Republic of Iraq
Ministry of Higher Education & Scientific Research
University of Karbala
College of Medicine
Department of Microbiology

Study of Interleukins 5, 12 and 18 in Infertile Female: Correlation with Reproductive Hormonal Levels, Oocyte Maturity and Embryonic Development IVF-ICSI Cycle

A Thesis

Submitted to the Council of the College of Medicine/University of Karbala in Partial Fulfillment of the Requirements for the Degree of Master in Medical Microbiology

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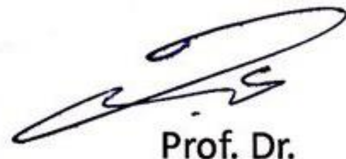

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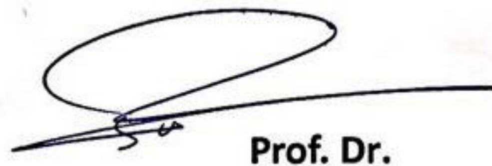

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Dedication

To the Master of Martyrs, Imam Hussein and his brother Abu Al-Fadhl Al-Abbas (peace be upon them)
I dedicate this thesis with gratitude .

Dhuhaa

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Acknowledgments

First, I would like to thank my Creator, who gave me the health and strength to complete this work.

Also, I would like to thank Professor **Dr. Abeer Thaher Al-Hasnawi** and Assistant Professor **Dr. Ali Ibrahim Rahim,** supervisors of this thesis, for their advice and keenness to complete this work in the best possible manner during the specified time.

I want to thank all faculty members and staff of the Microbiology Branch of the College of Medicine University of Karbala, particularly the Head of the Department, Professor **Dr. Sawsan Mohammed Jabbar,** for her support, continuous encouragement, and kindness throughout the study.

Morever, I would like to thank the doctors and staff of the Fertility Center in Najaf, Al-Kafeel Super-speciality Hospital and Al-Ameer International Hospital

My faithful-filled thanks and gratitude extend to my family for their unlimited support and permanent encouragement.

All thanks and gratitude to patients and their relatives of their contribution to the study: I wash them to have good children.

Finally, all thanks and appreciation to everyone who helped and advised me to complete this thesis.

To all, please accept my truthful thanks.

Dhuhaa 2023

Summary

Infertility is the incapacity to fulfill pregnancy after reasonable time of sexual intercourse with no contraceptive measures taken. Ovulation disorders are one of the female factors that causes of infertility, including polycystic ovaries Syndrome (PCOS) and poor Ovarian reserve (POR). The ovarian tissue in addition to the immune cells produces Cytokines. Cytokine function in the ovary described as promoting processes of follicular growth, steroidogenesis, recruitment and activation of leukocytes necessary for ovulation and tissue remodelling during ovulation, luteinization, and luteolysis. The current study has aimed to evaluate interleukin 5 (IL-5), interleukin 12 (IL-12) and interleukin 18 (IL-18) in follicular fluid and serum and studied the correlation between these markers and Intracytoplasmic sperm injection (ICSI) outcomes.

This study is a cross sectional and subjects of this study are divided into three groups : infertility due to male factor (n=40), PCOS (n=30) and POR (n=30) enrolled in AL-Sader Teaching Medical City, Al-Kafeel Super-speciality hospital and Al-Ameer International Center for Fertilization and IVF during the period from November 2022 to April 2023. The samples of blood were collected at cycle day two and detected the level of follicle stimulating hormone, luteinizing hormone, prolactin hormone, estradiol hormone, anti-mullerian hormone and estradiol hormone at day hCG injection by MINI VIDS. Serum and follicular fluid collected at oocytes retrieval day and detected levels of IL-5, IL-12 and IL-18 by ELISA technique.

The result of present study showed that day 2; FSH was higher level while, E2, AMH and Estradiol hormone (E2) at day of HCG injection were lower level in POR and there were significantly different from male factor and PCOS cases with (P=0.005, P=0.008, P=0.005, P=0.005) respectively.

LH was low level in Male factor cause and there was significantly different from PCOS and POR patients with (P=0.006). While, prolactin was high level in PCOS cause and there was significantly different from male factor and POR causes with (P=0.005). In addition, the low mean of Total follicle number, Embryo Grade l (GI), Embryo Grade ll (GII) of POR cases were significantly different from male factor and PCOS cases with (P=0.005, P=0.005 and P=0.003) respectively. Whereas, the high mean of Embryo Grade III (Glll) of PCOS cases was significantly different from male factor and POR patients with (P= 0.030), the low mean of Transferred embryo in POR was significantly different from male factor and PCOS patients with (P=0.015).

Regarding, the serum and follicular levels of cytokines (IL-5, IL-12 and IL-18), there were no significant difference between groups except higher median of IL-12 level in the follicular fluid and serum of Male factor was significantly different from PCOS and POR patients with (P=0.005). Also, about the low mean of transferred embryo in non pregnant women, there was a significant difference between B-HCG groups with (P=0.013). Furthermore, higher median of follicular fluid and serum levels of IL-5 in negative group were a significant difference between B. HCG groups with (P=0.005). In addition, higher median of the follicular fluid and serum levels of IL- 18 in positive group were a significant difference between B-HCG groups with (P=0.005).

Moreover, in this study there were a positive correlation between IL- 18 serum level and fertilization rate in male factor cases with (P= 0.033). While in PCOS women, there were a negative correlation between IL-5 in follicular fluid and fertilization rate with (P= 0.020) and a positive correlation between IL12 in follicular fluid and embryo grade II with (P= 0.014). Concerning the POR there were a negative correlation between IL-5 in follicular fluid and

embryo grade II with (P= 0.033) and positive correlation between IL-18 in follicular fluid and embryo grade II with (P= 0.021), also, positive correlation between IL-5 serum level with maturity rate with (P=0.044). About IL-12 serum level, there was a negative correlation with fertilization rate with (P= 0.002), while, IL-18 serum level was positive correlation with embryo grade II with (P= 0.024).

The results of this study have shown a non significant difference between groups regarding cytokines levels in follicular fluid and serum except elevation of IL-12 level in follicular fluid and serum for male factor patients compared with PCOS and POR patients . Therefore, these markers might have no important role in the intracytoplasmic sperm injection (ICSI) outcomes.

Table of Contents

Sequence	Subject	Page No.
	Summary	I
	List of Content	IV
	List of Tables	XIV
	List of Figures and Pictures	XIII
	List of Abbreviations	XVI
Chapter One : Introduction and Literature Review		
1.1	Introduction	1
	Aim of the Study	3
1.2	Literature Review	4
1.2.1	Definition of infertility	4
1.2.2	Epidemiology of infertility	4
1.2.3	Types of infertility	6
a.	Primary infertility	6
b.	Secondary infertility	6
1.2.4	Risk factors of infertility	6
1	Age	6
2	Body mass index	6

3	Duration of infertility	7
4	Smoking	7
5	Alcohol	8
6	Infection	8
7	Chronic disease	8
1.2.5	Etiology of infertility	8
1.2.5.1	Male factor	9
A	Pre-testicular deficiency	10
B	Testicular deficiency	10
C	Post-testicular deficiency	11
1.2.5.1.1	Diagnosis of male factor	11
1.2.5.2	Female factor	12
I	Hormonal Disorders	12
II	Ovarian disorders	12
III	Fallopian Tube Damage or Blockage	12
IV	Uterine factors	13
1.2.5.3	Combined factor	13
1.2.5.4	Unexplained infertility	13
1.2.5.2.1	Polycystic ovary syndrome. (PCOS)	14
1.2.5.2.2	Poor Ovarian reserve (POR) or Diminished Ovarian reserve (DOR)	15
1.2.8	Treatment Modalities of Infertility	17

1.2.8.1.	**Intra-Uterine Insemination (IUI)**	**17**
1.2.8.2	**Assisted Reproductive Technology (ART)**	**18**
1	**In-Vitro Fertilization (IVF)**	**18**
2	**Zygote Intra-fallopian Transfer (ZIFT) and Gamete Intrafallopian Transfer (GIFT)**	**18**
3	**Intracytoplasmic Sperm Injection (ICSI)**	**18**
4	**Assisted Hatching**	**18**
5	**Donor Eggs and Sperms**	**19**
6	**Gestational Carrier**	**19**
7	**Adoption**	**19**
1.2.9	**Intra-Cytoplasmic Sperm Injection (ICSI)**	**19**
1.2.9.1.	**Indication of ICSI**	**19**
A)	**Male Factor Infertility**	**19**
B)	**Non Male Factor Infertility**	**20**
1.2.10	**Follicular Fluid (FF)**	**21**
1.2.11	**Immunological markers of infertility**	**22**
1.2.11.1	**Cytokines**	**22**
1.2.11.2	**Role of some cytokines in the infertility**	**23**
1.2.11.2.1	**Interleukin-5 (IL-5)**	**23**
1.2.11.2.2	**Interleukin-12 (IL-12)**	**25**
1.2.11.2.3	**Interleukin-18 (IL-18)**	**26**

Chapter Two : Study Design, Material and Method

2.	Study design, Materials and Methods	28
2.1	Study design	28
2.1.1	Subjects	30
2.1.2	Ethical issue	30
2.1.3	Exclusion criteria	30
2.1.4	Inclusion criteria	30
2.2	Materials	31
2.2.1	Instruments and Equipment	31
2.2.2	Chemical and biological materials	34
2.2.3	ELISA Kit used in the study	34
2.2.3.1	ELISA Kit Content of Human IL-5 , IL-12 and IL-18	35
2.3.	Methods	36
2.3.1	Sample collection	36
2.3.1.1	Follicular Fluid (FF)	36
2.3.1.2	Serum Sample	36
2.3.2	Hormone evaluation	38
2.3.2.1	Test principle of (FSH, LH, Prolactin, E2, AMH and B-HCG)	38
2.3.2.1.1	Assay Procedure for Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Prolactin(PRL).	39
2.3.2.1.2	Assay Procedure for Estradiol II (E2)	40

2.3.2.1.3	Assay Procedure for Anti-Mullerian Hormone (AMH)	41
2.3.2.1.4	Assay Procedure for Beta-Human Chronic Gonadotrpin (B-HCG)	42
2.3.3	Estimation of IL-5 ,IL-12 and IL-18 concentration in serum and follicular fluid	42
2.3.3.1	Test principle of IL-5 ,IL-12 and IL-18	42
2.3.3.2	Reagent Preparation	43
2.3.2.3	Assay procedure	43
2.3.2.4	Results Calculation	44
2.3.4.	Female Preparation for ICSI	45
2.3.4.1	Controlled Ovarian Hyper-stimulation (COH)	45
2.3.4.2	Measurement of E2 for Follow up	45
2.3.4.3	Oocyte Pick Up	46
2.3.5	Assisted Reproduction Technique	47
2.3.5.1	Dishes Preparation	47
1.	Preparation of collection Dish for ICSI	47
2.	Preparation of Denudation Dish	47
3.	Preparation of Injection Dish	47
4.	Culture Dish	47
2.3.5.2	Collection of Oocytes on the Day of Oocytes Retrieval	49
2.3.5.3	Oocyte Denudation	49
A	Enzymatic Denudation	49
B	Mechanical Denudation	49

2.3.5.4	Assessment of Denuded Oocyte	49
2.3.5.5	Sperm Preparation	49
2.3.5.6	Set up of the ICSI Micromanipulator	50
2.3.5.7	Sperm Immobilization	50
2.3.5.8	The Injection of Oocyte (Day 0)	51
2.3.5.9	Incubation of the Injected Oocyte (Day 0)	51
2.3.5.10	Assessment of Fertilization (Day 1)	53
2.3.5.11	Assessment of Embryo Quality (Day2-3)	53
2.3.5.12	Embryo Transfer	54
2.3.6	Statistical analysis	54
Chapter Three : Result		
3	Results	54
3.1	Demographic characteristics among studied groups according to the infertility causes	54
3.2	Mean Hormonal levels in studied groups	55
3.3	Clinical characteristics of different infertility groups.	57
3.4	Cytokine levels among studied groups	59
3.5	Mean differences of Demographic characteristics according to the result of B HCG (pregnancy result)	60
3.6	Hormonal study according to the result of B HCG (pregnancy result)	61
3.7	Clinical characteristics according to the result of B-HCG (pregnancy result)	62

3.8	**Cytokines according to the result of B HCG (pregnancy result)**	**63**
3.9	**Correlation of study cytokines in male factor patients**	**65**
3.9.1	**Correlation between study follicular cytokines with BMI and clinical characteristics**	**65**
3.9.2	**Correlation between study serum cytokines with BMI and clinical characteristics**	**66**
3.10	**Correlation of study cytokines in PCOS patients**	**67**
3.10.1	**Correlation between study follicular cytokines with BMI and clinical characteristics**	**67**
3.10.2	**Correlation between study serum cytokines with BMI and clinical characteristics**	**68**
3.11	**Correlation of study cytokines in POR patients**	**69**
3.11.1	**Correlation between study follicular cytokines with BMI and clinical characteristics**	**69**
3.11.2	**Correlation between study serum cytokines with BMI and clinical characteristics**	**71**
3.12.1	**Correlation between study serum and follicular cytokines of male factor**	**72**
3.12.2.	**Correlation between study serum and follicular cytokines of PCOS**	**73**
3.12.3.	**Correlation between study serum and follicular cytokines of POR**	**74**
3.13.	**Receiver Operating Characteristic Curves ROC**	**76**
3.13.1.	**ROC measurement to study cytokines in PCOS cause**	**76**
3.13.2.	**ROC measurement to study cytokines in POR cause**	**77**

Chapter Four: Discussion		
4	Discussion	79
4.1	Demographic characteristics among studied groups according to the infertility causes	79
4.2	Mean Hormonal levels in studied groups	81
4.3	Clinical characteristics of different infertility groups	86
4.4	Cytokine levels among studied groups	88
4.5	Demographic characteristics according to the result of B HCG (pregnancy result)	90
4.6	Hormonal study according to the result of B HCG (pregnancy result)	91
4.7	About the clinical characteristics according to the result of B HCG (pregnancy result)	92
4.8	Regarding the Cytokines according to the result of B HCG (pregnancy result)	92
4.9	Correlation of serum and FF cytokines level with BMI and clinical characteristics in male factor, PCOS and POR patients.	93
4.10	Correlation between study serum and follicular cytokines of male factor, PCOS and POR cases	95
4.11	ROC measurement to study cytokines in PCOS and POR causes	96
Conclusion and Recommendations		
	Conclusions	97
	Recommendations	98

References		
	References	99
Appendix		
I	Male factor, PCOS and POR patients questionnaires	
II	ELISA instruments	
III	ELISA curve for IL-5, IL-12 and IL-18 in Follicular fluid (FF) and serum of patients	
الخلاصة		

List of Figures and Pictures

Figure Number	Subject	Page Number
2.1	Study Design	28
2.2	Steps of the duplication dilution.	42
2.3	Oocyte maturity(GV, MI and MII oocyte)	48
2.4	Sperm immobilization(a) and intra-cytoplasmic sperm injection (b, c and d) of embryos	50
2.5	Fertilization assessment of oocyte	51
3.1	Receiver Operating Characteristic Curve of cytokines in detecting PCOS cause in relation to male factor	77
3.2	Receiver Operating Characteristic Curve of cytokines in detecting POR cause in relation to male factor	78

