Sinjin; Zhuyin, C. and Ming, X. (2004)**. Fast Dipstick Dye  Immunoassay for Detection of IgG and IgM Antibodies of human Toxoplasmosis. Clin. and Diagnostic Lab. Immuno. 12(1): pp. 198-201.  141. **Wilson, M. and Remington, J.S. (1997)**. Evaluation of six commercial kits for detection of human immunologlobulin M antibodies *T.gondii* the FDA Toxoplasmosis Ab, HoC working group. J.Microbiol. 35: pp. 3112-3115.  142. **Burg, J.L.; Perelman, D. and Kasper, L.H. (1989).** Molecular analysis of the gene encoding the major surface antigen of *T.gondii* J.immunol. 141: pp. 3584-3591.  143. **Pinon, J.; Dumon, H. and Chemla, C. (2001).** Strategy for diagnosis of congenital toxoplasmosis: evaluation of methods. Comparing mothers and newborns and Standard methods for post-natal detection of immunoglobulin G, M and IgA antibodies. J. Clin Microbiol. 39: pp. 2267-2271.  144. **Thulleiz, P.; Daffos F. and Forestier, F. (1992).** Diagnosis of *Toxoplasma* infection in the pregnant women and the unborn child, current problems. Scand. J. Infec. Dis. 84: pp. 18-22.  145. **Berrebi, W. and Kobuch, E. (1994).** Toxoplasmosis in pregnancy. The lancet. 334: p. 950.  146. **Burg, J.L.; Grover, C.M.; Pouletty, P. And Boothroyd, J.C. (1989).** Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii,* by polymerase chain reaction. J. Clin. Microbiol. 27: pp. 1787–1792.  147. **Mahalakshima B.; Therese K.L.; Shyamala G.; Devipriya, U. and Madhavan, H.N. (2007).** *Toxoplasma gondii* detection by nested polymerase chain reaction in lens aspirate and peripheral blood leukocyte in congenitalcataract patients: The first report from a tertiary eye hospital in India. Curr. Eye Res. 32, pp. 653–657.  148. **Nagy B.; Lázár L.; Nagy G.; Bán Z. and Papp Z. (2007).** Detection of *Toxoplasma gondii* in amniotic fluid using quantitative real-time PCR method. Orv. Hetil. 148, pp. 935–938.  149. **Makar, R.S. and Toth, T.L. (2002).** "The evaluation of infertility". Am. J. Clin. Pathol. 117 Suppl: S. pp. 95–103.  150. **Cooper, T.G.; Noonan, E. and von Eckardstein, S*.* (2010).** "World Health Organization reference values for human semen characteristics". Hum. Reprod. Update. 16(3): pp. 231–45.  151. **Fertility: Assessment and Treatment for People with Fertility Problems.** London: RCOG Press. 2004. [ISBN](http://en.wikipedia.org/wiki/International_Standard_Book_Number) [1-900364-97-2](http://en.wikipedia.org/wiki/Special:BookSources/1-900364-97-2).  152. **Bamigbowu, E. O. and Adegoke, O. A. (2005):** Female infertility, diagnosis and management. Nigeria Biomedical Science Journal.1(4): pp. 121 – 130.  153. **World Health Organization (2004).** Infecundity, infertility, and childlessness in developing countries. DHS Comparative Reports No 9. Calverton, Maryland, USA: ORC Macro and the World Health Organization.  154. **Sciarra J.** **(1994).** Infertility: an international health problem. Int J Gynaecol. Obstet*.* 46 : pp. 155-63  155. **Fathalla, M.F. (1991).** Reproductive health: a global overview. Ann. NY. Acad. Sci*.* 626*:* pp. 1-10.  156. **van Balen F. and Gerrits T. (2001)**. Quality of infertility care in poor-resource areas and the introduction of new reproductive technologies. Hum. Reprod. 16*:* pp. 215-9.  157. **Ombelet, W.; Cooke, I.; Dyer, S.; Serour, G. and Devroey, P.** **(2008).** Infertility and the provision of infertility medical services in developing countries. Hum. Reprod. Update. 14: pp. 605-21.  158. **Khan, Khalid; Janesh K. Gupta and Gary Mires (2005).** Core clinical cases in obstetrics and gynaecology: a problem-solving approach. London: Hodder Arnold. pp. 152.  