**Ministry of Higher Education and Scientific Research**

**University of Babylon – College of Science**

**Department of Biology**

# Association of Some Cytokines and Signaling Enzymes in Infertile Women with *Toxoplasma gondii*

**Thesis Submitted to the Council of College of Science - University of Babylon as a Partial Fulfillment of the Requirements for the Degree of Doctorate Philosophy of Science in Biology/Zoology**

***By***

**Noor Abed Al Reda Ibrahim Ahmed**

B.Sc. Microbiology /University of Babylon /College of Science/ Department of Biology-2011

M.S.C. Biology/ University of Babylon /College of Scienc / Department of Biology - 2020

**Supervised by**

**Prof. Dr. Maher Ali Jatan Al – quraishi**

**2023 AD** **1445 AH**

بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

﴿لِلَّهِ مُلْكُ السَّمَاوَاتِ وَالْأَرْضِ يَخْلُقُ مَا يَشَاءُ يَهَبُ
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صدق الله العلي العظيم

[الشورى: ٤٩ – ٥٠]

## Dedication

**To :**

Light of my eyes, the soul of my heart, endless support, and the source of compassion and The Spring that never stops giving

**My father and mother**

The source of love, inspiration and help me in everything…

**My husband(Mustafa)**

To the strength source and the loving partner of my life

**My brother and sister (Ahmed and Bassma)**

Those who their love flows in my veins, and my heart always remembers them…

**My children (Haider and Jumana)**

**Noor**

## Acknowledgements

First of all, thanks to Allah for giving me the power and the insistence to proceed up to complete this work

I would like to express my heartiest and sincerest sense of gratitude to my worthy Supervisor prof. Dr. **Maher Ali Jatan AL-quraishy for** providing their valuable guidance, supervision, and moral support from the very early stage till the end of my thesis work.

I express my deep thanks to prof **Dr.Alla Tarek Shakir** for help me for there continuous interest to complete this work.

I want to thank the staff of the Department of Biology in the college of Science at the University of Babylon Furthermore, much appreciation is dedicated to the Dean of the College of Science for his crucial role and to the Head of the Biology Department , **Assist. Prof. Dr. Adi Jasim Abd Al-Razzaq** who offered a permission to use all the required equipment and the necessary material for the successful completion of the present work .

and to my dear friends **Worood Ali Hathal & Basma Abed Al-Kalic**

I would like to express my sincere thanks and gratitude to the patients and their women who donated as control samples.

Finally ,I would like to thank all those who helped me and supported me even with a word if I could not mention him.

**Noor**

## Summary

*Toxoplasma gondii* can infect any warm-blooded animal, including humans. Toxoplasmosis in females can significantly affect the results of their reproductive processes, particularly their fertile capacity. However, there is insufficient research that investigates a connection between female *T. gondii* infection and infertility, particularly in our region.

This case-control study was conducted to ascertain the seroprevalence of anti-Toxoplasmas antibodies and some immunomodulatory factors among infertile women. The study included 250 infertile and 50 appaerantly women, who had no history of abortion, was fertile, and with negative results for *Toxoplasma gondii* IgG and IgM. The study was performed during the period from 1st February to 25 October 2022 from the private laboratories and gynecology outpatient clinics of two of Babylon's leading hospitals: Babil Teaching Hospital for Maternity and Childhood, and Al-Imam ul-Sadiq Teaching Hospital.

After careful selection of the study participants, based on specific inclusion and exclusion criteria, the current work was conducted using a questionnaire that asked about the name and age of the infertile female, the length of the marriage, any male factors of infertility, the type of infertility (primary or secondary), the presence of any field or feline pets, and a hormonal profile.

The serological evaluation of all participants included Toxoplasma immunoglobulins (IgG and IgM), interleukins (IL-12 and IL-17), TNF- α, TGF-β, cyclophilin-A, and phospholipase serum levels.

The study results revealed that the average age of the patients was 31.6 ±4.3 years. And only 62 (24.8%) of the 250 infertile females met the requirements for the research study. The average duration of marriage was 3.9±1.3 years. Thirty-three

(13.2) of the infertile women had additional infertility reasons (male factors) in their partners. Out of the total 250 participants, 152 individuals (60.8%) reported having field or feline animals and 68 participants (27.2%) reported having a period infection of toxoplasma. The serological and immunological parameters were higher in the patients, either considerably (IL-12 and cyclophilin-A) or highly significantly (IL-17, TGF-β, TNF-α, and phospholipase), respectively. The study's variables have varying degrees of correlation with one another.

The study concluded that for patients with Toxoplasma, none of the study parameters were strongly diagnostic or had good predictions. As well, none of the investigated indicators may be used as a biomarker to identify or distinguish healthy women from *T. gondii* infertile females.

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# List of Abbreviations

| Abbreviation | Meaning |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| BOH | Bad Obstetric History |
| CMI | Cellular mediate immunity |
| CBC | Complete Blood Count |
| CT | Congenital toxoplasmosis |
| CS | Cyclosporine |
| DCs | Dendritic cells |
| DNA | Deoxynucleic Acid |
| DHEA | Dehydroepiandrosterone |
| DALYs | Disability adjusted life years |
| ELISA | Enzyme Linked Immunosorbent Assay |
| EGFR | Epidermal growth factor receptor |
| EDTA | Ethylene Diamine Tetra Acetic Acid |
| GPI | Glycosylphosphatidylinositol |
| GRA | Granular antigen |
| HIV | Human Immunodeficiency Virus |
| HUC-PVSCs | Human umbilical cord perivascular cells |
| IgM | Immunoglobulin M |

| | |
|---|---|
| **IgG** | **Immunoglobulin gamma** |
| **IFNGR1** | **Interferon-gamma receptor1** |
| **IL-17** | **Interleukin-1 7** |
| **IL-12** | **Interleukin-12** |
| **MAMPs** | **Microbes associated molecular pattern** |
| **PV** | **Parasitophorous vacuoles** |
| **PAMPs** | **Pathogens associated molecular pattern** |
| **PRRs** | **Pattern recognition receptor** |
| **PPIase** | **Peptidyl-poly *cis-trans* isomerase** |
| **PPID** | **Peptidylprolyl Isomerase D** |
| **PCOS** | **Polycystic ovary syndrome** |
| **PE** | **Preeclampsia** |
| **PAH** | **Pulmonary arterial hypertension** |
| **RBC** | **Red Blood Corpuscle** |
| **ROPs** | **Rhoptry antigen** |
| **SAG** | **Surface antigens** |
| **TLR** | **Toll like receptor** |
| ***T. gondii*** | ***Toxoplasma gondii*** |
| **TGF** | **Transforming growth factor** |
| **TGFβ** | **Transforming growth factor beta** |
| **TNF** | **Tummer necrosis factor** |

| | |
|---|---|
| **TNFα** | **Tummer necrosis factor alpha** |
| **SUSA** | **Unrelated surface antigens** |
| **WBC** | **White Blood Cell** |
| **WHO** | **World Health Organization** |

# Chapter one
# Introduction

## Introduction

The obligate intracellular parasite *Toxoplasma gondii* is the primary cause of the preventable infectious illness Toxoplasmosis, an opportunistic zoonotic infection that is widesprea d throughout the world ( Elaadli et al., 2023). Around the world, up to one-third of the population is seropositive for T. gondii and at risk of infection (Kolören and Dubey, 2020). The incidence of primary maternal T. gondii infection during pregnancy ranges from about 1 to 310 per 10,000 pregnancies in different populations in Europe, Asia, Australia, and the Americas (Mohammed , 2019). Human can become infected by consuming raw or undercooked meat with tissue cysts (bradyzoites), drinking water, or eating food contaminated with cat excrement containing sporulated oocysts ( Almeria and Duby, 2021). Handling raw meat and consuming unpasteurized milk containing the rapidly growing stage (tachyzoites) may also provide risks for human T. gondii infection (Markon et al., 2021).

Human are frequently infected by toxoplasmosis, which is practically universally prevalent. The two main methods of transmission to humans are oral and transplacental (Sadegi et al., 2023). Toxoplasmosis in females can have a major impact on the outcomes of their reproductive processes, in particular, congenital toxoplasmosis and its effects on the fetus. The consequences brought on by this parasite include infertility, miscarriage, severe abnormalities, developmental delays, hydrocephalus, intracerebral calcification, blindness, epilepsy, and intrauterine fetal death (Arora et al., 2017). It has a wide spectrum of clinical symptoms, and the pattern of those symptoms depends on the host's immunological condition and age (Khan and Moretto, 2022).

*T. gondii* infection in healthy humans becomes asymptomatic because the host's innate and adaptive immunity resists its initial proliferation and eradicates most of

the parasites. Infection of monocytes by a *T. gondii* tachyzoite strongly induces innate immune responses such as the production of pro-inflammatory cytokines (interleukins, tumor necrosis factors, transforming growth factors, and others), resulting in the activation of adaptive immune responses mediated by T and B cells ( Miwa Sasai, 2018; Khan and Moretto, 2022). The activation of adaptive immunity further stimulates cell-autonomous immune responses (different CD subsets and others) in infected cells, inducing T. gondii stage conversion into a bradyzoite that eventually leads to chronic infection. Thus, the immunological balance between a healthy host and *T. gondii* is a key step in *T. gondii* immunobiology (Khan and Moretto, 2022).

Seroimmunological tests of *T. gondii* identify the antibodies in the serum. IgM antibodies are the first sort of immunoglobulins detected earlier after getting a primary infection and decrease quicker than IgG antibodies, which remained for prolonged durations (Alkubaisi et al., 2023). The influence of toxoplasmosis on reproductivity in humans has been the subject of several studies yielding discordant results. A positive effect of latent toxoplasmosis on the risk of miscarriage has been shown in a study performed on 5 033 obstetric patients but was not confirmed by others ( Kaňková et al., 2015). Researchers have reported that among females with an average of 23± 5.24 years, IgG anti-*T. gondii* antibody was detected in 22.1% of pregnant women. Earlier Iraqi study has shown a significant relationship between Toxoplasmosis and anti Mullerian hormone level as a predictor of ovarian reserve in females (Salman, 2014). latent Toxoplasmosis is associated with an increase in autoimmune thyroid diseases in pregnancy (Kaňková et al., 2014) and gestational diabetes mellitus (Oliveira-Scussel et al., 2022).

This case-control study was conducted to ascertain the seroprevalence of anti Toxoplasmas antibodies among infertile women due to insufficient research on the frequency of toxoplasmosis infection in our county and the high incidence of associated abnormal reproductive outcomes, particularly infertility.

**Aims of the study:**

1- Study the profile of Cytokine in patient with toxoplasmosis.
2- To compare the serum levels of some of the immunological factors (Interleukin-12, Interleukin-17, Transforming Growth Factor–β, Tumors necrosis factor -α, Phospholipase, and Cyclophilin-A) between infertile females with T. gondii infection and the healthy controls.
3- To evaluate the diagnostic and predictive ability of serum levels of these immune regulatory factors (Interleukin-12, Interleukin-17, Transforming Growth Factor–β, Tumors necrosis-α factor, Phospholipase A, and Cyclophilin-A) as a biomarker for *T. gondii* infection among infertile females.

# Chapter Two
# Literature Review

# LITERATURE REVIEW

## 2-1: History of parasite

*Toxoplasm* Spp is the protozoa paraites, There are three main important species that belongs to genus toxoplasma these are: *T. gondii, T. hammondi*, *T. bahaiensis* .It infects many animals such as dogs, cats, cattle, rodents and birds. It is the only type that causes Toxoplasmosis in human. *Toxoplasm gondii* spread all over the world specially in tropical areas where the percentage of the infection by this parasite is about 30% from the people of the world. The percentage of infection for children rises in the places where there are many cats and their feces. It is high for adults where they have meats are not well cooked (AL-Quraishi,2011)

*Toxoplasma gondii* is worldwide distribution protozoan parasite that is estimated to infect one-third of the world's human population. Many species it can infected a warm-blooded animals and is a significant zoonotic and veterinary pathogen (Almeria and Dubey,2021). The National Institutes of Health, Bethesda and USA are recognized as a category B priority pathogen. In several of its hosts, *T. gondii* is associated with congenital infection and abortion. In addition, *T. gondii* can cause encephalitis or systemic infections in the immunocompromised, particularly individuals with HIV/AIDS. It has been 100 years since *T. gondii* was first discoveredgy70 in the tissues of *Ctenodactylids gundii*, a North African rodent, by Nicolle and Manceaux (1908). In the same year Splendore (1908), in Brazil, was identified on the organism in the tissues of a rabbit. The genus was named by Nicolle and Manceaux as Toxoplasma for its bow-like shape (from Greek: toxo = bow or arc; plasma = creature) (Halonen and Weiss,2009).

Further insight into the great prevalence of this parasite and the discovery of the Toxoplasma life cycle during the 1970s should have depicted Toxoplasma as a relatively harmless microorganism, or model pathogen, during this time (Robert-Gangneux and Dardé,2012). However, the rise of HIV epidemic during the 1980s, and the increasing number of reports of pathology as a consequence of chronic toxoplasmosis during the 1990s and beginning of this millennium, had successively swayed both researchers and clinicians to re-evaluate the clinical significant and economic impact of *Toxoplasma*. (Kadhim *et al.*,2015).

## 2-2: The life cycle of the parasite

Toxoplasma is an intracellular obligate parasitic pathogen that is capable of infecting and replicating within any nucleated mammalian or avian cells *T. gondii* infects most species of warm-blooded animals, including humans. The definitive host is the domestic cat or their relatives (family Felidae), while rodents, particularly mice and rats, act as intermediate hosts (Gilot-Fromont *et al.*,2012). *T. gondii* can be found anywhere in the world and in human's infection can cause life-threatening encephalitis in immunocompromised individuals such as HIV/AIDS patients or organ transplant recipients (Woyesa and Taylor-Robinson ,2021). Infection acquired during pregnancy may spread into the foetus and cause severe damage to foetal development (Waldorf and McAdams,2013).

The *T. gondii* life cycle starts in felids usually through the consumption of infected prey containing tissue cysts. The cyst wall is digested in the stomach and intestines, liberating bradyzoite stage parasites that penetrate the

epithelial cells of the small intestine. This initiates the progressive development of asexual and by gametogony sexual forms of the parasite (Robert-Gangneux and Dardé,2012). The complex life cycle of this pathogenic parasite alternates between feline and non-feline infections where sexual and asexdxual reproduction takes place, respectively (Woyesa and Taylor-Robinson,2021). Tachyzoites (tachos = fast) and bradyzoites (brady = slow) are rapidly and slowly growing stages of *T. gondii*, respectively (Dubey,2013). Domestic cats are infected through consuming either sporulated oocysts or intermediate hosts containing tissue cysts. To be infective, an unsporulated oocyst is converted into a sporulated one within 1-5 days (Attias *et al*., 2020). Tachyzoites, also termed endozoites, replicate inside cells for a 6 to 8 h generation time (observed *in vitro*) till regress to infect neighbouring cells. This stage may be identified in the blood during acute phase Toxoplasmosis (Woyesa *et al.*,2021). Tachyzoites multiply slowly in infected host cells and differentiate into bradyzoites (cystozoites) (Dogga *et al.*, 2022).

In order to remain life-long in their hosts, tachyzoites reside predominantly in brain, eyes, skeletal and cardiac muscle and it takes 7 to 10 days' post infection to detect tachyzoites (Mangal *et al.*, 2022). Tissue Cysts develop within the Cytoplasm of host cells and they enclose hundreds of crescent-shaped bradyzoites (Lindsay and Dubey ,2020).

The chronic phase of *Toxoplasmosis* is indicated by development of tissue cysts of the asexual cycle. While digestion is ongoing rupturing may happen to cysts ingested with infected tissues. This rel eases bradyzoites that

infect the epithelium of intestinal lumen where they can differentiate back to the rapidly dividing tachyzoite stage to disseminate throughout the body, thereby completing the asexual cycle (Smith *et al.*,2021*).*

## 2-3: Classification of *T. gondii*

*T. gondii* is apicomplexan parasite. It belongs to the family of the Sarcocystidae in the class of the coccidia and is the only species in the Toxoplasma genus. Coccidia are obligate, intracellular and cyst forming parasites that infect their hosts through the gastrointestinal tract, Other genera in the family of the Sarcocystidae are : Besnoitia, Cystoisospora, Frenkelia, Hammondia, Hyaloklossia, Sarcocystis and Neospora (Genova and Knoll, 2020)

*T. gondii*, is classified according to (Bandyopadhyay *et al.*,2022) as follows:

Kingdom: Animalia

Sub Kingdom: Protozoa

Phylum: Apicomplexa

Class: Sporozoea

Subclass: Coccidia

Order: Eucoccidea

Suborder: Eimeriina

Family: Sarcocystidae

Genus: Toxoplasma

Species*: T.gondii*

## 2-4: Epidemiology of *Toxoplasma gondii*

*T. gondii* infects about 30 to 50% of human populations worldwide and more than one million cases of infection contracted through drinking oocyst-contaminated water or eating tissue cyst-contaminated food, primarily raw or undercooked pork and lamb, are reported every year in Europe alone (Woyesa and Taylor-Robinson,2021). Hence, it is extremely prevalent in low-income countries this widespread distribution means that toxoplasmosis not considered as a classic 'disease of poverty'. Globally, geographical differences and food cultural habit variation from one country to another, even from one area to another within the same country, contribute greatly to regional variation in prevalence of *T. gondii* human infection. In particular, food cultural variation among ethnic groups in the same location is the main reason for their different observed rates of infection of *T. gondii*. For instance, the highest prevalence of toxoplasmosis was reported from France (54.3%) when compared with other European countries in earlier studies (Robinson *et al*., 2021).

A two-year survey in the US revealed the seroprevalence of *T. gondii* among persons ≥6 years was 13.2%, and the age-adjusted prevalence was 12.4%, with the largest prevalence (9.1%) among women in the 15 to 44 years age group (Firdaousse *et al*., 2021). Data analyzed for 19 years (1995-2008) from 88 countries, 29 of which were in Europe, indicated the correlation of toxoplasmosis with specific diseases burden estimated as age-standardized disability adjusted life years (DALYs) and also with mortality of women of childbearing age. According to this published survey, the prevalence of

toxoplasmosis correlated with 23% of various diseases burden in Europe (Flegr and Kuba, 2016).

Studies conducted for 16 years at three intervals (1988- 1994, 1999-2004 and 2009-2010) among US women of child-bearing age (15-44 years) revealed a declining ageadjusted prevalence of *Toxoplasmosis* (15, 11 and 9%) from earliest to most recent years. These findings indicate that there was an incremental increase in awareness over time to control *Toxoplasmosis* transmission (Rani ,2020). Some African nations reported highest prevalence of *Toxoplasmosis* among child-bearing age women that indicates the highest rate of transmission and lack of awareness to control toxoplasmosis. For instance, the prevalence of *Toxoplasmosis* among child-bearing age women reported from Ghana was 92.5% (Al-Adhroey *et al*., 2019). *T. gondii* infects children not only after birth but also congenitally in utero. Between 40 and 400 children born each year in Canada are infected congenitally by *T. gondii* (University of Guelph Centre for Public Health and Zoonoses, 2009).

The highest seroprevalence of *T. gondii* has been reported from developing countries like Ethiopia. For example, the seroprevalence of *T. gondii* recorded in northern Ethiopia was 76.5% (Chaklu *et al*., 2020). The prevalence of *Toxoplasmosis* was similar among pregnant women with previous histories of normal delivery and abortion (21.5% vs. 24.6%, respectively) (Yousefvand *et al*., 2021).

An investigation in Sudan showed the highest prevalence of *T. gondii* among HIV patients (75%), then aborting women (58.3%) and suspected cases of *T. gondii* (55.5%) (Mohamed *et al*., 2016). There is a risk of toxoplasmic encephalitis in HIV-positive patients and among HIV-negative

immunosuppressed patients. Hence, immune status improvement among immunosuppressed patients such as those with HIV/AIDS reduces the chance of being infected by *T. gondii*. For instance, studies from Iran on HIV/AIDS patients indicated a reduction of prevalence of *Toxoplasmosis (*from 49.75%) by more than 50% over several years based on improvement of the immune status of patients (Foroutan *et al*., 2016). A study reported from Germany indicated that being in frequent contact with domestic cats and male obesity (body mass index ≥ 30 kg/m3 ) were independent predictors to acquire

*T. gondii* infection. Similarly, the rate of *T. gondii* infection increased with age of individuals (20% among 18-29 age group versus 77% among 70-79 age group) (Wilking *et al*., 2016).

## 2-5: Seroprevalence of Toxoplasmosis

*Toxoplasma gondii* is an obligate intracellular protozoan able to infect different species (Zhao *et al*., 2022). Sexual forms of the parasite are found in the intestinal epithelium of definitive hosts such as domestic cats: where they transform into oocysts which are subsequently shed to the environment. Oocysts, remarkably stable environmentally, are transmitted to other hosts through inadvertent ingestion (Opsteegh *et al.*,2022). Humans acquire*T. gondii* through ingestion of undercooked meat, contact with feline faeces and rarely through drinking contaminated water or through transplantation of a contaminated organ (Pal *et al.*, 2021).

*T. gondii* is a ubiquitous parasite of warm-blooded animals that causes one of the most common parasitic infections in humans. The seroprevalence varies widely in different regions of the globe, measuring between 30% and 60% in most countries (Sint *et al.*,2023). The prevalence changes according to

social and cultural habits, geographic factors, climate, and transmission route, and it typically increases with age (Semenza and Besteiro, 2021). *Toxoplasmosis* is most dangerous to two populations: immunocompromised patients and fetuses whose mothers acquire acute infection during pregnancy (Pleyer *et al*.,2019).

Under normal immune conditions, *T. gondii* infection is frequently asymptomatic, but in individuals who are immunocompromised, such as in patients with AIDS, the parasites can become widely disseminated, causing severe *Toxoplasmosis* and encephalitis ( Zhou *et al.*,2021). Primary infections acquired during pregnancy may also result in severe damages to the fetus, manifested as mental retardation, seizures, blindness, and death (Abdulsamad and Manhal,2019).

*T. gondii* infection, when acquired during pregnancy, can lead to fetal infection, which may ultimately result in the loss of the fetus, or in lesions which normally involve the brain and eyes (Acharya, 2020). The risk of maternal fetal transmission of infection increases with gestational age at the time of exposure, whereas the incidence of severe disease decreases (Bălălău *et al*.,2020). Seroprevalence of *T.gondii* infection in women at childbearing age is found to be between 4%-100%. Incidence of primary maternal infection during pregnancy varies in a range of 1 to 310 per 10.000 pregnancies in the populations in Europe, Asia, Australia, and the Americas (Kalantari *et al*.,2021).

Toxoplasmosis seroprevalence in women who are pregnant or of childbearing age originating from Asia and Oceania, once more there is an over-representation of certain countries such as Iran, while information from other, geographically larger, countries may be limited. There exists no

obvious gradient in *Toxoplasmosis* seroprevalence in Asia. High prevalence foci exist in the Middle East including Turkey, Iran, Iraq and Kuwait. (Molan *et al*.,2019). Primary infection during pregnancy may cause spontaneous abortion or stillbirth. In utero infection may cause congenital *Toxoplasmosis* with ocular and neurological manifestations. (Dubey *et al*.,2021). laboratory animals reported that infection with *T. gondii* could be a cause of infertility in experimental animals (Saki *et al*.,2020).

Protozoan parasitic diseases are endemic in many countries worldwide, especially in developing countries, where infertility is a major burden. It has been reported that such infections may cause infertility through impairment in male and female reproductive systems (Al-Mussawi *et al*.,2020). The high rate of infertility, inability to conceive offspring, is one of the most important and underappreciated reproductive health problems in many countries. According to data from a comprehensive meta-analysis study by the World Health Organization (WHO) until 2010, almost 50 million couples suffered from infertility worldwide (Al-kremy and Al-hassnawi, 2020). several studies on laboratory animals have shown that infection with *T. gondii* could be a cause of infertility in experimental animals.

In female mice, chronic Toxoplasmosis causes endometritis, ovarian dysfunction, impaired folliculogenesis, ovarian and uterine atrophy, decrease in reproductive organs' weight and reproductive performance, adrenal hypertrophy, vasculitis, cessation of estrus cycling, and reproductive failure in experimental mice (Rostami *et al*.,2020).