List of Tables

Table number	Subject	Page Number
2.1	Instruments and Equipment	30
2.2	Chemical and biological materials with their Manufacturing company and country of origin	34
2.3	MIMIVIDUS Kits used in the study	33
2.4	ELISA Kits used in the study	33
2.5	Kit components and Storage of IL-5, IL-12 and IL-18	34
3.1	Demographic characteristics among studied groups according to the infertility causes	55
3.2	Mean Hormonal levels in studied groups	56
3.3	Clinical characteristics of different infertility groups	58
3.4	Cytokine levels among studied groups	59
3.5	Demographic characteristics according to the result of B HCG (pregnancy result)	61
3.6	Mean differences of hormonal study according to the result of B HCG (pregnancy result)	62
3.7	Mean differences of clinical characteristics according to the result of B HCG (pregnancy result)	63
3.8	Median differences of cytokines according to the result of B HCG (pregnancy result)	64

3.9	The correlation between follicular cytokines with BMI and clinical characteristics regarding male factor group	65
3.10	The correlation between serum cytokines with BMI and clinical characteristics regarding male factor group	66
3.11	The correlation between follicular cytokines with BMI and clinical characteristics regarding PCOS group	67
3.12	The correlation between serum cytokines with BMI and clinical characteristics regarding PCOS group	68
3.13	The correlation between follicular cytokines with BMI and clinical characteristics regarding POR group	70
3.14	The correlation between serum cytokines with BMI and clinical characteristics regarding POR group	71
3.15	The correlation between serum and follicular cytokines regarding male factor	72
3.16	The correlation between serum and follicular cytokines regarding PCOS	73
3.17	The correlation between serum and follicular cytokines regarding POR	75
3.18	Area under the curve of cytokines in detecting PCOS cause in relation to male factor	76
3.19	Area under the curve of cytokines in detecting POR cause in relation to male factor	77

List of Abbreviations

Code	Word
AFC	Antral follicle count
AMH	Anti mullerian hormone
ART	Assisted Reproductive Technology
ASRM	American Society of Reproductive Medicine
B-HCG	Beta-Human Chronic Gonadotrpin
COC	Cumulus Oocyte Complex
DOR	Diminished Ovarian reserve
E2	Estradiol II
ELISA	Enzyme-Linked immunoSorbent Assay
FF	Follicular fluid
FSH	Follicle-stimulating hormone
FMR1	Frgil X messenger ribonucleoprotein
GI	Grade 1
GII	Grade II
GIII	Grade III
GV	Germinal Vesicle
HEPES	Hydroxy ethyl piperazine ethan sulfonic acid
HPG	Hypothalamic-pituitary-gonadal
ICSI	Intracytoplasmic sperm injection

IL	**Interleukin**
IVF	**In Vitro Fertilization**
LH	**Luteinizing hormone**
MI	**Metaphase 1**
MII	**Metaphase 1I**
ng/ml	**Nanogram per milleter**
OD	**Optical density**
ORT	**Ovarian Reserve Test**
ORT	**Ovarian Reserve Test**
PCOM	**Polycystic ovary morphology**
PCOS	**Polycystic ovary syndrome**
POR	**Poor Ovarian Reserve**
PVP	**Polyvinylpyrrolidone**
Pg/ ml	**Pikogram per milleter**
Rpm	**Rotation per minute**
WHO	**World Health Organization**

Chapter One
Introduction
and
Literature Review

1.1 Introduction:

Infertility is a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse. (Borght and Wyns, 2018). The most common causes of infertility are Female factor 40% and male factor 30%. (El Adlani *et al*., 2021). Anovulation and abnormalities in the semen quality are the most prevalent reasons for infertility. Polycystic ovary syndrome (PCOS) is the most prevalent etiology of anovulatory infertility in women, accounting for around 40% of female infertility (Sharif, Yasamin and Hamza, 2022). It is an endocrinological problem that affects about 5%–10% of women of childbearing age all over the world. Oligo-anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovary morphology (PCOM) on ultrasonography are the most important clinical manifestations of PCOS. It was reported that approximately 80% of anovulation infertility was caused by PCOS. (Gao *et al*., 2023)

Regarding, Poor Ovarian reserve (POR). It is refers to the decline in fertility caused by the loss of normal ovarian function. (Wang *et al*., 2023). The age for the prediction of POR in women were less than 40 years old. Furthermore, anti mullerian hormone (AMH) is the only independent factor that is significantly related to POR in women.(Laqqan and Yassin, 2023)

The Intracytoplasmic sperm injection (ICSI) is a micromanipulation technique that allows the injection of one spermatozoon into the ooplasm of a metaphase II oocyte. In humans, ICSI has been utilized for more than a quarter of a century and has become one of the most widely used assisted reproductive technologies for reproduction in humans. The widespread use of ICSI is the result of the capacity for this procedure to be utilized in addressing many reproductive problems, such as lack of sperm motility and globozoospermia, which are mainly related to male infertility. Furthermore, ICSI has been utilized to overcome some problems when there is female

infertility, such as less-than-optimal quality oocytes and/or when it is only possible to collect a small number of oocytes (Briski and Salamone, 2022)

Cytokines can modulate the function of immune cells within the ovary in the context of reproduction. The ovarian interleukins, which are secreted by the granulosa cells and other immune cells within the ovaries and follicles along with hormonal changes, regulate various functions, including folliculogenesis, oogenesis, ovulation, fertilization, embryonic development, implantation, formation, and regression of the corpus luteum. One of the success factors of in vitro fertilization (IVF) is the quality of the obtained oocytes. (Aleksandra *et al*., 2022).

Moreover, Numerous cytokines were detected in follicular fluid, the role of which in reproductive physiology seems crucial. They influence the development and maturation of the follicle, ovulation, and corpus luteum formation, as well as embryo implantation and maintenance of pregnancy. (Adamczak *et al*., 2021)

The elevated intrafollicular concentrations of IL-5 seem to be a negative predictor to the pregnancy outcome in ICSI cycles.(Alhilali *et al*., 2019). While, the Interleukin-12 is a disulphide-linked heterodimeric cytokine, produced by B-cells, phagocytic cells, and other antigen-presenting cells. Increase IL12 concentration may be correlated with fragmented embryos and found at a low concentration in PCOS. (Piccinni *et al*., 2021). In addition, the concentration of IL-18, both in the blood serum and follicular fluid (FF), positively may be correlated with the number of oocytes collected from patients qualified for IVF. (Adamczak *et al*., 2021).

Aim of the Study

The aim of this study was planned to estimate the association between some immunological markers (IL-5, IL-12 and IL-18) in infertile females with different causes (male factor, PCOS and POR) with oocytes maturity and embryonic development of IVF-ICSI Cycle Outcomes in a group of couples; the following objectives achieved this:

1. Determination of Hormonal levels (FSH, LH, Prolactin, E2, AMH and E2 at HCG injection day) according to the cause of infertility in patients groups.
2. Detection of follicular fluid and serum levels of IL-5, IL-12 and IL-18 by ELISA technique in three patients groups.
3. Determination of clinical variables according to the cause of infertility in cases.
4. Evaluation of cytokines according to the result of B-HCG (pregnancy result).
5. Determination of hormones and the cytokines according to results of B.HCG .
6. Determination of the correlation between serum cytokines with BMI and clinical characteristics (Maturity rate, Fertilization rate, GI, GII, GIII and embryo transfer) regarding three infertility groups.

1.2. Literature Review

1.2.1. Definition of infertility:

Infertility, can be described as a couple based disease that prevents women from becoming pregnant after one year during regular, sexual relations without protection. (Thoma *et al*., 2021; WHO, 2022). It is often in two terms, (infertility and sub-fertility) are overlapping (Mustafa *et al*., 2019).

Infertility is not synonymous with sterility (A permanent state of Infertility) (Kadir and Veleva, 2020). It is linked to negative health consequences like poor mental health, persistent conditions like cardiovascular disease, and complications associated with infertility treatments. (Thoma *et al*., 2021; WHO, 2022)

1.2.2. Epidemiology of infertility:

The World Health Organization (WHO) estimates that there are between 45–80 million infertile couples globally. (Agarwal *et al*., 2015; WHO, 2022). Generally, (20-35%) of infertility cases are due to female cause, (20-30%) of cases are due to male cause, (25-40%) of cases due to both of male and female (combined) etiologies and 10% unexplained infertility. (Mustafa *et al*., 2019; El Adlani *et al*., 2021).

For decades the burden of couples' infertility has been often and disproportionately supposed as the women responsibility, as such, for biological and social causes, a couple's infertility has been unequally shared, with the tendency to investigate the female partner over the male (Pozzi *et al*., 2021).

Infertility is a global public health issue with a high impact on individuals of both sexes and society. Infertility is ranked as the 5 highest serious global disability with a negative impact on the self-esteem of those who are involved. These negative side effects remain a higher social burden for women than men (Zayed and El-Hadidy, 2020).

In Iraq, the percentage of women who did not give birth over the age of 30 years increased from 11% in 2000 to 26% in 2016 . In 2018 The reasons

behind female infertility may differ in terms of their relative importance from one country to another, for example, because of differences in the base rates of the prevalence of sexually transmitted diseases or the different Ages of the population groups being studied. (Alakkam, Mohammed and Musa, 2022).

In addition, the infertility rate is 10-15% for women younger than 35 years, this rate increases to about 33% for women in 35-40 age group, while 87% of 45 years old women have no possibility of conceiving a child, after 35 years of age. Infertility is present in 50% of couples, and the percentage of those with primary infertility increases. (Al-Ani, Al-Kasser and Al-Aboosy, 2021)

Moreover, the prevalence of Infertility in reproductive-aged women has been estimated to be one in every seven couples in the western world and one in every four couples in developing countries. In some regions of the world, including South Asia, some countries of sub-Saharan Africa, the Middle East and North Africa, Central and Eastern Europe and Central Asia infertility rates may reach 30%. (Borght and Wyns, 2018). It is estimated to affect between 8 and 12% of reproductive-aged couples worldwide. Males are found to be solely responsible for 20–30% of infertility cases but contribute to 50% of cases overall. Secondary infertility is the most common form of female infertility around the globe, often due to reproductive tract infections. (Borght and Wyns, 2018).

1.2.3. Types of infertility:

Two types of infertility have been identified :

a. Primary infertility: is defined as infertile couples with no pregnancy history.

b. Secondary infertility: is defined as the inability to have a pregnancy after previously successful conception (Hanson *et al*., 2017 ; Zayed and El-Hadidy, 2020 and Chenfeng *et al*., 2022). It infertility is the failure to conceive following a previous pregnancy in the absence of contraception, breastfeeding, or postpartum amenorrhea. Primary infertility is more typical than secondary infertility in affluent nations; however, the turnaround is valid in developing nations. (Magdum *et al*., 2022)

1.2.4. Risk factors of infertility:

Risk factors of infertility includes:

1. Age

The prevalence of infertility of women increases from one per cent at age 25 to 55 percent at age 45years old. (Schmidt *et al*., 2011; American College of Obstetricians and Gynecologists, 2020). Increasing male age is a risk factor for infertility. Declining male fertility is related to falling androgen levels, decreased sexual activity, alterations in Sperm motility and morphology, and deterioration in sperm quality and DNA integrity . (Sartorius and Nieschlag, 2010 ; du Fosse *et al*., 2020).

2. Body mass index

Obesity is common in women with polycystic ovary syndrome. Polycystic ovary syndrome and obesity are associated with reduced fertility. The effect of metabolic syndrome on the success of infertility treatment and pregnancy outcomes in women with polycystic ovary syndrome undergoing ovulation induction has not been investigated. (Arya *et al*., 2021).

The most common cause of infertility in underweight women، Chronic energy deficiency can impair the hypothalamic-pituitary-gonadal (HPG) axis and lead to hypothalamic anovulation in underweight women, the syndromes related to underweight status that are associated with infertility, and the utilization of drugs can cause excessive weight loss. They may result in

fertility problems due to the loss of adipose tissue and the subsequent hormonal disturbances. (Boutari *et al.*, 2020)

3. Duration of infertility

The largest study identified that 85% of women would conceive within 12 months. Based on this study's findings, fecund ability is 25% in the first three months of unprotected intercourse and then decreased to 15% for the remaining nine months. The American Society of Reproductive Medicine (ASRM) recommends initiating an evaluation for infertility after failing to achieve pregnancy within 12 months of unprotected intercourse or therapeutic donor insemination in women younger than 35 years or within 6 months in women older than 35 (Infertility Workup for the Women's Health Specialist, 2019). A nonlinear relationship was observed between infertility duration and IVF fertilization rate, which decreased with infertility years up to the turning point (4.8 years). (Zhang *et al.*, 2022)

4. Smoking

These are modifiable risk factors which are affecting human reproductive function. Smoking is linked to premature menopause in addition to decreased levels of ovarian reserve markers, mediated by an diminishing of antral follicle progress and growth, resultant in cytotoxicity and making of poor quality oocytes. In men alterations in morphology and decreased concentration, motility and viability of Sperm have been observed among smokers (De Angelis *et al.*, 2020).

5. Alcohol

Alcohol consumption is associated with higher levels of estrogens and lower levels of progesterone, as well as decreased luteinizing hormone (LH), hCG receptor expression, granulosa cell expression, reduced oviductal smooth muscle cell contractility, irregular menstrual cycles, and ovulatory

dysfunction (Akison, Moritz and Reid, 2019). In men shown a significant decline in testosterone levels, seminal fluid volume and sperm concentration in chronic alcoholics. (Azam *et al.*, 2019; De Angelis, *et al*, 2020).

6. Infection

Many microbial causes infertility of male and female example Gonorrhea, chlamydia, trichomonas, brucellosis, ureaplasma, mycoplasma, coliforms bacteria, adenovirus, enterovirus and mumps.(Manju *et al.*, 2020; Shah *et al.*, 2021).

7. Chronic disease

In male, the association between male infertility and the risk of the two out of three major non malignant chronic conditions according to WHO (diabetes and cardiovascular diseases) and all mortality cause. (Clara *et al.*, 2017). In female, many chronic illness associated with female infertility such as, Polycystic Ovaries syndrome ,Endometrioses and thyroid disorders (Mallikarjuna and Rajeshwari, 2015).

1.2.5. Etiology of infertility:

Infertility may occur due to:

- Male factors .
- Female factors .
- A combination of male and female factors .
- Unexplained. For both women and men

However, environmental and lifestyle factors such as age ,smoking, excessive alcohol intake, obesity and exposure to environmental pollutants have been associated with lower fertility rates. (WHO, 2022). The most common causes of infertility are ovulatory dysfunction, male factor infertility, and tubal disease. The remaining 15% of infertile couples have “unexplained infertility. (Carson and Kallen, 2021)

1.2.5.1 . Male factor

Male infertility is defined as the inability of a male partner to accomplish a pregnancy in a fertile female partner. (Shah *et al.,* 2021). It is accounting for 40–50% of all infertility cases. (Bold and Swinburne, 2022)

A male reproductive impairment could be due to many different factors affecting sperm production, which can ultimately result in oligozoospermia (sperm concentration lower than 15×10^{6} ml^{-1}), asthenozoospermia (sperm total motility lower than 40% or progressive motility lower than 32%), teratozoospermia (normal forms lower than 4%), or a combination of these three (oligoasthenoteratozoospermia (OAT), as indicated by the 2010 World Health Organization reference values (Assidi, 2022).

Severe male factor (SMF) infertility involves severe oligozoospermia (<5 $\times10^{6}$ sperms per ml-1 of ejaculate), cryptozoospermia (the condition by which spermatozoa cannot be observed in a fresh semen sample, but can be found after centrifugation and microscopic observation of the pellet), or even an absence of spermatozoa in the ejaculate. The latter is defined as azoospermia, a condition affecting 1% of the general male population and up to 10%–15% of the infertile male population (Bold and Swinburne, 2022).