159. **Altmäe, S.; Stavreus-Evers, A.; Ruiz, J.; Laanpere, M.; Syvänen, T.; Yngve, A.; Salumets, A. and Nilsson, T. (2010).** "Variations in folate pathway genes are associated with unexplained female infertility". Fertility and sterility. 94(1): pp. 130–137.  160. **Feng, H.L. (2003).** Molecular biology of male infertility. Arch. Androl. 49: pp. 19-27.  161. **World Health Organization (2010).** WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 5th ed. Cambridge: Cambridge University Press. Available at:  http://www.who.int/reproductivehealth/publications/infertility/9789241547789/en/index.html  162. **G.R. Dohle; K. Zsolt; A. Jungwirth; T. Diemer; A. Giwercman and C. Krausz (2004).** Guidelines for the investigation and treatment of male infertility. Eur. Urol. 46(5): pp. 555-8.(Text update April 2010) Available at: <http://www.uroweb.org>.  163. **Kalbasi S. (2011). Endocrine aspects of infertility.** Iranian Journal of Reproductive Medicine, Vol.9, Suppl.1.( Abstract)  164. **Speroff, L.; Glass, R.H. and Kase, N.G. (1994).** Female infertility in: Clinical Gynecologic Endocrinology and Infertility*.* Speroff, L, Glass, R.H., and Kase, N.G. (eds) 6th ed. Lippincott, Williams and Wilkins Philadelphia, U.S.A.  165. **Solomon, B.A.; Adegoke, O. Adebayo; Bamigbowu, E. Olugbenga; Ayodele, Martins, B. O. (2011).** Gonadotrophic Hormones, Progesterone and Prolactin Levels among Infertile Women Attending University of Port Harcourt Teaching Hospital. 57(2): pp.366-372. Available at: <http://www.eurojournals.com/ejsr.htm>  166. <http://www.fertilityfaq.org/_pdf/magazine1_v4.pdf> .(Internet)  167. **Abha Maheshwari; Mark Hamilton and Siladitya Bhattacharya. (2009).** Effect of female age on the diagnostic categories of infertility. Human Reproduction. 23(3): pp. 538–542.  168. **Dechanet, C.; Anahory, T.; Mathieu Daude, J. C.; Quantin, X.; Reyftmann, L.; Hamamah, S.; Hedon, B. and Dechaud, H. (2010).** "Effects of cigarette smoking on reproduction". Human Reproduction Update. 17 (1): pp. 76.  169. **Kds, A. and Nguyen, T. (1997).** Infertility in developing countries. Reproductive Health Outlook. 15: pp. 1-14.  170. **American Society for Reproductive Medicine (ASRM) (2009).** [Fertility Fact, Female Risks](http://www.protectyourfertility.com/femalerisks.html). Retrieved on Jan 4, 2009.  171. **Sloboda, D. M.; Hickey, M. and Hart, R. (2010).** "Reproduction in females: the role of the early life environment". Human Reproduction Update. 17 (2): pp.210–227.  172. **Rosendahl, M.; Andersen, C.; La Cour Freiesleben, N.; Juul, A.; Løssl, K. and Andersen, A. (2010).** "Dynamics and mechanisms of chemotherapy-induced ovarian follicular depletion in women of fertile age". Fertility and sterility 94(1): pp.156–166.  173. SOGC (Society of Obstetricians and Gynaecologists of Canada) Committee Opinion 2011. Genetic considerations for a woman’s pre-conception evaluation. J. Obstet. Gynaecol. Can. 33(1): pp.57-64.  174. **Bayrak, A.; Saadat, P.; Mor, E.; Paulson, R.J. and Rebecca Z. (2005).** Pituitary imaging is indicated for the evaluation of hyperprolactinemia. Fertility and Sterility.84: pp.181–5.  175. **Tomassetti, C.; Meuleman, C. and Pexsters, A. (2006).** "Endometriosis, recurrent miscarriage and implantation failure: is there an immunological link?". Reprod. Biomed. Online. 13 (1): pp.58–64.  176. **Guven, M.A.; Dilek, U.; Pata, O.; Dilek, S. and Ciragil, P. (2007).** "Prevalence of Chlamydia trochomatis, Ureaplasma urealyticum and Mycoplasma hominis infections in the unexplained infertile women". Arch. Gynecol. Obstet. 276 (3): pp.219–23.  177. **García-Ulloa, A.C. and Arrieta, O. (2005).