Proved evidence is available which shows that infection by Toxoplasma induces foetal loss in women. This agent has predilection for nucleated cells of muscle, intestinal epithelium and placenta. It can be congenitally acquired

by transfer through the placenta, if the mother contracts the disease during pregnancy, causing abortions, stillbirths, congenital malformations (Elsheikha *et al.*,2020). *T. gondii* is an important cause of bad obstetric history (BOH) leading to habitual abortions and now its role as a cause of infertility has also been established (Affan ,2020). Moreover, studies showed a relation between positivity for toxoplasmosis and female sterility. Furthermore, visual impairment, hearing loss and malignant neoplasms were major coincidental diseases in *T. gondii* seropositive cases (Flegr and Kaňková, 2020).

The Toxoplasmosis has some unfavorable effects on reproductive capacity in both men and women. The data obtained from limited studies performed in animal models as well as in infertile couples, have supported the relationship between Toxoplasmosis and infertility (Siddiqui *et al.*,2014).

The hypothesis concerning infertility mechanisms due to *T.gondii* in females include development of endometritis and fetal rejection due to local release of *T.gondii* from latently located cysts in endometrial tissue in the stimulation during plansenta formation; impaired folliculogenesis in ovaries and uterine atrophy and reproductive failure due to hypothalamic dysfunction as a result of chronic toxoplasmosis. (Zamaniyan *et al.*,2023). The infection of *T.gongii* more common in warm climates and in low-lying areas than in cold climates and mountainous regions, where conditions for sporulation and survival of oocysts are less favourable .

The prevalence of *T. gondii* infection also varies between ethnic groups, and it is thought that this is largely due to sanitary and cooking habits rather than genetic differences. A seroprevalence of 80% has been reported from Paris where undercooked meat is often consumed . Lower seroprevalences

(10–40%) have been reported in countries from Southeast Asia where meat is cooked thoroughly (Desmettre,2020).

## 2-6: Pathogenicity of *Toxoplasma gondii*

*Toxoplasma gondii* infection in most immunocompetent adults is asymptomatic because of effective protective immunity, include extracellular antibody and intracellular T-cell factors. Endogenous interferon gamma appears to be an important mediator of host resistant to *Toxoplasma* (Fereig *et al*.,2022 ) In immunocompetent patients almost 90% of the infections are asymptomatic, while the rest exhibit a self-limiting, flu-like illness that does not require any treatment .Clinical infection with *T. gondii* depends on the immune status of the patient, (Al-Kaeebi and Al-Jubouri, 2022).*Toxoplasma* parasite can be responsible for acute disease in immunocompromised patients and congenitally infected fetuses and newborns (Giugno *et al*., 2020).

Symptomatic infection is usually characterized by lymphadenopathy and reticular cell hyperplasia. In immunocompromised patients such as AIDS, toxoplasmosis almost always happens as a result of reactivation of chronic infection. In these patients, clinical symptoms consist of mental status changes, seizures, sensory abnormalities, cerebellar signs, movement disorders, and neuropsychiatric findings (Al-Muskakeh *et al.*, 2022).

Humans may remain infected for life and will stay asymptomatic unless immunosuppression occurs (Herrmann *et al*., 2010). Primary infection of toxoplasmosis in immunocompetent subject is usually asymptomatic or associated with self-limited symptoms such as fever, malaise, and cervical lymphadenopathy (Khalid *et al*.,2023) Infection acquired during pregnancy is

often associated with transmission of *T. gondii* to the fetus, resulting in congenital disease., *T. gondii* infection causes severe manifestation, including splenomegaly, chorioretinitis, pneumonitis, encephalitis, multisystem organs failure, and even death In immunocompromised patients (Daher *et al.*,2021). *T. gondii* has a worldwide distribution and is of both medical and veterinary importance. Most of the *T. gondii* infections in humans are asymptomatic and have a benign course. However, in congenitally infected or immune-suppressed people, the consequence could be a serious illness or death (Vado-Sol´ıs *et al.*,2014). Cats are the definitive hosts for *T. gondii*; therefore, a major route of parasite transmission in humans and animals is via ingestion of fruit, vegetables, soil, or water contaminated with oocysts shed by cats. In addition, the parasite may be transmitted by handling or consumption of undercooked or raw meat containing tissue cysts (Vado-Sol´ıs *et al.*,2014).

*Toxoplasma gondii* may also cause spontaneous abortions during the first 3 month of pregnancy, when most of the fetal organogenesis is occurring. The probability of an abortion diminishes when infection occurs in the second or third trimester of pregnancy, but the probability of a child with congenital malformations increases (Curcio *et al.*,2020). The belief that long-lasting immunity is produced if a woman is infected before pregnancy is changing. It is now known that many atypical *T. gondii* genotypes exist and that those genotypes can differ in pathogenicity and transmissibility from the typical genotypes (Fabiani *et al.*,2022). Congenital infection is one of the most important sequels of toxoplasmosis in pregnant women. Congenital transmission of *T. gondii* predominantly occurs at the first time during pregnancy. The approximate incidence rate of congenital toxoplasmosis is 1.5

cases per 1000 live births with a global incidence rate of 190,100 cases annually (Abdoli *et al*,2017).

Frequency of transplacental transmission and severity of congenital toxoplasmosis correlates with the gestational age of infected mothers. The highest rates of transplacental transmission occur in the third trimester of pregnancy; which usually results in asymptomatic infections at birth. (Abdoli *et al*.,2017). Although several studies have reported an association between *T. gondii* infection and spontaneous abortion Congenital toxoplasmosis is particularly important during 2 periods of pregnancy. First, early (first trimester) maternal toxoplasmosis infection affects pregnancyinless than5% of cases, but may cause severe fetoplacental infections that generally lead to a miscarriage or, in a few continuing pregnancies, to major fetal lesions,mainly of the central nervous system(Kalantari *et al*.,2021).

## 2-7: Toxoplasmosis and pregnancy

Toxoplasmosis is a widely-distributed zoonosis caused by *Toxoplasma gondii* protozon. Although there is a high prevalence of unapparent infections, toxoplasmosis can develop into a severe systemic illness when in its congenital form, in which the mother, when infected for the first time during pregnancy, can present a temporary parasite with focal lesions generated within the placenta, thereby infecting the fetus (Lopes *et al*.,2007). Many diseases are more severe and dangerous to the fetus and Trans placental transmission may occur during pregnancy. As an immune-privileged organ, the placenta can tolerate the introduction of antigens without inducing a strong inflammatory response that would lead to abortion. However, for the

control of intracellular pathogens, a strong Th1 response characterized by the production of interferon-γ is needed ( Arranz-Solís *et al*.,2021).

Thus, invasion of the placenta by intracellular parasites puts the maternal immune system in a quandary: The proinflammatory response needed to eliminate the pathogen can also lead to abortion. *T. gondii* is a highly successful parasite that causes lifelong chronic infections and is a major cause of abortions in humans and livestock. Here, the study discuss how *T. gondii* strain type and parasite effectors influence host cell signaling pathways, and the study speculate about how this might affect the outcome of gestation ( Arranz-Solís *et al* .,2021)

The impact of toxoplasmosis on the health of the mother and the newborn should not be neglected. The surveillance, prevention, and control of toxoplasmosis are based mainly on research on IgM and IgG antibodies against *T. gondii* (Laboudi *et al*.,2021). The screening of the disease must be conducted early during pregnancy to allow the early detection of seroconversion that can lead to congenital toxoplasmosis. The clinical manifestations of congenital toxoplasmosis can be particularly severe when fetal contamination occurs during the first trimester of pregnancy (Laboudi *et al*.,2021). The most important concern associated with diagnosing congenital toxoplasmosis is to determine when the mother has been infected. The importance of using serological diagnostic tests such as IgG-avidity assay which can distinguish between recent and past infections has been proven (Azimi *et al*.,2022).

Toxoplasmosis is usually benign. However, it has adverse consequences in immunocompromised people and fetuses-newborns from women who have

contracted toxoplasmosis during their pregnancy. Congenital toxoplasmosis (CT) is the result of mother-to-child vertical transmission of the parasite, and the risk increases with gestational age (Boucoiran *et al.*,2020). The severity of the encountered disorders is inversely related to the pregnancy period at maternal infection. The disorders may range from severe abnormalities (mainly neurological and ophthalmic) or abortion during the first trimester of gestation, to manifestations of variable severity during the second trimester and asymptomatic traces at the third trimester (Dambrun *et al*.,2021).

Pregnant females in general are more susceptible to infection than non-pregnant females. Increase disease susceptibility and severity during pregnancy have been documented for a variety of diseases, including bacterial, viral and parasitic. It is the change in immune function during pregnancy that alters susceptibility and severity to these infections (Littauer and Skountzou ,2018).

Diagnosis of *Toxoplasmosis* in pregnant women represents a challenge for the obstetrician due to subclinical course in the majority of pregnant women and it is unknown what the long-term outcomes of congenital infection (Khalil *et al*., 2020). Infection throug\h placental transmission occurs in 10 - 80% cases where maternal infection is verified and this depends on the age of the foetus . Clinical severity of the infection has been found to decrease as the time of maternal infection progresses (Al Beloushi *et al*., 2021).

A woman that infected with *T. gondii* before conception rarely transmits the parasite to her fetus, but women who are acutely infected or experience a reactivation of *T. gondii* infection during pregnancy may transmit the organism trans placentally (Faral-Tello *etal.*,2023). Congenital toxoplasmosis

occurs secondary to an acute maternal infection acquired during pregnancy. In a congenital infection, the organism, after invading the pregnant mother's gastrointestinal tract, enters her bloodstream and invades multiple tissues, including the placenta (Kota and Shabbir, 2019).

Following multiplication in the placenta, the parasite infects the fetus. The likelihood and severity of fetal infection depend on when the mother is infected. The risk of congenital disease is lowest (10 to 25 percent) when maternal infection occurs during the first trimester and highest (60 to 90 percent) when maternal infection occurs during the third trimester (Souza *et al.*,2023).

Clinical toxoplasmosis manifests itself in several forms: disease at birth; disease occurring during the first month of life; sequelae or relapse of an undiagnosed infection during infancy, childhood or adolescence; or subclinical infection (Gilvaz ,2021). Previous studies showed that as a result of congenital infections approximately 90% of infants are asymptomatic (Nicloux *et al.*,2020).

During the first year of life or in early adulthood individuals were at their highest risk of developing retinochoroiditis (Lago *et al*., 2020). These studies showed evidence that 15% - 80% of children with prenatal *Toxoplasmosis* develop ocular disease. To prevent this, it is recommended that postnatal diagnosis is required and treatment should begin rapidly (Saso *et al*., 2020).

A hypothesis of relaxed quality control explains these phenomena by postulating that *T. gondii* could relax the stringency of some 'quality control' mechanism, which, under normal conditions, is responsible for miscarriage of

embryos with developmental defects and thus with a (statistically) slower foetal growth. This hypothesis can also explain an earlier observation of extremely high prevalence of *Toxoplasmosis* in mothers of children with Down syndrome (Kaňková *et al.*,2015).

## 2-8: Immunity against *Toxoplasma gondii*

The innate immune system is the first to respond to infection with production of interleukin (IL)-12 by neutrophils, dendritic cells (DCs), and monocytes but not macrophages t hat have phagocytosed *T.gondii* The innate immune system is the first to respond to infection with production of interleukin (IL)-12 by neutrophils, dendritic cells (DCs), and monocytes but not macrophages that have phagocytosed *T.gondii* ( Fisch and Frickel 2019).

The human cellular response to *T.gondii* -infection is highly dependent on cell type and the infecting strain of *T.gondii* (Saeij and Frickel ,2017). Interestingly, although the principal cytokine controlling *T. gondii* -infection is IFNγ, other cytokines have been implicated. For example, brain microglial cells control *T. gondii* growth by production of tumour necrosis factor α (TNFα) and IL-6 .TNFα is proposed to mediate *T.gondii* killing in patients with IFNγ receptor 1 (*IFNGR1*) deficiency, partially compensating for lack of IFNγ-responsiveness ( Fisch and Frickel, 2019).

Furthermore, IFNγ-independent control of *T.gondii* has been reported via cluster of differentiation (CD)40-induced autophagy of parasitophorous vacuoles (PV) in human macrophages , with the caveat that *T.gondii* activates epidermal growth factor receptor (EGFR) to combat its own autophagic

clearance . It is likely that several different host response pathways act in concert to control *T. gondii* -infection (Muniz-Feliciano *et al*.,2013).

One of the most distinctive immunologic features of *T. gondii* infection is the strong and persistent CMI elicited by the parasite, resulting in host protection against rapid tachyzoite growth and consequent pathologic changes (Sanchez and Besteiro ,2021).

Another interesting aspect of Toxoplasma-triggered immunity is that it is normally harmless to the host. Thus, in contrast to many other parasites and several experimental models of toxoplasmosis, *T. gondii* under normal conditions fails to elicit significant immunopathologic changes in immunocompetent hosts and is usually accompanied by symptoms no more severe than fever, fatigue, and lymphadenopathy (Saeij and Frickel ,2017).

In recent years, several trials on DNA-based and protein-based vaccines have focused on *T. gondii* antigens belonging to several major protein families, such as the glycosylphosphatidylinositol (GPI)-anchored proteins named SAG (surface antigens), SRS (SAG1-related sequences), and SUSA (SAG-unrelated surface antigens), rhoptry antigens (ROPs), dense granule antigens (GRAs), and micronemal proteins (MICs) (Dodangeh *et al*.,2019).

The most common antigens used for experimental cocktail vaccines together with SAG1 were ROP2 and SAG2. In addition, the most parasite strains used were RH and ME49. Freund's adjuvant and cholera toxin have been predominantly utilized. Furthermore, regarding the animal models, route and dose of vaccination, challenge methods, measurement of immune

responses and cyst burden have been discussed in the text (Pagheh *et al.*,2020).

Most of these experimental vaccines induce immune responses and have a high degree of protection against parasite infections, increase survival rates and duration and reduce cyst burdens. The data demonstrated that SAG1 antigen has a high potential for use as a vaccine and provided a promising approach for protecting humans and animals against toxoplasmosis (Pagheh *et al.*,2020).

Toxoplasmosis in immunocompromised individuals (AIDS, bone marrow transplant and neoplasia). The antigens that have been proposed to be used in vaccine candidate in various studies include surface antigens and secretory excretions that have been synthesized and evaluated in different studies. In some studies, secretory antigens play an important role in stimulating the host immune response. Various antigens such as SAG, GRA, ROP, ROM, and MAG have been from different strains of *T. gondii* have been synthesized and their protective effects have been evaluated in animal models in different vaccine platforms including recombinant antigens, nanoparticles, and DNA vaccine (Mamaghani *et al* .,2022).

## 2-9 Immune response against *T. gondii* in the intermediate host

The ability of *T. gondii* to persist in a wide range of intermediate hosts is the result of a balance between the host immune system and the parasite's own escape mechanisms. Noticeably, cell responses to infection are dependent on species and cell types infected by the parasite ( Sanchez and Besteiro ,2021). It is also known that the different parasite strains will not induce the same

immune response depending on the presence and the polymorphism of their effectors. Most of the *in vivo* infection data for *T. gondii* were generated in mice, not only because they are well-characterized models for mammalian immune function in general, but also because they are natural hosts of the parasite ( Arranz-Solís *et al*.,2021).

Although the findings described in this part mostly focus on mice as the archetypal model for mammalian response to *T. gondii,* it should be kept in mind that there are marked differences between humans and mice in sensor and effector proteins that determine host resistance to this parasite ( Mayoral ,2020).In the early stages of *T. gondii* infection, dendritic cells (DCs), macrophages, and monocytes are the first host cells to respond.

Classically, during pathogen infection the host will first identify the "non-self" via receptors called PRRs (pattern recognition receptors) located on the cell surface or inside the cell (like Tolllike receptors – TLR-). These receptors will generally recognize components of microbes or pathogens called MAMPs or PAMPs (microbes/pathogens associated molecular patterns). This way, innate immune cells will trigger the production of IL-12, a cytokine that plays an early and main role in the resistance to bacterial and parasitic infections (Rossella *et al*.,2021).

In mice, the main mechanism driving IL-12 production in response to *T. gondii* infection is through the recognition of *T. gondii* profilin by TLR11 and TLR12 (Mahmoudzadeh *et al*.,2021). However, other proteins or parasite molecules, such as glycosylphosphatidylinositols, can also activate TLRs (Costa *et al*.,2019). Humans do not have functional equivalents to all murine

TLRs, and thus may not use the exact same mechanism for parasite sensing (Sher *et al*.,2017).

## 2-10: The effect of parasite on human fertility

Latent infection of the globally spread parasite *Toxoplasma gondii* in humans has been associated with changes in personality and behavior. Numerous studies have investigated the effect of toxoplasmosis on depression (Hlaváčová *et al*.,2021). About a third of people in the world are infected with *Toxoplasma gondii*. This parasite has been found in the reproductive organs and semen of males of many animal species as well as humans. The effects of toxoplasmosis on sperm count, motility and morphology were confirmed in rats. A higher prevalence of toxoplasmosis has been observed in infertile men (Hlaváčová *et al*.,2021) .

Clonal strains of *T. gondii* are types I, II, and III, Type I, the hypervirulent strain, causes infections leading to death in immunocompetent mice. Type II and III strains cause nonlethal infections due to their avirulent nature; the chronic latent infections are the main manifestations of these strains. Adults usually have no manifestations following infection with *T. gondii*; nevertheless, Toxoplasmosis is associated with devastating outcomes in a developing fetus (Saki *et al*.,2020). Apoptosis is a type of programmed cell death that is morphologically homogeneous, apoptosis morphologically shrinks nuclei and cytoplasms, condenses nuclear chromatin, dilates endoplasmic reticulum, and blebs membrane (Chen *et al*.,2006). It is necessary in physiological conditions to regulate and fine-tune organelle function and architecture, it also can be induced or impeded during

pathological conditions such as infections, inflammations, and cancer (Aitken *et al*.,2011).

A dynamic and synchronized maturation of stem spermatogonia into mature spermatozoa is called spermatogenesis; the testicular seminiferous tubules are the major site of spermatogenesis. The first wave of spermatogenesis occurs as soon as the gonocytes differentiate into spermatogonia; apoptosis increases also occur in this phase (Houda *et al*.,2021).

Some studies have investigated the rate of apoptosis in infertile males and shown interestingly the higher rate of apoptosis in the individuals. Moreover, they have also reported the percentage of apoptotic sperm is more abundant in ejaculated semen samples from infertile men. Therefore, it seems apoptosis can be regarded as one of the etiological molecular pathways involved in male infertility (Saki *et al*.,2020).

Infections may be infertile through damage to the female reproductive system. There are some parasitic primary animals such as *Trichomonas vaginalis* which may cause genital tract abnormalities, cervical tumors and tubal and non-anomalous pelvic infections in women. *T. gondii* causes endometriosis, dysplasia of reproductive tract and synthetic disorders such as intrauterine adhesions (Al-Mussawi *et al*.,2020).

So the incidence of the *T. gondii* present and manifestations of some sex hormones at infertile persons.Follicle-stimulating hormone (FSH), 17-β Estradiol (E2), and testosterone are key regulators for the development and progress of germline cells. Also affect many defensive functions to the

immune system. *T.gondii* attempts to manipulating these Hormones concentration to their survive ( Al-Ardi ,2021).*T. gondii* is an important member of the infections group "TORCH" (Toxoplasma ,Rubella, Cytomegalovirus, and Herpes viruses ( Gyang *et al* .,2015)

The tissue cysts are used for the diagnosis of infection and also in immune compromised patients (e.g. Acquired immunodeficiency syndrome). the tissue cysts act as a source of organisms when these tissue cysts rupture for a fetus the severity of the disease depends upon the gestational stage of pregnancy in which the infection occurs, in fetuses the most significant congenital *T. gondii* infections can be caused in the first trimester (Ullah *et al*.,2022). About 35 to 45% of mothers who had been infected and developed serum antibodies to *T. gondii* infection before their first pregnancy, their fetus has no risk of infection, *T. gondii* antibodies "IgM" detection is useful especially in acute or primary infection diagnosis in "risk" individuals such as immunocompromised patients, pregnancy, and organ transplantation (Dupont *et al.*, 2021) .

Toxoplasmosis modifies various hormones and cytokines in the infected hosts which may result in several disorders. Also the hormons testosterone, DHEA, and prolactin concentration levels among *T. gondii* infected women (Bayani *et al.*,2022*). T. gondii* infections are also associated with modulation of circulating hormones, including testosterone and glucocorticoids, both positive and negative associations have been reported in the literature these hormones are known to target the amygdala and other interconnected regions of the brain, including the hippocampus and hypothalamus, which in turn regulate circulating steroid hormone levels that influence behavior (Heany *et al*.,2016).

The clinical implications of *T. gondii* infection in pregnant women are manifold. Unlike acute toxoplasmosis in pregnancy, which could result in congenital toxoplasmosis and serious damages to fetuses and newborns (Lucignani *et al.*,2022). latent toxoplasmosis of the mother seems to have no significant negative impact on the health of the offspring. However, pregnant women with latent toxoplasmosis have been reported to have prolonged pregnancy and seemingly younger (less developed) fetuses, especially at the 16th week of pregnancy (Flegr and Kaňková ,2020)**.**

*Toxopalasma gondii* could cause endometritis, impaired folliculogenesis, ovarian and uterine atrophy, adrenal hypertrophy, vasculitis, and cessation of estrus cycling in female and also decrease in semen quality, concentration, and motility in male (Rostami *et al.*,2020). A 1.9 % of women aged between 20 and 44 suffered from primary infertility, inability of young women to have their first live birth, and 10.5 % of women who already had one childsuffered from secondary infertility that is inability in the have another baby (Barrera *et al*., 2022).

Female mice developed more severe brain inflammation than male mice following infection. Moreover, a direct role for sex hormones was demonstrated in experiments which found that gonadectomy increased resistance, whereas estrogen administration exacerbated disease in mice. Similarly, simultaneous gonadectomy and estrogen treatment predisposed guinea pigs to increased parasite burdens compared with non-treated control animals (Zghair and Fadhel, 2015). It has been suggested that toxoplasmosis has some unfavorable effects on reproductive capacity in both men and women. The data obtained from limited studies performed in animal models

as well as in infertile couples, have supported the relationship between Toxoplasma and infertility (Nazarlu *et al.*,2020).

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 (Holec-Gąsior and Sołowińska ,2022).The data obtained from limited studies performed in animal models as well as in infertile couples, have supported the relationship between Toxoplasma and infertility (Niknamian,2019). *T. gondii* positive women reported to take a significantly longer time to conceive and to have more frequent or more serious fertility problems than *T. gondii*-free women ( Kaňková *et al.*,2015).

Many studies correlated between reproductive performance and Toxoplasmosis. *T. gondii* deteriorated the male and female reproductive performance in many experimental animals. Toxoplasmosis decreased hypothalamic, pituitary and gonadal secretion. Furthermore, Toxoplasmosis also caused profound adverse effect on human reproductive functions (Choudhari,2022).

Acute toxoplasmosis infection caused hypogonadotrophic gonadal insufficiency in male patients regardless of the course of the disease. The women with toxoplasmosis may complain spontaneous abortions, stillbirths, intrauterine growth retardation, preterm deliveries, or fetal anomalies (Ogbera and Anaba ,2021).

## 2-11: Cyclophilin A in human body

Cyclophilins are a family of proteins from vertebrates and other organisms that bind to cyclosporine (cyclosporine A), an immunosuppressant used to suppress rejection after organ transplantations. These proteins exhibit peptidyl proline isomerase activity, which catalyzes the trans to cis isomerization of peptide bonds on proline residues and facilitates protein folding (Liao *et al*., 2020). At present, 17 types of cyclophilins with different structures have been identified in human cells, among which the most abundant one is cyclophilin A (CypA), which accounts for 0.1–0.6% of the total cytoplasmic protein (Harikishore and Sup Yoon, 2015).

Cyclophilins have important homeostatic roles, but following tissue injury, cyclophilin A (CypA) can promote leukocyte recruitment and inflammation, while CypD can facilitate mitochondrial-dependent cell death **(**Leong *et al*.,2021). Cyclophilins contribute to many pathologic processes, and cyclophilin inhibitors demonstrate therapeutic activities in many experimental models. However, no drug with cyclophilin inhibition as the primary mode of action has advanced completely through clinical development to market (Kuo *et al*.,2019).