Most of the scientific studies investigated the effect of SMF in In Vitro Fertilization (IVF), unfortunately without considering the female factor. As azoospermic couples acquire earlier in their lives an indication of IVF, they tend to be characterized by a younger female counterpart with a good ovarian reserve. Naturally, lower ovarian reserve and response to the ovarian simulation protocol worsen the IVF outcome, defined as the chance to achieve a live birth per intention to treat . (Maheshwari, McLernon and Bhattacharya, 2015; Mazzilli *et al*., 2022).

The cause of male infertility is commonly divided into 3 main categories:

A . Pre-testicular deficiency

As a less common etiology than other causes of male infertility ,hormonal disorders leading to abnormalities in hormones produced by the pituitary gland, hypothalamus and testicles. Hormones such as testosterone regulate sperm production. Example of disorders that result in hormonal imbalance include pituitary or testicular cancers. hypogonadotropic hypogonadism (HH) is caused by insufficient gonadotropin-releasing hormone (GnRH) and/or FSH and LH secretion. These insufficiencies result in deficient androgen secretion and spermatogenesis. HH can arise from congenital GnRH deficiency, hemochromatosis, genetic disorders, hormonal abnormalities, or medications. In addition, systemic disorders such as chronic illnesses, nutritional deficiencies, and obesity have been identified as causes of HH. (Assidi, 2022)

B . Testicular deficiency

Testicular failure to produce sperm , sometimes referred to as nonobstructive azoospermia, is spermatogenic failure caused by conditions other than obstruction or hypothalamic-pituitary gonadal axis (HPG) dysfunction. This category of dysfunction can be further subdivided into congenital, acquired, or idiopathic testicular failure. Congenital failure can manifest as anorchia, testicular dysgenesis, cryptorchidism, or genetic abnormalities. Acquired testicular failure can be the result of trauma, testicular torsion, orchitis, exogenous factors (e.g., medications example medical treatments that impair sperm-producing cells (such as chemotherapy), systemic diseases, varicocele), or surgeries that damage the vascular structure of the testes. About 15% of the general male population and approximately 40% of men presenting with male infertility have varicoceles. (Sharma, 2017)

C. Post-testicular deficiency

This form of male infertility is less common than non-obstructive azoospermia, but occurs in approximately 40% of men presenting with azoospermia obstruction of the reproductive tract causing dysfunctionalities in the ejection of semen or obstruction of sperm delivery. This blockage can occur in the tubes that carry semen (such as ejaculatory ducts and seminal vesicles). Blockages are commonly due to injuries or infections of the genital tract. The obstruction can arise from the epididymis, vas deferens, or ejaculatory duct and can be acquired or congenital. (Sharma, 2017; Assidi, 2022)

Male fertility is largely determined in spermatogenesis, the development of spermatozoa from spermatogonia in the testes. This meticulous developmental process is marked by both mitotic and meiotic divisions, followed by extensive morphological and biochemical differentiation, leading to a mature spermatozoan. Male infertility is attributed to abnormal spermatozoa parameters (spermatogenic failure), such as total absence (azoospermia), low count (oligozoospermia), abnormal morphology (teratozoospermia), and/or abnormal motility (asthenozoospermia). (Agarwal *et al.*, 2015 ; Agarwal *et al.*, 2021)

1.2.5.1.1 Diagnosis of male factor:

Components of the initial evaluation should include at minimum medical history, physical examination, and semen analysis. Semen microbiological examination, endocrine assessment, and imaging are suggested in most men and recommended when specific risk factors for infertility exist or first-step analyses showed abnormalities. Full examination including genetic tests, testicular cytology/histology, or additional tests on sperm is clinically oriented and based on the results of previous investigations. For treatment purposes, the identification of the specific cause and the pathogenetic mechanism is advisable. At least, distinguishing

pre-testicular, testicular, and post-testicular forms is essential. Treatment should be couple-oriented, including lifestyle modifications, etiologic therapies, empirical treatments, and assisted reproduction techniques (ART) on the basis of best evidence and with a gradual approach. (Ferlin *et al*., 2022)

1.2.5.2. Female factor

According to the Center for Diseases Control (CDC), 1.5 million women in the US (6%) are infertile, and 25% of infertile couples have more than one factor that contributes to their infertility. (Sala *et al*, 2018) The cause of Female infertility is commonly divided into 4 main categories:

I .Hormonal Disorders

It is an essential reason of anovulation, hormonal disorders that affect ovulation include a hypothalamic pituitary cause such as hypogonadotropic hypogonadism, or hyperthyroidism, hypothyroidism, and hyperprolactinemia, women with hormonal disproportion impair producing the folliculogenesis to ensure the development of an oocyte. (Koyyada and Orsu, 2020; Nasser, Al-Jumaili and Alhusni, 2021).

II .Ovarian Disorders

Disorders of ovulation are often present with irregular periods (oligomenorrhoea) or an absence of periods (amenorrhoea), Studies done worldwide prove that polycystic ovarian syndrome and primary Ovarian insufficiency are the most common cause of female factor of infertility. (Deshpande and Gupta, 2019; Man, 2022).

III .Fallopian Tube Damage or Blockage

The most common cause is genital tract infection, which may result in pelvic inflammatory diseases (Gonorrhea and Chlamydia), endometriosis in most cases leads to pelvic adhesions and then tubal obstruction (Reekie *et al*., 2019 ; Ketki *et al*., 2022).

IV .Uterine Factors

Uterine factor infertility (UFI) is defined as a complete lack of a uterus (Absolute Uterine Factor Infertility or AUFI) or as a nonfunctional uterus (Non-Absolute Uterine Factor Infertility or NAUFI). The exact prevalence of UFI is currently unknown. Early studies, which have been repeatedly conducted over the years, suggest that it affects 3–5% of the world's female population and that AUFI affects up to 1 in 500 women of childbearing age. (Hur *et al.*, 2019).

There are many causes of UFI, cogenital and acquired: uterine agenesis, hysterectomies, uterine malformations, polyps, myomas, adenomyosis, synechiae, uterine irradiation. There is no reliable data of the prevalence of UFI and its various causes among women under 40 years old. (Sallée *et al.*, 2022)

The uterus has a vital role in allowing for a woman to attain pregnancy and carry it to term successfully, there are many etiologies, but by categorizing the diagnoses into either congenital due to structural defect of uterus or acquired due to fibroids (Daolio *et al.*, 2020).

1.2.5.3. Combined factor

In 30-15% of couples, both the male and female are infertile. (Mustafa *et al.*, 2019).

1.2.5.4. Unexplained infertility

Fertility testing hasn't found a reason that a person or couple is unable to get pregnant. (Penzias *et al.*, 2020). The failure of conception not explained by anovulation, poor sperm quality, tubal pathology or any other causes of infertility, the two most useful treatments for unexplained infertility are intra-uterine insemination and in vitro fertilization (Mol *et al.*, 2018). In 15–30% of patients, infertility remains unexplained. (La Marca and Mastellari, 2020).

1.2.5.2.1 Polycystic ovary syndrome. (PCOS)

Polycystic ovary syndrome (PCOS) is the most common chronic reproductive and metabolic endocrine disorder affecting women of childbearing age, with prevalence estimated to be 4%–21% worldwide (Belenkaia *et al*., 2019). It is a heterogeneous disorder characterized by a triad of hyperandrogenism, menstrual irregularities and polycystic ovaries. Therefore, patients can present with different manifestations of this triad depending on the disease phenotype, patient's age, and lifestyle (Joham *et al.*, 2022). This syndrome is also characterized by variation in the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, serum androgens (testosterone and and rostenedione), high anti-Mullerian hormone (AMH) and hyperprolactinemia. (Teede *et al*., 2018)

The etiology of this syndrome remains largely unknown, but mounting evidence suggests that PCOS might be a complex multigenic disorder with strong epigenetic and environmental influences, including diet and lifestyle factors. PCOS is frequently associated with abdominal adiposity, insulin resistance, obesity, cancers, metabolic disorders and cardiovascular risk factors. (Thursby and Juge, 2017; Escobar, 2018 ; Wolf *et al.*, 2018 ; Abdalla *et al*., 2020). Insulin resistance is also associated with this disorder, where excess androgen leads to the reduced sex hormone-binding globulin (SHBG) by inhibiting its synthesis in the liver. However, low serum SHBG levels are considered a biomarker of metabolic abnormalities and linked with insulin resistance, hyperandrogenism, and abnormal glucose metabolism in PCOS patients, which is why the patients require long-term screening in addition to insulin-sensitizing medications such as metformin .(Sendur and Yildiz, 2020)

The therapeutic options available for PCOS include a change in lifestyle, diet, exercise, and pharmacotherapy. However, the standard pharmacological approaches have not given satisfactory results, and PCOS prevalence is still on the rise . In recent years, the strong association of gut

microbiota with physiology of female reproductive functions has been reported. (Rababa'h, Matani and Yehya, 2022)

The 2018 PCOS guidelines from the National Health and Medical Research Council (NHMRC), Australia, recommended the inclusion of ethnic variation for better management of the disease. According to the guideline, health professionals and scientists should consider ethnic variation in the manifestations of PCOS, including differences in hirsutism patterns. (Wolf *et al.*, 2018)

1.2.5.2.2. Poor Ovarian reserve (POR)

Poor ovarian reserve (POR) is defined as a decrease in the number and/or quality of oocytes in the ovary, accompanied by decline in the level of anti-Müllerian hormone (AMH), a decrease in the number of antral follicles, and an increase in the level of follicle-stimulating hormone (FSH).(Cohen, Buffet and Darai, 2015). In particular, several terminologies such as primary ovarian insufficiency (POI), poor ovarian response (POR), and other common diseases in reproductive medicine are all closely related to Diminished Ovarian reserve (DOR.). (Fàbregues *et al.*,2020)

According to the consensus elaborated by the European Society of Human Reproduction and Embryology (ESHRE) in 2011, to define POR, at least two of the following three features must be present:

- Advanced maternal age (≥40 years) or any other risk factor for POR.
- A previous POR (≤3 oocytes with a conventional stimulation protocol).
- An abnormal ovarian reserve test (i.e., AFC of 5–7 follicles or AMH of 0.5–1.1 ng/ml).

Two episodes of POR after maximal stimulation are sufficient to define a patient as a poor responder in the absence of advanced maternal age or abnormal ovarian reserve test (ORT). From that time, according to the literatures, the prevalence of POR after ovarian stimulation ranged from 5.6 to 35.1% worldwide. The American Society of Reproductive Medicine

(ASRM) put forward an expert consensus in 2020 that POR has no diagnostic criteria, but it is mainly manifested in the decline in the quality and quantity of oocytes and the decline in reproductive potential. (Alviggi *et al*., 2016)

A number of risk factors for POR have been reported in the literature, including advanced age (over 35 years old), family history of early menopause, genetic factors (45, X chromosome mosaicism, frgil X messenger ribonucleoprotein (FMR1) gene mutation, etc.), diseases that may cause ovarian damage (endometriosis, pelvic tuberculosis, pelvic infection, etc.) or history of ovarian surgery, chemotherapy for ovarian-related diseases, pelvic radiotherapy and autoimmune diseases, smoking, and environmental factors. (Haydardedeoglu and Zeyneloglu, 2015; Rasool and Shah, 2017; Bunyaeva *et al*., 2021).

POR is characterized by decreased fertility and poor fertility outcomes even when assisted reproductive techniques (ARTs) are used. Improving the clinical outcome of patients with POR is still one of the most challenging tasks of in vitro fertilization (IVF) clinical practice (Wald *et al*., 2020). Therefore, while continuously improving IVF treatment strategies, researchers have also extensively tried to identify specific medication to improve ovarian functions in patients with POR to obtain better treatment results. Researchers have obtained preliminary results showing increased pregnancy outcomes in ART treatment. (Chang *et al*., 2018 ; Liu *et al*., 2019; Liu *et al*., 2020)

The ovarian reserve is determining the capacity of the ovary to provide egg cells which was assessed based on age, the baseline FSH level, and the baseline antral follicle count (AFC), besides baseline anti-Mullerian Hormone (AMH) level. AMH is produced by granulosa cells of small, growing follicles in the ovary. Serum levels of AMH are strongly Correlate with the number of growing follicles, and consequently AMH has

established increasing attention as a marker for ovarian reserve (Moolhuijsen and Visser, 2020).

Beside the age which has an important role influencing egg quality and ovarian reserve. Reduced ovarian reserve describes the diminished of various ages have decreased ovarian reserve. These patients also complain of increasing use of ovulation stimulants, ovarian hypo-response, and a high rate of ovulation cancellation, in addition to having fewer and lower-quality surviving oocytes (Mamsen *et al.*, 2021).

Following treatment with assisted reproductive technology (ART), these women may still experience a high rate of miscarriage, a decrease in the number of eggs obtained, and a decrease in clinical pregnancy and live Births. The physiological and psychological difficulties on these women are considerably increased by recurrent ovulation cancellation, post-ovulation Fertilization failure, and failures of implantation (Chang *et al.*, 2018).

1.2.8.Treatment Modalities of Infertility

Infertility treatment depends on the cause, duration, both partners age, and personal preferences. The couple should be explained that some of the causes of infertility cannot be corrected. Financial, physical, and time commitment is required for infertility treatment (Anwar and Anwar, 2016). Infertility is typically treated with fertility drugs, medical procedures, surgery, or a combination of these (Dayan *et al.*, 2019).

1.2.8.1.Intra-uterine Insemination (IUI)

This could be used for unexplained infertility and female cases with minimal endometriosis and mild male factor infertility problems. In this, healthy sperms that have been collected and concentrated are placed directly in the uterus around the time of ovulation. The timing of IUI can be coordinated with the normal cycle or by using fertility medications. (Boomsma, Cohlen and Farquhar, 2019).

1.2.8.2. Assisted Reproductive Technology (ART)

Assisted Reproductive Technology are a group of medical procedures for treating the infertile human in which both male and female gametes are used outside the body (in vitro) to attain pregnancy. It includes : (Lepore and Petruzziello, 2021)

1. In-Vitro Fertilization (IVF): In IVF, multiple mature eggs from a woman are retrieved, and fertilized with a man's sperm outside the womb and inside a laboratory. Then, the fertilized embryos are implanted in the uterus after three to five days of fertilization. (Bain, 2019)

2. Zygote Intra-fallopian Transfer (ZIFT) and Gamete Intrafallopian Transfer (GIFT): In ZIFT, the fertilized egg is directly transferred into the fallopian tube; whereas, in GIFT a mixture of sperms and eggs is placed in the fallopian tube and fertilization occurs there. (De Jongh, 2019)

3. Intracytoplasmic Sperm Injection (ICSI): In ICSI, a single healthy sperm is injected directly into a mature egg. ICSI is used when there is a problem with the quality of the semen, or there are few sperms, or prior IVF cycles have failed. .(Geng *et al.,* 2020).

4. Assisted Hatching: Through this technique, implantation of the embryo into the uterus is assisted by breaking the outer covering of the embryo. This helps the embryo to smoothly implant**.** (Hammadeh, Fischer-Hammadeh and Ali, 2011)

5. Donor Eggs and Sperms: Assisted reproductive technology mostly uses the married couple's eggs and sperms, but when there are severe issues with the eggs and sperms then donor sperms or even embryo is taken to enhance fertility. (Banerjee and Singla, 2018)

6. Gestational Carrier: This is sometimes called as surrogate pregnancy, when a woman who does not have a uterus or if the uterus is not functional and to whom the pregnancy can endanger health, the couple can decide to have a gestational carrier, who carries the couple's embryo in the uterus. (Murugappan *et al*., 2018)

7. Adoption: This can be an option for couples who have multiple unexplained IVF failure cycles. (Anwar and Anwar, 2016)

1.2.9. Intra-Cytoplasmic Sperm Injection (ICSI)

It is a type of (ART), and has become the most commonly used technique of in vitro fertilization. It was primarily presented to manage male infertility with severely impaired characteristics such as azoospermia or severely compromised sperm parameters (concentration, motility, and morphology), although the reliability of ICSI has made it an attractive option even in non-male factor couples suffering infertility worldwide .(Geng *et al.,* 2020).