** "Tubal occlusion causing infertility due to an excessive inflammatory response in patients with predisposition for keloid formation". Med. Hypotheses. 65 (5): pp.908–14.  178. **Raga, F.; Bauset, C.; Remohi, J.; Bonilla-Musoles, F.; Simón, C. and Pellicer, A. (1997).** "Reproductive impact of congenital Müllerian anomalies".Hum.Reprod.12(10):pp.2277–81.  179. **Tan, Y. and Bennett, M.J. (2007).** "Urinary catheter stent placement for treatment of cervical stenosis". The Australian & New Zealand journal of obstetrics & gynaecology. 47(5): pp.406–9.  180. **Francavilla, F.; Santucci, R.; Barbonetti, A. and Francavilla, S. (2007).** "Naturally-occurring antisperm antibodies in men: interference with fertility and clinical implications. An update". Front. Biosci. 12 (8–12): pp.2890–911.  181. **Rock, J.A. and Schlaff, W.D. (1985).**The obstetrical consequences of utero-vaginal anomalies, Fertil. Steril*.* 43: pp.681.  182. **Marshal, W. J. and Bangert, S. K.** **(2008).** Clinical chemistry. Sixth edition, MOSBY, ELSEVIER, chapter 10, pp. 191.  183. **Marks, D.B.; Marks, A.D. and Smith, C.M. (2006).** Basic medical biochemistry, a clinical approach. Section Six. Chapter 34. pp. 647.  184. **Murray**, **R. K.; Granner, D.K.; Mayes, P.A. and Rodwell, V.W. (2003).** Harper’s Illustrated Biochemistry*.* McGraw-Hill Companies, Inc., twenty-sixth edition, chapter 42, pp. 444.  185. **American Academy of Pediatrics. (2000):** Evaluation of the newborn with developmental anomalies of the external genitalia. Pediatrics.106: pp.138-142. 186. **Grumbach, M. and Styne, D. (1998).** Puberty: Ontogeny, neuroendocrinology, physiology, and disorders. In: Wilson, Foster, Kronenberg, et al. eds. Williams Textbook of Endocrinology. 9th ed. Philadephia, PA: W.B. Saunders Company: pp.1550-1625.  187. **Griffin, J.E. and Wilson, J.D. (1998).** Disorders of the testes and the male reproductive tract. In: Wilson, Foster, Kronenberg, et al. eds. Williams Textbook of Endocrinology. 9th ed. Philadephia, PA: W.B. Saunders Company. pp. 819-875.  188. **Ajayi, A.A. and Halushka P.V. (2005).** "Castration reduces platelet thromboxane A2 receptor density and aggregability". QJM. 98 (5): pp.349-56.  189. **Haddad, R.M.; Kennedy, C.C.; Caples, S.M.; Tracz, M.J.; Boloña E.R.; Sideras, K.; Uraga, M.V.; Erwin, P.J. and Montori, V.M. (2007).** "Testosterone and cardiovascular risk in men: a systematic review and meta-analysis of randomized placebo-controlled trials". Mayo. Clin. Proc*.* 82 (1): pp.29–39.  190. **Swerdloff, R.S.; Wang, C. and Bhasin, S. (1992).** "Developments in the control of testicular function". Baillieres Clin. Endocrinol. Metab. 6 (2): pp.451–83.  191. **Gardner**, **D. G. and Shoback, D. (2007). Greenspan's** Basic & Clinical Endocrinology. 8th edition. McGraw-Hill Companies*.* Chapter13. USA. (eboock)  192. **Mohan, K. and Sultana, A. M. (2010).** Follicle stimulating hormone, luteinizing hormone and prolactin levels in infertile women in north Chennai, Tamilnadu. J. Biosci. Res. 1 pp. 279 – 284.  193. **E. Loumaye; M. Dreano; A. Galazka; C. Howles; L. Ham; A. Munafo and A. Eshkol. (1998).** Recombinant follicle stimulating hormone: development of the first biotechnology product for the treatment of infertility. Human reproductive update. 4(6): pp. 862 – 881.  194. **Steven M. Pincus; Johannes D.Veldhuis; Thomas Mulligan; Ali Iranmanesh and WilliamS. Evans. (1997).** Effects of age on the irregularity of LH and FSH serum concentrations in women and men. J. Physiol. Endocrinol. Metab. 273: pp. 989 – 995.  195. **Ehteram Ibrahim AL-Mawajdeh. (2011).