Cyclophilin A (CypA), which was initially discovered in 1984, is the primary target molecule of the immunosuppressant cyclosporine A. CypA is involved in numerous biological activities, including protein folding , inflammation , immunosuppression (Li *et al* .,2012).

A previous study conducted by demonstrated that CypA is able to promote myocardial hypertrophy and exacerbate the severity of Ang II-induced

myocardial hypertrophy. In rats, CsA effectively blocks or alleviates Ang II-induced myocardial hypertrophy by binding with CypA to form a dimer complex and inhibiting Ang II activity by binding to calcineurin (Venkatesan *et al* ,.2010).

Cyclophilin (CYP) is the major intracellular binding protein for the immunosuppressive drug cyclosporine (CS) (Gurung *et al.*,2023). CYP distribution was investigated in human tissues by solid-phase immunoassay, Western and Northern blot analysis as well as immunohistochemistry. CYP was found in all tissues examined at concentrations in the range of 1 microgram/mg protein. Furthermore, mRNA specific for CYP was found in every tissue, indicating local production of the protein. Immunohistochemical investigations revealed preferential parenchymal and only little stromal localization. Within certain organs, e.g. kidneys, regional differences of immunoreactive CYP was evident. The presence of CYP was also investigated in several lymphoid and non-lymphoid cell lines and was found at comparable concentrations. Immunogold staining confirmed cytosolic, but revealed also nuclear localization of CYP (Favretto *et al.*,2023).

Cyclophilin A is linked to diverse human diseases including viral infections. inhibitors of cyclophilin A (CypA), which is a ubiquitous, cytosolic protein with peptidyl–prolyl *cis–trans* isomerase (PPIase) activity first described in 1989, belonging to the immunophilin family. There is abundant experimental evidence suggesting that CypA is crucially involved in human diseases including not only cardiovascular diseases and cancers but also viral infections ( Han *et al.*,2022). Cyclophilin A is widely expressed by all prokaryotic and eukaryotic cells. Upon activation, CyPA can be released

into the extracellular space to engage in a variety of functions, such as interaction with the CD147 receptor, that contribute to the pathogenesis of cardiovascular diseases. CyPA was recently found to undergo acetylation at K82 and K125, two lysine residues conserved in most species, and these modifications are required for secretion of CyPA in response to cell activation in vascular smooth muscle cells (Rosa *et al*.,2022)**.**

The major forms of cyclophilins reported in humans include CyPA (PPIA), CyPB (PPIB), CyPC (PPIC), CyP40 (PPID), CyPE (PPIE), and PPIF (Hadpech and Thongboonkerd,2022). Herein, the study avoid using an acronym CyPD, which is somewhat confusing as it can refer to PPID (encoded by the *PPID* gene on chromosome 4) and/or PPIF (encoded by the *PPIF* gene on chromosome 10). Most of the cyclophilins (CyPA, CyPB and CyPC) are found in cytoplasm and extracellularly, while CyPE is found in nuclear compartment and PPIF is identified as a mitochondrial cyclophilin . Among them, CyPA is the most abundant cyclophilin accounting for about 0.1-0.6**%** of total protein in the cytoplasmic compartment (Hadpech and Thongboonkerd,2022).

## 2-12: Relation between cyclophilin A and *T.gondii* in infertility women

*Toxoplasma gondii* is a protozoan parasite that can infect all mammals, that serving as intermediate hosts. The cause of congenital toxoplasmosis is transplacental transmission of the parasite to the foetus, resulting in wide range of manifestations from mild chorioretinitis to miscarriage. Its frequency can be reduced by early screening of pregnant women which is based mainly on tests for anti-Toxoplasma antibodies **(**Khan *et al*.,2020). Parasites

belonging to the Apicomplexa phylum still represent a major public health and world-wide socioeconomic burden that is greatly amplified by the spread of resistances against known therapeutic drugs. Therefore, it is essential to provide the scientific and medical communities with innovative strategies specifically targeting these organisms (Fréville *et al* .,2022) .

Cyclophilin A plays important roles in inflammation and oxidative stress and is significantly increased in serum of preeclampsia (PE) patients. Preeclampsia (PE) is the most complicated type of pregnancy-related hypertensive disorder ( Leslie and Papageorghiou, 2011). A 3 to 5% of pregnancies in the United States and up to 8% of pregnancies worldwide are reported affected by PE. Combined with other hypertensive disorders PE acts as a main cause of maternal and fetal mortality and morbidity . The clinical manifestations of PE include severe high blood pressure, proteinuria, and complications such as renal and heart insufficiency, liver involvement, hematological disorders, preterm birth and fetal intrauterine growth restriction. It has been reported that PE is associated with inherited susceptibility, oxidative stress, immune regulation, and superficial implantation of placenta (sun *et al*.,2019).

Cyclophilin A is known as an inflammatory mediator that is secreted by various types of cells in response to inflammatory stimuli. Previous studies have shown that immunohistochemical expressions and/or circulating levels of CyPA are high in many diseases that cause inflammatory conditions in the body. Patients with PCOS have lower circulating levels of CyPA than women with normal ovaries. Decreased CyPA levels may be related to increased insulin resistance in PCOS patients (Usta *et al*.,2018).

Cyclophilin A is a 18 kDa protein with *cis–trans* isomerase activity that has intracellular and secreted forms. Cytosolic CypA is a multi-functional chaperone that takes part in folding, transport, and assembly of proteins, and in the regulation of cell proliferation (Wang and Heitman , 2005). and signal transduction from a T-cell receptor ,As CypA is also the main ligand for cyclosporine A (CsA), it thus mediates the action of this immunosuppressive drug (Kalinina *et al*.,2019). Both extracellular and intracellular CypA have been shown to play pathological roles in animal models of many diseases. data in humans show that the level of plasma CypA correlates with disease progression and severity. Cardiovascular diseases in which CypA plays a potential pathogenic role include carotid intima–media thickness, coronary artery disease, peripheral artery disease, PAH, and blood–brain barrier dysfunction. This diversity of diseases supports the concept that extracellular CypA is a pathogenic mediator of cardiovascular disease **(**Xue *et al* .,2018) .

Asherman's syndrome (AS) is characterized by intrauterine ad-hesions or fibrosis resulting from scarring inside the endometrium. AS is associated with infertility, recurrent miscarriage,and placental abnormalities. Although mesenchymal stem cells show therapeutic promise for the treatment of AS, pharmacologic and genetic tools,we uncovered that hUC-PVSCs secrete paracrine factors, such as Cyclophilin A (CYP-A), that improved compromised uterine environments via hypoxia inducible factor 1a (HIF1a)-dependent angiogenesis in mice with AS ( Park *et al*.,2020) .

Also Women with polycystic ovary syndrome (PCOS) are more likely to suffer from obesity, insulin resistance, and chronic low-grade inflammation than other women. Cyclophilin A is known as an inflammatory mediator that is secreted by various types of cells in response to inflammatory stimuli. The

immunohistochemical expressions and/or circulating levels of CyPA are high in many diseases that cause inflammatory conditions in the body. The patients with PCOS have lower circulating levels of CyPA than women with normal ovaries. Decreased CyPA levels may be related to increased insulin resistance in PCOS patients. Further research is needed to evaluate the association between CyPA and PCOS (Usta *et al*.,2018).

*Toxoplasma gondii* infection is one of the most prevalent infectious disease with worldwide distribution. Congenital toxoplasmosis is annually responsible for 1.20 million disability-adjusted life years around the world, but often it is overlooked many countries. *T. gondii* is associated with several brain related disorders in both mothers and newborns, and also it is cause of several abnormalities in reproductive organs ( Fallah *et al*.,2021).

CypA also plays a critical role in infection or the life cycle of certain parasites or host immune regulation. They are also candidate drug targets, in particular for the immunosuppressant cyclosporine A. In addition, cyclosporine is known to exhibit anti-parasitic effects on a wide range of organisms including several apicomplexa ( Park *et al*.,2019).

## 2-13/ The lipid phospholipase in the human body

Enzymes of the phospholipase superfamily are involved in lipid metabolism, as well as regulation of membrane composition, cell signaling, and inflammation. an insight into the structure, functional properties, and biotechnological application of phospholipase A2 and phospholipases in general (Filkin *et al*.,2020). Mammalian generates phosphatidic acid, a dynamic lipid secondary messenger involved with a broad spectrum of cellular functions including but not limited to metabolism, migration, and

exocytosis (Bowling *et al*.,2021). Historically, only one mammalian $PLA_2$ enzyme, which is abundantly present in pancreatic juice, was known before 1986 .The second $PLA_2$, which is stored in secretory granules of platelets and other immune cells and is markedly induced in various inflamed sites such as in synovial fluid of rheumatoid arthritis, was cloned in 1989 .Because of their sequence similarities to soluble $PLA_2$s present in snake venoms, pancreatic and synovial $PLA_2$s were termed groups I and II(Kudo and Murakami,2002).

Phospholipase $A_2$s constitute a wide group of lipid-modifying enzymes which display a variety of functions in innate immune responses The phospholipase $A_2$ ($PLA_2$) superfamily consists of a broad range of enzymes defined by their ability to catalyze the hydrolysis of the ester bond at the sn-2 position of glycerophospholipids. The hydrolysis products of this reaction, free fatty acid and lysophospholipid, serve as precursors for a variety of bioactive lipid mediators with important biological roles (Nagarajan *et al*.,2021) .

The $PLA_2$s are systematically classified according to sequence homology criteria, and include 16 groups (I-XVI), most of them with several subgroups, comprising more than 30 proteins. An alternative classification also exists that groups these enzymes into six major classes on the basis of biochemical similarities and/or cell regulation properties. These are the $Ca^{2+}$-dependent cytosolic $PLA_2$s, the $Ca^{2+}$-dependent secreted $PLA_2$s ($sPLA_2$), the $Ca^{2+}$-independent cytosolic $PLA_2$s, the platelet-activating factor acetyl hydrolases, the lysosomal $PLA_2$, and the adipose-specific $PLA_2$ (Murakami,2019). The $sPLA_2$ family represents the largest class of $PLA_2$ enzymes and possesses, as a common motif, a conserved His-Asp

catalytic dyad **(**Lambeau and Gelb, 2008). The $sPLA_2$s are widely distributed in pancreatic secretions, inflammatory exudates, and also in arthropod and snake venoms. A variety of biological activities have been described for $sPLA_2$s, including digestive actions, toxic activities (neurotoxic, myotoxic, hypotensive, etc.) and immune roles (Rodríguez *et al*.,2020) .

Throughout its evolution, *T. gondii* has developed mechanisms that enable a long-lasting parasite -host interaction to assure its survival without inducing life-threatening disease in the intermediate host, making it extremely adapted for infection in humans (Lang *et al*.,2007). Phospholipases $A_2$ ($PLA_2$) play an important role in *T. gondii* host cell penetration. They are also key enzymes in the host cell response to the parasite invasion. $PLA_2$ hydrolyse cellular phospholipids, releasing multiple inflammatory lipidic mediators (Teixeira *et al*.,2022).

These phospholipid-hydrolyzing esterases are crucial for membrane dynamics during host cell infection and egress by the parasite as well as for replication and cell signaling, and thus they are considered important virulence factors ( Flammersfeld *et al*.,2018).

Also the new way of evicting *Toxoplasma gondii* from cells. In resting cells, *T. gondii* creates a vacuole surrounded by a membrane, inside which it can replicate and grow without being destroyed by the immune system. However, when the immune system stimulates the cell with a protein called interferon gamma (IFNγ) multiple genes are activated, including a gene called RARRES3 which codes for a phospholipase enzyme and is regulated by a transcription factor called IRF1 (Sánchez-Arcila and Besteiro,2021).

## 2-14/ The immunity aspect in human body against the parasite infection

Twenty-first-century witnesses significantly rapid development in science and technology sectors. The 21st -century skill is required to master critical thinking skill very desirable in labor force in the education sector. Education will enable the students to acquire competency in skill considered as an important part of the curriculum high-quality education is student-centered and enables the students to achieve the level higher than the one expected to make them thinking critically, doing critical thinking, improving imagination, being creative, solving the problem, and making positive critique (Elisanti *et al*.,2018).

So the immune system is an important system for human health. The function of immune system is to protect the body from various pathogens such as viruses, bacteria, parasites. If the immune system is good, the protection from various pathogens that cause disease will be increasingly. However, the immune system can also be weakened. Factors that influence the work of the immune system are age, nutrition, exercise, hormones, and emotions. Eating nutritional food is one way to improve the immune system (Pratiwi and Pratiwy ,2020).

The discovery of interleukin (IL)-6 and its receptor subunits provided a foundation to understand the biology of a group of related cytokines: IL-12, IL-23, and IL-27. These family members utilize shared receptors and cytokine subunits and influence the outcome of cancer, infection, and inflammatory diseases (Wojno *et al*.,2019).

IL-6 and the heterodimeric cytokines IL-12, IL-23, and IL-27 belong to the class I hematopoietin family of cytokines, based on their shared four-α-helix-

bundle motif. This motif is oriented in an up-up-down-down topology and is only found in helical cytokines. The β subunits of the heterodimers are linked to the α subunits through a disulfide bond. The receptor binding partners for these Cytokines belong to the largest class of cytokine receptors, the hematopoietin receptor family ( Wojno *et al*.,2019).

Interleukin-17 (IL-17) is a pro-inflammatory cytokine secreted by activated T-cells. Recently discovered related molecules are forming a family of cytokines, the IL-17 family. The prototype member of the family has been designated IL-17A. Due to recent advances in the human genome sequencing and proteomics five additional members have been identified and cloned: IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. The cognate receptors for the IL-17 family identified thus far are: IL-17R, IL-17RH1, IL-17RL (receptor like) (Moseley *et al*.,2003).

The IL-17 family contains six isoforms of 20–30 kDa molecular weight and is a group of secreted and glycosylated proteins. All other members of the IL-17 family show 20–55% sequence homology to IL-17A, with IL-17E exhibiting the lowest homology with other family members . IL-17A and IL-17F could exist as heterodimers or homodimers and are co-expressed by linked genes . Structurally, IL-17 family proteins have a conserved C-terminus with four cysteine residues, which form intramolecular disulfide bridges (Ge *et al*.,2020).

During the acute phase of the *T. gondii* infection, tachyzoites multiply rapidly within the nucleated cells; finally, they lyse host cells and are hematogenously distributed throughout the body. After the acute phase, the host immune system develops a protective response; finally, it is altered from tachyzoite to bradyzoite form, and the formation of cysts occurs.The parasite

generates cysts in muscle and neural cells, including neurons, glial cells, and especially astrocytes . because the brain is an immune-privileged site for having a long life of T. gondii cysts ( Fallah et al.,2021).

IL-17A can be secreted by other cell subsets, such as γδT cells, cytotoxic CD8+ T cells, innate tissue-specific cells, innate lymphoid cells (ILCs), and myeloid cells . IL-17A-mediated inflammation is required for host protection and survival against infection . IL-17A can also exacerbate fetal inflammatory responses, and has been implicated in immunopathology. IL-17A levels are elevated in various inflammatory conditions, including sepsis, pneumonia, systemic lupus erythematosus, rheumatoid arthritis, allograft rejection, and cancer (Ge, and Yao,2020) IL-17A is produced mainly by lymphoid cells, including Th17, Tc17 and γδ T cells, and ILC3, in the course of various infections. Several pathogens also induce IL-17A production by neutrophils ( Ge and Yao,2020). While the induction of a cell-mediated response is essential for protection against *T. gondii*, the initial innate immune response led by neutrophils has also been reported to be critical for successful resolution of the infection . The factors involved in the development of this neutrophil response against infection have not been well studied. Recently, interleukin 17 (IL-17) has been shown to be one of the major Cytokines involved in the development and recruitment of neutrophils ( Kelly et.al.,2005) .

## 2-15/ The effect of Tumor necrosis factor inhibitors on the infected women

Tumor Necrosis Factor is a polypeptide protein which initially manufacture as a pro peptide by an enzyme TNF-α converting enzyme (TACE) to turn into a full form secretary TNF- alpha consist from 157 amino acids, mainly produced from activation of macrophage, dentretic cell and T-lymphocyte and less producing from BLymphocyte at T. gondii infection (Hilal and Hamad ,2019). TNFα (tumor necrosis factor) is both a pro-inflammatory and anti-inflammatory cytokine that is central to the development of autoimmune disease, cancer, and protection against infectious pathogens. As well as a myriad other activities, TNFα can be a product of T cells and can act on T cells.( Mehta *et al*.,2018).

Some macrophage-secreted cytokines are considered indirect biomarkers for toxoplasmosis , including IFN-γ, TNF-α and IL-10 That TNF-α was found to be increasing during infection and decreasing during the treatment back to its healthy level (Hussein and Ali,2022). TNFα is a dynamic pro inflammatory cytokine having pleiotropic actions on numerous cell kinds and an important role in the chronic disease pathogenicity. TNFα is secreted by different cell kinds including immune cells like (B cells and T cells, natural killer cells, basophils, dendritic cells, eosinophil, neutrophil and mast cells), nonimmune cells (astrocytes, granuloma cells, fibroblasts, glial cells, and keratinocytes) (Saheb *et al* .,2020).

Primary infection during pregnancy can be complicated by maternal-fetal transmission, responsible for congenital Toxoplasmosis. After the acute infection phase, toxoplasmosis evolves into a chronic infection during which the parasites become encysted in the brain and other organs where they remain

in a latent form, probably for lifetime of the infected person. Reactivation of these encysted parasites can occur in cases of immunosuppression and can result in pulmonary , cerebral , or ocular disease, the latter also being observed in immunocompetent patients ( Denis et al .,2022).

Despite the infections often being subclinical, *T. gondii* infection induces a strong immune response, as was demonstrated in animal studies, Recognition of the parasite through Toll-like receptor (TLR) signaling activates the production and secretion of many proinflammatory Cytokines that play a major role in the infection control , such as interleukin 1 (IL-1), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), l IL-6, IL-12 or, more controversially, IL-17A (Fisch et al., 2019). the infection with T. gondii enhances both humoral and cell-mediated immunity . The innate response to T. gondii includes the involvement of a wide range of cytokines such as interleukins, Interferon gamma (IFN-y), Tumour Necrosis Factor (TNF), Nitrogen monoxide (NO), a Reactive Oxygen species (ROS) and many other factors (El-Sherbin et al .,2019).

Alterations in levels of antibodies and cytokines during the reactivation of T. gondii infection happen, the role and function of cytokines, in cellular mediation, in the humoral response, as well as their impaired action during pregnancy and abortion processes need to be investigated. The present study was aimed to determine the levels of Interferon gamma (IFN-γ), Tumor Necrosis Factor alpha (TNF-α), and Interleukin 10 (IL-10) in the blood of aborted pregnant women (Al-Dorry *et al*.,2021) Recurrent spontaneous abortion (RSA) is defined as 2 or more times of pregnancy loss in the first 20 weeks of pregnancy. It is a common complication of pregnancy, which accounts for 2% to 4% (Al-Dorry *et al*.,2021) .

## 2-16/ Transforming growth factor beta and its relation with infertility

Jack of all trades, master of everything' is a fair label for transforming growth factor β1 (TGF-β) – a cytokine that controls our life at many levels. In the adult organism, TGF-β1 is critical for the development and maturation of immune cells, maintains immune tolerance , homeostasis, and regulates various aspects of immune responses. Following acute tissue damages, becomes a master regulator of the healing process with impacts on about every cell type involved (Lodyga and Hinz, 2020). Transforming growth factor (TGF)-β is a crucial enforcer of immune homeostasis and tolerance, inhibiting the expansion and function of many components of the immune system ( Batlle and Massagué, 2019).

The three TGF-β isoforms (TGF-β1, -β2, and –β3), are central regulators of cell differentiation, migration, proliferation, and gene expression and have been implicated in both reparative and fibrotic responses. The notion that TGF-βs mediate tissue fibrosis is supported by cell biological studies, animal model experiments, and clinical evidence (Frangogiannis, 2020).

Recurrent spontaneous abortion (RSA) is a common health problem in women of reproductive age . It is defined by the loss of three or more consecutive pregnancies before the 20th week of gestation (Motedayyen et al.,2018) .

Although a variety of factors such as infections and abnormalities including genetic, chromosomal, anatomic, immunologic, and endocrine have been reported for their attribution into the disease, no identifiable etiology was diagnosed in about 40–60% of patients (Motedayyen et al.,2018). Its distribution in worldwide and about 20-90% of world adult population has serum Toxoplasma antibodies. Several factors affected on the distribution of

this parasite such as cultural levels, age, residency, sanitation, nutritional habits, gender, modes and cat bearing houses (Saeed and Al-Aubaidi,2018).

Parasites will proliferate within the host cells lysing them, which they can disseminate through the body by blood circulation and infect any cells. Fetus through the placenta of infected mothers can acquire infection; congenital Toxoplasmosis may cause serious damage to the fetus (Saeed and Al-Aubaidi,2018).

*Toxoplasma gondii* excreted-secreted antigens (ESA) cause spontaneous abortion or fetal teratogenesis during the pregnancy in mice, especially in the early stage. Those adverse pregnancy outcomes are due to the deficit in regulatory T cells (Tregs) (Chen et al.,2019). only TGF-β1, TGF-β2, and TGF-β3 are present in mammals; the three isoforms elicit similar responses in vitro. TGF-β1, the most abundant isoform (Lodyga and Hinz ,2020) .

Transforming growth factor-β (TGF-β) family of proteins that have attracted much attention because of their ability to control cellular functions. TGF-β3 also contributes to tissue remodeling which occurs after infections and injuries, this cytokine contributes to development of Th17 and T regulatory lymphocytes, activation and suppression of immune which play significant roles in parasite responses, against infection (Hammadi and Hamad.,2021).

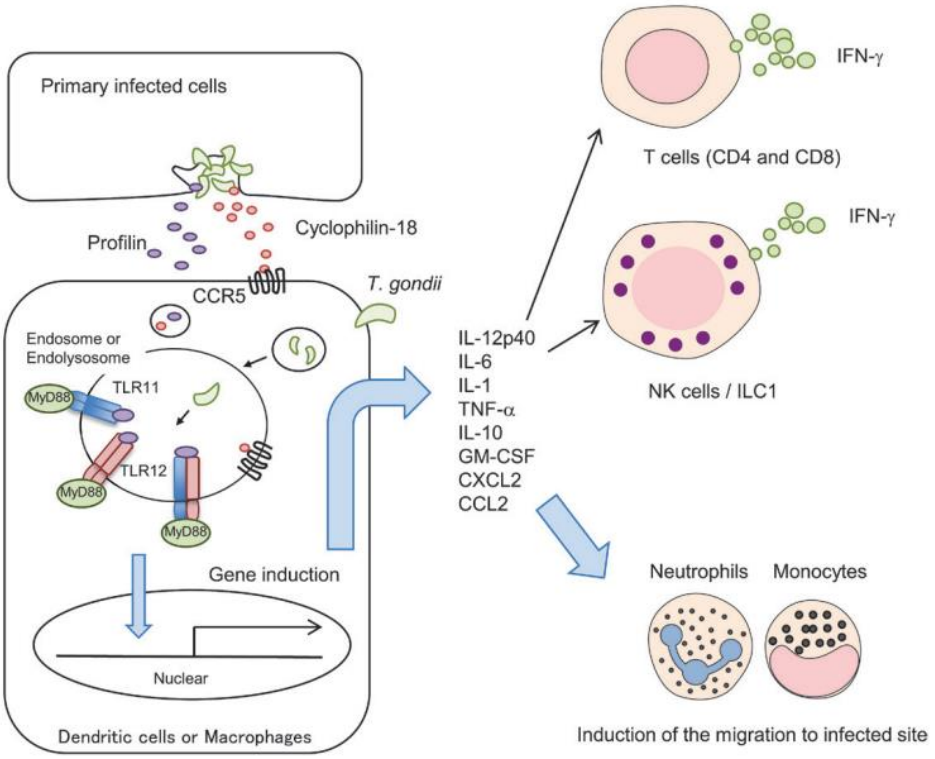
Multiplication of T.gondii in maternal tissues may induce a systemic immune response that harms cells in the fetal brain and course inflammatory necrosis. Similarly, elevated levels of interferon (IFN)-γ, transforming growth factor (TGF)-β and tumor necrosis factor (TNF)-α have been measured in the serum of pregnant women in the acute infection which has a harmful effect and cause abortion. Maternal infections induce clinical signs such as Ocular lesions, a different type of hydrocephalus and sometimes children are born with acute

Toxoplasmosis that characteristic by petechial rash, fever, jaundice, microphthalmia, hepatosplenomegaly, myocarditis and cataract ( Alsailawi et al .,2022) .