1.2.9.1. Indication of ICSI

A) Male Factor Infertility: Include any factor that inhibit the spermatozoon from normally entering and fertilizing an oocyte due to either**:**

1- A low number (Sever Oligozoospermia) of spermatozoa,

2- Impaired motility(Sever Asthenozoospermia)

3- An abnormal morphology(Sever Teratozoospermia)

4- Combination of all (Sever Oligoasthenoteratozoospermia) (O'Neill *et al.*, 2018).

5- If there is no sperm (Azoospermia) spermatozoa can be obtained by testicular sperm extraction (TESE or micro-TESE) (Lacey *et al.*, 2021).

6- Reflux of semen into the bladder occurs when the bladder neck fails to close during the expulsion phase (retrograde ejaculation).

7- Electro ejaculation in men with neurologic impairment and present with abnormalities or failure of ejaculation.

8- Immunological infertility presence of anti-sperm antibody in either male or female partner (Haddad *et al.*, 2021**)**.

B) Non-male Factor Indications

1- Fertilization of poor-quality or dysmorphic oocytes.

2- Poor responders to maximize fertilization rate.

3- Cryopreservation oocytes or sperm to postpone conception.
4- In conjunction with preimplantation genetic testing (PGT) to evaluate the genetic status of embryos in addition to increase the likelihood of implantation.
5- Advanced maternal age patients and
6- Unexplained infertility (Pereira and Palermo, 2018)

Although several tests and techniques to enhance fertility have recently become available, the real cause of infertility must first be identified before any procedures are effectively selected. As a very first step, fertility doctors usually advise a couple to prevent risk factors such as reducing excess weight, abstaining from alcohol, smoking and sexual intercourse during the ovulation period to increase their chances of being pregnant. If infertility can't be corrected after a full lifestyle and sexual habit modification, fertility procedures that might help are intracytoplasmic sperm injection (ICSI), intrauterine insemination (IUI), gamete intrafallopian transfer (GIFT) and in vitro fertilization (IVF). Certain fertility techniques will be carefully chosen based on the age and health status of the couple. Premarital counselling and planning, combined with reproductive system evaluations, greatly improve the odds of getting pregnant. If any reproductive abnormalities, such as chocolate cysts and endometriosis, have been found, effective and appropriate treatments must first be provided prior to becoming pregnant. Premarital health screening plays a significant role in fertility preparation. The sooner abnormal findings are discovered and treated, the better outcomes for making family plans could be successfully achieved. (Geng *et al.,* 2020).

1.2.10. Follicular Fluid (FF)

Follicular fluid (FF) surrounds the granulosa cell-oocyte complex and is one of the mediating factors in the communication between the cells within the follicle. Literature reveals that human FF and its components are key

factors to the success of natural fertilization. Among other substances, FF consists of multiple cytokines and immune cells, including interleukins, macrophages, NK cells and lymphocytes. Together, these cells and cytokines might influence the oocyte-granulosa-cell complex. Altered balances of immune content might be involved in changes on folliculogenesis, oocyte maturation, oocyte quality and ovulation (Prince *et al.*, 2020).

These altered balances are possibly involved in infertility associated with immune-mediated diseases such as PCOS and POR. The human follicular fluid which is the microenvironment of the oocyte during its development and maturation, is a semi-viscous, hypocoagulable fluid comprising a wide variety of biologically active molecules. It is a product of transfer of plasma constituents across the blood-follicular barrier and the secretory activity of granulosa and theca cells. It is important in ovarian physiology, including steroidogenesis, growth of the follicle and ovulation, maturation of the oocyte and its transport to the oviduct. (Wu *et al.*, 2015 ; Usman *et al.*, 2021).

It can be obtained by trans-vaginal oocyte retrieval for in-vitro fertilization (IVF). It is extracellular fluid that forms during development of the antral follicle under the influence of follicle stimulating hormone (FSH). FF bathes the developing oocyte. It is constituents can impact oocyte development and maturation, necessary events for fertilization and eventually live birth. A majority of an embryo's cytoplasm and a substantial portion of it is DNA is derived from the oocyte. As such, FF measurements have the potential to offer greater insight into biologically effective doses affecting fecundity than either blood or urine(Butts *et al.*, 2020).

However, FF obtain only by invasive techniques for in vitro Analysis. Therefore, an oocyte pick-up (OPU) is required, which is an Invasive procedure, there is no possibility of leaving the developing egg in Vivo and un-disturb it, FF is essential in ovarian physiology, and steroidogenesis,

development of the follicles, maturation of the oocytes, ovulation, As well as their transport to the oviduct. (Güngör and Güngör, 2021)

1.2.11. Immunological markers of infertility

1.2.11.1 Cytokines

Cytokines were originally identified as products of immune system cells and are important immune response mediators. Cytokines can stimulate or inhibit cell growth, regulate cell differentiation, induce cell chemotaxis, and modulate the expression of other cytokines. (Gunther *et al.*, 2016)

Cytokines have been found to regulate the reproductive process by influencing the immune environment within the follicle itself, as well as the uterus (Lu *et al*., 2018). It consist of smaller water-soluble proteins and glycoproteins. They are classified into lymphokines, interleukins, and chemokines based on their function, cell origin, and target cells. Cytokines can modulate the function of immune cells within the ovary in the context of reproduction. (Prince *et al*., 2020)

The ovarian Interleukins, which are secreted by the granulosa cells and other immune cells within the ovaries and follicles along with hormonal changes, regulate various functions, including folliculogenesis, oogenesis, ovulation, fertilization, embryonic development, implantation, formation, and regression of the corpus luteum (Yogesh *et al*., 2016).

One of the success factors of in vitro fertilization (IVF) is the quality of the obtained oocytes. The quality of the oocyte is significantly affected by the environment in which it is located or the so-called microenvironment. Defining certain parameters of the microenvironment, which can be easily and quickly detected, which enable the differentiation of oocytes that are of better or worse quality, could potentially increase the success of IVF. In the follicles, oocytes undergo growth and maturation. The follicular wall consists of granulosa and theca cells, which are separated by the basal membrane. The maturation process is carried out through several stages

within follicles. During follicular growth, its interior is filled with follicular fluid that is made by the filtration of the blood plasma constituents and by the secretory activity of granulosa and theca cells (Prince *et al*., 2020).

The interleukins that Play crucial roles during decidualization, implantation, placentation as well as during the earlier and later stages of pregnancy, namely IL-11, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13 as Well as IL-27. (Piccinni *et al*., 2021)

1.2.11.2. Role of some cytokines in the infertility :-

1.2.11.2.1. Interleukin-5 (IL-5)

Interleukin-5 is a cytokine with a length of 115 amino acids and a molecular weight of 15.2 kDa that is derived from T-cells with hematopoietic functions predominantly associated with antigen-induced eosinophilia. IL-5 induces differentiation of B-cells to immunoglobulin secreting cells and is an important factor in growth, differentiation and activation of eosinophils. IL-5, GM-CSF and IL-3 comprise the β-common (βc) cytokine family, so named because the receptors share a common β chain complexes with cytokine-specific α chains. IL-5 and IL-5R are the targets of therapeutic antibodies for treatment of eosinophilic asthma and are involved in type 2 inflammation in the mucosal allergic reaction to grass pollen (Varricchi and Canonica, 2016). It is produced by type 2 T helper cells (Th2), mast cells, and eosinophils, and non-hematopoietic cells, and it is responsible for the maturation and release of eosinophils in the bone marrow. In humans, interleukin 5 is a very selective cytokine as a result of the restricted expression of the interleukin 5 receptor on eosinophils and basophils. IL-5 has pleiotropic actions, from enhancing the homeostatic proliferation and survival of B-1 cells through noncognate stimulation and driving the differentiation of B-1 and B-2 cells into terminally differentiated plasma cells to augmenting the survival and activation of eosinophils. (Varricchi and Canonica, 2016; Nagase, Ueki and Fujieda, 2020).

IL-5 propagates cellular signals via JAK–STAT (Janus kinase/ signal transducer and activator of transcription) and Ras/ MAPK (Mitogen-activated protein kinase) pathways. (Zielińska *et al.*, 2020).

Th2 cells produce and secrete IL-5 through a complex activation process induced by inhaled allergens. IL-5 is a pro-inflammatory factor that plays a very important role in eosinophil biology. It is the factor responsible for the differentiation, growth, activation, survival and recruitment of eosinophils into the airways. It also prevents apoptosis of these cells. Eosinophils secrete numerous mediators of type 2 inflammation, including granule proteins, enzymes, cytokines, chemokines, growth factors, lipids, and oxidation products. Due to its properties, IL-5 may prolong the survival of eosinophils, which is important in the development of inflammation. The association of IL-5 with most eosinophil-induced diseases is indicated. (Roufosse, 2018; Pelaia *et al.*, 2019; Kuang, 2020; Gevaert *et al.*, 2022 and Palacionyte *et al.*, 2022).

Serum interleukin 5 could be used as an indicator for eosinophilic esophagitis diagnosis (Elkholy *et al.*, 2022). Variety of cytokines, chemokines and growth factors were shown to be present in human semen, such as: interleukins (IL-1α and -1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-11, IL-12, IL-13, IL-17, IL-18, IL-23) (Fraczek and Kurpisz, 2015) .The altered immune response to the inflammatory status in the PCOS could be related to the diminished concentration of IL-3 and IL-5 as Th2 cytokines (Ghodsi, Hojati and Armin, 2021). Also, IL-5 may be elicits local inflammation in women with endometriosis and may act at the earlier stages of this inflammatory condition to develop endometrial lesions. (Monsanto *et al.*, 2016).

1.2.11.2.2. Interleukin-12 (IL-12).

Interleukin-12 is a disulphide-linked heterodimeric cytokine, produced by B-cells, phagocytic cells, and other antigen-presenting cells.

(Youssef, Mohammad and Ezz-El-Arab, 2015). IL-12 consists of two subunits, which are connected by disulphide-bonds, The smaller p35 monomer (35 kDa α-chain) is encoded on chromosome 3, while the gene for the larger p40 monomer (40 kDa β-chain) is located on chromosome 5, Co-expression results in the formation of the biologically active p70 heterodimer. IL-12 is primarily produced by professional antigen-presenting cells (APCs) such as B cells and dendritic cells DCs as well as phagocytes including monocytes, macrophages and granulocytes, While the production of IL-12 p35 is predominantly regulated at the translational level, transcriptional regulation is responsible for the amount of IL-12 p40 expressed. The initial signal triggering IL-12 expression is the exposure of the above mentioned cells to bacteria, viruses, fungi or parasites. Pathogen associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) or CpG DNA expressed or contained in such commensals or pathogens are recognized by pattern recognition receptors (PRRs) of the toll like receptor (TLR) family. This leads to the activation of several transcription factors regulating IL-12 production, most importantly NF-κB and interferon regulatory elements (IRFs). (Ullrich *et al.*, 2020)

During implantation, several classes of interleukins ILs are secreted by epithelial and stromal endometrial cells, including IL-6, IL-10, IL-12, IL-15, IL-18 and the leukemia inhibitory factor. These is create a perplexing network that orchestrates both proliferation and maturation of uterine natural killer cells, controls the function of regulatory T and B cells inhibiting the secretion of antifetal antibodies, and supports trophoblast invasion and decidua formation (Pantos *et al.*, 2022).

IL-12 constitutes a pro-inflammatory cytokine that also regulates the activation of natural killer (NK) cells, while also promotes the secretion of IFN-γ in high doses (Ma *et al.*, 2015). Several studies found that IL-12 levels correlate positively with the total sperm count and normal morphology,

suggesting that this interleukin might play a certain biological role in male fertility/infertility. In turn, IL-12 in combination with IL-18 may be critically dangerous for sperm membranes and DNA integrity under in vitro conditions. (Fraczek and Kurpisz, 2015).

1.2.11.2.3.Interleukin-18 (IL-18)

Interleukin-18 is a pro-inflammatory cytokine starting the cascade of additional inflammatory cytokines. It was discovered quite recently and was initially described as an interferon-gamma (IFNγ) inducing factor. IL-18 can stimulate responses mediated by T-helper type1 (TH1) and T-helper type2 (TH2). Interleukin-18 in Serum and Follicular Fluid have role during in vitro fertilization and intracytoplasmic sperm injection (Veronika *et al*., 2016).

Polycystic ovary syndrome is associated with higher circulating levels of C-reactive protein (CRP), interleukin (IL)-6, IL-18, tumor necrosis factor (TNF)-α and monocyte chemotactic protein (MCP)-1 (Stokkeland *et al*., 2022). It has been found that PCOS is associated with low-grade systemic inflammation as evidenced by elevated level of multiple markers of inflammation such as C-reactive protein, IL-18 and IL-6 (panelLang *et al*., 2015).

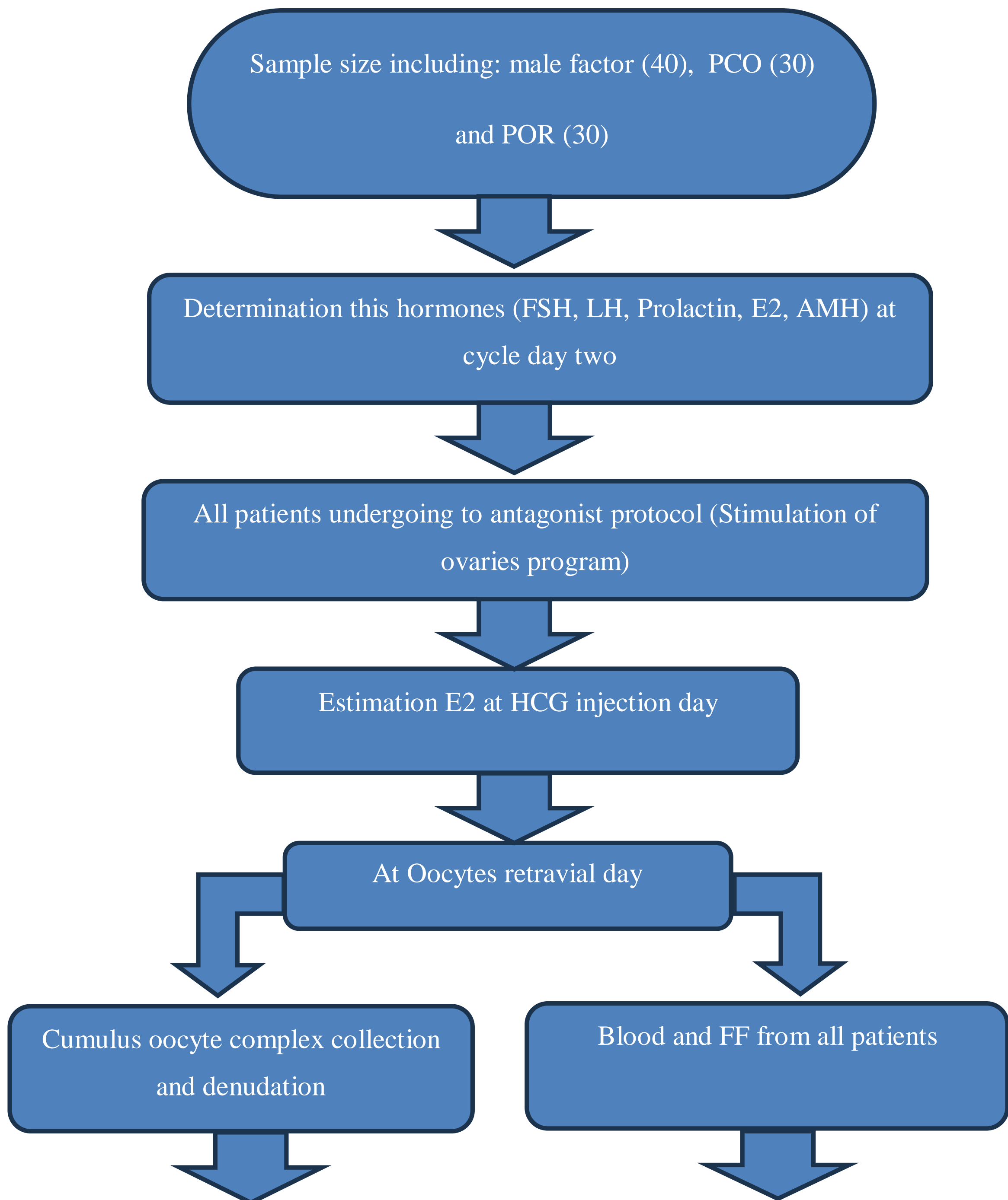
In addition, IL-18 is secreted by epithelial and stromal endometrial cells and have a role in the maturation and proliferation of uterine natural killer cells , control of regulatory T and B cells functions and supports the decidua formation (Pantos *et al*., 2022). In turn, IL-12 in combination with IL-18 may be critically dangerous for sperm membranes and DNA integrity under in vitro conditions. (Fraczek and Kurpisz, 2015).