** Correlation of Thyroid Hormones, Gonadotropins and Age in Infertile Women in the South of Jordan. European Journal of Scientific Research. 63 (2): pp.219-230.  196. **Weiske WH (1994).** Minimal invasive Vasektomie mittels Fulgurationstechnik. Erfahrungen bei 1000 Patienten in 12 Jahren. Urologe. 34: pp.448-452.  197. **G.R. Dohle; T. Diemer; A. Giwercman; A. Jungwirth; Z. Kopa and C. Krausz (2010).** Guidelines on Male Infertility. European Association of Urology. pp. 8-9.  198. **Adam H.Balen; Joop S.E. Laven; Seang-Lin Tan and Didier Dewailly (2003).** Ultrasound assessment of the polycystic ovary: international consensus definitions. Human Reproduction Update. 9(6): pp. 505-514.  199. **Evanthia Diamanti-Kandarakis; Jean-Pierre Bourguignon; Linda C. Giudice; Russ Hauser; Gail S. Prins; Ana M. Soto; R. Thomas Zoeller and Andrea C. Gore (2009).** Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. Endocrine Reviews. 30(4): pp. 293-342.  200. **Mosher, W.D. and Pratt, W.F. (1991).** Fecundity and infertility in the United States: Incidence and trends. J. Fertil. Steril. 56: pp.192–193.  201. **Lafferty, K. D. and Kuris, A. M. (2009).** Parasitic castration: the evolution and ecology of body snatchers. Trends in Parasitology. 25 (12): pp. 564.  202. **Stahl, W.; Kaneda, Y. and Noguchi, T. (1994).** Reproductive failure in mice chronically infected with *Toxoplasma gondii*. Parasitol. Res. 80: pp.22-28.  203. **Nguyen, T.D.; Bigaignon, G.; Markine-Goriaynoff, D.; Heremans, H.; Nguyen, T.N.; Warnier, G.; Delmee, M.; Warny, M.; Wolf, S.F. and Uyttenhove, C. (2003)** Virulent *Toxoplasma gondii* strain RH promotes T-cell-independent overproduction of proinflammatory cytokines IL-12 and g-interferon. J. Med. Microbiol. 52: pp.869-876.  204. **Kalra, P.S.; Edwards, T.G.; Xu, B.; Jain, M. and Kalra, S.P. (1998)** The antigonadotropic effects of cytokines: the role of neuropeptides. Domest. Anim. Endocrinol. 15: pp.321-332.  205. **Aron, D.C. (1989).** Endocrine complications of the acquired immunodeficiency syndrome. Archives of internal medicine. 149: pp. 330-333.  206. **Suresh Babu, P.C.; Nagendra, K.; Sarfaraz Navaz, R. And Ravindranath, H.M. (2007).** Congenital toxoplasmosis presenting as hypogonadotropic hypogonadisim. Indian J. Pediatr. 74 (6): pp. 577-579.  207. **Zhang, X.; Li, Q.; Hu, P.; Cheng, H. and Huang, G. (2002).** Two case reports of pituitary adenoma associated with *Toxoplasma gondii* infection. Journal of Clinical Pathology. 55: pp.965–966.  208. **Dardé, M.L.; Vilena, I.; Pinon, J.M. and Beguinot, I. (1998).** Severe toxoplasmosis caused by a *Toxoplasma gondii* strain with anew isoenzyme type acquired in French Guiana. Journal of Clinical Microbiology. 36: pp. 324.  209. **Liu Run-fang and Wang Xin-cai (2007)**. [Detection and Analysis of IgM Antibodies Against *Toxoplasma gondii* in Infertile Patients](http://en.cnki.com.cn/Article_en/CJFDTOTAL-LYYZ200704018.htm). J. Henan Univ. Sci. Tech. (Med. Sci.). **27:** pp. 123-144 (In Chinese with English abstract). 210.[Moraes](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract)[a](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract" \l "aff1" \o "),E.P.B.X.;[Freitas](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract)[b](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract" \l "aff2" \o "),A.C.;[Gomes-Filho](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract)[c](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract" \l "aff3" \o "),M.A.;[Guerra](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract)[a](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract#aff1), M.M.P.; [Silva](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract)[b](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract#aff2), M.A.R.;  [Pereira](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract)[a](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract#aff1), M.F.; [Braga](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract)[d](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract" \l "aff4" \o "),V.A., and [Mota](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract)[a](http://www.journals.elsevierhealth.com/periodicals/anirep/article/S0378-4320(10)00365-9/abstract#aff1),R.A. (2010). Characterization of reproductive disorders in ewes given an intrauterine dose of *Toxoplasma gondii* tachyzoites during the intrauterine insemination. Animal reproductive science, Elsevier B.V. (Abstract). 211. **Dass, S.A.H.; Vasudevan, A.; Dutta, D.; Soh, L.J.T.; Sapolsky, R.M., and Vyas, A. (2011).** Protozoan Parasite Toxoplasma gondii Manipulates Mate Choice in Rats by Enhancing Attractiveness of Males. PLoS ONE 6(11): e27229.  212. **De Paepe, M.E. and Waxman, M. (1989).** Testicular atrophy in AIDS: A study of 57 autopsy cases. Hum. Pathol. 20: pp.210-214.  213. **Stewart,C. E. and Koepke, J. A.(1987).** Basic quality assurance practices for clinical laboratories. In Howanitz, J. F. (eds). Laboratory quality assurance. 4th ed. pp.217. J. B. Lippincott, Philadelphia.  214. **World Health Organization. (2010).** WHO laboratory manual for the examination and processing of human semen - 5th ed. , 20 Avenue Appia, 1211 Geneva 27.  215. **Roller, A.; A. Bartlett, and D.E. Bidwell. (1987).** Enzyme Immunoassay with Special Reference ELISA Technique. J. Clin. Path. 31: pp. 507-520.  216. **Turune, H.J.; P.O. Leinikke, and K.M. Saari (1983).** Demonstration of intraocular Synthesis of Immunoglobin G *Toxoplasma* Antibodies for Specific diagnosis of Toxoplasmic Chorioretinitis by Enzyme Immunoassay. J. Clin. Microbiol. 17: pp.988-992.  217. **Butt, W.R. (1983).** "Gonadotropin" In: Hormones in blood. Edited by Gray CH. & James WTH; 3rd Ed., Academic Press, London, Chapter 7. pp. 147-177.  218. **Bardin, W.E.T., and Pauls, C.A. (1981).**Textbook of endocrinology-Ed Williams, H.R., Saunders W.B. 6th Ed. Philadelphia - The tenses. 6: pp.293-354.  219. **Litwack, G. (1992).** Biochemistry of Hormones II: Steroid Hormones. In: Devlin, T.M. Textbook of Biochemistry with Clinical Correlations, 3rd ed. Wiley, J. and sons. pp. 901-925.  220. **Sorlie, D.E.** **(1995).** Medical biostatistics & epidemiology: Examination & Board Review.1st ed. Norwalk, Connecticut, Appleton & Lange: pp.47-88.  221. **Xin-cai ; Liu Run-fang and Zhao Rong-po (2006).** [Detection on Infection of *Toxoplasam gondii* in Infertile Couples](http://en.cnki.com.cn/Article_en/CJFDTOTAL-LYYZ200602006.htm). J. Henan Univ. Sci. Tech. (Med. Sci.). **25:** pp. 143-144 (In Chinese with English abstract).  222. [**Liu, S.G**](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Liu%20SG%22%5BAuthor%5D)**.;** [**Qin, C**](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Qin%20C%22%5BAuthor%5D)**.;** [**Yao, Z.J**](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Yao%20ZJ%22%5BAuthor%5D)**. and** [**Wang, D**](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Wang%20D%22%5BAuthor%5D)**.** **(2006).** Study on the transmission of *Toxoplasma gondii* by semen in rabbits. [Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi.](http://www.ncbi.nlm.nih.gov/pubmed/17094613) 24 (3): pp.166-70.  223. **Scarpelli, L.C. (2001).