Infection of the placental tissue can result in a placentitis and can lead to subsequent infection of trophoblast cells, which are at the interface with the foetal compartment and may let the parasites proceed . This important process has two main consequences: (i) placental infection may adversely affect this tenuous equilibrium between maternal and foetal compartments; and (ii) the placenta is directly involved in parasite transmission to the foetus, making it a main therapeutic and diagnostic target (Ander *et al*., 2019).

This opinion paper focuses on these different aspects and places emphasis on the recovery of the placenta to diagnose congenital toxoplasmosis. Human trophoblast cells produce interleukin 10 (IL-10) and transforming growth factor b1 (TGF-b1) , which promote a Th-2 immune response to ensure maternal–foetal tolerance but induce a significant increase in both T. gondii intracellular replication and invasion(Brenier-Pinchart et al .,2011) TGF-β might be important in maintaining the balance between control and clearance of infectious organisms on the one hand and prevention of immune-mediated pathology on the other ( Poznansky et al.,2023).

(Figure2 -1) illustrate some of the immunological response of the human immune system to the *T. Gondii* parasite that was abovementioned.




**Fig( 2- 1): Recognition of *T. gondii* by innate immune cells leads to activation of acquired immunity. Macrophages and DCs produce various inflammatory cytokines and chemokines to promote IFN-γ Production from T cells, NK cells, or ILC1 and the recruitment of neutrophils and inflammatory monocytes to the infected sites.**

Additional well-designed, epidemiological, and experimental larger cohorts are required about the role of *T. gondii* in female infertility, recurrent miscarriages, and other abnormal reproductive outcomes, particularly in Iraq. Understanding the exact immunorespones of the host cells in humans or animals to the *Toxoplasma* parasites and the detailed cytokines interrelationships will promote clarification of exact intracellular protozoal behavior, recognize the innate response during early infection and the adaptive immunity for long-term protection, and will also provide important

insights for the development of therapeutic agents for immunocompromised subjects that exhibit severe susceptibility to the infection.

# Chapter Three
# Material and Method

# Materials and Methods

## 3-1: Study design

This work was a retrospective (descriptive-analytical) case-control study that included 250 patients and 50 subjects as a healthy control. The data were obtained based on a specific survey formula to exclude cases who had any medical disorders, information from the subjects was gathered via an interviewer-managed questionnaire (Table 1). The study was performed during the period from 1st February to 25th October 2022 from the Private laboratories and gynecology outpatient clinics of the two Babylon's leading hospitals: Babil teaching hospital for Maternity and Childhood, and Al-Imamul-Sadiq Teaching Hospital. A workflow of the present study is shown in Fig. 1.

The study questionnaire was based on identifying the name and age of the infertile female, the length of the marriage, any male factors of infertility, the kind of infertility (primary or secondary), the presence of any field or feline pets, the hormonal profile of sex hormones, thyroid and pituitary hormones, history of toxoplasma infection, history of uterine or other Mullerian anomalies, history of any hereditary and chronic disorders including diabetes mellitus, cystic fibrosis, asthma, emphysema, tuberculosis, pleurisy or other diseases of the lungs, albumin or sugar in the urine or other endocrine problems, and presence of pelvic inflammatory diseases. As well, the regularity of the menstrual cycle was assessed.

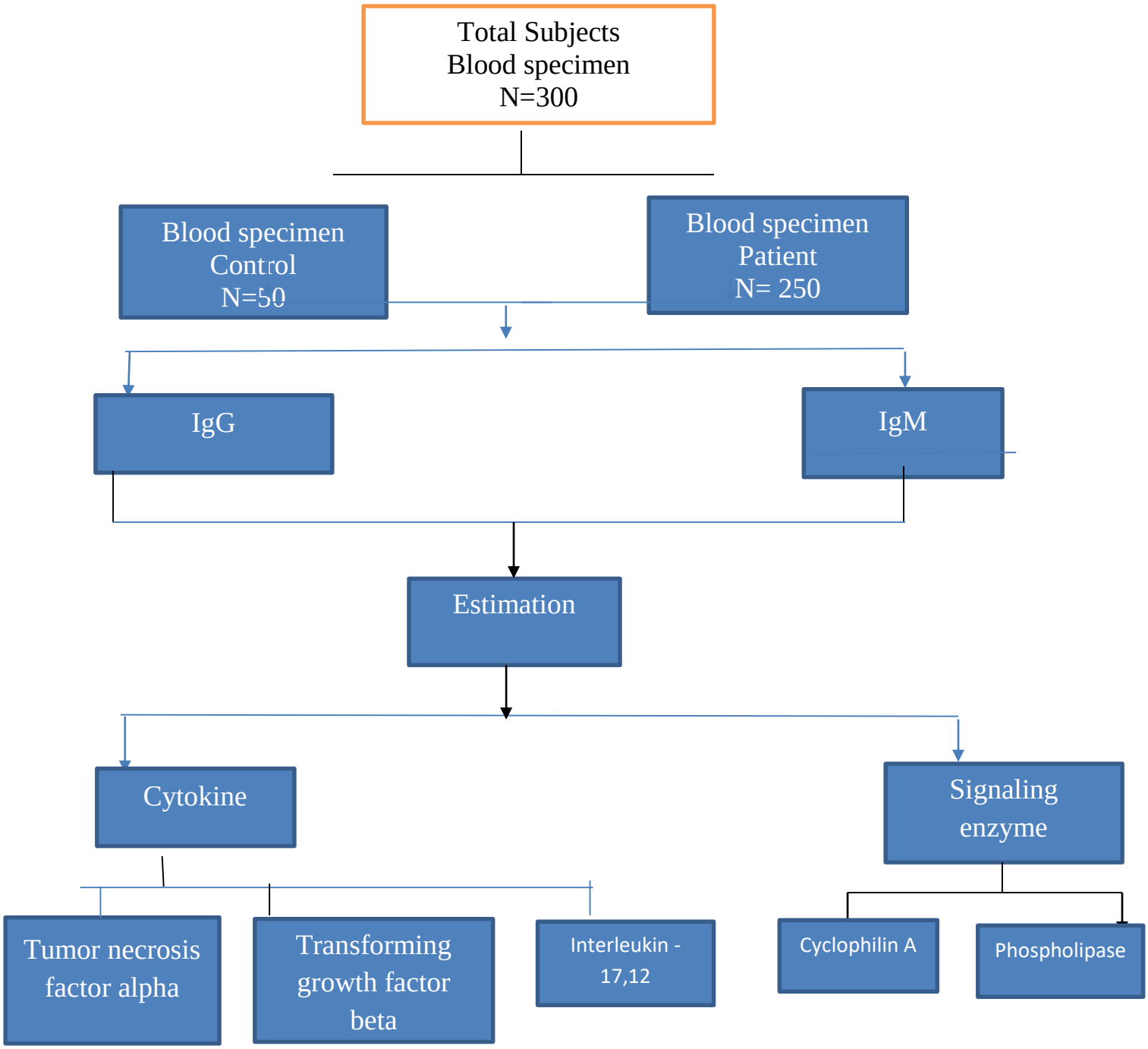



**Fig (1) Study design**

### 3-2: Types of infertility

Primary infertility is the inability to have any pregnancy, while secondary infertility is the inability to have a pregnancy after a previously successful conception ( world Health Organization, 2023 ) Women whose pregnancy spontaneously miscarries, or whose pregnancy results in a stillborn child, without ever having had a live birth would present with primary infertility (Mohammed *et al.*, 2016 )

### 3-3: Research parameters

The following parameters are being measured for both groups of the research: Phospholipase, cyclophilin A, IL-12, IL- 17, TNF-α, and TGF-β.

### 3-4: Instruments and Kits

The instruments and kits used in this study and their sources are presented in table (3-1), in the appendix consequently

**Table (3-1): Instruments and equipment for laboratories**

| NO. | Instruments | Company/ origin |
|---|---|---|
| **1** | Disposable syringes | Jiangsu/China |
| **2** | ELISA reader | Biotech/ USA |
| **3** | Eppendorf Tubes® | Germany |
| **4** | Gel tube | Germany |
| **5** | High speed centrifuge | Hettich/ Germany |
| **6** | Human Phospholipase, IL-12, IL- 17, TNF-α, and TGF-β ELISA Kit | Elabscience/ USA |
| **7** | Human Cyclophilin A, CYPA ELISA Kit | BT LAB/ Chaina |

| | | |
|---|---|---|
| **8** | Refrigerator | Kiriazi/ Egypt |
| **9** | Vidas | BioMerieux/France |

### 3-5: Sampling size procedure

The size was calculated based on the formula mentioned below.

$$n = 4 X p X q / d2$$

Where p = Prevalence (Prevalence of the disease which was taken as 50% as no records were available regarding the study).

q = 100 – p

d = Absolute error taken as 10%

n = 4 X 50 X 50 / 102

n = 100 ( Lijffijt *et al.*, 2009).

### 3-6: Ethical consideration

All infertile women were told of the importance of this research and fully explain the aims of this study and written consent were also obtained. The protocol of ethics was approved by the local ethical guide committee of the hospital and by the College of Science, University of Babylon. Interestingly, not all subjects were accepted to give their serum samples. The entire study protocol adhered to the recommendation of the Helsinki Declaration

### 3-7: Participants collection

#### 3-7-1: A sampling of the participants

A total of 250 hematological samples were taken from female patients. Patients were selected with a history of infertility. The diagnosis of *T gondii* infection among infertile females was performed by specialists and gynecologists based on history, clinical examination, laboratory investigation,

immunological, and appropriate imaging techniques. The control group included 50 age-matched healthy individuals from Hila City who were married, appaerantly healthy, has no history of abortion, were fertile, and revealed negative results for *Toxoplasma Gondii* IgG and IgM.

### 3-7-2: Selection criteria:

Inclusion criteria:

Females with either primary infertility or secondary infertility after recurrent abortions, during their reproductive age, have normal else other general medical conditions, regular menstrual cycle, and a probable or confirmed history of contact with any pets.

Exclusion criteria:

Extreme reproductive ages, females with male factors of infertility, abnormal hormonal profile, history of interrupted sexual relation, chronic severe debilitating disorders, females with advanced Mullerian anomalies, refuse to be enrolled in the study.

It is important to note that all infertile women with any of the aforementioned related diseases were initially disqualified from participating in this study; as a result, only 62 women who met the study's inclusion requirements could take part (N=62/250).

## 3-8: Immunological Assays

Five milliliters of blood were collected in plane tubes from the patient, then, serum was separated from whole blood by centrifugation at 2500 RPM for 10 min before being stored at -20ºC until use.

Serological assays

The detection of anti-Toxoplasma IgG and IgM in serum was performed by using enzyme-linked fluorescent and quantitative IgG tested by automated VIDAS® family instruments. This assay principle of the kit combines an enzyme immunoassay method by immunocapture with final fluorescent detection (ELFA). The procedure of this assay was accomplished based on manufacture instructions as recommended by BioMérieux Company® (France).

### 3-9: Immunological assays Phospholipase, IL-12, IL- 17, TNF-α, and TGF-β

Phospholipase, IL-17 and IL-12 were measured by using Enzyme-Linked Immunosorbent Assay (ELISA) technique by using Monobind Inc., Human Elisa kit (USA), according to the following procedure:

1. Select the wells for the sample, the blank, and the diluted standard. In the relevant wells, 100 µL of each dilution of the standard, blank, and sample was introduced.

2. The sealer included in the kit was used to cover the plate. The plate was then incubated at 37°C for 90 minutes.

3. Each well was immediately filled with 100 µL of the biotinylated detection Ab working solution after the liquid had been drained from each one. The plate was then given a fresh coat of sealant. At 37 °C, incubate for one hour.

4. the plate was removed the solution and filled each one with 350 µL of wash buffer. A clean absorbent surface was used to dry the plate after it had soaked for one minute and aspirated or drained the solution from each well.

5. Each well received 100 µL of the HRP conjugate working solution. At 37°C, the plate was incubated for 30 minutes.

6. The plate washed three times

7. Each well received 90 µL of substrate reagent. Then, incubated at 37°C for roughly 15 minutes. The last step was add 50 µL of stop solution to each well, followed by shielding the plate from light after the color has changed.

8. The results were recorded by set to 450 nm, the optical density of each well was calculated simultaneously.

**3-10: Measurement of Cyclophilin A protein**

This kit is also an Enzyme-Linked Immunosorbent Assay (ELISA), produced by Bioassay Technology Laboratory® (China), using Human Cyclophilin A (PPIA/CYPA) ELISA Kit. Human PPIA antibody has been pre-coated on the plate. PPIA from the sample is added and interacts with antibodies that have been coated on the wells. PPIA in the sample is then bound by a biotinylated human PPIA antibody that has been introduced. The biotinylated PPIA antibody is then bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed following incubation during a washing phase. Following the addition of the substrate solution, colour changes according to the concentration of Human PPIA. By adding an acidic stop solution, the process is stopped, and absorbance is measured at 450 nm.

Assay procedure of Cyclophilin A protein:

1. As directed, prepare all reagents, standard solutions, and samples. Before use, bring all reagents to room temperature. The experiment was carried out at room temperature.

2. Established the number of strips needed for the test. To use, place the strips in the frames. The strips should be kept between 2 and 8 °C.

3. The walls filled by 50 µL of standard. Since the standard solution already contains biotinylated antibodies, do not add additional antibodies to the standard well.

4. Forty ML of sample were poured of material into the sample wells, followed by 10 µL of Human PPIA antibody and 50 µL of streptavidin-HRP (not in the blank control well). Mix thoroughly, seal the plate, and incubate for 60 minutes at 37°C.

5. Removed the sealant and use a wash buffer to wash the plate five times. For each wash, soak wells in 300 µL. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300 µL wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Added 50 µL substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. Added 50 µL stop solution to each well, the blue color will change into yellow immediately.

8. Determined the optical density of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

### 3-11: Statistical Analysis

All statistical analyses were performed using SPSS 22.0 software statistical package (SPSS Inc., Chicago, USA) suitable for window. Student's t-test was performed to determine statistical significance between any comparative statistic between healthy group and patients. Statistical differences among the quantitative variables were investigated by ANOVA test. Spearmen's Correlation coefficient was used to evaluate any relationships between the study variables. Quantitative continuous results and data were displayed as mean and standard deviation. Categorical variables were presented as frequencies and percentages. The significant variation is accepted at the level of differences ($p < 0.05$) between the control and treated groups. ( Daniel and Cross,2018)

# Chapter Four
# Result and Discussion

# Result and discussion

## 4-1: main characteristics of the studied variables

Only 62 (24.8%) of the 250 infertile females met the requirements for the research study and were thus enrolled as a patient group. Table 1 presented data related to age, marriage duration, infertility type, presence of certain health conditions, and other variables among the infertile participants. Based on the table, it seems that the average age of the patients was 31.6 ±4.3 years. Nearly equal numbers of infertile patients were distributed among the age categories. This finding is consistent with that of (Eltantawy *et al.*, 2014) who both found no significant differences in the Egyptian infection rates among the studied age groups in their study, which include 319 infertile female patients. Another Iranian study also demonstrated comparable results (Soltani *et al.*, 2021). They displayed no significant association between *T. gondii* infection and different age groups, although the seroprevalence rate in 41-50 years was higher than that in other groups. However, a prior larger Korean study included 1,265 patients who failed to repeat such observations and reported that the prevalence tended to increase with age, although this increase was not statistically significant and a peak seroprevalence was detected in the 40-49year old age group in a preceded survey (Shin *et al.*, 2009).

The literature suggests though it is not conclusive, that the duration of infertility is considered in an inverse relationship, and the longer the infertility, the lower the chance of achieving pregnancy ( Alakkam and Salim, 2022). In the current study, the average duration of marriage was 3.9±1.3 years. This means the duration of infertility preceding seeking medical advice is rather longer than what is expected in our society (Fayyad, 2012). Possible explanations include 1) delayed parenthood as

some couples may delay starting a family until later in their marriage due to various personal or professional reasons, this could potentially impact fertility, especially for women, due to age-related fertility decline ( Muhammed and Alsakee, 2021). 2) financial causes of the couples that delay consultation. 3) sociodemographic reasons (Hassan *et al*., 2022). 4) earlier ages of females at marriages.

Thirty-three (13.2%) of the infertile women had additional infertility reasons (male factors) in their partners, which was harmonious with the results published by other Iraqi studies (Fayyad, 2012, Mohammed, 2021). Meanwhile, there were 111 (44.4%) participants with primary infertility and 139 (55.6%) participants with secondary infertility.

Out of the total participants, 152 individuals (60.8%) reported having field or feline animals and 68 participants (27.2%) reported having a period infection of toxoplasma. Several recent studies performed in human and animal models have demonstrated that latent toxoplasmosis is associated with reproductive organ disorders, and infertility, in pregnant women (Zamaniyan *et al.*, 2023). Accordingly, the authors advise that screening and treatment of Toxoplasma infection among infertile women must be favorably considered.

Forty-one participants (16.4%) were found to have an abnormal hormonal profile and 71 (28.4%) participants were diagnosed with some sort of endocrine disorder, and more than half of the participants complained an irregular menses. Several pieces of evidence from the past literature demonstrated that many cases of female infertility can reflect numerous endocrine derangements. Stress, (Lewinski, 2023), cortisol (Karunyam *et al.*, 2023), growth hormone, (Chang *et al*., 2022), thyroid hormones (Mazzilli *et al.*, 2023), ovarian (Alam *et al*., 2023; Al-Bdairi,

2021; Al-Bdairi 2021), pituitary disorders (Bendarska-Czerwińska *et al*., 2023), as well as several other endocrine disorders.

Twenty-two participants (8.8%) were diagnosed with any Mullerian anomalies. Congenital uterine anomaly is a type of female genital malformation that results from abnormal formation, fusion, and resorption of the Müllerian duct during the early process of development in the fetus (Albalushi *et al.*, 2023). A meta-analysis of studies on mullerian anomalies, found higher rates of infertility and spontaneous abortion (Venetis *et al*., 2014) among the studied females.

**Table (4-1): Main characteristics of the studied variables among all-participant patients (N=250)**

| Variables | | Descriptives |
|---|---|---|
| Age/ years (mean ± SD) | | 31.6 ± 4.3 |
| Age categories N= 62 | 20 – 29 | 33 (53) |
| | 30 – 39 | 29 (47) |
| Period of marriage (mean ± SD) | | 3.9 ±1.3 |
| Male factors (mean ± SD) | | 33 (13.2) |
| Type of infertility | Primary | 111 (44.4) |
| | Secondary | 139 (55.6) |
| Have field or feline animals N (%) | | 152 (60.8) |
| Have period infection of toxoplasma N (%) | | 68 (27.2) |
| Abnormal hormonal profile N (%) | | 41 (16.4) |
| Any Mullerian anomalies N (%) | | 22 (8.8) |
| Chronic disease N (%) | | 11 (4.4) |
| Sugar or albumin in urine N (%) | | 6 (2.4) |
| Endocrine disorders N (%) | | 71 (28.4) |
| Irregular menstrual cycle N (%) | | 140 (56) |
| Pelvic inflammatory diseases N (%) | | 100 (40) |

Pelvic inflammatory diseases (PID) were found in 100 (40%) participants. For women of childbearing age, PID is a significant cause of illness and reproductive problems. To better evaluate and discuss the various treatment methods that are available if spontaneous conception does not occur, couples trying to conceive should assess their PID history as early as possible (Hunt and Vollenhoven, 2023).

**4-2: Seroprevalence of Toxoplasma antibodies (IgG and IgM) among the participants**

Immunological verification of *T. gondii* antibodies (IgG and IgM) among all participants (250 patients and 50 controls) revealed an overall infection rate of Toxoplasma was 24% among infertile women, which indicated by a positive serological result for IgG and/or IgM antibodies, Table (4-2).

Unlike the relatively low prevalence rate in our study, a recent 10 years Iranian study that included 520 infertile women was referred to the infertility center during 2010-2019. The revealed a high seroprevalence rate of *T. gondii* equal to anti-*T. gondii* IgG, IgM, and both IgM & IgG antibodies were detected among 65.8%, 0.8%, and 0.19% infertile females, respectively (Zamaniyan *et al*., 2023).

**Table (4- 2): Seroprevalence of Toxoplasma antibodies (IgG and IgM) among all the participants (N=300)**

| Serological test N (%) | Positive | Negative |
|---|---|---|
| *Toxoplasma gondii* (IgG) | 22 (8.8%) | 228 (91%) |
| *Toxoplasma gondii* (IgM) | 40 (16.0%) | 210 (84%) |
| Total | 62 (24.8%) | 188 (75%) |

## 4-3: Seroprevalence distribution of *Toxoplasma gondii* according to age groups

The table (4-3) presents the presence of *T. gondii* infection among infertile patients. It divided the infertile patients into two categories: "*T.gondii* positive" and "*T. gondii* negative", and distributes *T. gondii* IgG and IgM Antibodies within these categories. These antibodies are specific to *T. gondii* infection and are significant for diagnosis and monitoring. IgG avidity is a vital test for screening females who need treatment (Sharifi *et al*., 2018)

**Table (4- 3): Seroprevalence distribution of *Toxoplasma gondii* according to age group**

| Infertile patients | 30-39 years | | 20-29 years | | Total |
|---|---|---|---|---|---|
| ***Toxoplasma gondii* positive** | 29 (47%) | | 33 (53%) | | 62 |
| | IgG | IgM | IgG | IgM | |
| | 14 (22.6%) | 15 (24.2%) | 8 (12.9%) | 25 (40.3%) | |
| ***Toxoplasma gondii* negative** | 79 (42%) | | 109 (58%) | | 188 |
| | IgG | IgM | IgG | IgM | |
| | Negative | | Negative | | |
| **Total** | 108 (43.2%) | | 142 (56.8%) | | 250 |

The result showed that in the age group (20-29) a relative increase in the percentage of *Toxoplasmosis* infection compared with other age groups. This finding seems similar to several prior studies (Sharifi *et al*., 2018). In contrast, such a finding was disliked that reported by a preceded study, which revealed that although there was no significant association between *T. gondii* infection and different age groups, the seroprevalence rate in 41-50 years was higher than that in other groups (Soltani *et al.*, 2021) and another study (Alvarado-Esquivel *et al*., 2009) also published comparable results.

The prevalence of the parasite *T. gondii* in women varies depending on the climatic conditions, dietary and health practices, socioeconomic status, degree of education, and age in various countries throughout the world (Mizani *et al.*, 2017).

Serum samples from the current study tested positive for anti-Toxoplasma antibodies, with positive results identified as (15) for anti-IgM antibodies and (14) for anti-IgG antibodies. This study contrasts with one by Farhan (Farhan, 2022), which found that 21 abortion women tested

positive for anti-*Toxoplasma* antibodies, with positive results identified as (9) for anti-IgM antibodies and 12 (57.14%) for anti-IgG antibodies.

The current study also conflicts with (Al-Awadi *et al*., 2022), which found that among women in Baghdad with a history of spontaneous recurrent miscarriage, the seroprevalence of *Toxoplasma* IgG antibody was 59% and seroprevalence of *Toxoplasma* IgM antibody was 8%. The age group (20–29) had the largest percentage of infected patients (53%) and the age group (30–39) had the lowest percentage (47%).

This study agreed with (Ubaid Hamza, 2022) that infection rates were highest among those aged 26-35 and lowest among those aged 35-45. This high incidence of seroprevalence in the 20-29 age group could be attributed to more frequent contact with cats or infected vegetables and also similar with Similar to the result of (Nazari *et al.*, 2019), a high prevalence of seropositivity was seen in the 25-30 age range in Iran.

There is clear evidence from the past kinds of literature that usually, to show if a person has been infected in the past, or has recently been infected; a combination (IgM and IgG) of serological tests is required (Elmore *et al*., 2010). These tests frequently detect "nonspecific IgM" as well as "residual IgM" (associated with a stable positive IgG titer). In such cases and the absence of a preceding positive result, a complementary test done by specialized laboratories (such as ISAgA IgM or IgA, IgG avidity test, differential agglutination, etc.) is indispensable to prevent any error of interpretation. These complementary tests as well as the serological evolution (control done in 15 days) permit, in a large number of cases, to reassure the patient with full confidence (Sharifi *et al*., 2019).