Chapter Two
Materials and Methods

2. Study design, Materials and Methods

2.1. Study design

The cross sectional study was done in the Al-Najaf and Karbala province from November 2022 to April 2023,as in figure (2-1).



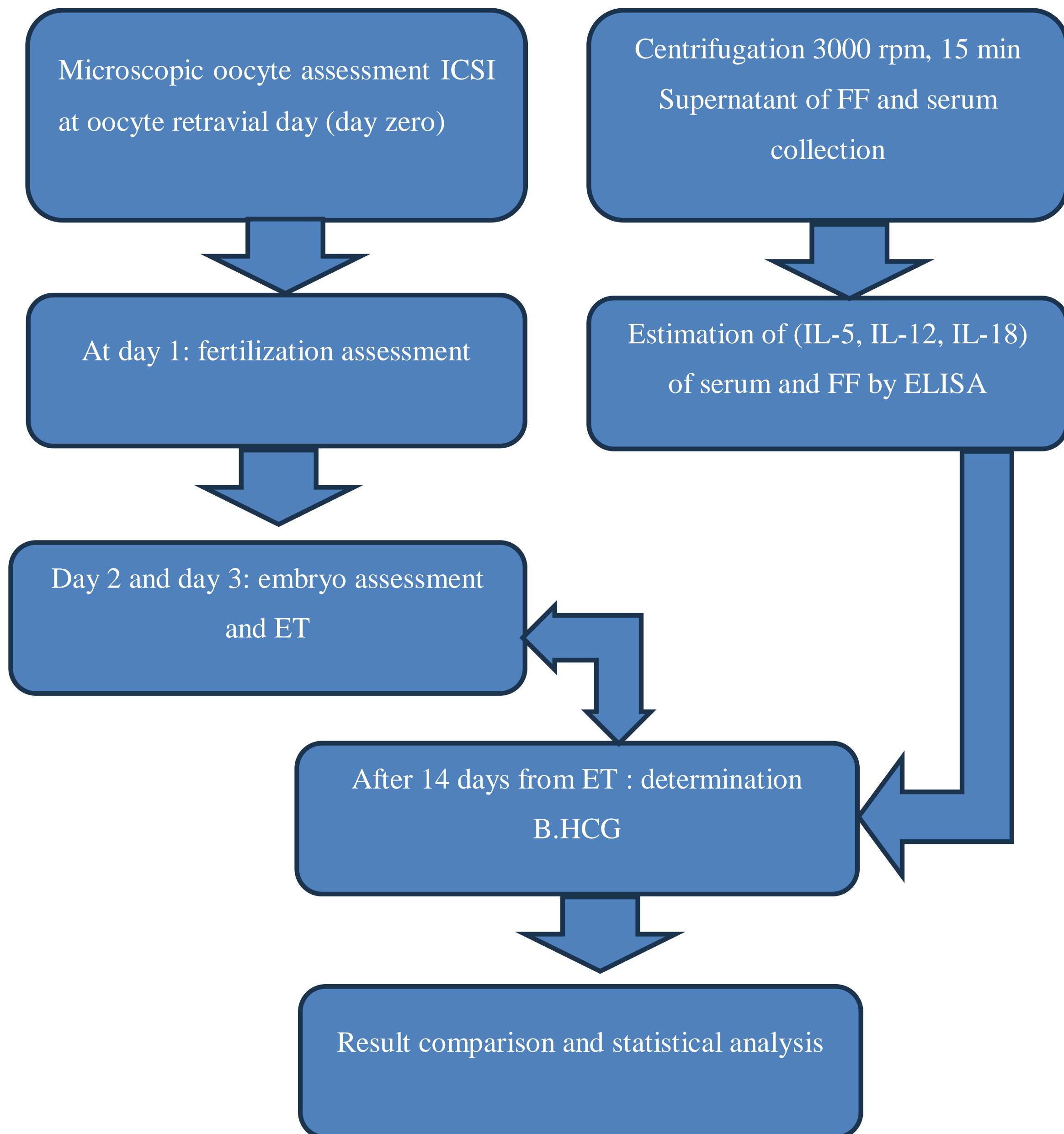


Figure (2-1) study design

PCOS: polycystic ovary syndrome. POR: poor ovarian reserve, FSH: follicle stimulating hormone, LH: Luteinizing hormone ,E2: Estradiol II , AMH: Anti mullerian hormone, FF: follicular fluid, ET: embryo transfer , B.HCG: Beta-Human Chronic Gonadotrpin,

2.1.1. Subjects

The study included 100 subjects divided into three groups depended on the most common causes of infertility: The first group is the male factor, which consists 40 patients. The second group is PCOS, which consists 30 patients. The third group is POR, which consists 30 patients who ICSI attenders in the Fertility Center in AL-Sader Teaching Medical City in AL-Najaf governorate, Al-Kafeel supper-specialty hospital in AL- Karbala governorate and Al-Ameer International Center for Fertilization and IVF in AL-Najaf governorate and were assessed by specialized gynecologists and Embryologists. The age group of the patients ranged from (18-40) years old. Information of case sheets involving (Age, Body mass Index, Types of infertility and others were carried for each participant (appendix I).

2.1.2. Ethical issue

Ethical approval was obtained from Karbala Medical College Ethical Committee. Also, verbal approval was taken from the patients before taking on the sample. During sample collection, health measures and safety was taken.

2.1.3. Exclusion criteria:

1. Female factor due to endometriosis .
2. Cycles end with ovarian hyper stimulation syndrome.
3. Cancelled cycles.
4. Steroid treatment.
5. Autoimmune diseases, tumor disease, cardiovascular disease, hepatitis and kidney diseases.
6. Azoospermic patients or sperm source from testicular biopsy.

2.1.4 Inclusion criteria:

1-Age (18-40years).

2-Women with anovulatory disorders (PCOS and POR) and women with

male factor of infertility
3-All cycles that end with ova pick up and sperm injections.

2.2. Materials

2.2.1. Instruments and Equipment

The main instruments and Equipment used in this study are listed in the table (2-1).

Table (2-1): Instruments and Equipment

Equipment and instruments	Company	Source
Air coda	GenX	USA
Air incubator	Heraeus, Kelvitrout®	Germany
Automatic pipette	Thermo-scientific	USA
Center well dish	Thermo scientific	USA
Centrifuge	Universal 16 A	Germany
CO2 incubator	Genx International	USA
CO2 incubator analyser	GEOTEC	India
Codan syringe 1 ml	Codan	Denmark
Conical tube	Falcon	USA
Deep freeze refrigerator	New Brunswick	UK
Disposable syringe (5ml)	Medi	China
Disposable Tips for automatic pipette Finntip	Thermo scientific	USA

Distillator	**GFL**	**Germany**
Double lumen	**GYNETICS**	**Belgium**
Electrophoresis unit	**Biometra**	**Germany**
ELISA automated washer	**Paramedical**	**Italy**
ELISA reader and printer	**Paramedical**	**Italy**
Embryo transfer catheter	**GYNETICS**	**Belgium**
Eppendrof tubes (0.2& 1.5 ml)	**Sterellin Ltd**	**UK**
EZ tip (155-135)	**RI**	**UK**
Face mask	**PRO. Care**	**China**
Falcon® Tissue Culture Flasks	**Falcon**	**USA**
Filter paper	**MEHECO**	**China**
Four 4- well dish	**Nunc**	**Denmark**
Gel Tube	**AFCOVAC**	**Jordan**
Glass Cylinders (250, 500 & 1000 ml)	**Isolab**	**Germany**
Handling pipette	**GYNETICS**	**Belgium**
Height scale Floor type	**EVERICH**	**China**
High-speed cold Micro-centrifuge	**Hettich**	**Germany**
Holding pipette for ICSI	**Cook**	**Australia**
Incubator	**Bender**	**Germany**

Injecting needle for ICSI	Cook	Australia
Inverted microscope	Olympus Optical Co Ltd.	Japan
Laminar air flow hood	Gelman instrument	Germany
Latex gloves	Comfit	Malaysia
Light Microscope Olympus	Optical Co Ltd.	Japan
Micromanipulator	Narshiege	Japan
Micropipette (different size)	Eppendorf	Germany
Micropipette tips (different sizes)	Human	Germany
Microscopical slides and cover slides	Marienfeld	Germany
MINI VIDAS®	BioMérieux	France
Petri Dish 90 cm	Nunc	Denmark
Petri Dish 60 cm	Nunc	Denmark
Plane test tube	ALS	China
Plastic pasture pipette	India MART	India
Refrigerator	Concord	Lebanon
Round bottom tube 14 mL	Nunc	China
Thermometer	Thermo scientific	USA
Ultrasound device	MediSON	Korea
Vortex	Thermo Scientific	Singapore
Water pump (Aspirator 3)	LABOTECT	Germany

2.2.2. Chemical and biological materials

The main chemicals used in this study are in table (2-2).

Table (2-2): Chemical and biological materials with their

Manufacturing company and country of origin

Chemicals and biological materials	Manufacturing Company	Country
Aspiration media	Fertipr	Belgiu
Gain Early stage media	Fertipro	Belgiu
HEPES media	Fertipro	Belgium
Hyaluronidase	Fertipro	Belgium
Mineral oil	Fertipro	Belgium
Oosafe® Disinfectant of CO2 incubator	SparMCD	Denmark
Oosafe® hand Disinfectant	SparMCD	Denmark
Polyvinylpyrrolidone 10% (PVP)	Fertipro	Belgium

2.2.3. MIN VIDUS Kit used in the study

MIN VIDUS Kit used in this study are listed in the table (2-3).

Table (2-3): MIN VIDUS Kits used in the study

MIN VIDUS Kit	Manufacturing Company	Country
1. FSH Kit **2. LH Kit** **3. Prolactin Kit** **4. E2 Kit** **5. AMH Kit** **6. B.HCG Kit**	**bioMerieux®**	**France**

2.2.4.ELISA Kit used in the study

ELISA Kit used in this study are listed in the table (2-4).

Table (2-4): ELISA Kits used in the study

ELISA Kit & Catalog No.	Manufacturing Company	Country
1.HumanIL-5 (Interleukin 5) ELISA Kit of serum Catalog No: E-EL-H0191 **2.HumanIL-5 (Interleukin 5) ELISA Kit of follicular fluid** Catalog No: E-EL-H0191 **3.HumanIL-12 (Interleukin 12) ELISA Kit of serum** Catalog No: E-EL-H0150 **4.Human IL-12 (Interleukin 12) ELISA Kit of follicular fluid** Catalog No: E-EL-H0150 **5.Human IL-18 (Interleukin 18) ELISA Kit of serum** Catalog No: E-EL-H0253 **6.Human IL-18 (Interleukin 18) ELISA Kit of Follicular fluid** Catalog No: E-EL-H0253	**Elabscience®**	**USA**

2.2.3.1. ELISA Kit Content of Human IL-5, IL-12 and IL-18

The kit of ELISA contents used in this study is listed in table (2-4).

Table (2-5): Kit components and Storage of IL-5, IL-12 and IL-18

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	**96T: 8Wells ×12 strips 96T*5:5 plate, 96T**	**-20°C, six months**
Reference Standard	**96T: 2 Vials 96T*5: 10 Vial**	
Concentrated Biotinylated Detection Ab (100×)	**96T:1 Vial, 120µL 96T*5: 5 Vials, 120Ml**	
Concentrated HRP Conjugate (100×)	**96T:1 Vial, 120Ml 96T*5: 5 Vials, 120µL**	**-20°C(Protect from light), 6 months**
Reference Standard & Sample Diluent	**96T/48/24T: 1Vial, 20mL 96T*5: 5 Vials, 20mL**	**2-8 °C, 6 Months**
Biotinylated Detection Ab Diluent	**96T/48/24T 1Vial, 14mL 96T*5: 5 Vials, 14mL**	
HRP Conjugate Diluent	**96T/48/24T: 1Vial, 14mL 96T*5: 5 Vials, 14mL**	
Concentrated Wash Buffer (25×)	**96T/48/24T: 1Vial, 30mL 96T*5: 5 Vials, 30mL**	
Substrate Reagent	**96T/48/24T: 1Vial, 10mL 96T*5: 5 Vials, 10mL**	**2-8 °C(Protect from light)**
Stop Solution	**96T/48/24T: 1Vial, 10mL 96T*5: 5 Vials, 10mL**	**2-8 °C**
Plate Sealer	**96T/48/24T: 5 Pieces 96T*5: 25 Pieces**	
Product Description	**1copy**	
Certificate of Analysis	**1copy**	

2.3.Methods

2.3.1.Sample collection

At cycle day two, 5 ml of Blood samples were drawn from the veins of 100 subjects using a disposable syringe and a sterilization technique . Blood sample collected in gel tube and allowed to clot after that serum was separated by centrifugation at 3000 rpm for 5 minutes. Serum was used to determine the FSH, LH, prolactin, E2, A.M.H and concentrations by MINI VIDAS®.

At day of Oocytes retravial, Serum and follicular fluid samples were collected .

2.3.1.1 Follicular Fluid (FF): Follicular fluid were obtained from ovarian follicles at the time of oocyte pick up, with ultrasound guided aspiration needle and emptied into planed tube. Transparent Follicular fluid collected after removal of oocytes from collection step in ICSI lab into plane tube ,centrifuged immediately at 15 min for . 3000 rpm and taken to get out of the debris and the supernatant stored at -80ºC for an ELISA test to determine the concentration of IL-5, IL-12 and IL-18.

2.3.1.2 Serum Sample: 3 ml of Blood samples were drawn from the veins of 100 subjects at the day of Oocytes Retrieval by using a disposable syringe and a sterilization technique. Blood was collected in gel tube blood was allowed to clot for 15 min after that serum separated by centrifugation at 3000 rpm for 5 minutes. Two ml of serum was collected in an Eppendorf tube and then stored at -20 °C for an ELISA test to determine the concentration of IL-5, IL-12 and IL- 18 .