** Viabilidate da transmissăo venérea do *Toxoplasma gondii* em bovinos. Dissertaçăo de Mestrado, FCAV, Universidade Estadual Paulista, Jaboticabal, SP. 128.  224. **Cosme Alvarado-Esquivel; Olga-Patricia Alanis-Quiñones; MiguelÁngel Arreola-Valenzuela; Alfredo Rodríguez-Briones; Luis-Jorge Piedra- Nevarez; Ehecatl Duran-Morales; Sergio Estrada-Martínez; Sergio- Arturo Martínez-García and Oliver Liesenfeld (2006).** Seroepidemiology of *Toxoplasma gondii* infection in psychiatric inpatients in a northern Mexican city. BMC Infectious Diseases. 6(178): pp. 5.  225. **Martinez-Garcia, F.; Regadera, J.; Mayer, R.; Sanchez, S. and Nistal M (1996)**. Protozoan infections in the male genital tract. J. Urol.  156: pp. 340-349.  226. **Flegr J.** **and Stříž I. (2011).** Potential immunomodulatory effects of latent toxoplasmosis in humans. BMC Infectious Diseases. 11(27): pp. 1-7.  227. **Styrt, B. and B. Sugarman. (1990).** Estrogens and infection. Rev. Infect. Dise. 13: pp.1139-1148.  228. **Shirbazou, S.; Abasian, L. and Talebi Meymand, F.** **(2011).** Effects of *Toxoplasma gondii* infection on plasma testosterone and cortisol level and stress index on patients referred to Sina hospital, Tehran. Jundishapur J. Microbiol. 4(3): pp.167-173.  229. **Li Shu-hong; Dai Pei; Cui Li-ming; Zong Shan and Zuo Wen-jing (2006).** [Infection rate of *Toxoplasma gondii* and age distribution in female patients with sterility](http://en.cnki.com.cn/Article_en/CJFDTOTAL-BQEB200601068.htm). J. Jilin Univ. Sci. Tech. **6:** pp. 526-527 (In Chinese with English abstract).  230. **Zahida Tasawar; Fariha Aziz; Mushtaq Hussain Lashari; Shaista Shafi; Munir Ahmad; Vijay Lal and Chaudhary Sikandar Hayat (2012).** Seroprevalence of Human toxoplasmosis in Southern Punjab, Pakistan. Pak. j. life soc. Sci. 10(10): pp. 1-5.  231. **Jones J.L.; D. Kruszon-Moran; K. Won; M. Wilson and P.M. Schantz (2008).** *Toxoplasma gondii* and *Toxocara* spp. co-infection. American Journal of Tropical Medical Hygiene. 78: pp. 35-39.  232. **Al- Najjar, Saif Abdulelah Mustafa (2005).** Detection of anti-*Toxoplasm*a antibodies among patients with acute leukemia or lymphoma using latex agglutination test and ELISA. M. Sc., thesis, College Of Medicine. Mosul university.  233. **Hossein Ali Khazaei; Behzad Narouie; Mohammed Bokaeian and Agheel Miri (2011).** Evaluation of IgM against *Toxoplasma gondii* in under marriage women and its pathogenicity relation with demographic factors. Afr. J. Microbiol. Res. 5(29): pp. 5221-5225.  234. **Roberts, C.W.; Walker, W. and Alexander, J. (2001).** Sex-associated hormones and immunity to protozoan parasites. Clinical Microbiology Reviews. 14: pp. 476–488.  235. **Barakat, A.M.A. (2007).** Some diagnostic studies on male New Zealand rabbit experimentally infected with *Toxoplasma gondii* strain. Global veterinaria. 1(1): pp. (17-23).  236. **Cagatay Oktenli; Levent Doganci; Taner Ozgurtas; R.Engin Araz; Mehmet Tanyuksel; Ugur Musabak; S.Yavuz Sanisoglu; Zeki Yesilova; M.Kemal Erbil and Ali Inal (2004).** Transient hypogonadotrophic hypogonadism in males with acute toxoplasmosis: suppressive effect of interleukin-1β on the secretion of GnRH. Human Reproduction. 19(4): pp. 859-866.  237. **Antonios, S.N.; Ismail, H.I. and Essa, T. (2000)**. Hypothalamic origin of reproductive failure in chronic experimental toxoplasmosis. J Egypt Soc  Parasitol. 30: pp. 593-599.  238. **Boothroyd, J.C. and Grigg, M.E. (2002)**. Population biology of *Toxoplasma gondii* and its relevance to human infection: do different strains cause different disease? Curr. Opin. Microbiol. 5: pp. 438-442. |
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