Toxoplasma IgM antibodies had more appearance than the IgG antibodies in acute cases and this agreed with Flori *et al*., 2009, who said:

"The IgM antibodies appear sooner after infection than the IgG antibodies and disappear faster than IgG antibodies after recovery"

## 4-4: Serological evaluation of Interleukin-12, Interleukin-17, Transforming Growth F actor–β, Tumors necrosis factor -α, Phospholipase A, and Cyclophilin-A

All the serological parameters of the study were higher among the patients either highly significantly (interleukin-17, transforming growth factor–β, tumors necrosis factor-α, and phospholipase A) or significantly (interleukin-12 and Cyclophilin-A), Table 4-4.

**Table (4- 4): Concentration of Interleukin-12, Interleukin-17, Transforming Growth Factor–β Tumors necrosis factor-α, Phospholipase A, and Cyclophilin-A between toxoplasma patients and the control**

| **Variables** M ±SD | Patients (N=62) | Control (N=50) | P-Value |
|---|---|---|---|
| **Interleukin-12** | 71.4 ± 11.9 | 64.9 ± 13.8 | 0.041* |
| **Interleukin-17** | 250.1± 66.3 | 207.5± 61.9 | 0.008* |
| **Transforming Growth Factor–β** | 56.3 ±26.6 | 29.2 ± 16.7 | 0.001** |
| **Tumors necrosis factor-α** | 78.9 ±45.3 | 49.8 ± 2.1 | 0.001** |
| **Phospholipase A** | 250.1 ± 66.3 | 207.5 ±61.9 | 0.008* |
| **Cyclophilin-A** | 22.9 ± 2.2 | 20.0 ±2.9 | 0.02 |

**P> 0.05**

The levels of serum IL-12 in toxoplasmosis patients were higher significantly compared to the control group (p-0.041). This was supporting earlier findings, which disclosed a highly significant level of IL-12 in both sexes with latent toxoplasmosis in comparison with free-toxoplasmosis groups. Correspondingly these findings also agreed with another recent study in Erbil by Muhammed and Alsakee 2021, which revealed a

relatively high IL-12 level and significantly elevated in the sera of women with toxoplasmosis. And also agree with the study showing that anti-Toxoplasma IgM serum levels and IL-12 serum levels were higher compared with controls (Al-Kuraishi *et al.*, 2020). As well, the placental IL-12 level was higher in the pregnant women with acute *T. gondii* compared with controls.

It is known that Natural Killer cells (NK cells), as a type of cytotoxic lymphocyte, have critical protective roles in innate immunity during the *T. gondii* infection by releasing interferon-gamma (IFN-γ) ( Mahmoudzadeh *et al.*, 2021). IFN-γ is a multifaceted molecule produced by NK cells and is connected to anti-proliferative, pro-apoptotic, and antitumor processes (AL-Shimmery *et al.*, 2023). Interleukin-12 (IL-12) is a pivotal critical cytokine for the generation of IFN-γ-producing NK cells. Several studies have shown cytokines' impact on NK cell activation; and IL-12 has an important role as a potent stimulatory factor for NK cells ( Mahmoudzadeh *et al.*, 2021).

The release of the innate cytokine IL-12, produced by DCs, macrophages, and neutrophils is dominant to host protection against *T. gondii* infection (Khan and Moretto, 2022). The role of IL-12 which is primarily produced by T-follicular helper cells (TFH), a subset of CD4-T cells, has also been implicated in regulating antigen-specific CD8-T cell dysfunction during chronic Toxoplasma infection. Additionally, cytokines that modulate the TFH subset have been shown to regulate the antibody response against *T. gondii* (Moretto *et al.*, 2017).

Furthermore, IL-12 was found to increase the production of IgG but not to inhibit IgE. IFN-*γ*, IL-2, and IL-12 are involved in the protection against parasitic invasions. IL-12 stimulates the production of IFN-*γ* and TNF-*α* and activates lymphocyte Cytotoxicity (Evering and Weiss 2006).

Harmonious to previous studies, the present study expressed significantly higher interleukin-17 serum levels among the toxoplasmosis patients compared to the healthy controls. Several lines of evidence give reason to believe that IL-17 stimulates protective immunity against the intracellular pathogen in particular *T. gondii* ( Vesely *et al.*, 2020).

$CD8^+$ T cells play an essential role in the protection against both acute as well as chronic *T. gondii* infection. Immune CD8+ T cells from *T. gondii* infected host are an important source of IFN-γ, a cytokine that is critical for survival against both acute as well as chronic phases of infection ( López-Yglesias et al .,2019). Moreover, CD8+T cells from *T. gondii*-infected hosts can exhibit in vitro cytotoxic activity against parasite-infected targets ( Kongsomboonvech *et al.*, 2020). The Cytotoxic function of these antigen-specific CD8+T cells has been reported to play an important role in keeping chronic infection under control *(* Khan *et al*.,2019). Depletion of either IFN-γ or CD8+T cells abrogates protective immunity against *T. gondii* infection leading to morbidity or mortality of the infected host ( Bhadra and Khan, 2010).

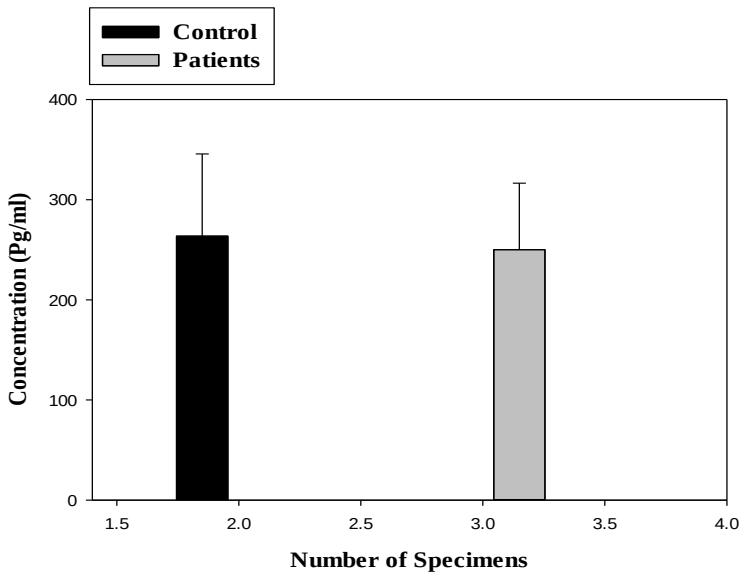



**Fig (4-1) Comparisons between patient and control (IL-17)**

However, a lack of IL-17 alone has minimal impact on splenic CD8+ T cell maturation or effector function development during acute

Toxoplasmosis. While, the absence of both IL-17 and IL-15 only in the context of infection severely down-regulates the development of a potent CD8+ T cell response ( Bhadra and Khan, 2010).

Immunological studies have revealed several parasite virulence factors regulated by the disengagement of immune activity. IFN-γ is essential for host cell resistance and acts by upregulating the expression of IFN-γ-activated effectors that destroy *T. gondii* (Sturge and Yarovinsky, 2014). Several parasite factors block the proper functioning of IFN-γ. One of these is the dephosphorylation of STAT1 by the suppressor of cytokine signaling-1 ( Ahmadpour *et al.*,2023).

Additionally, dendritic cells (DCs) act as carriers of systemic parasites during infection. It has been shown that the *T. gondii* parasite can transmit from DCs to NK cells. Rapid transfer of *T. gondii* from infected DC to effector natural NK cells may contribute to the parasite's sequestration and shielding from immune recognition shortly after infection ( Ahmadpour *et al.*,2023).

*T. gondii* can also be killed independently of IFN-γ by autophagy in mouse macrophages by the involvement of the TNF receptor superfamily, (Bendarska-Czerwińska *et al*.,2023). demonstrated that murine congenital toxoplasmosis increases skull apoptotic index and skull apoptosis associated with increased IFN-γ expression, but it decreases TNF-α expression (Suwanti and Mufasirin, 2018).

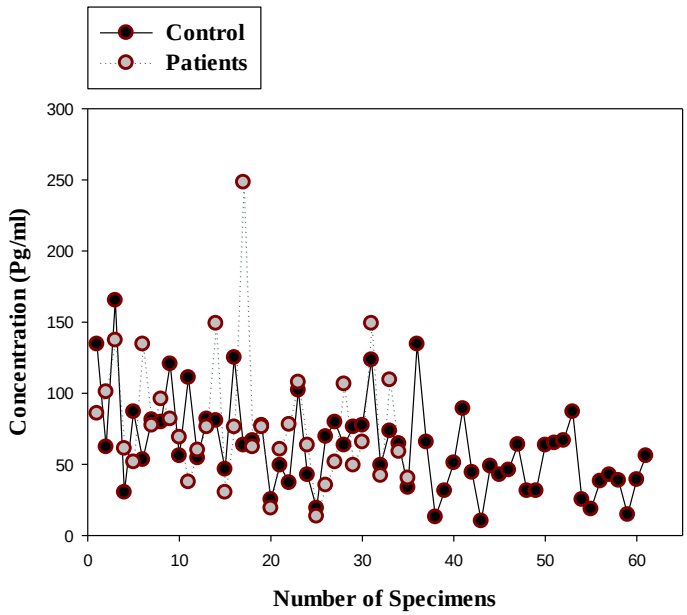



**Fig (4-2) Comparisons between patient and control (tnf α)**

However, the existing study disagreed with (Matowicka-Karna, 2009), which illustrates that in the course of *Toxoplasmosis*, the levels of TNF-*α* did not change and also disagree with what was reported by (Lang *et al.*,2007) The latter suggested that *T. gondii* inhibits the production of TNF-*α* and together with IL-6 can enhance the proliferation and differentiation of B lymphocytes (Lang *et al.*,2007). TNF-*α* activates eosinophil cytotoxicity toward protozoa and induces secretion of acute phase proteins via IL-6 production (Al-Shimmery *et al.*, 2023). Mutually, the effects of TNF-*α* and IFN-*γ* have antiproliferative properties. Thus, in toxoplasmosis, TNF-*α* appears to be essential for macrophage activation and inhibition of parasite replication possible only in cooperation with IFN-*γ*.

An increasing body of literature suggests a characteristic immunoregulatory role of TGF-*β* that inhibits T-cell activation directly and indirectly and promotes immunosuppressive modalities (Al-Hindy, 2020, Dleikh, 2020; Ramadan, 2022)

Recent clinical trials have established an unusual role for TGF-β in promoting the formation of a new generation of T-helper (Th) cells known as Th17 cells, acting in conjunction with IL-6 and other inflammatory cytokines (Veldhoen *et al.*, 2006). The mean TGF- β concentration in the patient's serum in this investigation was noticeably higher than that of the control.

This result was consistent with a study (Baneen *et al.*,2021)that revealed a significant association of TGF-β levels during Toxoplasma infection. The study concluded serum TGF-β levels as a candidate biomarker for the pathological effect of *T. gondii* during pregnancy.

A previous study reported that the level of placental TGF-β level was decreased in mice including adverse pregnancy outcomes after *Toxoplasmosis* infection (Liu *et al*., 2014). Other studies reported that TGF-β levels were raised in pregnant with acute *T. gondii* contagion in contrast to those of uninfected pregnant (Marchioro *et al.*, 2018).

TGF-β1 was higher in females with anti-Toxoplasma antibodies ( Abdulkhaliq *et al.*,2017). During pregnancy, TGF-β1 and TGF-β2 induce endometrial cell apoptosis, while TGF-β3 promotes endometrial cell proliferation. The differential regulation of TGF-β subtypes on endometrial cells may be the key regulatory mechanism of endometrial decidualization (Yang *et al.*, 2021).

On the other hand, TGF-β induces immune responses by the development of Th17 lymphocytes and mucosal immunity. Despite this, the role of immunomodulatory cytokines such as TGF-β is controversial, suggesting that it inhibits inflammation, in this way diminishing tissue damage, but allowing the course of chronic infection. The relationship between TGF-β and immune responses against *T. gondii* is not fully understood (Gómez-Chávez *et al.*, 2020; Sana *et al.*, 2022).

Meanwhile, other recent observational, and epidemiological studies have failed to confirm these associations between TGF-β and immune responses against *T. gondii* and they attributed this sort of association to other regulatory mechanisms (Zare-Bidaki *et al.*, 2016).

The data generated by the current study reported in Table (4-3) revealed a significantly high means of TNF-α concentration in the serum of the patient compared to the control. These results were complied with (Baneen. et al., 2021). which showed that the TGF-β levels and their relationship during infection are significant. T regulatory cells provoke secretion of TGF-β and IL-10 that compromise immune response against the intracellular parasites. TGF-β induces the evolution of Th17 responses and produces the immune response.

During the past five years, numerous researchers, principally in Iraq and Kurdistan, have conducted frequent diagnostic techniques for early detection of congenital Toxoplasmosis in pregnant and aborted women and applied the most recent tests and procedures to reach results that can be trusted. These techniques include numerous serological and molecular techniques. Our research validates that patients with *Toxoplasma* infections have higher concentrations of phospholipase A. This work supports a prior observation that phospholipase promotes *T.gondii* host cell penetration ( Najm, 2021).

Phospholipase A2 greatly enables *T.gondii* entrance into host cells (PLA2). They play a critical role in the host cell's reaction to the invasion of the parasite. The cellular phospholipids of PLA2 are hydrolyzed, generating several inflammatory lipidic mediators (Cassaing *et al*., 2000). These phospholipid-hydrolyzing esterases are significant virulence factors because they are essential for membrane dynamics during parasite invasion

and egress from the host cell, as well as for replication and cell signaling (Al-Kremy and Al-quraishi, 2022).

Infections caused by several bacteria often include phospholipase A2 (PLA2), which has been linked to host cell invasion and has a substantial pathogenic function. PLA2 plays a crucial role in the cellular invasion by the tachyzoite stage of the intracellular Toxoplasma gondii protozoa a process that involves multiple phases (Al-Kremy and Al-quraishi, 2022). Human cells now contain 17 different types of cyclophilins, with cyclophilin A being the most common and accounting for 0.1-0.6% of the total cytoplasmic protein. These cyclophilins have different structural properties (Harikishore and Sup Yoon, 2016)

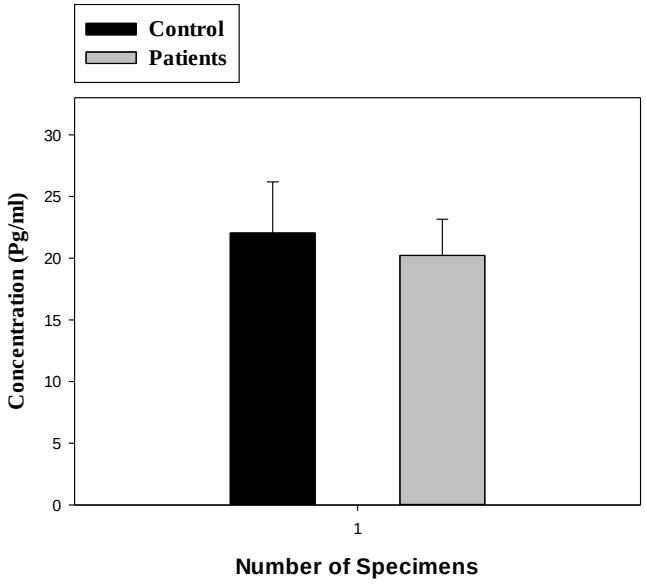



Fig (4-3) Comparisons between patient and control (cyclophilin)

Cyclophilin A, which is significantly higher in the serum of preeclampsia patients (the most challenging kind of pregnancy-related hypertension disease in pregnancy), plays crucial functions in inflammation and oxidative stress (Leslie and Papageorghiou, 2011).

**4-5: Effects of the age on the study variables**

There were negative significant correlations between the age and both TNF-α and TGF-β, otherwise there were non-significant negative correlations with all other study variables other than Cyclophilin-A.

Duality of functional interactions between TGF-β and TNF-α has been reported in a preceded reports (Liu *et al.*, 2022).

Contrary to earlier reports, the negative relationships between age and TNF-α and TGF-β were observed in the current study. Peripheral levels of TNF-α in plasma were predicted to rise with age over time based on a larger body of research on inflammation and ageing (Lindsay and Dubey,2020). TNF-α and IL-12 levels did not vary during the duration of toxoplasmosis, however, it has been noted that the prevalence of *T. gondii* rises with age (Matowicka-Karna and Kemona, 2009). In a previous study, TGF-β expression increased in the placental tissue of *T. gondii*-infected aborted women, but this rise was not correlated with the ageing of the infected females. The risk of toxoplasmosis was significantly correlated with age, though.

The negative correlations between the age and both TNF-α and TGF-β were inconsistent with what were reported previously. On the basis of the broader literature on inflammation/aging, peripheral levels of TNF-α in plasma were expected to increase with age over time (Lindbergh and Dubey,2020). However, in the course of toxoplasmosis the levels of TNF-*α* and IL-12 did not change, but the prevalence of *T. gondii* has been reported to increase with age of 40 years (Matowicka-Karna *et al.*, 2009). There was an increase in expression of TGFβ, in the placental tissue of aborted women infected with *T. gondii* in a prior study but not associated with increasing ages of the infected females. However, the age showed a significant correlation with Toxoplasmosis and the risk of infection increased with age ($P<0.05$) ( Alsailawi *et al.*, 2022).

**Table(4- 5): Effects of the age on the study variables**

| | Pearson Correlation | TNF-α | TGF-β | Phospholipase-A | IL-12 | IL-17 | Cyclophilin-A |
|---|---|---|---|---|---|---|---|
| Age/years | *R* | - 0.365 | - 0.521 | - 0.236 | - 0.193 | - 0.236 | 0.068 |
| | *P* | 0.001 | 0.001 | 0.052 | 0.109 | 0.052 | 0.757 |

## 4-6: Correlations among various study parameter

Table (4-6) revealed a positive highly significant correlation of TNF-α with (TGF-B, phospholipase, and IL-17) and a significant correlation with cyclophilin A, but not with IL-12 among the study participants.

**Table (4- 6): Correlation of study variables among the study participants**

| Pearson Correlation | | TNF-α | TGF-B | IL-12 | IL-17 | Cyclophilin A | Phospholipase A |
|---|---|---|---|---|---|---|---|
| **Phospholipase A** | R | 0.366 | 0.445 | 0.165 | 1.000 | - 0.056 | 1 |
| | P | 0.002 | 0.000 | 0.179 | 0.000 | 0.801 | 1 |
| **IL-12** | R | 0.188 | 0.193 | 1 | 0.165 | - 0.283 | 0.165 |
| | P | 0.119 | 0.110 | 1 | 0.179 | 0.190 | 0.179 |
| **IL-17** | R | 0.366 | 0.445 | 0.165 | 1 | - 0.056 | 1.000 |
| | P | 0.002 | 0.001 | 0.179 | 1 | 0.801 | 0.001 |
| **Cyclophilin A** | R | 0.425 | 0.109 | - 0.283 | - 0.056 | 1 | - 0.056 |
| | P | 0.043 | 0.621 | 0.043 | 0.621 | 1 | 0.801 |
| **TGF-B** | R | 0.391 | 1 | 0.193 | 0.445 | 0.109 | 0.445 |
| | P | 0.000 | 1 | 0.110 | 0.000 | 0.621 | 0.000 |
| **TNF-α** | R | 1 | 0.391 | 0.188 | 0.366 | 0.043 | 0.002 |
| | P | 1 | 0.001 | 0.119 | 0.002 | 0.043 | 0.002 |

Table (4-6) revealed a positive highly significant correlation of TGF-B with (TNF-a, phospholipase A, and IL-17) and a significant correlation but not with IL-12 and cyclophilin A, among the study participants. revealed a positive highly significant correlation of Phospholipase A with (TNF-a, TGF-B, and IL-17) and but no significant correlation with cyclophilin A and IL-12 among the study participants.

Table-(4-6) revealed a non-significant correlation of IL-12 with all other study parameters among the study participants. and revealed a positive highly significant correlation of IL-17 with TNF-a, TGF-B, and phospholipase-A but no significant correlation with cyclophilin A and IL-12 among the study participants.also revealed a non-significant correlation of cyclophilin-A with all other study parameters apart from TNF-a, among the study participants.

Generally, the immune system of immunocompetent individuals builds a protective immune response upon the interaction of antigen with antigen-presenting cells, which induces the translocation of nuclear factor kappa (NF-κB) to initiate the production of pro-inflammatory cytokines (IL1b, IL12, IL18, and IFNγ) (Fisch et al., 2019). The host lymphocytes and myeloid cells not only secrete a network of cytokines for signaling pathways upon exposure to antigen but also up-regulate certain chemokines (CXC, C, and $CX_3C$) and toll-like receptors (TLR) on their surfaces for acting as signal-recipients against any antigen (Fisch, 2019; Costantini and Colonna 2010). In response to specific intracellular signals, various pro-inflammatory (IL1β, IL12, IL18, TNFα, IFNγ) and anti-inflammatory (IL4, IL10, TGF-β) Cytokines give rise to a Cytokinome that acts for specific immunological stimulus to develop an immune response for susceptibility or resistance to toxoplasmosis (Sana *et al*., 2022).

## 4-7: Receiver-Operating Characteristic Analysis for Evaluating Diagnostic Tests and Predictive Models of study parameters

Receiver-Operating Characteristic analysis was tested on the studied variables to scrutinize their diagnostic and predictability to differentiate Toxoplasma from the healthy females. All the study parameters had not the ability to diagnose or poor predictability for toxoplasma patients, although TNF-α revealed a significant (p-0.02) high AUC (0.838), sensitivity, (0.0832), and specificity (0.822); but with not enough confidence (95%CI = 0.675-1.000)

**Table (4-7): Receiver-Operating Characteristic Analysis for Evaluating Diagnostic Tests and Predictive Models of study parameters**

| | AUC | P-value | Sensitivity | Specificity | 95% CI | |
|---|---|---|---|---|---|---|
| **TNF-a** | 0.838 | 0.02 | 0.832 | 0.822 | 0.675 | 1.000 |
| **TGF-B** | 0.701 | 0.2 | 0.656 | 0.649 | 0.413 | 0.989 |
| **Phospholipase A** | 0.686 | 0.2 | 0.727 | 0.586 | 0.446 | 0.927 |
| **IL-12** | 0.196 | 0.03 | 0.495 | 0.513 | 0.014 | 0.378 |
| **IL-17** | 0.686 | 0.2 | 0.724 | 0.643 | 0.446 | 0.927 |
| **Cyclophilin A** | 0.667 | 0.2 | 0.992 | 0.512 | 0.372 | 0.962 |

Based on several studies conducted in a mouse model, it can be stated that *T. gondii* infection evokes a strong immune response (both innate and

adaptive). Innate immunity is not only critical for controlling parasite multiplication during the early phase of acute infection but also helps in the shaping of the adaptive immune response, however, the long-term protection is dependent on adaptive immunity. One important question that needs attention is that, due to CD8 T cell exhaustion, a mild constant reactivation may take place during chronic infection of immune-competent individuals. In that scenario, the recruitment of functional CD8 T cells may be able to control the spread of reactivation. Additionally, it will be equally important to assess if some of the memory CD8 T cell subsets that are prone to exhaustion during chronic infection are continuously being replaced by an influx of non-exhausted population due to a constant low-grade reactivation of the parasite (Khan and Moretto, 2022).

The first possibility is the crucial role of CD [CD4, which stimulate cytokines like IFNγ, in acute infection (Liesenfeld, 1996 ), while the CD8 T cell subset is critical in a chronic state ( Zander *et al*., 2019)] T cell that might recruit other CD subsets, Chemokines, and other proinflammatory cytokines including TNF-a, TGF-B, several interleukins, phospholipases, TLRs subsets, and/or Cyclophilins and represents washing shot or the immunological links that might explain the changeable correlations of these different immunoregulatory factors together (Khan and Moretto, 2022, Sturge and Yarovinsky, 2014; Yousif and Alsakee, 2021).

A second possibility is the presence of an unidentified sensor(s) that recognizes *T. gondii* infection in humans, independent of TLRs and CCR5. Further investigations are required to identify this sensor ( Sasai *et al.*, 2018).