2.3.2. Hormone evaluation

2.3.2.1. Test principle of (FSH, LH, Prolactin, E2, AMH and B-hCG)

The MINI VIDAS® (FSH, LH, Prolactin, E2, AMH and B-hCG) assay is an enzyme-linked fluorescent immunoassay (ELFA) performed in an automated instrument. The instrument controls all assay steps and assay temperature. A pipette tip-like disposable device, the Solid Phase Receptacle (SPR), serves as a solid phase for the assay and a pipetting device. The SPR® is coated at manufacture with mouse monoclonal anti-FSH antibodies. The MINI VIDAS hormone assay configuration prevents nonspecific reactions with the SPR. Reagents for the assay are located in the sealed Reagent Strips. The sample is transferred into the well containing an anti-hormone antibody conjugated with alkaline phosphatase. The sample/conjugate mixture is cycled in and out of the SPR, and the hormone will bind to antibodies coated on the SPR and to the conjugate forming a "sandwich." Wash steps and remove unbound conjugate. A fluorescent substrate, 4-methylumbelliferyl phosphate, is cycled through the SPR. Enzymes remaining on the SPR wall will catalyze the conversion of the substrate to the fluorescent product 4-methylumbelliferone. The optical scanner in the instrument measures fluorescence intensity; it is proportional to the hormone concentration in the sample. When the MINI VIDAS hormone assay is completed, the results are analyzed automatically by the instrument, and a report is printed for each sample.

2.3.2.1.1. Assay Procedure for Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Prolactin (PRL).

1. Necessary components were extracted from the kit, and all unused components were returned to storage at 2-8°C.

2. Components were allowed to reach room temperature(approximately 30 minutes).

3. One "FSH" strip and one "FSH" SPR have been used for each sample, control, or calibrator to be tested. The storage pouch has been carefully resealed after removing the required SPRs.

4. The test has been identified by the "FSH" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control is to be tested, it should be identified by "C1".

5. The "FSH" Reagent Strips have been labeled with the appropriate sample identification numbers.

6. The calibrator, control, and sample have been mixed using a vortex-type mixer (for serum or plasma separated from the pellet).

7. The calibrator, control, and sample test portion for this test is 200 µl.

8. The "FSH" Reagent Strips and SPRs have been inserted into the appropriate position on the instrument.

9. The assay processing has been initiated as directed in the Operator's Manual. All the assay steps are performed automatically by the instrument.

10. The vials have been enclosed and returned to the required temperature after pipetting.

11. The assay will be completed in approximately 40 minutes. After the assay was completed, the SPRs and strips were removed from the instrument.

12. The used SPRs and strips have been disposed of in an appropriate recipient.

2.3.2.1.2. Assay Procedure for Estradiol II (E2)

1. Necessary components were extracted from the kit, and all unused components were returned to storage at 2-8°C.

2. Components were allowed to reach room temperature (approximately 30 minutes).

3. One "E2II" strip and one "E2II" SPR have been used for each sample, control, or calibrator to be tested. Ensure the storage pouch has been carefully resealed after removing the required SPRs.

4. The test has been identified by the "E2II" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control needs to be tested, it should be identified by C1.

5. The "E2II" Reagent Strips have been labeled with the appropriate sample identification numbers.

6. The calibrator, control and samples have been mixed using a vortex-type mixer (for serum or plasma separated from the pellet).

7. The calibrator, control, and sample test portion for this test is 200 µl.

8. The "E2II" SPR® S and "E2II" Reagent strips have been inserted into the appropriate position on the instrument.

9. The assay processing has been initiated as directed in the Operator's Manual. All the assay steps are performed automatically by the instrument.

10. The vials have been reclosed and returned to the required temperature after pipetting.

11. The assay will be completed in approximately 60 minutes. After the assay was completed, the SPRs and strips were removed from the instrument.

12. The used SPRs and strips have been disposed of in an appropriate recipient.

2.3.2.1.3. Assay Procedure for Anti-Mullerian Hormone (AMH)

1. Necessary components were extracted from the kit, and all unused components were returned to storage at 2-8°C.

2. Components were allowed to reach room temperature (approximately 30 minutes).

3. One "AMH" strip and one "AMH" SPR have been used for each sample, control, or calibrator to be tested. Ensure the storage pouch has been carefully resealed after removing the required SPRs.

4. The test has been identified by the "AMH" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control needs to be tested, it should be identified by C1.

5. The "AMH" Reagent Strips have been labeled with the appropriate sample identification numbers.

6. The calibrator, control and samples have been mixed using a vortex-type mixer (for serum or plasma separated from the pellet).

7. The calibrator, control, and sample test portion for this test is 200 µl.

8. The "AMH"SPR® S and "AMH" Reagent strips have been inserted into the appropriate position on the instrument.

9. The assay processing has been initiated as directed in the Operator's Manual. All the assay steps are performed automatically by the instrument.

10. The vials have been reclosed and returned to the required temperature after pipetting.

11. The assay will be completed in approximately 35 minutes. After the assay was completed, the SPRs and strips were removed from the instrument.

12. The used SPRs and strips have been disposed of in an appropriate recipient.

2.3.2.1.4. Assay Procedure for Beta-Human Chronic Gonadotrpin (B-HCG)

1. Necessary components were extracted from the kit, and all unused components were returned to storage at 2-8°C.

2. Components were allowed to reach room temperature (approximately 30 minutes).

3. One "HCG" strip and one "HCG" SPR have been used for each sample, control, or calibrator to be tested. Ensure the storage pouch has been carefully resealed after removing the required SPRs.

4. The test has been identified by the "HCG" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control needs to be tested, it should be identified by C1.

5. The "HCG" Reagent Strips have been labeled with the appropriate sample identification numbers.

6. The calibrator, control and samples have been mixed using a vortex-type mixer (for serum or plasma separated from the pellet).

7.The calibrator, control, and sample test portion for this test is 100 µl.

8.The "HCG"SPR® S and "HCG" Reagent strips have been inserted into the appropriate position on the instrument.

9.The assay processing has been initiated as directed in the Operator's Manual. All the assay steps are performed automatically by the instrument.

10. The vials have been reclosed and returned to the required temperature after pipetting.

11. The assay will be completed in approximately 30 minutes. After the assay was completed, the SPRs and strips were removed from the instrument.

12. The used SPRs and strips have been disposed of in an appropriate recipient.

2.3.3. Estimation of IL-5 ,IL-12 and IL-18 concentration in serum and follicular fluid

2.3.3.1.Test principle of IL-5 ,IL-12 and IL-18

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody

specific to Human (IL-5, IL-12 and IL-18). Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for human (IL-5, IL-12 and IL-18) and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain human (IL-5, IL-12 and IL-18) biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color .The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm . The OD value is proportional to the concentration of human (IL-5, IL-12 and IL-18). Calculation of the concentration of human(IL-5, IL-12 and IL-18), in the samples by comparing the OD of the samples to the standard curve as in (Appendix III)

2.3.3.2. Reagent Preparation

1. All the reagents were brought to room temperature (18-25Cº) before use.

2. Wash buffer was prepared by adding 30 ml of Buffer to 720 ml of distilled water to form 750 ml of wash buffer.

3. Standard working solution**:** Centrifuge the standard at 3000 rpm for 1 min. After 1 mL of Reference Standard and Sample Diluent has been added, let it stand for 10 min and invert it gently several times. After it had completely dissolved, I thoroughly mixed it with a pipette. This reconstitution produces a working solution of 2000 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL.

Dilution method: 7 EP tubes were taken, and 500 µL of Reference Standard and Sample Diluent were added to each tube. Pipetted 500 µL of the 2000 pg/mL working solution into the first tube and mixed it to make a 1000

pg/mL working solution. Pipetted 500 µL of the solution from the former tube into the latter one according to these steps, as in figure(2-2).

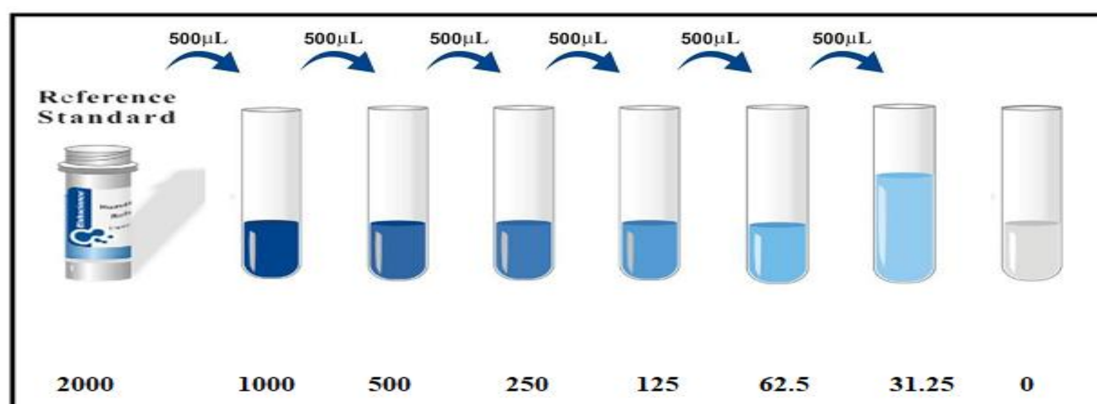


Figure (2-2): Steps of the duplication dilution.

4. Biotinylated Detection of Ab working solution, each well needs 100 µl for 96 wells prepared more than 9600 µl.

5.Concentrated Horseradish peroxidase (HRP) Conjugate working solution: It also needs 100 µl per well, so it is prepared slightly more than the calculated amount.

2.3.2.3. Assay procedure

1. Sample: 100µL of Standard, Blank, or Sample was added per micro ELISA plate well. The blank well was filled with reference standards and Sample diluent. After that, solutions are mixed gently, covered on the plate with sealer, and then incubated for 90 minutes at 37°C and made wash to remove unbound Reagent. (Appendix II)

2. Biotinylated Detection Ab: The liquid of each well was removed, and immediately 100µL biotinylated Detection Ab working solution was added to each well and covered with the plate sealer incubated for 1 hour at 37°C.

3. Wash: All plate wells were aspirated and washed, repeated three times. The wash was done by filling each well with Wash Buffer (approximately 350 µL) using a squirt bottle.

4. HRP Conjugate: 100μL of HRP Conjugate working solution was added to each well, covered with the plate sealer, and then incubated for 30 minutes at 37°C.

5. Wash: The wash process was repeated five times in step3.

6. Substrate Reagent: 90 μL of substrate Solution was added to each well and covered with a new Plate sealer, then incubated for about 15 minutes at 37°C.

7. Stop: 50 μL of Stop Solution was added to each well. Then, the color turns yellow immediately.

8. OD Measurement: for determining the optical density (OD value) of each well at once, a microplate reader set at 450 nm was used.

2.3.2.4. Results Calculation

The ELISA results were calculated based on the readings for each standard and the sample's optical density. Then create a standard curve by plotting the OD value for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph in the excel office program.

1. First, calculate the OD value for each standard and sample, then construct the standard curve.

2. Determined the amount of (IL-5, IL-12 and IL-18) in each sample by locating the OD according to their site in the curve. (Appendix III)

2.3.4. Female Preparation for ICSI

2.3.4.1. Controlled Ovarian Hyper-stimulation (COH)

On day two of female cycle checked the female patients, by vaginal ultrasound to evaluate the antral follicles count (follicles with diameter of 2-10mm), to rule out any ovarian cyst, and to measure the endometrial thickness. In the same day, the Gynecologists sent the patient for hormonal

analysis including FSH, LH, Prolactin, E2 and AMH with mini VIDAS immunoassay technique, then according to the finding on examination, U/S, hormonal analysis and patient's information, they underwent COH (Malathi, Balakrishnan and Lakshmi, 2021).

*Controlled ovarian hyper stimulation (COH) comprises 3 basic elements:

- Exogenous gonadotrophins to stimulate multiple follicular maturation.
- Co-treatment by either gonadotropin-releasing hormone (GnRH) agonist or antagonists to suppress pituitary action and inhibit premature LH surge.
- Triggering of final oocyte maturation (36 to 38) hours prior to oocyte Retrieval. (Gallos *et al.*, 2017)

2.3.4.2. Measurement of E2 for Follow up

The serial measurement of E2 level (same process mentioned before) was used to evaluate the patient's response to the treatment together with endometrial thickness and follicles number. This help in the estimation of the day of hCG injection.

Antagonist protocol:

All patients in this study underwent for GnRH antagonists (cetrotide0.25 mg, Merck Serono) involve a shorter duration of use, absence of vasomotor symptoms, minimizing of ovarian hyper-stimulation syndrome, and a significant lower dose of gonadotropin per cycle, make GnRH antagonists not only well-tolerated by patients but also clinicians', and are started a few days after initiation of stimulation, until day of hCG injection. (Eftekhar and Tabibnejad, 2021).

Ovulation Triggers :

By human chorionic gonadotropin (hCG) (Lupi-HCG 10,000 IU) trigger shot intramuscularly when at least 2 dominant follicles have reached 18–20 mm, which is used to mimic the natural endogenous LH surge to initiate the

process of final oocyte maturation and better timing before ovulation around 36 hours. (Salama *et al*, 2021).

2.3.4.3. Oocyte Pick Up

Which done by gynecologist occur within 36-38 hours after ovulation induction, by trans-vaginal ultrasound-guided trans-vaginal puncture that passed through vaginal wall to the ovary for oocyte retrieval, this was done under general anesthesia. One end of the needle was attached to suction pump which creates a negative pressure not exceeded (140 – 150) mmhg to avoid oocyte rupture for aspirating follicles. "Flushing tube with 5ml syringe holding aspiration medium (HEPES, heparin containing) was utilized for this purpose (De Roo and Tilleman, 2021).

Follicular fluid collection by Pre-warmed tubes and these tubes then transferred to IVF lab for cumulus oocyte complex (COC) searching and collection by Embryologist, by using a Petri dish and 1 ml syringe with adapter For searching of COC under dissecting Stereomicroscope then the COC transferred to a four-well dish Containing culture medium, which was prepared overnight and incubated at 37ċ with 5% CO2 till time of oocyte denudation (D’Angelo *et al*, 2019).

2.3.5. Assisted Reproduction Technique

2.3.5.1. Dishes Preparation

1. Preparation of collection Dish for ICSI

Four wells dish filled by 600 µl of Hydroxy ethyl piperazine ethan sulfonic acid (HEPES) media each well and covered by mineral oil ,prepared and

incubated overnight in incubator conditions of 5% Co2 and 37ºC. (Johansson , 2014).

2. Preparation of Denudation Dish

A drop of about (100-200) µl of hyaluronidase solution with multiple drops (100-200) µl of flashing media were placed on a petri dish and placed in the hood on heated area to keep it warm. Stripper tips must be prepared with inner diameters of 290, 170,155 and 135 µm respectively . (Naji *et al*, 2018).

3.Preparation of Injection Dish

Number of drops according to oocytes number, each one is 5 µL of HEPES buffered medium, arranged in a shallow falcon dish, add 5 µL of Polyvinylpyrrolidone 10% (PVP) to the central droplet and covered with oil the sperm will be placed in it to decrease sperm movement due to PVP viscous nature, so facilitating sperm manipulation. The dish was placed on heated area in the hood. (Johansson, 2014).

4. Culture Dish

A special IVF media with mineral oil were incubated in culture dishes overnight in incubator conditions of 5% Co2 and 37ºC. The culture system for embryo includes droplets under oil: 5 drops of culture media of 50-µl volume were arranged with one central drops surrounded by four drops covered by 5 ml of mineral oil then placed in the incubator. (Johansson, 2014).

2.3.5.2. Collection of Oocytes on the Day of Oocytes Retrieval

In lCSI lab, the tubes received containing the follicular fluid to look for the oocytes. For the collection of COC, the embryologist had already prepared an adaptor, a 1 ml syringe(codan), a petri dish (90cm) and 4- well dish (A dish prepared in advance the day before). The scanning for COC had

been achieved under a stereoscopic dissecting microscope. The petri dish was examined under a low power lens (6x-12x) while the confirmation of the oocyte presence had been done under a higher magnification (25x-50x) by the fining of COC under dissecting stereomicroscope. Oocytes isolated in 4-well dish and kept incubator .

2.3.5.3 Oocyte Denudation

Oocytes are enclosed by the cumulus cells forming COC these COC may block the injector needle and so interfere with the gentle oocyte micro-injection. Furthermore, because only mature oocytes that have reached the MII stage are appropriate for ICSI, ideal optical circumstances to allow assessment of the meiotic status of the oocytes are necessary, and these become restricted in the existence of the cumulus cells (Naji *et al*, 2018).

Denudation done by two steps:

A. Enzymatic Denudation: Because of one of the main component of COC is hyaluronic acid so hyalurodinase enzyme is usually used for removal of glycol-protein granules of the hyaluronic matrix so, low concentrations of the enzyme for instance 80 IU/ml is used (no more than 1 minute).

B. Mechanical Denudation: Further denudation was done mechanically by gentle pipetting through capillaries of successively narrower inner diameters of 290 ,170,155 and 135 µm respectively in enzyme free HEPES -buffered media, then oocytes washed off and all cumulus cells had been detached. Lastly, the denuded oocytes were transferred to the 4 well dish droplets and their meiotic status and morphology were estimated (Carvalho *et al.*, 2020).

2.3.5.4 Assessment of Denuded Oocyte

Under an inverted microscope the oocytes morphologic features were classified according to maturity if MII (mature with polar body), MI

(immature no polar body) or germinal vesicle GV (immature with oocyte nucleus), and the existence of intra- and extra- cytoplasmic abnormalities (Faramarzi, Khalili and Ashourzadeh, 2017). As found in figure (2-3).

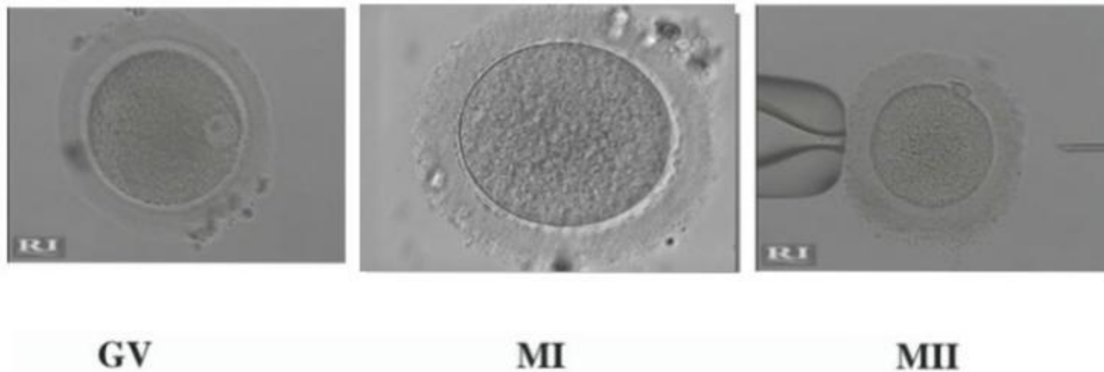


Figure (2-3): Oocyte maturity(GV, MI and MII oocyte in ICSI dish under inverted microscope, magnification 40X)

2.3.5.5 Sperm Preparation:

The most widely used method for the sperms preparation for ICSI was the Centrifugation swim up technique. It is summarized in the following steps:

- After abstinence time between 2-7 days, the semen was collected by masturbation in special room near the IVF lab and ejaculated into wide mouth, clean container labeled with the name, age of patient and the time of collection. The patients were instructed to collect the whole sample.
- The sample was left at room temperature for liquefaction completely for about 30-60 minutes. The macroscopic and microscopic examination were done .
- After completing the liquefaction, the sample then was transferred to conical test tube, to be diluted with HEPES-buffered media, and centrifuged.

- After centrifugation, the supernatant was discarded and the pellet overlaied with HEPES media.
- The conical tube was incubated at 30-45 minutes.
- After the incubation time, the sample need aspiration the upper half. (McVay and Cooke, 2021)

2.3.5.6. Set up of the ICSI Micromanipulator

An inverted microscope equipped with micromau: pulator for micro-injector pipette and micro-holder pipette used for ICSI procedure with a special heated stage to maintain work temperature at 37ºC. The two manipulator permit three dimensional movement. The micro-holder was used for fixing and releasing the oocyte while the micro-injector pipette used for immobilization of sperms and injecting the sperm inside the oocyte.

2.3.5.7. Sperm Immobilization

The sperm as much as possible of normal morphology and motility was selected to be immobilized. The immobilization had been done by mechanical pressure of the sperm against the flour of the ICSI dish. The sperm was place at 90 degrees to the tip of the injector. Then, the injector was lowered to compress the sperm tail (figure 2-4a). Perfectly immobilized sperm should keep the shape of the tail normal. If any damage or kinking happened to the tail, the sperm was discarded and the procedure was repeated to another sperm. (Fleming, 2021).

2.3.5.8. The Injection of Oocyte (Day 0)

The oocyte has been held in place by pipette suction force and so the polar body was placed at 6 o'clock so that reduce the damage to the meiotic spindle. The injector, which already contained the immobilized sperm near its tip, was moved close the held oocyte and injected to the ooplasm of the MII egg at a place of 3 o'clock. Applying the minimal suction was necessary to allow the ooplasm entering the injection pipette. (De Coster, *et al*., 2022). The suction was immediately stopped and the sperm was released to the oocyte gently with head entering first, now the pipette can be carefully withdrawn figure (2-4).

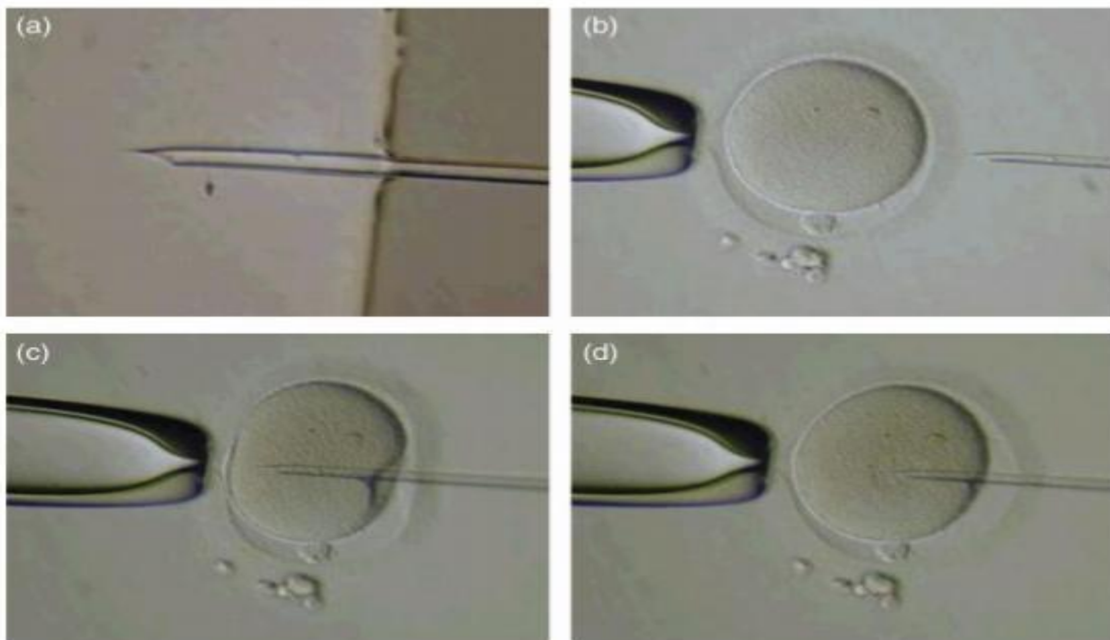


Figure 2-4. Sperm immobilization(a) and intra-cytoplasmic sperm injection (b, c and d) of embryos. (in ICSI dish under inverted microscope, magnification 40X , at 6 o'clock)

2.3.5.9. Incubation of the Injected Oocyte (Day 0)

After the injection, the oocytes had been cultured and incubated in droplet of culture media covered with mineral or paraffin oil. Oocytes then kept in atmosphere of 5% Co2 and 37ºC. (Sciorio & Rinaudo, 2023).

2.3.5.10. Assessment of Fertilization

After 16-18 hour of ICSI, the injected oocytes were checked for intactness and fertilization. The normally fertilized oocyte should have two polar bodies along with two visible pronuclei, which have the nucleoli, (Tepla, *et al*., 2022) as demonstrated in figure (2-5).

Figure 2-5. Fertilization assessment of oocyte (in ICSI dish under

inverted microscope, magnification 400X)

2.3.5.11. Assessment of Embryo Quality (Day2-3)

After assessing the oocytes for fertilization, the morphological evaluation of embryo occurred around 48 hour subsequent to oocyte pick up depending on: cleavage, symmetry of blastomeres, and presence and percentage of fragmentation and state of nucleation . Next to ICSI, more than 90% of 2-PN zygotes will enter cleavage, leading to multicellular embryos. If the embryo was of a good quality, it will reach four cell stage on day 2 and 8 cell stage on day 3 after injection. The resulting embryos are scored according to the number of blastomeres, how equal in size are they and the percentage of fragmentation. (Eastick, 2023).

2.3.5.12. Embryo Transfer

The human embryo was chosen for transfer depending on its morphology at the zygote, cleavage, and blastocyst stage. Good quality embryos were surely the best for transfer firstly. The embryos with grade A (good quality) and B (fair quality) have been transferred to the uterus while other embryos without upward grading system were left over. 48-72 hours after oocyte pick up, the best embryos 'around three' were transferred. The loading catheter (inner part) was bathed two time in the transfer media then loaded as follows: 5-7 microliter of air, then ~20 microliter of transferring media together with the embryos that is often bracketed with air, and finally 5-7 microliter of media for sealing the catheter. The catheter with the embryos were given to the gynecologist to transfer the embryos gently to the uterus where the outer part was sited in the uterus. This was followed by pulling out the whole catheter and checking it for retained embryos. (Swadi, Edan and Al-Dulaimi, 2023)

2.3.6.Statistical analysis

Statistical analyses were performed using SPSS statistical package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA). Distribution was tested using Kolmogorov-Smirnov test; IL5, IL12 and IL 18 in follicular and serum distribution were found to be non-normal. Quantitative data are represented as mean, SD for the normal distribution data and median, IQR (Inter-quartile range) for the non-normal distribution data. ANOVA test (analysis of variance) and LSD (least significant difference) was used to test the difference between the three studied groups in the normal distribution data. Student’s t-test was used to study the difference between two groups. Kruskal-Wallis test was used to study the difference between the three groups in the non-normal distribution data.

Mann-Whitney U test was used to study the difference between two group. Chi-square test was used to test the relation between qualitative data. Pearson's correlation test was used to test the relation between cytokines and studied clinical characteristics. ROC (receiver operating characteristic curve) was used to test the sensitivity, specificity, AUC (area under the curve) and cut-off values for the studied cytokines in differentiating the PCOS causes from male causes and POR causes from the male causes.

P value of <0.5 was considered as significant.

Chapter Three

Results

3. Results:

3.1.Demographic characteristics among studied groups according to the infertility causes:

Demographic characteristics of the subjects according to the cause of infertility are presented in table (3-1).

The ages of patients ranged from (18-40) with mean values (30.00), (29.97) and (31.50) among male factor, PCOS and POR patients respectively. There was no significant difference between the patient groups (*P* =0.439) regarding age. In male factor patients, the body mass index (BMI) with mean values (28.82) and (26.80) of PCOS while the BMI of POR with mean value (28.69). There was no significant differences in BMI between patients groups (*P* =0.474). About Duration of infertility, the mean values of male factor, PCOS and POR were (9.19), (8.00) and (9.20) respectively with a non significant differences (*P= 0.532*).

Concerning the types of infertility, the male factor count of primary infertility was (33), while the counts of PCOS and POR were (23), (22) respectively. Whereas, the male factor count of secondary infertility was (7), while the counts of PCOS and POR were (7), (8) respectively There was no significant differences about infertility types between patients

groups (*P* =0.684).

Table (3.1): Demographic characteristics among studied groups according to the infertility causes.

	Cause of infertility						*P value*
	Male factor (40)		PCOS (30)		POR (30)		
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	

Age (year)	30.00	5.88	29.97	4.72	31.50	5.36	0.439
BMI (Kg/m^2)	28.82	10.36	26.80	3.47	28.69	5.53	0.474
Duration (year)	9.19	4.93	8.00	4.73	9.20	5.06	0.532
	Count	**%**	**Count**	**%**	**Count**	**%**	
Infertility pri.	33	41.0	23	30.8	22	28.2	0.684
Infertility sec.	7	31.8	7	31.8	8	36.4	

Infertility pri.: Primary infertility, Infertility sec.: Secondary infertility, ANOVA test (LSD test), Chi-square test, SD: Standard deviation

3.2.Mean Hormonal levels in studied groups:

The mean of follicle stimulating hormone (FSH) in male factor cases was (5.63) and in PCOS group was (5.72), while in POR group was (7.42). However, POR was significantly different from male and PCOS causes (*P=0.005*), as shown in table (3-2).

Also, the mean of luteinizing hormone (LH) in male factor group was (3.43) and PCOS group was (6.27), while in POR group was (7.01). However, Male factor cause was significantly different from PCOS and POR causes with (*P=0.006*). On the other hand, the mean of prolactin in male factor was (18.79), in PCOS cases was (31.29) and in POR was (16.74). However, PCOS cause was significantly different from male and POR causes with (*P=0.005*).

Regarding Estradiol hormone (E2)at cycle day two, the mean in male factor was (48.07), in PCOS cases was (51.97) and in POR was (33.59) However, POR was significantly different from male and PCOS causes with (*P=0.008*). The anti mullerian hormone (AMH) mean in male factor was (3.28) , in PCOS group was (4.13) and in POR was (0.87). However, Male

causes was significantly different from PCOS and POR. Also, PCOS was significantly different from POR (*P=0.005).*About the mean of Estradiol hormone (E2) at day of HCG injection in male factor was (2262.58), in PCOS cases was (1951.83) and in POR was (1282.20) However, POR was significantly different from male and PCOS causes with (*P=0.005*).

Table (3.2): Mean Hormonal levels in studied groups.

Variables	**Cause of infertility**						***P value***
	Male factor (40)		PCOS (30)		POR (30)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
FSH (mIU/ml)	5.63	1.16	5.72	1.84	7.42	3.75	**0.005***
LH (mIU/ml)	3.43	0.99	6.27	4.09	7.01	7.70	**0.006***
Prolactin (ng/ml)	18.79	6.62	31.29	16.32	16.74	7.04	**0.005***
E2 day 2 (pg/ml)	48.07	18.93	51.97	35.56	33.59	11.48	**0.008***
AMH (ng/ml)	3.28	1.17	4.13	2.09	0.87	0.29	**0.005***
E2 HCG (pg/ml)	2262.58	832.43	1951.83	770.88	1282.20	705.68	**0.005***

FSH: follicle stimulating hormone, LH: Luteinising Hormone, E2 at day2: Estradiol hormone at cycle day two, AMH: Anti Mullerian Hormon, E2 HCG: Estradiol Hormone at Human Chorionic Gonadotropin injection . ANOVA test (LSD test), (*): significant at $p<0.05$

3.3. Clinical characteristics of different infertility groups.

The mean of Intracytoplasmic sperm injection (ICSI)attempt in male factor cases was (0.41) and in PCOS group was (0.35), while in POR group was (0.77). However, no significant difference between groups (*P=0.060*). About the mean of Total follicle number in male factor cases was (12.33)

and in PCOS group was (13.65), while in POR group was (4.43). However, POR was significantly different from male and PCOS causes (*P=0.005*).