The third possibility is the *T. gondii* infection in healthy humans becomes asymptomatic because the host's innate and adaptive immunity resists its initial proliferation. Infection of monocytes by a *T. gondii* tachyzoite strongly induces innate immunity such as the production of several subsets

of pro-inflammatory cytokines, resulting in the activation of adaptive immune responses mediated by T and B cells. This activation further stimulates cell-autonomous immunity to disrupt intracellular growth of the protozoa and mediate its clearance, inducing *T. gondii* stage conversion into a bradyzoite that eventually leads to chronic infection. Thus, the immunological balance between a healthy host and *T. gondii* is a key step in *T. gondii* immunobiology ( Sasai *et al.*, 2018; Gómez-Chávez *et al.*, 2020; Sturge, 2014; Yousif and Alsakee 2021).

Fourthly, apart from the CD-TLR signals and Cytokine milieu, one of the most important factors governing the immunorespones is the balance between positive signals from co-stimulatory receptors and negative signals from inhibitory receptors ( Chen and Flies, 2013).

# Conclusion and Recommendation

## Conclusions

- The *Toxoplasma gondii* causing acute infection in most patient

- The parasite induce innate immunity that increase IL-12 in micro environment

- The parasite induce proinflammatory cytokine TNFα and IL-17 . - Toxoplasma gondii increase cytoplasmic signaling cyclophilin and phospholipase in cells

- There was a significant correlation of phospholipase-A with TNF-α , TGF-B, and IL-17, but not with IL-12 and cyclophilin-A, among the study participants.

- All the studied parameters cannot be used as a biomarker to diagnose *Toxoplasma Gondii* among infertile females.

## Recommendations

- Study regulatory cytokine in toxoplasma in infertile patient .

- Study the profile of these cytokine in male

- Study the genetic on cytokine in infertile women that associated with toxoplasma

# References

# References

**Abdoli, A., Dalimi, A., Soltanghoraee, H., & Ghaffarifar, F. (2017).** Molecular detection and genotypic characterization of *Toxoplasma gondii* in paraffin-embedded fetoplacental tissues of women with recurrent spontaneous abortion. *International Journal of Fertility & Sterility*, *10*(4), 327.

**Abdulkhaliq, R. J., Mohammed, S., & Abbas, A. A. H. (2017**). The Role of Some Cytokines inwomen withRecurrent Abortion in Iraqi Women.

**Abdulsamad, S. S., & Manhal, S. H. (2019).** The Incidence of parasite *Toxoplasmosis* in pregnant women.

**Acharya, M. R. (2020**). Study of *Toxoplasma gondii*, Rubella, CMV and HSV antibodies among pregnant women in pokhara, Nepal. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN*, 2279-0853.

**Affan, F. (2020).** A Cross-sectional study on Maternal and Congenital *Toxoplasma gondii* infections among patients attending a tertiary care centre (*Doctoral dissertation, Stanley Medical College, Chennai).*

**Ahmadpour, E., Babaie, F., Kazemi, T., Mehrani Moghaddam, S., Moghimi, A., Hosseinzadeh, R., & Pagheh, A. S**. **(2023).** Overview of Apoptosis, Autophagy, and Inflammatory Processes in *Toxoplasma gondii* Infected Cells. *Pathogens*, *12*(2), 253.

**Aitken, R. J., Findlay, J. K., Hutt, K. J., & Kerr, J. B. (2011).** Apoptosis in the germ line. *Reproduction*, *141*(2), 139.

# References

**Al Beloushi, M., Kalache, K., Ahmed, B., & Konje, J. C. (2021).** Ultrasound diagnosis of infections in pregnancy. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *262*, 188-197.

**Al-Adhroey, A. H., Mehrass, A. A. K. O., Al-Shammakh, A. A., Ali, A. D., Akabat, M. Y., & Al-Mekhlafi, H. M. (2019).** Prevalence and predictors of *Toxoplasma gondii* infection in pregnant women from Dhamar, Yemen. *BMC Infectious Diseases*, *19*(1), 1-9.

**Alakkam, E. M. & Salim ,A. (2022).** Study Of Risk Factors For Infertility For Iraqi Patients (A Cross-Sectional Study Of Women Aged 20-40 Years). *Eurasian Medical Research Periodical 14, 9.*

**Alam, Z., Abdalla, M. A., Alseiari, S., Alameemi, M., Alzaabi, M., Alkhoori, R., Östlundh, L. & Al-Rifai, R. H. (2023).** Polycystic Ovarian Syndrome Among Women Diagnosed With Infertility In The Gulf Cooperation Council Countries: A Protocol For Systematic Review And Meta-Analysis Of Prevalence Studies. Womens Health (Lond), 19, 17455057231160940.

**Al-Ardi, M. H. (2021).** The Role of Hormones and *Toxoplasma gondii* Infection to Change the Secondary Sex Ratio. *Iraqi Journal of Science*, 2850-2860.

**Al-Awadi, A. A. H., Abdul-Ghani, R. & Al-Mekhlafi, A. M. (2022).** *Toxoplasma Gondii* Infection Among Institutionalized Children With Down Syndrome In Sana'a City, Yemen: Implications Of Low Igg Seroprevalence. *Acta Parasitol, 67, 530-534.*

# References

**Albalushi, H., Ba-Alawi, A., Aljabri, R. & Al Khaduri, M. (2023).** Prevalence Of Congenital Uterine Anomalies And Tubal Blockage In Infertile Omani Women: A Retrospective Study. *Oman Med J, 38, E463.*

**Al-Bdairi, A. A., Al-Hindy, H. A. A. M., Alkhudair, S. H., & Alkadhim, K. H. (2021).** Serum and Seminal Plasma concentrations of Inhibin B and FSH: A Case-Control Comparison Study between Fertile and Infertile Males. *History of Medicine*, *8*(2), 22-28.

**Al-Bdairi, A., Al-Hindy, H. A. A. M., & Al-Shalah, M. A. (2021).** Preoperative Measures of Serum Inhibin B, and FSH Levels Predict Sperms Retrieval Outcome in Non-Obstructive Azoospermic Males. *Clinical Schizophrenia & Related Psychoses*, *15*..

**Al-Dorry, Z. A., Yaseen, N., & Molan, A. L. (2021).** Immune response in *Toxoplasma gondii* seropositive Iraqi aborted pregnant women in comparison with seronegative women. *American Journal of Life Science Researches*, *9*(1), 1-7.

**Al-Hindy, H. A. A., Mousa, M. J., & Shaker, A. K. (2020).** No Significant Relationship of Ferritin Levels to the Levels of Platelet-derived Growth Factor (PDGF) in the Peripheral Blood of Transfusion-dependent β-Thalassemia Major Patients with Growth Retardation. *International Journal of Pharmaceutical Research (09752366)*, *12*(3).

**Al-Hindy, H. A. A., Mousa, M. J., Shaker, A. K., Al-Saad, R.Z., Al-Dujaili, W. H. S. (2020).** Relationship Of Levels Of Transforming Growth Factorbeta1 (Tgf-B1) To The Levels Of Ferritin In Blood Of

# References

Transfusion Dependent B-Thalassemia Major Patients With Growth Retardation: A Case-Control Study. *Eurasian Journal Of Biosciences*, *14*, *521-527*.

**Al-Kaeebi, S. R. A., & Al-Jubouri, G. A. J.( 2022)** Use GRA6 gene for genotyping of *Toxoplasma gondii* from aborted women in Al-Diwaniyah Governorate *HIV Nursing*, *22*(2), 3455-3461.

**Al-kremy, N. A. R., & Al-hassnawi, A. T. S. (2020).** The considerable evidence between latent Toxoplasma infection with testosterone and total antioxidant among infertile women. *Eurasian Journal of Biosciences*, *14*(1).

**Al-Kremy, N. A. R., & Al-quraishi, M. A. (2022).** Estimation of Cyclophilin and Phospholipase Enzyme in Women Infected with *Toxoplasmosis. Medical Archives*, *76*(6), 443.

**Alkubaisi, S. A., Sadeq ,M., and Al.-Zubiady, I. A.H.(2023).** Toxoplasmosis In Females From Al-Anbar, Iraq. *Journal Of The Faculty Of Medicine Baghdad, 65, 74-78.*

**Al-Kuraishi, A. H., Khalil ,H.I., Hassan ,H.H. and Al-Kuraishy ,H.M. (2020).** Placental Dysfunction And Acute Toxoplasmosis: The Role Of Melatonin In Relation To Inflammatory Cytokines Interleukin-10 And Interleukin-12. *J Microsc Ultrastruct 41, 126-9.*

**Almeria, S., & Dubey, J. P. (2021).** Foodborne transmission of *Toxoplasma gondii* infection in the last decade. An overview. *Research in veterinary science*, *135*, 371-385.

# References

**Al-Muskakeh, M. K., Yaseen, A. N., & Aldabagh, M. A. (2022).** Assessment of Soluble PD-1 and PD-L1 in Iraqi Women Patients with Breast Cancer with Toxoplasmosis. *Indian Journal of Forensic Medicine & Toxicology*, *16*(1), 1395-1404.

**Al-Mussawi, K. A., Radhi, I. J., & Abdulwahid, H. H. (2020).** Relationship between Parasitic Infections in Protozoa and Female Secondary Infertility. *Indian Journal of Public Health Research & Development*, *11*(2).

**AL-Quraishi, M. A. (2011).** Epidemiology Study of Toxoplasmosis to Patient Women of Middle Euphrates Hospitals of Iraq. *journal of kerbala university*, *9*(3).

**Alsailawi, H. A., Ayat, S. S., Mudhafar, M., Mohammed, R. K., Dhahi, S. J., Abdulrasool, M. M., & Raheem, H. A. (2022).** Immunohistochemical study of TGF-β, PDL-1, and IL-10 in patients with *Toxoplasma gondii*. *World Journal of Advanced Research and Reviews*, *14*(1), 436-448.

**Al-Shimmery, A. H. S., Al-Hindy, M., Z. A., Al- Mammori, H.A.M., Obayes,R. T. Mokif, T. A., Al-Dahmoshi, H. O. M.and Al-Khafaji, N.S .K.( 2023).** Immunological Study Of Ifn-Γ, Icam-4, And Vitamin D3 Markers Among Gastrointestinal Tumor Patients In Babylon Province, Iraq. *The Asian Pacific Journal Of Cancer Prevention, 24, 253-257.*

**Al-Shimmery, A. H. S., Al-Alwany, M. H. O., Chabuck, Z. A. G., Al-Mammori, R. T. O., Mokif, T. A., Mahdi, Z. A. A., & Abdulabbas,**

# References

**H. S. (2023).** Assessment of tumor necrosis factor-α, interleukin-17, and vitamin D3 levels on a group of gastrointestinal tumor patients in Babylon Provence, Iraq. *Medical Journal of Babylon*, *20*(2), 362-7.

**Alvarado-Esquivel, C., Torres-Castorena, A., Liesenfeld, O., García-López, C., Estrada-Martínez, S., Sifuentes-Alvarez, A., Marsal-Hernández, J., Esquivel-Cruz, R., Sandoval-Herrera, F. & Castaneda, J. J. J. O. P. (2009).** Seroepidemiology Of *Toxoplasma Gondii* Infection In Pregnant Women In Rural Durango, Mexico. 95, 271-274.

**Ander, S. E., Diamond, M. S., & Coyne, C. B. (2019).** Immune responses at the maternal-fetal interface. *Science immunology*, *4*(31), eaat6114.

**Arora, N., Sadovsky, Y., Dermody, T. S. & Coyne, C. B.(2017).** Microbial Vertical Transmission During Human Pregnancy. Cell Host Microbe, 21, 561-567.

**Arranz-Solís, D., Mukhopadhyay, D., & Saeij, J. J. (2021).** Toxoplasma effectors that affect pregnancy outcome. *Trends in parasitology*, *37*(4), 283-295.

**Attias, M., Teixeira, D. E., Benchimol, M., Vommaro, R. C., Crepaldi, P. H., & De Souza, W. (2020).** The life-cycle of *Toxoplasma gondii* reviewed using animations. *Parasites & vectors*, *13*, 1-13.

**Azimi, A., Parsaei, M., Soleymani, E., Mehranzadeh, E., & Maghsood, A. H. (2022).** Diagnosis of Acute *Toxoplasmosis* in the First Trimester

# References

of Pregnancy by IgG Avidity. *Avicenna Journal of Clinical Medicine*, *29*(1), 50-56.

**Bălălău, O. D., Olaru, O. G., Dumitru, A. V., Păunică, I., & Stănescu, A. D. (2020).** Maternal infections with an increased risk of transmission to the foetus; a literature review. *J Clin Invest Surg*, *5*(2), 66-72.

**Bandyopadhyay, P. K., Das, N. R., & Chattopadhyay, A. (2022).** Protozoan Parasites. In *Biochemical, Immunological and Epidemiological Analysis of Parasitic Diseases* (pp. 9-90). Singapore: Springer Nature Singapore.

**Baneen, A., Mohammed, A. D. & Al-Attabbi ,A. S.( 2021).** Study Levels Of Pdl-1, Il-10 And Tgf-B In Patients With *Toxoplasmosis*. Annals Of The Romanian Society For Cell Biology 25, 1899–1913.

**Barrera, N., Omolaoye, T. S., & Du Plessis, S. S. (2022).** A contemporary view on global fertility, infertility, and assisted reproductive techniques. In *Fertility, Pregnancy, and Wellness* (pp. 93-120). Elsevier.

**Batlle, E., & Massagué, J. (2019).** Transforming growth factor-β signaling in immunity and cancer. *Immunity*, *50*(4), 924-940.

**Bayani, M., Kalantari, N., Esmailzadeh, S., Ghaffari, S., Mahjoub, S., Ghofrani, F., & Ghaffari, T. (2022).** An evaluation of the level of testosterone, DHEA and prolactin among *Toxoplasma gondii* infected and uninfected infertile couples attending to Fatima Al-Zahra

infertility treatment center, Babol, Northern Iran. *Acta facultatis medicae Naissensis*, *39*(1), 37-47.

**Bendarska-Czerwińska, A., Zmarzły, N., Morawiec, E., Panfil, A., Bryś, K., Czarniecka, J. & Grabarek, B. O. (2023).** Endocrine disorders and fertility and pregnancy: An update. *Frontiers in Endocrinology*, *13*, 970439.

**Bhadra ,R.H. G. & Khan .,I. A. (2010).** Absence Of Both Il-7 And Il-15 Severely Impairs The Development Of Cd8+ T Cell Response Against *Toxoplasma gondii*. Plos One 5, E10842.

**Bishr, N. M., Abdel-Rahman, A. A. H., Ashour, A. M., & Ibrahim, H. M. (2020).** Biochemical effects of *Toxoplasma gondii* and Neospora caninium infection on diary bovine models in Menoufia Province, Egypt. *Adv. Anim. Vet. Sci*, *9*(3), 379-386.

**Boucoiran, I., Kakkar, F., & Renaud, C. (2020).** Maternal infections. *Handbook of Clinical Neurology*, *173*, 401-422.

**Bowling, F. Z., Frohman, M. A., & Airola, M. V. (2021).** Structure and regulation of human phospholipase D. *Advances in Biological Regulation*, *79*, 100783.

**Brenier-Pinchart, M. P., Gangneux, J. P., & Pelloux, H. (2011).** The placenta: a main role in congenital toxoplasmosis?. *Trends in Parasitology*, *27*(12).

# References

**Cassaing, S., Fauvel, J., Bessières, M. H., Guy, S., Séguéla, J. P. & Chap, H. (2000).** *Toxoplasma Gondii* Secretes A Calcium-Independent Phospholipase A(2*). Int J Parasitol, 30, 1137-42.*

**Chaklu, M., Tarekegn, Z. S., Birhan, G., & Dagnachew, S. (2020).** *Toxoplasma gondii* infection in backyard chickens (Gallus domesticus): seroprevalence and associated risk factors in Northwest Ethiopia. *Veterinary Parasitology: Regional Studies and Reports*, *21*, 100425.

**Chang, C. W., Sung, Y. W., Hsueh, Y. W., Chen, Y. Y., Ho, M., Hsu, H. C., Yang, T. C., Lin, W. C. & Chang, H. M. (2022).** Growth Hormone In Fertility And Infertility: Mechanisms Of Action And Clinical Applications. 13. *Frontiers in Endocrinology*, *13*, 1040503.

**Chen ,Z., Hauser ,R., Trbovich ,A.M., Shifren ,J.L., Dorer ,D.J. & Godfrey-Bailey ,L. (2006).**The relationship between human semen characteristics and sperm apoptosis: a pilot study. *J Androl;27:112–120*

**Chen, J., Hu, L., Wang, J., Cao, Y., Zhu, D., Chen, L., & Duan, Y. (2019**). *Toxoplasma gondii* excreted-secreted antigens suppress Foxp3 via PI3K-AKT-mTOR signaling pathway. *Journal of cellular biochemistry*, *120*(9), 16044-16051.

**Chen, L., & Flies, D. B. (2013).** Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nature reviews immunology*, *13*(4), 227-242.

**Choudhari, R. H. (2022).** Multidimensional Impact of Climate Change on Human Reproduction and Fertility: A Medical Perspective on Changing

# References

Dynamics. In *Research Anthology on Environmental and Societal Impacts of Climate Change* (pp. 1672-1709). IGI Global.

**Costa, M.N., Lopes,D. F., Ricci-Azevedo, R., Sardinha-Silva, A., Figueiredo Pinzan, C., Paiva Alegre-Maller, A. C., & Roque-Barreira, M. C. (2019).** Receptor heterodimerization and co-receptor engagement in TLR2 activation induced by MIC1 and MIC4 from *Toxoplasma gondii*. *International Journal of Molecular Sciences*, *20*(20), 5001.

**Costantini, S. C. G.,& Colonna G**. **(2010).** Human Cytokinome: A New Challenge For Systems Biology. *Bioinformation 5, 166–167*.

**Curcio, A. M., Shekhawat, P., Reynolds, A. S., & Thakur, K. T. (2020).** Neurologic infections during pregnancy. *Handbook of Clinical Neurology*, *172*, 79-104.

**Daher, D., Shaghlil, A., Sobh, E., Hamie, M., Hassan, M. E., Moumneh, M. B., & El Hajj, H. (2021).** Comprehensive overview of *Toxoplasma gondii*- induced and associated diseases. *Pathogens, 10(11), 1351*.

**Dambrun, M., Dechavanne, C., Guigue, N., Briand, V., Candau, T., Fievet, N., & Migot-Nabias, F. (2022).** Retrospective study of toxoplasmosis prevalence in pregnant women in Benin and its relation with malaria. *PloS one*, *17*(1), e0262018. ..

**Daniel, W. W., & Cross, C. L. (2018**). *Biostatistics: a foundation for analysis in the health sciences*. Wiley.

**Daryani, A., Sarvi, S., Aarabi, M., Mizani, A., Ahmadpour, E., Shokri, A. & Sharif, M. (2014).** Seroprevalence of *Toxoplasma gondii* in the

# References

Iranian general population: a systematic review and meta- analysis. *Acta tropica*, *137*, 185-194.

**Denis, J., Gommenginger, C., Strechie, T., Filisetti, D., Beal, L., Pfaff, A. W., & Villard, O. (2022).** Dynamic immune profile in French toxoplasmosis patients. *The Journal of Infectious Diseases*.

**Desmettre, T. (2020).** *Toxoplasmosis* and behavioural changes. *Journal Francais d'Ophtalmologie*, *43*(3), e89-e93.

**Dleikh, F. S., Al-Aaraji, A. J., Mohin, R., Mousa, M. J., Al-Hindy, H. A. A. M., & Al-Ka'abi, B. A. (2020).** Possible cause-and-effect linkage of transforming growth factor-beta1 and platelets derived growth factor-AB with delayed anthropometric parameters in adolescent patients with Cooley's anemia: Cases vis control research strategy. *EurAsian Journal of BioSciences*, *14*(1).

**Dodangeh, S., Daryani, A., Sharif, M., Aghayan, S. A., Pagheh, A. S., Sarvi, S., & Rezaei, F. (2019).** A systematic review on efficiency of microneme proteins to induce protective immunity against *Toxoplasma gondii*. *European Journal of Clinical Microbiology & Infectious Diseases*, *38*(4), 617-629

**Dogga, S. K., Lunghi, M., Maco, B., Li, J., Claudi, B., Marq, J. B., & Hammoudi, P. M. (2022).** Importance of Aspartyl Protease 5 in the establishment of the intracellular niche during acute and chronic infection of *Toxoplasma gondii*. *Molecular Microbiology*.

**Dubey, J. P., Murata, F. H. A., Cerqueira-Cézar, C. K., Kwok, O. C. H., & Villena, I. (2021).** Congenital toxoplasmosis in humans: an

# References

update of worldwide rate of congenital infections. *Parasitology*, *148*(12), 1406-1416.

**Dubey,J.P. (2013).** "Duration of Immunity to Shedding of *Toxoplasma Gondii* Oocysts by Cats OF *Toxoplasma gondii* To Shedding Duration Of Immunity Oocysts By Cats*." 81(3):410–15.*

**Dupont, D., Fricker-Hidalgo, H., Brenier-Pinchart, M. P., Garnaud, C., Wallon, M., & Pelloux, H. (2021).** Serology for Toxoplasma in immunocompromised patients: still useful?. *Trends in parasitology*, *37*(3), 205-213.

**Elaadli, H., Abo El-Makarem, H., Abd Elrahma, A. H., Shaapan, R. M., & Bessat, M. (2023).** Prevalence and associated risk factors of Toxoplasma gondii infection in sheep and aborted women in Egypt. *Iraqi Journal of Veterinary Sciences*, *37*(2), 437-445.

**Elisanti, E., Sajidan, S., & Prayitno, B. A. (2018).** The Effectiveness Of Inquiry Lesson-Based Immunity System Module To Empower The Students'critical Thinking Skill. *EDUSAINS*, *10*(1), 97-112.

**Elmore, S. A., Jones, J. L., Conrad, P. A., Patton, S., Lindsay, D. S. & Dubey, J. P. (2010).** *Toxoplasma gondii*: Epidemiology, Feline Clinical Aspects, And Prevention. *Trends Parasitol*, *26*, *190-6.*

**Elsheikha, H. M., Marra, C. M., & Zhu, X. Q. (2020).** Epidemiology, pathophysiology, diagnosis, and management of cerebral *Toxoplasmosis*. *Clinical microbiology reviews*, *34*(1), e00115-19.

# References

**El-Sherbini, M. S., El-Aal, A., Amany, A., El-Sherbiny, W. S., Attia, S. S., Abdel Aziz, I. Z., & Badr, M. S. (2019).** *Toxoplasmosis* and abortion: pro-and anti-inflammatory cytokines gene expression of the host immune cells. *Egyptian Journal of Medical Human Genetics*, *20*(1), 1-10.

**Eltantawy, N., Taman, A. & Shalaby**, **H.( 2014).** Toxoplasmosis And Female Infertility: Is There A Co-Relation? *American Journal Of Epidemiology And Infectious Disease*, *2, 29-32*.

**Evering, T., &Weiss, L.M. (2006).** The immunology of parasite infections in immunocompromised hosts. *Parasite immunology*, *28*(11), 549-565.

**Fabiani, S., Caroselli, C., Menchini, M., Gabbriellini, G., Falcone, M., & Bruschi, F. (2022).** Ocular Toxoplasmosis, an overview focusing on clinical aspects. *Acta Tropica*, *225*, 106180.

**Fallah, M., Ehsan, T. S., Salehi, I., Maghsood, A. H., Matini, M., & Pooyandehravan, A**. **(2021).** Effect of Acute *Toxoplasmosis* on Anxiety and Cortisol and Interleukin-17 Levels in Male Rats: An Experimental Study. *Zahedan Journal of Research in Medical Sciences*, *23*(3).

**Faral-Tello, P., Pagotto, R., Bollati-Fogolín, M., & Francia, M. E. (2023).** Modeling the human placental barrier to understand *Toxoplasma gondii´* s vertical transmission. *Frontiers in Cellular and Infection Microbiology*, *13*, 1130901.

**Farhan, B. A.( 2022).** Seroprevelance Of Toxoplasma Gondii (Igm,Igg) Among Aborted Women In Some Karbala Hospital By Using Elisa

# References

Technique And Microscopic Examination. *Indian Journal Of Forensic Medicine & Toxicology, 14, 950.*

**Fayyad, H. N. (2012).** Fertility In Iraq Trends, Evolution And Influential Factors. Jstor: *Arab Center For Research & Policy Studies.*

**Fereig, R. M., Omar, M. A., & Alsayeqh, A. F. (2022).** Exploiting the Macrophage Production of IL-12 in Improvement of Vaccine Development against *Toxoplasma gondii* and *Neospora caninum* Infections. *Vaccines*, *10*(12), 2082.

**Filkin, S. Y., Lipkin, A. & Fedorov, A. J. B. (2020).** Phospholipase Superfamily: Structure, Functions, And Biotechnological Applications. *Biochemistry (Moscow)*, *85*(1), 177-195.

**Firdaousse, A., Azerki, M., Chraibi, A., Jeraf, N., Bezad, R., Alami, M., & Filali, A. (2021).** Toxoplasmosis And Pregnancy.

**Fisch D, C. B. & Frickel E.M. (2019).** Human Immunity To *Toxoplasma Gondii Pathogens Plos One*, 15, E1008097.

**Flammersfeld, A., Lang, C., Flieger, A., & Pradel, G. (2018).** Phospholipases during membrane dynamics in malaria parasites. *International Journal of Medical Microbiology*, *308*(1), 129-141.

**Flegr, J. and Kuba, R. (2016).** "The Relation of Toxoplasma Infection and Sexual Attraction to Fear, Danger, Pain, and Submissiveness." *Evolutionary Psychology* 14(3):1–10.

# References

**Flegr, J., & Kaňková, Š. (2020).** The effects of toxoplasmosis on sex ratio at birth. *Early Human Development*, *141*, 104874.

**Flori, P., Chene, G., Varlet, M. N., & Sung, R. T. M. (2009)** *Toxoplasma gondii* serology in pregnant woman: characteristics and pitfalls. In *Annales de biologie clinique* (Vol. 67, No. 2, pp. 125-133).

**Foroutan-Rad, M., Majidiani, H., Dalvand, S., Daryani, A., Kooti, W., Saki, J., & Ahmadpour, E. (2016).** Toxoplasmosis in blood donors: a systematic review and meta-analysis. *Transfusion medicine reviews*, *30*(3), 116-122.

**Frangogiannis, N. G. (2020).** Transforming growth factor–β in tissue fibrosis. *Journal of Experimental Medicine*, *217*(3).

**Fréville, A., Gnangnon, B., Khelifa, A. S., Gissot, M., Khalife, J., & Pierrot, C. (2022).** Deciphering the role of protein phosphatases in apicomplexa: the future of innov ativtherapeutics? *Microorganisms*, *10*(3), 585.

**Ge, Y., Huang, M., & Yao, Y. M. (2020).** Biology of interleukin-17 and its pathophysiological significance in sepsis. *Frontiers in Immunology*, *11*, 1558.

**Genova ,M.D. & Knoll, L. J. (2020).** Comparisons of the sexual cycles for the coccidian parasites Eimeria and Toxoplasma. *Frontiers in cellular and infection microbiology*, *10*, 604897.

# References

**Gilot-Fromont, E., Lélu, M., Dardé, M. L., Richomme, C., Aubert, D., Afonso, E., & Villena, I. (2012).** The life cycle of *Toxoplasma gondii* in the natural environment. *Toxoplasmosis-recent advances*, *10*, 2845.

**Gilvaz, S. (2021).** TORCH Infections in Pregnancy. *Clinical Cases in Obstetrics & Gynecology*, 169.

**Giugno, S., Monteleone, M. M., Insalaco, G., Leanza, G., Palumbo, M., & Leanza, V. (2020).** Congenital toxoplasmotic chorioretinitis following reinfection. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *251*, 263-265.

**Gómez-Chávez, F., Cañedo-Solares, I., Ortiz-Alegría, L. B., Flores-García, Y., Figueroa-Damián, R., Luna-Pastén, H., Gómez-Toscano, V., López-Candiani, C., Arce-Estrada, G. E., Bonilla-Ríos, C. A., Mora-González, J. C., García-Ruiz, R. & Correa, D. (2020).** A Proinflammatory Immune Response Might Determine *Toxoplasma gondii* Vertical Transmission And Severity Of Clinical Features In Congenitally Infected Newborns. *Front Immunol, 11, 390*.

**Gurung, D., Danielson, J. A., Tasnim, A., Zhang, J. T., Zou, Y., & Liu, J. Y. (2023**). Proline Isomerization: From the Chemistry and Biology to Therapeutic Opportunities. *Biology*, *12*(7), 1008.

**Gyang, V. P., Akinwale, O. P., Lee, Y. L., Chuang, T. W., Orok, A., Ajibaye, O., & Fan, C. K. (2015*).*** *Toxoplasma gondii* infection: seroprevalence and associated risk factors among primary schoolchildren in Lagos City, Southern Nigeria. *Revista da Sociedade Brasileira de Medicina Tropical*, *48*, 56-63.

# References

**Hadpech, S., & Thongboonkerd, V. (2022).** Current update on theranostic roles of cyclophilin A in kidney diseases. *Theranostics*, *12*(9), 4067.

**Halonen, S. K., & Weiss, L. M. (2009).** *Toxoplasma gondii* presentations at the 10th International Workshops on Opportunistic Protists: 100 years and counting. *Eukaryotic Cell*, *8*(4), 437-440.

**Hammadi, A. A., & Hamad, H. K. (2021).** Evaluation Of Some Cytokines And Growth Factors In Serum Of Heart Disease Patients Infected With *Toxoplasmosis*. *Plant Archives*, *21*(1), 1399-1403

**Hamza, U. M., Hameed, M., Al-Zubaidi,N. H. S., Abulkassim, R., Mohamed,B.Z., Mahmood, S. S., & A Hamad, D. (2022).** *Toxoplasma gondii* Seroprevalence Among Pregnant Women in Baghdad During 2021-2022. *Journal of Obstetrics, Gynecology and Cancer Research*, *7*(6), 563-568.

**Han, J., Lee, M. K., Jang, Y., Cho, W. J., & Kim, M. (2022).** Repurposing of cyclophilin A inhibitors as broad-spectrum antiviral agents. *Drug Discovery Today*

**Harikishore, A. & Sup Yoon, H. (2016**). Immunophilins: structures, mechanisms and ligands. *Current molecular pharmacology*, *9*(1), 37-47.

**Hassan, S. U., Zahra, A., Parveen, N., Iqbal, N., Mumtaz, S. & Batool, A. (2022).** Quality Of Infertility Care Services And Emotional Health Of South Asian Women. *Psychol Res Behav Manag, 15, 1131-1146.*

# References

**Heany, S. J., van Honk, J., Stein, D. J., & Brooks, S. J. (2016).** A quantitative and qualitative review of the effects of testosterone on the function and structure of the human social-emotional brain. *Metabolic brain disease*, *31*(1), 157-167.

**Herrmann, D. C., Pantchev, N., Vrhovec, M. G., Barutzki, D., Wilking, H., Fröhlich, A., & Schares, G. (2010).** Atypical *Toxoplasma gondii* genotypes identified in oocysts shed by cats in Germany. *International journal for parasitology*, *40*(3), 285-292.

**Hilal, A. M., & Hamad, H. K. (2019).** The role of some cytokines in diabetic patients infected with toxoplasmosis. *Plant Archives*, *19*(2), 602-605.

**Hlaváčová, J., Flegr, J., Fiurašková, K., & Kaňková, Š. (2021).** Relationship between latent toxoplasmosis and depression in clients of a center for assisted reproduction. *Pathogens*, *10*(8), 1052.

**Holec-Gąsior, L., & Sołowińska, K. (2022).** IgG Avidity Test as a Tool for Discrimination between Recent and Distant *Toxoplasma gondii* Infection—Current Status of Studies. *Antibodies*, *11*(3), 52.

**Houda, A., Nyaz, S., Sobhy, B. M., Bosilah, A. H., Romeo, M., Michael, J. P., & Eid, H. M. (2021).** Seminiferous tubules and spermatogenesis. *Male Reproductive Anatomy*.

**Hunt, S. & Vollenhoven, B. (2023).** Pelvic Inflammatory Disease And Infertility. Aust J Gen Pract, 52, 215-218.

# References

**Hussein, A. M., & Ali, H. Z. (2022).** Detection of TNF Alpha Level as Biomarker in Different Stages of Cutaneous Leishmaniasis Infection. *Iraqi Journal of Science*, 3313-3321.

**Hwang, S., & Khan, I. A. (2015).** CD8+ T cell immunity in an encephalitis model of *Toxoplasma gondii* infection. In *Seminars in immunopathology* (Vol. 37, pp. 271-279). Springer Berlin Heidelberg.

**Kadhim, I., A. and Hamedon, T. R. (2015).** "The Sociodemographic Characteristics of Pregnant Women With *Toxoplasmosis* in Al-Qadisiyah-Iraq." *International Journal of Public Health and Clinical Sciences Novenber Atheer Kadhim Ibadi Titi Rahmawati Hamedon* 2(59):2289–7577.

**Kalantari, N., Gorgani-Firouzjaee, T., Moulana, Z., Chehrazi, M., & Ghaffari, S. (2021).** *Toxoplasma gondii* infection and spontaneous abortion: A systematic review and meta-analysis. *Microbial Pathogenesis*, *158*, 105070.

**Kalinina, A., Zamkova, M., Antoshina, E., Trukhanova, L., Gorkova, T., Kazansky, D., & Khromykh, L. (2019).** Analyses of the toxic properties of recombinant human Cyclophilin A in mice. *Journal of Immunotoxicology*, *16*(1), 182-190.

**Kaňková, Š. P. L., Flegr, J., Calda, P., Springer, D., Potluková, E.(2014).** Effects Of Latent *Toxoplasmosis* On Autoimmune Thyroid Diseases In Pregnancy. *Plos One 9, E110878*.

# References

**Kankova, S., Flegr, J., & Calda, P. (2015).** The influence of latent *Toxoplasmosis* on women's reproductive function: four cross-sectional studies. *Folia parasitologica*, *62*, 1..

**Karunyam, B. V., Karim, A. K. A., Mohamed, I. N., Ugusman, A., Mohamed, W. M., Faizal, A. M., & Kumar, J. (2023).** Infertility and cortisol: a systematic review. *Frontiers in Endocrinology*, *14*..

**Kelly, M. N., Kolls, J. K., Happel, K., Schwartzman, J. D., Schwarzenberger, P., Combe, C., & Khan, I. A. (2005).** Interleukin-17/interleukin-17 receptor-mediated signaling is important for generation of an optimal polymorphonuclear response against *Toxoplasma gondii* infection. *Infection and immunity*, *73*(1), 617-621.

**Khalid, S., Memon, S. F., Jumani, L., Memon, S. A., & Siddiqui, M. S. (2023).** Neurotoxoplasmosis in the Immunocompetent: A Rare Occurrence. *Cureus*, *15*(3).

**Khalil, A., Sotiriadis, A., Chaoui, R., da Silva Costa, F., D'Antonio, F., Heath, P., & Ville, Y. (2020).** ISUOG Practice Guidelines: The Role of ultrasound in Congenital Infections. *Ultrasound in Obstetrics & Gynecology*.

**Khan, I. A. & Moretto, M. (2022**). Immune Responses To *Toxoplasma gondii. Curr Opin Immunol, 77, 102226.*

Khan, I. A. & Moretto, M. (2022) Immune Responses To Toxoplasma Gondii. Curr Opin Immunol, 77, 102226.

# References

**Khan, I. A., Ouellette, C., Chen, K., & Moretto, M. (2019).** Toxoplasma: immunity and pathogenesis. *Current clinical microbiology reports*, *6*, 44-50..

**Khan, K., Khan, W., Khan, T., Naaz, G., Naheda, A., & Aqeel, S. (2020).** Seroprevalence of low avidity anti-Toxoplasma IgG in pregnant women and its relationship with their age and contact with cats. *Tropical Biomedicine*, *37*(4), 1038-1049.

**Kolören, Z. & Dubey, J. P.( 2020).** A Review Of *Toxoplasmosis* In Humans And Animals In Turkey. Parasitology, 147, 12-28.

**Kongsomboonvech, A. K., Rodriguez, F., Diep, A. L., Justice, B. M., Castallanos, B. E., Camejo, A., & Jensen, K. D. (2020).** Naïve CD8 T cell IFNγ responses to a vacuolar antigen are regulated by an inflammasome-independent NLRP3 pathway and Toxoplasma gondii ROP5. *PLoS pathogens*, *16*(8), e1008327.

**Kota, A. S., & Shabbir, N. (2019).** Congenital Toxoplasmosis.

**Krücken, J., Greif, G., & von Samson-Himmelstjerna, G. (2009).** In silico analysis of the cyclophilin repertoire of apicomplexan parasites. *Parasites & Vectors*, *2*, 1-24.

**Kudo, I., & Murakami, M. (2002).** Phospholipase A2 enzymes. *Prostaglandins & other lipid mediators*, *68*, 3-58.

**Kuo, J., Bobardt, M., Chatterji, U., Mayo, P. R., Trepanier, D. J., Foster, R. T., & Ure, D. R. (2019).** A pan-cyclophilin inhibitor,

CRV431, decreases fibrosis and tumor development in chronic liver disease models. *Journal of Pharmacology and Experimental Therapeutics*, *371*(2), 231-241.

**Laboudi, M., Taghy, Z., Duieb, O., Peyron, F., & Sadak, A. (2021).** *Toxoplasma gondii* seroprevalence among pregnant women in Rabat, Morocco. *Tropical Medicine and Health*, *49*, 1-8.

**Lago, E. G., Endres, M. M., Scheeren, M. F. D. C., & Fiori, H. H. (2020).** Ocular outcome of Brazilian patients with congenital toxoplasmosis. *The Pediatric Infectious Disease Journal*, *40*(1), e21-e27.

**Lijffijt, M., Lane, S. D., Meier, S. L., Boutros, N. N., Burroughs, S., Steinberg, J. L., & Swann, A. C. (2009).** P50, N100, and P200 sensory gating: relationships with behavioral inhibition, attention, and working memory. *Psychophysiology*, *46*(5), 1059-1068.

**Lambeau, G., & Gelb, M. H. (2008).** Biochemistry and physiology of mammalian secreted phospholipases A2. *Annual review of biochemistry*, *77*(1), 495-520

**Lang, C., Groß, U., & Lüder, C. G.( 2007).** Subversion Of Innate And Adaptive Immune Responses By *Toxoplasma gondii*. Parasitology Research, 100, 191-203.

**Leong, K. G., Ozols, E., Kanellis, J., Badal, S. S., Liles, J. T., Nikolic-Paterson, D. J., & Ma, F. Y. (2020).** Cyclophilin inhibition protects

against experimental acute kidney injury and renal interstitial fibrosis. *International Journal of Molecular Sciences*, *22*(1), 271.

**Leslie K, T. B., and Papageorghiou A. (2011).** Early Prediction And Prevention Of Pre-Eclampsia. *Best practice & research Clinical obstetrics & gynaecology*, *25*(3), 343-354

**Lewinski A., B. M**. **(2023).** Female Infertility As A Result Of Stress-Related Hormonal Changes. *Gynecological And Reproductive Endocrinology & Metabolism, 02-03/2022, 094-098*.

**Li, H., Pink, M. D., Murphy, J. G., Stein, A., Dell'Acqua, M. L., & Hogan, P. G. (2012).** Balanced interactions of calcineurin with AKAP79 regulate Ca2+–calcineurin–NFAT signaling. *Nature structural & molecular biology*, *19*(3), 337-345.

**Liao, Y., Luo, D., Peng, K., & Zeng, Y. (2021).** Cyclophilin A: a key player for etiological agent infection. *Applied microbiology and biotechnology*, *105*, 1365-1377.

**Lindbergh, C. A., Casaletto, K. B., Staffaroni, A. M., Elahi, F., Walters, S. M., You, M., & Kramer, J. H**. **(2020).** Systemic tumor necrosis factor-alpha trajectories relate to brain health in typically aging older adults. *The Journals of Gerontology: Series A*, *75*(8), 1558-1565.

**Lindsay, D. S., & Dubey, J. P**. **(2020).** Neosporosis, *Toxoplasmosis*, and sarcocystosis in ruminants: an update. *Veterinary Clinics: Food Animal Practice*, *36*(1), 205-222.

# References

**Littauer, E. Q., & Skountzou, I. (2018).** Hormonal regulation of physiology, innate immunity and antibody response to H1N1 influenza virus infection during pregnancy. *Frontiers in immunology*, *9*, 2455.

**Liu, Y., Zhao, M., Xu, X., Liu, X., Zhang, H., Jiang, Y., Zhang, L. & Hu, X.(2014).** Adoptive Transfer Of Treg Cells Counters Adverse Effects Of *Toxoplasma gondii* Infection On Pregnancy. J Infect Dis, 210, 1435-43.

**Liu, Z. W., Zhang, Y. M., Zhang, L. Y., Zhou, T., Li, Y.-Y., Zhou, G.-C., Miao, Z.-M., Shang, M., He, J.-P., Ding, N.-. & Liu, Y. Q. (2022).** Duality Of Interactions Between Tgf-B And Tnf-A During Tumor Formation. 12.

**Lodyga, M., & Hinz, B. (2020)** TGF-β1–a truly transforming growth factor in fibrosis and immunity. In *Seminars in cell & developmental biology* (Vol. 101, pp. 123-139). Academic Press.

**Lopes, F. M. R., Gonçalves, D. D., Mitsuka-Breganó, R., Freire, R. L., & Navarro, I. T. (2007).** *Toxoplasma gondii* infection in pregnancy. *Brazilian Journal of Infectious Diseases*, *11*, 496-506.

**López-Yglesias, A. H., Camanzo, E., Martin, A. T., Araujo, A. M., & Yarovinsky, F. (2019).** TLR11-independent inflammasome activation is critical for CD4+ T cell-derived IFN-γ production and host resistance to *Toxoplasma gondii*. *PLoS Pathogens*, *15*(6), e1007872.

**Lucignani, G., Guarnera, A., Rossi-Espagnet, M. C., Moltoni, G., Antonelli, A., Figà Talamanca, L., & Longo, D. (2022).** From fetal to

neonatal neuroimaging in TORCH infections: a pictorial review. *Children*, *9*(8), 1210.

**Mahmoudzadeh, S., Nozad Charoudeh, H., Marques, C. S., Bahadory, S., & Ahmadpour, E. (2021).** The role of IL-12 in stimulating NK cells against *Toxoplasma gondii* infection: a mini-review. *Parasitology Research*, *120*(7), 2303-2309.

**Mahmoudzadeh, S., Nozad Charoudeh, H., Marques, C. S., Bahadory, S., & Ahmadpour, E. (2021).** The role of IL-12 in stimulating NK cells against *Toxoplasma gondii* infection: a mini-review. *Parasitology Research*, *120*(7), 2303-2309.

**Mamaghani, A. J., Fathollahi, A., Arab-Mazar, Z., Fathollahi, M., Spotin, A., Bashiri, H., & Bozorgomid, A. (2022).** *Toxoplasma gondii* vaccine candidates: A concise review. *Irish Journal of Medical Science (1971-)*, 1-

**Mangal, S., Agarwal, N., & Singh, N. (2022).** *Toxoplasmosis* in Pregnancy. In *Infections and Pregnancy* (pp. 177-189). Singapore: Springer Singapore.

**Marchioro, A. A., Colli, C. M., De Souza, C. Z., Da Silva, S. S., Tiyo, B. T., Evangelista, F. F., Higa, L., Conchon-Costa, I. & Falavigna-Guilherme, A. L.( 2018).** Analysis Of Cytokines Ifn-Γ, Tnf-A, Tgf-B And Nitric Oxide In Amniotic Fluid And Serum Of Pregnant Women With Toxoplasmosis In Southern Brazil. Cytokine, 106, 35-39.

# References

**Markon, A. O., Ryan, K. A., Wadhawan, A., Pavlovich, M., Groer, M. W., Punzalan, C., Gensheimer, K., Jones, J. L., Daue, M. L., Dagdag, A., Donnelly, P., Peng, X., Pollin, T. I., Mitchell, B. D. & Postolache, T. T. (2021).** Risk Factors For *Toxoplasma gondii* Seropositivity In The Old Order Amish. *Epidemiology & Infection, 149, E89.*

**Matowicka-Karna J, D. P. V. & Kemona H. (2009).** Does *Toxoplasma gondii* Infection Affect The Levels Of Ige And Cytokines (Il-5, Il-6, Il-10, Il-12, And Tnf-Alpha) *Clinical And Developmental Immunology, 2009, 374696.*

**Mayoral, J. A. (2020).** A Diverse Toolkit: Studies on Secreted Proteins from the Parasite *Toxoplasma gondii*. Albert Einstein College of Medicine.

**Mazzilli, R., Medenica, S., Di Tommaso, A. M., Fabozzi, G., Zamponi, V., Cimadomo, D., Rienzi, L., Ubaldi, F. M., Watanabe, M., Faggiano, A., La Vignera, S. & Defeudis, G.( 2023).** The Role Of Thyroid Function In Female And Male Infertility: *A Narrative Review. J Endocrinol Invest, 46, 15-26.*

**Mehta, A. K., Gracias, D. T., & Croft, M. (2018).** TNF activity and T cells. *Cytokine*, *101*, 14-18

**Mizani, A., Alipour, A., Sharif, M., Sarvi, S., Amouei, A., Shokri, A., Rahimi, M.-T., Hosseini, S. A. & Daryani, A. (2017).** Toxoplasmosis Seroprevalence In Iranian Women And Risk Factors Of The Disease: A Systematic Review And Meta-Analysis. *Tropical Medicine And Health, 45, 7.*

# References

**Mohamed, K., Adil, B., Degnah, N., Basuni ,S., Al Bagir, M., Al Malki, A., & Babalghith, A. (2016).** "Detection of *Toxoplasma gondii* Infection and Associated Risk Factors among Pregnant Women in Makkah Al Mukarramah, Saudi Arabia." *Asian Pacific Journal of Tropical Disease* 6(2):113–19.

**Mohammed, A. J. M.( 2019**). Seroprevalence Of Toxoplasmosis In Aborted Women In Babylon Province, Iraq. *Med J Babylon, 16, 188-91*.

**Molan, A., Nosaka, K., Hunter, M., & Wang, W**. **(2019).** Global status of *Toxoplasma gondii* infection: systematic review and prevalence snapshots. *Trop Biomed*, *36*(4), 898-925.

**Moretto, M. M., Hwang, S. & Khan, I. A. (2017).** Downregulated Il-21 Response And T Follicular Helper Cell Exhaustion Correlate With Compromised Cd8 T Cell Immunity During Chronic *Toxoplasmosis*. Front Immunol, 8, 1436.

**Moseley, T. A., Haudenschild, D. R., Rose, L., & Reddi, A. H**. **(2003).** Interleukin-17 family and IL-17 receptors. *Cytokine & growth factor reviews*, *14*(2), 155-174.

**Motedayyen, H., Zarnani, A. H., Tajik, N., Ghotloo, S., & Rezaei, A**. **(2018).** Immunomodulatory effects of human amniotic epithelial cells on naive CD4+ T cells from women with unexplained recurrent spontaneous abortion. *Placenta*, *71*, 31-40

# References

**Muhammed,Y.,N. & Alsakee , H. M. A. (2021).** Investigating Immunological Parameters And Quantification Of Rhoptry Pseudokinases Gene Expression In *Toxoplasmosis Indian Journal Of Pharmaceutical Sciences, 123, 231-8.*

**Muniz-Feliciano ,L., Van Grol ,J., Portillo ,J.A.C., Liew ,L., Liu ,B., Carlin ,C.R.(2013).** *Toxoplasma gondii*-induced activation of EGFR prevents autophagy protein-mediated killing of the parasite. *PLoS Pathog. 19;9[12]:*

**Murakami, M. (2019).** Novel functions of phospholipase A2s: Overview. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1864*(6), 763-765.

**Nagarajan, S. R., Butler, L. M., & Hoy, A. J. (2021).** The diversity and breadth of cancer cell fatty acid metabolism. *Cancer & Metabolism*, *9*, 1-28.

**Najm, R. (2021)**. Molecular characterization of bradyzoite invasion and evaluation of vaccine candidates targeting the moving junction of *Toxoplasma gondii* (Doctoral dissertation, Université Montpellier).

**Nazari, N., Bozorgomid, A., Janbakhsh, A., & Bashiri, F. (2018).** *Toxoplasma gondii* and human immunodeficiency virus co-infection in western Iran: A cross sectional study. *Asian Pacific Journal of Tropical Medicine*, *11*(1), 58-62.

**Nazari, N., Shojaee, S., Mohebali, M., Teimouri, A., Ghadiri, K., Raeghi, S., Shiee, M. R., Azarakhsh, Y. & Bozorgomid, A.( 2019).**

# References

*Toxoplasma gondii* And *Neospora Caninum* In Brain Tissue Of Rodents In North-West Iran. *Vet Med (Auckl), 10, 223-227.*

**Nazarlu, Z. H. A., Matini, M., Bahmanzadeh, M., & Foroughi-Parvar, F. (2020).** *Toxoplasma gondii*: A possible inducer of oxidative stress in reproductive system of male rats. *Iranian Journal of Parasitology*, *15*(4), 521.

**Nicloux, M., Peterman, L., Parodi, M., & Magny, J. F. (2020).** Outcome and management of newborns with congenital cytomegalovirus infection. *Archives de Pédiatrie*, *27*(3), 160-165.

**Niknamian, S. (2019).** Spermatogoniaapoptosis Induction as a Possible Mechanism of *Toxoplasma gondii* Induced Male Infertility.

**Ogbera, A. O., & Anaba, E. (2021).** Protozoa and Endocrine Dysfunction.

**Oliveira-Scussel, A. C. D. M., Ferreira, P. T. M., Resende, R. D. S., Ratkevicius-Andrade, C. M., Gomes, A. D. O., Paschoini, M. C., De Vito, F. B., Farnesi-De-Assunção, T. S., Silva, M. V. D., Mineo, J. R., Rodrigues, D. B. R. & Rodrigues, V. ( 2022).** Association Of Gestational Diabetes Mellitus And Negative Modulation Of The Specific Humoral And Cellular Immune Response Against *Toxoplasma gondii*. *Front. Immunol., 13, 18.*

**Opsteegh, M., Cuperus, T., Dam-Deisz, C., Smits, C. B., Verhaegen, B., Schares, G. & Wisselink, H. J. (2022).** In vitro assay to determine inactivation of *Toxoplasma gondii* in meat samples.

# References

**Pagheh, A. S., Sarvi, S., Sharif, M., Rezaei, F., Ahmadpour, E., Dodangeh, S., & Daryani, A. (2020).** *Toxoplasma gondii* surface antigen 1 (SAG1) as a potential candidate to develop vaccine against toxoplasmosis: A systematic review. *Comparative Immunology, Microbiology and Infectious Diseases*, *69*, 101414.

**Pal, M., Berhanu, G., Steinmetz, C. H., & Durglishvili, N. (2021).** *Toxoplasmosis:* An emerging and re-emerging zoonosis of global public health concern. *American Journal of Infectious Disease and Microbiology*, *9*, 32-38.

**Park, H. S., Lee, S. C., Cardenas, M. E., & Heitman, J. (2019).** Calcium-calmodulin-calcineurin signaling: a globally conserved virulence cascade in eukaryotic microbial pathogens. *Cell host & microbe*, *26*(4), 453-462.

**Park, M., Hong, S. H., Park, S. H., Kim, Y. S., Yang, S. C., Kim, H. R., & Song, H. (2020).** Perivascular stem cell-derived Cyclophilin A improves uterine environment with Asherman's syndrome via HIF1α-dependent angiogenesis. *Molecular Therapy*, *28*(8), 1818-1832.

**Pleyer, U., Gross, U., Schlüter, D., Wilking, H., & Seeber, F. (2019).** Toxoplasmosis in Germany: Epidemiology, diagnosis, risk factors, and treatment. *Deutsches Ärzteblatt International*, *116*(25), 435.

**Poznansky, S. A., Yu, M., Deng, K., Fu, Q., Markmann, J. F., & LeGuern, C. (2023).** Leveraging the tolerogenic potential of TNF-α and regulatory B cells in organ transplantation. *Frontiers in Immunology*, *14*, 1173672.

# References

**Pratiwi, D. Y., & Pratiwy, F. M. (2020).** A review: The potential of algae to increase immunity system.

**Ramadan, G. M., Al-Hindy, H. A. A. M., Mousa, M. J., & Al-Sadi, H. I. (2022).** Evaluation of Transforming Growth Factor-Beta 1 (TGFβ1) Levels in Cases with Multiple Myeloma: Case-Control Study among Iraqis. *Journal for ReAttach Therapy and Developmental Diversities*, *5*(2s), 516-523..

**Rani, S. (2020).** Evaluation and Modeling of Food Safety Risk Factors Associated with *Toxoplasma gondii* Infection in the Farm-to-Fork Framework (Doctoral dissertation, University of Maryland, College Park).

**Robert-Gangneux, F., & Dardé, M. L. (2012).** Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical microbiology reviews*, *25*(2), 264-296.

**Robinson, E., de Valk, H., Villena, I., Le Strat, Y., & Tourdjman, M. (2021).** National perinatal survey demonstrates adecreasing seroprevalence of *Toxoplasma gondii* infection among pregnant women in France, 1995 to 2016: impact for screening policy. *Eurosurveillance*, *26*(5), 1900710.

**Rodríguez, J. P., Leiguez, E., Guijas, C., Lomonte, B., Gutiérrez, J. M., Teixeira, C., & Balsinde, J. (2020).** A lipidomic perspective of the action of group IIA secreted phospholipase A2 on human monocytes: Lipid droplet biogenesis and activation of cytosolic phospholipase A2α. *Biomolecules*, *10*(6), 891.

# References

**Rosa, A., Butt, E., Hopper, C. P., Loroch, S., Bender, M., Schulze, H., & Zernecke, A. (2022).** Cyclophilin A Is Not Acetylated at Lysine-82 and Lysine-125 in Resting and Stimulated Platelets. *International Journal of Molecular Sciences*, *23*(3), 1469.

**Rossella, S., Trovato, M., & Manco, R. (2021).** Exploiting viral sensing mediated by Toll-like receptors to design innovative vaccines. *NPJ Vaccines*, *6*(1).

**Rostami, A., Riahi, S. M., Gamble, H. R., Fakhri, Y., Shiadeh, M. N., Danesh, M., & Gasser, R. B. (2020).** Global prevalence of latent *Toxoplasmosis* in pregnant women: a systematic review and meta-analysis. *Clinical Microbiology and Infection*, *26*(6), 673-683.

**Sadegi ,H., S., Heidari, Z., Habibzadeh, S. & Shahbazzadegan, S. (2023).** Seroprevalence Of *Toxoplasma gondii* Among Pregnant Women In Ardabil, Iran (2021-2022). *Iran J Parasitol, 18, 93-99.*

**Saeed, S. A., & Al-Aubaidi, I. K. (2018).** Seroprevalence of Toxoplasmosis Antibodies among Diabetes Mellitus Patients and Assessment some Biochemical Markers. *Ibn AL-Haitham Journal For Pure and Applied Science*, 539-551

**Saeij ,J.P and Frickel ,E.M.(2017).** Exposing *Toxoplasma gondii* hiding inside the vacuole: a role for GBPs, autophagy and host cell death. Curr Opin Microbiol. 40:72–80. pmid:29141239

**Saheb, E. J., Al-Issa, Y. A., Mussa, I. S., & Zghair, K. H. (2020).** Incidence of *Toxoplasmosis* in psoriasis patients and possible correlation

with tumor necrosis factor-α. *Baghdad Science Journal*, *17*(1 (Suppl.)), 0214-0214. .

**Saki, J., Sabaghan, M., Arjmand, R., Teimoori, A., Rashno, M., Saki, G., & Shojaee, S. (2020).** Spermatogonia apoptosis induction as a possible mechanism of *Toxoplasma gondii*-induced male infertility. *Iranian Journal of Basic Medical Sciences*, *23*(9), 1164.

**Salman, Y. J. (2014).** Correlation Between *Toxoplasma gondii* And Anti-Mullerian Hormone Levels In Sera Of Women In Kirkuk City Using Elisa Method. Int. J. Curr. Microbiol. App. Sci, 3, 85-92.

**Sana, M., Rashid, M., Rashid, I., Akbar, H., Gomez-Marin, J. E. & Dimier-Poisson, I. (2022).** Immune Response Against Toxoplasmosis-Some Recent Updates Rh: *Toxoplasma gondii* Immune Response. Int J *Immunopathol Pharmacol, 36, 3946320221078436.*

**Sanchez, S. G., & Besteiro, S. (2021).** The pathogenicity and virulence of *Toxoplasma gondii*. *Virulence*, *12*(1), 3095-3114.

**Sánchez-Arcila, J. C., & Jensen, K. D. (2022).** Parasites: Eviction notice served on Toxoplasma. *Elife*, *11*, e76246.

**Sasai, M., Pradipta, A., & Yamamoto, M. (2018).** Host immune responses to *Toxoplasma gondii*. *International Immunology*, *30*(3), 113-119.

# References

**Saso, A., Bamford, A., Grewal, K., Noori, M., Hatcher, J., D'Arco, F., & Lyall, H. (2020).** Fifteen-minute consultation: Management of the infant born to a mother with *Toxoplasmosis* in pregnancy. *Archives of Disease in Childhood-Education and Practice*, *105*(5), 262-269.

**Semenza, J. C., & Paz, S. (2021).** Climate change and infectious disease in Europe: Impact, projection and adaptation. *The Lancet Regional Health-Europe*, *9*, 100230.

**Sharifi, K., Hosseini Farash, B. R., Tara, F., Khaledi, A., Sharifi, K. & Shamsian, S. A. A. (2019).** Diagnosis Of Acute *Toxoplasmosis* By Igg And Igm Antibodies And Igg Avidity In Pregnant Women From Mashhad, Eastern Iran. *Iran J Parasitol, 14, 639-645.*

**Sharifi, K., Sharifi, K., Hoseini Farrash, R. & Khaledi, A. J. N. N. (2018).** Evaluate The Sero-Epidemiology Of *Toxoplasma gondii* In Patients Referred To Ghaem Hospital, Mashhad, Iran. 21, 42-48.

**Sher, A., Tosh, K., & Jankovic, D. (2017).** Innate recognition of *Toxoplasma gondii* in humans involves a mechanism distinct from that utilized by rodents. *Cellular & molecular immunology*, *14*(1), 36-42.

**Shin, D. W., Cha, D. Y., Hua, Q. J., Cha, G. H. & Lee, Y. H. (2009).** Seroprevalence Of *Toxoplasma gondii* Infection And Characteristics Of Seropositive Patients In General Hospitals In Daejeon, Korea. *Korean J Parasitol, 47, 125-30.*

**Siddiqui, N., Shujatullah, F., Khan, H. M., Rabbani, T., & Khan, P. A. (2014).** IgG avidity antibodies against *Toxoplasma gondii* in high risk

females of reproductive age group in India. *The Korean Journal of Parasitology*, *52*(5), 487.

**Sint, N. H., Htun, Y. M., Win, T. T., Mon, A. S., Lwin, T. Z., Maung, L. O., & Tun, K. M. (2023).** Seroprevalence and associated risk factors of *Toxoplasma gondii* infection among slaughterhouse workers in Yangon Region, Myanmar: A cross-sectional study. *Plos one*, *18*(4), e0284352.

**Sisquella, X., Ofir-Birin, Y., Pimentel, M. A., Cheng, L., Abou Karam, P., Sampaio, N. G., & Regev-Rudzki, N. (2017).** Malaria parasite DNA-harbouring vesicles activate cytosolic immune sensors. *Nature communications*, *8*(1), 1985.

**Smith, N. C., Goulart, C., Hayward, J. A., Kupz, A., Miller, C. M., &van Dooren, G. G. (2021).** Control of human *Toxoplasmosis*. *International Journal for Parasitology*, *51*(2-3), 95-121.

**Soltani, S., Ghaffari, Ali Dalir, Kahvaz, Mehdi Sagha , Sabaghan, M., Pashmforosh, M. & Foroutan, M. (2021).** Detection Of Anti-*Toxoplasma gondii* Igg And Igm Antibodies And Associated Risk Factors During Pregnancy In Southwest Iran. *Infectious diseases in obstetrics and gynecology*, *2021*

**Souza, J. S., Farani, P. S., Ferreira, B. I., Barbosa, H. S., Menna-Barreto, R. F., Moreira, O. C., & Mariante, R. M. (2023).**

# References

Establishment of a murine model of congenital Toxoplasmosis and validation of a qPCR assay to assess the parasite load in maternal and fetal tissues. *Frontiers in Microbiology*, *14*, 465.

**Sturge, C. R. and Yarovinsky, F**. **(2014).** Complex Immune Cell Interplay In The Ifn-Γ Response During *Toxoplasma gondii* Infection. *Infect. Immun., Iai, 01714–01722*.

**Sun, W., Xu, Y., Xin, Q., Zhang, Y., Cui, B., & Hong, F**. **(2019).** Association between polymorphism in Cyclophilin A gene and its serum and placental expression in Han Chinese women with severe preeclampsia. *Pregnancy hypertension*, *15*, 84-92.

**Suwanti, L. T. & Mufasirin (2018).** Increased Apoptosis Skull Of Pups Born To *Toxoplasma gondii*-Infected Mice Associated With Increased Expression Of Interferon Gamma, But Not Tumor Necrosis Factor Alfa. *Afr J Infect Dis*, *12*, *68-71*.

**Teimouri, A., Mohtasebi, S., Kazemirad, E., & Keshavarz, H**. **(2020).** Role of *Toxoplasma gondii* IgG avidity testing in discriminating between acute and chronic Toxoplasmosis in pregnancy. *Journal of clinical microbiology*, *58*(9), e00505-20.

**Teixeira, S. C., da Silva, M. S., Gomes, A. A. S., Moretti, N. S., Lopes, D. S., Ferro, E. A. V., & de Melo Rodrigues, V**. **(2022).** Panacea within a Pandora's box: the antiparasitic effects of phospholipases A2 (PLA2s) from snake venoms. *Trends in Parasitology*, *38*(1), 80-94.

# References

**Tuqa, M. S. A. B., Alasadi, N. A. H., & Kathem ,A. (2021).** Ratio Of Male To Female Infertility In Baghdad Alkarkh (2015-2020). *Teikyo Medical Journal, 44, 11.*

**Ubaid Hamza, M., Hameed, N., Al-Zubaidi ,H. S., Abulkassim, R., Basim, M. Z., Saad, M. S., & Hamad, D.( 2022)** . *Toxoplasma gondii* Seroprevalence Among Pregnant Women In Baghdad During 2021-2022. *Journal Of Obstetrics, Gynecology And Cancer Research, 7, 563-568.*

**Ullah, K., Zeb, M. A., Baqi, A., Sardar, J., Gul, A., & Shuaib, S. L. (2022).** Prevalence of Toxoplasma gondii and Brucella Species in Infertile Women In District Dir Lower, Khyber Pakhtunkhwa, Pakistan. *Pak-Euro Journal of Medical and Life Sciences*, *5*(2), 215-222. Sood R. Concise book of Medical Laboratory Technology. Jaypee Brothers Pvt. Limited; 2015.

**Usta, A., Taşkın, M. İ., Baykan, Ö., & Adalı, E. (2018).** Circulating levels of cyclophilin A in women with PCOS: Correlation with clinical and biochemical parameters.

**Vado-Solís, I. A., Suárez-Solís ,V., Jiménez-Delgadillo, B., Velázquez J. E., and José C..( 2013).** "*Toxoplasma gondii* Presence in Women with Spontaneous Abortion in Yucatan, Mexico ." *Journal of Parasitology* 99(2):383–85.

**Veldhoen, M., Hocking, R. J., Atkins, C. J., Locksley, R. M. & Stockinger, B. (2006).** Tgfbeta In The Context Of An Inflammatory

Cytokine Milieu Supports De Novo Differentiation Of Il-17-Producing T Cells. *Immunity, 24, 179-89.*

**Venetis, C. A., Papadopoulos, S. P., Campo, R., Gordts, S., Tarlatzis, B. C., & Grimbizis, G. F**. **(2014).** Clinical implications of congenital uterine anomalies: a meta-analysis of comparative studies. *Reproductive biomedicine online*, *29*(6), 665-683.

**Venkatesan, B., Valente, A. J., Prabhu, S. D., Shanmugam, P., Delafontaine, P., & Chandrasekar, B**. **(2010).** EMMPRIN activates multiple transcription factors in cardiomyocytes, and induces interleukin-18 expression via Rac1-dependent PI3K/Akt/IKK/NF-κB andMKK7/JNK/AP-1 signaling. *Journal of molecular and cellular cardiology*, *49*(4), 655-663.

**Vesely, M. C. A., Rodríguez, C., Gruppi, A., & Rodríguez, E. V. A. (2020).** Interleukin-17 mediated immunity during infections with Trypanosoma cruzi and other protozoans. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1866*(5), 165706.

**Waldorf, K. M. A., & McAdams, R. M**. **(2013).** Influence of infection during pregnancy on fetal development. *Reproduction (Cambridge, England)*, *146*(5), R151.

**Wang, P., & Heitman, J**. **(2005).** The cyclophilins. *Genome biology*, *6*(7), 1-6.

**Wilking, H., Thamm, M., Stark, K., Aebischer, T., & Seeber, F. (2016).** Prevalence, incidence estimations and risk factors of *Toxoplasma*

*gondii* infection in Germany: a representative, cross-sectional, serological study. *Scientific reports*, *6*(1), 22551.

**Wojno, E. D. T., Hunter, C. A., & Stumhofer, J. S. (2019).** The immunobiology of the interleukin-12 family: room for discovery. *Immunity*, *50*(4), 851-870.

**Woyesa, S. B., & Taylor-Robinson, A. W. (2021).** *Toxoplasma gondii*: Deeper understanding of epidemiology, virulence and pathophysiology enhances diagnosis and informs vaccine design. *Journal of Infectious Diseases and Immunity*, *13*(1), 7-19.

**Xue, C., Sowden, M. P., & Berk, B. C. (2018).** Extracellular and intracellular cyclophilin A, native and post-translationally modified, show diverse and specific pathological roles in diseases. *Arteriosclerosis, thrombosis, and vascular biology*, *38*(5), 986-993.

**Yang, D., Dai, F., Yuan, M., Zheng, Y., Liu, S., Deng, Z., Tan, W., Chen, L., Zhang, Q., Zhao, X. & Cheng, Y. (2021).** Role Of Transforming Growth Factor-B1 In Regulating Fetal-Maternal Immune Tolerance In Normal And Pathological Pregnancy. *Front Immunol, 12, 689181.*

**Yousefvand, A., Mirhosseini, S. A., Ghorbani, M., Mohammadzadeh, T., Moghaddam, M. M., & Mohammadyari, S. (2021).** Molecular and serological detection and of *Toxoplasma gondii* in small ruminants of southwest Iran and the potential risks for consumers. *Journal of Consumer Protection and Food Safety*, *16*, 117-127.

# References

**Zamaniyan, M., Fakhar, M., Tabaripour, R., Peivandi, S., Keighobadi, M., Ghasemi, S., & Montazeri, M. (2023).** Credible Serological Evidence of Latent *Toxoplasma* Infection Among Women with Primary Infertility: A Ten-Year Registry-Based Study. *Acta Parasitologica*, 1-6.

**Zander, R., Schauder, D., Xin, G., Nguyen, C., Wu, X., Zajac, A., & Cui, W. (2019).** CD4+ T cell help is required for the formation of a cytolytic CD8+ T cell subset that protects against chronic infection and cancer. *Immunity*, *51*(6), 1028-1042.

**Zare-Bidaki, M., Assar, S., Hakimi, H., Abdollahi, S. H., Nosratabadi, R., Kennedy, D. & Arababadi, M. K.(2016).** Tgf-B In Toxoplasmosis: Friend Or Foe? *Cytokine, 86, 29-35.*

**Zghair, K. H. & Fadhel, Z. H.(2015).** The Effect of Toxoplasmosis Infection on Interleukin-12Level During Human Maturity in Baghdad Province Iraqi *Journal of Science, 56, 1B,: 356-360*

**Zhao, W., Yu, H., Liu, X., Wang, T., Yao, Y., Zhou, Q., & Tan, F. (2022).** Systematic identification of the lysine lactylation in the protozoan parasite *Toxoplasma gondii*. *Parasites & Vectors*, *15*(1), 1-14.

**Zhou, Z., Lopez, H. I. A. O., Pérez, G. E., Burgos, L. M., Farina, J. M., Saldarriaga, C.,& Baranchuk, A. (2021).** Toxoplasmosis and the Heart.

## الخلاصه

د اء المقوسات  يمكن ان يصيب أي حيوان من ذوات الدم الحار، بما في ذلك البشر. يمكن أن يؤثر داء المقوسات لدى الإناث بشكل كبير على نتائج عملياتهن الإنجابية وخاصة قدرتها على الخصوبة. ومع ذلك، لا توجد أبحاث كافية تبحث في العلاقة بين عدوى داء المقوسات لدى الإناث والعقم، خاصة في منطقتنا

حيث أجريت دراسة الحالات والسيطره للتأكد من الانتشار المصلي للأجسام المضادة لمرض داء المقوسات وبعض العوامل المعدلة للمناعة بين النساء المصابات بالعقم.

شملت الدراسة 250 امرأة تعاني من العقم (مع نتائج إيجابية لـداء المقوسات و 50 ا مرأة سليمة تماماً، ليس لديها تاريخ للإجهاض، كانت لديها الخصوبة، وكانت نتائج سلبية لـداء المقوسات وتم إجراء الدراسة خلال الفترة من 1 شباط إلى 25 تشرين الاول 2022 في العيادات الخارجية لأمراض النساء و في اثنين من المستشفيات الرائدة في بابل: مستشفى بابل التعليمي للأمومة والطفولة، ومستشفى الإمام الصادق التعليمي.

بعد الاختيار الدقيق للمشاركين في الدراسة، بناءً على معايير إدراج واستبعاد محددة، تم إجراء العمل الحالي باستخدام استبيان حيث تم السؤال  عن اسم وعمر الأنثى الغير قادره على الانجاب، ومدة الزواج، وأي عوامل عقم عند الذكور، والنوع. العقم (الاولي أو الثانوي)، ووجود أي حيوانات أليفة حقلية أو قططية، وملف هرموني شمل التقييم المصلي لجميع المشاركين الجلوبيولين المناعي لداء المقوسات IgG وIgM، والإنترلوكينات -IL 12 وIL-17، وTNF-α، وTGF-β، وcyclophilin-A، ومستويات مصل الفوسفوليباز.

وكشفت نتائج الدراسة أن متوسط عمر المرضى كان 31.6 ±4.3 سنة. وفقط 62 (24.8٪) من 250 أنثى تعاني من العقم استوفت متطلبات الدراسة البحثية. وكان متوسط مدة الزواج 3.9 ± 1.3 سنة. ثلاثة وثلاثون (13.2) من النساء المصابات بالعقم كان لديهن أسباب عقم إضافية (عوامل ذكورية) لدى شركائهن. من إجمالي 250 مشاركاً

أبلغ 152 فردًا (60.8%) عن وجود حيوانات حقلية أو قططية، وأفاد 68 مشاركًا (27.2%) عن إصابتهم بعدوى داء المقوسات. كانت المعلمات المصلية والمناعية أعلى في المرضى، إما إلى حد كبير ( IL-12 cyclophilin-A ) وبشكل ملحوظ للغاية (IL-17، TGF-β، TNF-α، وphospholipase A) على التوالي. وتختلف متغيرات الدراسة في درجات الارتباط مع بعضها البعض.

لخصت الدراسة إلى أنه بالنسبة للمرضى الذين يعانون من داء المقوسات، لم تكن أي من معايير الدراسة تشخيصية قوية أو لديها تنبؤات جيدة. بالإضافة إلى ذلك، لا يمكن استخدام أي من المؤشرات التي تم بحثها كمؤشر حيوي لتحديد أو تمييز النساء الأصحاء من الإناث المصابات بالعقم من داء المقوسات.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل – كلية العلوم

قسم علوم الحياة

# علاقة بعض الحركيات الخلويه والانزيمات لدى النساء العقيمات والمصابه بالمقوسه الكونديه

رساله

مقدمة الى مجلس كلية العلوم – جامـــــعة بابل

وهي جزء من متطلبات نيل درجة الدكتوراه فلسفه في العلوم/ علوم الحياة / علم الحيوان

من قبل

نور عبد الرضا ابراهيم احمد

بكلوريوس علوم حياة-كلية العلوم-جامعة بابل-2011

ماجستير علوم حياة – كلية العلوم - جامعة بابل -2020

اشراف

أ.د . ماهر علي جتان القريشي

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2023 م