Regarding the mean of Maturity rate in male factor cases was (84.37) and in PCOS group was (76.74), while in POR group was (77.35). However, no significant difference was detected (*P=0.109*).The mean of Fertilization rate in male factor cases was (80.74) and in PCOS group was (79.52), while in POR group was (83.70). However, no significant difference was detected (*P=0.623*).

Also, the mean of embryo Gradel (GI) in male factor cases was (3.28) and in PCOS group was (2.48), while in POR group was (1.20). However, POR was significantly different from male and PCOS causes (*P= 0.005*). In addition, the mean of embryo Grade ll (GII) in male factor cases was (3.31) and in PCOS group was (3.39), while in POR group was (2.00). However, POR was significantly different from male and PCOS causes (*P =0.003*). About the mean of embryo Grade III (Glll) in male factor cases was (1.62) and in PCOS group was (2.29), while in POR group was (1.23). However, PCOS was significantly different from POR and male factor causes (*P=0.030*). Concerning the mean of Transferred embryo in male factor cases was (3.69) and in PCOS group was (3.39), while in POR group was (3.07). However, POR was significantly different from male causes (*P= 0.015*).

On the other hand, The count of Beta-Human Chorionic Gonadotropins (B-HCG) test, the male factor count of Negative result was (28), while the counts of PCOS and POR were (18), (21) respectively. Whereas, the male factor count of positive test was (12), while the counts of PCOS and POR were (12), (9) respectively. There were no significant difference between groups (*P=0.439*).

Table (3.3): Clinical characteristics of different infertility groups

Variables	Cause of infertility						P value
	Male factor (40)		PCOS (30)		POR (30)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
ICSI attempt	0.41	0.64	0.35	0.66	0.77	0.90	0.060
Total follicles	12.33	4.14	13.65	7.18	4.43	2.52	**0.005***
Maturity rate	84.37	14.53	76.74	18.52	77.35	17.70	0.109
Fertilization rate	80.74	14.59	79.52	18.06	83.70	19.40	0.623
Embryo GI	3.28	2.05	2.48	1.61	1.20	1.24	**0.005***
Embryo GII	3.31	1.64	3.39	2.22	2.00	1.31	**0.003***
Embryo GIII	1.62	1.55	2.29	1.68	1.23	1.43	**0.030***
Transferred embryo	3.69	0.61	3.39	0.92	3.07	1.08	**0.015***
	Count	**%**	**Count**	**%**	**Count**	**%**	
B HCG result -ve	28	71.8	18	58.1	21	70.0	0.439
+ve	12	28.2	12	41.9	9	30.0	

ICSI: Intracytoplasmic sperm injection, GI: Grade 1, B-HCG: Beta-Human Chorionic Gonadotropins, ANOVA test (LSD test), Chi-square test, (*): significant at p<0.05

3.4. Cytokine levels among studied groups

The median of IL-5 level in the follicular fluid of male factor cases was (337.18), while, in POCS and POR were (325.70), (401.27) respectively. There were no significant difference between three groups (*P= 0.629*). Whereas, the median of IL-5 serum level of male factor was (123.54), in POCS (106.50) and in POR (130.16). There were no significant difference between groups (*P=0.169*), as found in table (3-4).

Regarding the median of IL-12 level in the follicular fluid of male factor cases was (21.45), while, in POCS and POR were (9.13), (9.66) respectively. However, Male causes was significantly different from PCOS and POR causes (*P=0.005*). Whereas, the median of IL-12 serum level of male factor was (9.05), in POCS (5.69) and in POR (5.24). However, Male causes was significantly different from PCOS and POR causes (*P=0.005*).

On the other hand, the median of IL-18 level in the follicular fluid of male factor cases was (244.06), while, in POCS and POR were (435.96), (378.66) respectively. There were no significant difference between groups (*P=0.117*). Whereas, the median of IL-18 serum level of male factor was (580.20), in POCS (440.56) and in POR (508.37).There were no significant difference between groups (*P=0.342*).

Table (3.4): Cytokine levels among studied groups

Interleukins		**Causes of infertility**						***P value***
		Male factor (40)		PCOS (30)		POR (30)		
		Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	
IL5	FF	337.18	478.17	325.70	534.88	401.27	608.20	0.629
IL5	S	123.54	177.89	106.50	149.92	130.16	197.70	0.169
IL12	FF	21.45	12.97	9.13	6.16	9.66	3.29	**0.005***
IL12	S	9.05	2.93	5.69	2.56	5.24	3.32	**0.005***
IL18	FF	244.06	329.12	435.96	383.88	378.66	439.49	0.117

IL18 S	580.20	157.18	440.56	405.76	508.37	368.08	0.342

FF: Follicular fluid, S: Serum, IL: Interleukin, IQR: interquartile range, Kruskal-Wallis test, (*) : Significant at p<0.05

3.5. Mean differences of Demographic characteristics according to the result of B HCG (pregnancy result)

Regarding non pregnant women, the mean of age, body max index and duration of infertility were (30.67), (28.32) and (8.44) respectively, while, in pregnant women the mean of age, body max index and duration of infertility were (29.97), (27.82) and (9.61) respectively. There were no significant difference between B-HCG result groups with (*P=0.542, P=0.753 and P= 0.264*) respectively, as clarified in table(3-5).

About the infertility types in non pregnant women, the count of primary infertility was (52) and in secondary infertility was (15). while, in pregnant women, the count of primary infertility was (26) and in secondary infertility was (7). There was no significant relation to the B HCG result (*P=0.894*).

Concerning the infertility causes in non pregnant women, the count of male factor, PCO and POR were (28), (18) and (21) respectively, while, in pregnant women the count of male factor, PCO and POR were (12), (12) and (9) respectively. There was no significant relation to the B-HCG result (*P=0.439*).

Table (3.5): Demographic characteristics according to the result of B HCG (pregnancy result)

Variables	B HCG	

	Negative (non pregnant)		Positive (pregnant)		*P value*
	Mean	**SD**	**Mean**	**SD**	
Age (year)	30.67	5.44	29.97	5.31	0.542
BMI (Kg/m^2)	28.32	8.50	27.82	4.48	0.753
Duration (year)	8.44	4.88	9.61	4.89	0.264
	Count	**%**	**Count**	**%**	
Infertility Primary	52	66.7	26	33.3	0.894
Secondary	15	68.2	7	31.8	
Cause of infertility					
Male factor	28	71.8	12	28.2	0.439
PCOS	18	58.1	12	41.9	
POR	21	70.0	9	30.0	

BMI: Body Max Index, Student's t-test, Chi-square test, (*) :significant at $p<0.05$.

3.6. Hormonal study according to the result of B HCG (pregnancy result)

In non pregnant women, the means of FSH, LH, Prolactin, E2 day 2, AMH and E2 HCG were (5.84), (5.42), (22.92), (43.22), (2.74) and (1853.90) respectively. Whereas, in pregnant women the means of FSH, LH, Prolactin, E2 day 2, AMH and E2 HCG were (6.92), (5.32), (20.29), (48.42), (2.98) and (1909.16) respectively. There were no significant difference between B-HCG result groups with (*P=0.086, 0.924, 0.318, 0.422, 0.560 and 0.767*) respectively, as demonstrated in table (3-6).

Table (3.6): Mean differences of hormonal study according to the result of B HCG (pregnancy result)

Variables	B-HCG				P value
	Negative (non pregnant)		Positive (pregnant)		
	Mean	SD	Mean	SD	
FSH (mIU/ml)	5.84	1.98	6.92	3.26	0.086
LH (mIU/ml)	5.42	5.58	5.32	3.77	0.924
Prolactin (ng/ml)	22.92	12.60	20.29	11.70	0.318
E2 day 2 (pg/ml)	43.22	18.55	48.42	34.39	0.422
AMH (ng/ml)	2.74	1.99	2.98	1.74	0.560
E2 HCG (pg/ml)	1853.90	921.64	1909.16	771.66	0.767

Student's t-test.

3.7. Clinical characteristics according to the result of B-HCG (pregnancy result)

Current study found in non pregnant women, the mean of ICSI attempt, Follicles , Maturity rate, Fertilization rate, Embryo GI, Embryo GII, and Embryo GIII were (0.43), (10.87), (79.11), (79.66), (2.34), (2.79) and (1.60) respectively, while, in pregnant women the mean of the ICSI attempt, Follicles, Maturity rate, Fertilization rate, Embryo GI, Embryo GII and Embryo GIII were (0.64), (12.09), (81.51), (84.46), (2.55), (3.24) and (1.94) respectively. There were no significant difference between groups with (*P=0.201, 0.332, 0.509, 0.189, 0.619, 0.252 and 0.316*) respectively, while the mean of transferred embryo in non pregnant women was (3.25) and in pregnant women was (3.73). There were a significant difference between B HCG groups regarding transferred embryo (*P=0.013*), as clarified in table(3-7).

Table (3.7): Mean differences of clinical characteristics according to the result of B HCG (pregnancy result)

Variables	B HCG				*P value*
	Negative (non pregnant)		Positive (pregnant)		
	Mean	**SD**	**Mean**	**SD**	
ICSI attempt	0.43	0.70	0.64	0.82	0.201
Follicles	10.87	5.82	12.09	6.07	0.332
Maturity rate	79.11	17.33	81.51	16.51	0.509
Fertilization rate	79.66	18.15	84.46	14.60	0.189
Embryo GI	2.34	1.97	2.55	1.75	0.619
Embryo GII	2.79	1.81	3.24	1.92	0.252
Embryo GIII	1.60	1.52	1.94	1.75	0.316
Transferred embryo	3.25	0.91	3.73	0.80	**0.013***

Student's t-test**,** (*) :significant at $p<0.05$

3.8. Cytokines according to the result of B HCG (pregnancy result)

Regarding the median of IL-5 in follicular fluid, in non pregnant women was (517.17) and in pregnant women was (212.14), while the median of serum IL-5, in non pregnant women was (151.78) and in pregnant women was (67.71). However, there was a significant difference between B HCG groups regarding follicular and serum level of IL-5 with (*P=0.005*)

About the median of IL-12 in follicular fluid, in non pregnant women was (11.79) and in pregnant women(10.70). However, there was no significant difference between B-HCG groups (*P=0.953*), while the median

of serum IL-12, in non pregnant women was (7.05) and in pregnant women was (6.40). There was no significant difference between B HCG groups (*P=0.674*).

Concerning the median of IL-18 in follicular fluid, in non pregnant women was (279.77) and in pregnant women was (743.24), while The median of serum IL-18, in non pregnant women was (458.16) and in pregnant women was (795.53). However, there was a significant difference between B HCG groups regarding follicular and serum level of IL-18 with (*P=0.005*), as in table (3-8).

Table (3.8): Median differences of cytokines according to the result of B HCG (pregnancy result)

Interleukins	**B HCG**				***P value***
	Negative (non pregnant)		Positive (pregnant)		
	Median	**IQR**	**Median**	**IQR**	
IL-5 FF(pg/ml)	517.17	619.46	212.14	164.69	**0.005***
IL-5 S (pg/ml)	151.78	213.04	67.71	93.11	**0.005***
IL-12 FF (pg/ml)	11.79	9.72	10.70	10.09	0.953
IL-12 S (pg/ml)	7.05	4.00	6.40	4.38	0.674
IL-18 FF (pg/ml)	279.77	229.59	743.24	335.59	**0.005***
IL-18 S (pg/ml)	458.16	260.00	795.53	242.61	**0.005***

Kruskal-Wallis test, IQR: interquartile range, (*) :significant at p<0.05

3.9. Correlation of study cytokines in male factor patients

3.9.1. Correlation between study follicular cytokines with BMI and clinical characteristics

About table (3-9), there was no significant correlation between follicular cytokines with BMI and clinical characteristics (Maturity rate, Fertilization rate, Embryo GI, Embryo GII and Embryo GIII) with (*P>0.05*).

Table (3.9): The correlation between follicular cytokines with BMI and clinical characteristics in male factor group

Variables		IL 5 FF	IL 12 FF	IL 18 FF
BMI	R	-0.156	-0.152	-0.138
	P	0.343	0.356	0.403
	N	40	40	40
Maturity rate	R	0.012	0.011	0.160
	P	0.944	0.946	0.332
	N	40	40	40
Fertilization rate	R	-0.196	-0.095	-0.001
	P	0.233	0.565	0.997
	N	40	40	40
Embryo GI	R	-0.213	0.036	-0.087
	P	0.193	0.828	0.598
	N	40	40	40
Embryo GII	R	-0.119	0.118	-0.213

	P	0.472	0.473	0.194
	N	40	40	40
Embryo GIII	R	0.100	0.186	-0.079
	P	0.545	0.258	0.633
	N	40	40	40

r: Pearson Correlation, P: P value, N: Number

3.9.2. Correlation between study serum cytokines with BMI and clinical characteristics

There was no significant correlation between serum cytokines with BMI and clinical characteristics (Maturity rate, Fertilization rate, Embryo GI, Embryo GII and Embryo GIII) with (*P>0.05*) except a positive correlation between IL- 18 serum level with fertilization rate (*P= 0.033*), as in table (3-10).

Table (3.10): The correlation between serum cytokines with BMI and clinical characteristics regarding male factor group

Variables		IL 5 S	IL 12 S	IL 18 S
BMI	R	0.046	-0.004	-0.057
	P	0.782	0.982	0.731
	N	40	40	40
Maturity rate	R	0.013	0.062	0.280
	P	0.937	0.706	0.085
	N	40	40	40
Fertilization rate	R	0.012	0.127	**0.342**

	P	0.941	0.441	**0.033***
	N	40	40	**40**
Embryo GI	R	-0.268	-0.162	-0.034
	P	0.098	0.324	0.836
	N	40	40	40
Embryo GII	R	0.013	0.169	0.034
	P	0.939	0.304	0.837
	N	40	40	40
Embryo GIII	R	0.078	0.216	0.035
	P	0.638	0.186	0.833
	N	40	40	40

r: Pearson Correlation, P: P value, N: Number, (*) :significant at p<0.05

3.10. Correlation of study cytokines in PCOS patients

3.10.1. Correlation between study follicular cytokines with BMI and clinical characteristics

There was no significant correlation between follicular cytokines with BMI and clinical characteristics (Maturity rate, Fertilization rate, Embryo GI, Embryo GII and Embryo GIII) with (*P>0.05*) except a negative correlation between IL-5 Follicular fluid with fertilization rate (*P= 0.020)*, and a positive correlation between IL12 Follicular fluid with embryo grade II (*P= 0.014*), as in table (3-11).

Table (3.11): The correlation between follicular cytokines with BMI and clinical characteristics regarding PCOS group

Variables		IL 5 FF	IL 12 FF	IL 18 FF
BMI	R	0.098	-0.038	0.053
	P	0.601	0.838	0.779
	N	30	30	30
Maturity rate	R	0.070	-0.084	0.248
	P	0.708	0.652	0.178
	N	30	30	30
Fertilization rate	R	**-0.417**	0.199	0.216
	P	**0.020***	0.284	0.244
	N	**30**	30	30
Embryo GI	R	0.156	0.201	0.160
	P	0.402	0.277	0.389
	N	30	30	30
Embryo GII	R	0.052	**0.437**	0.207
	P	0.780	**0.014***	0.265
	N	30	**30**	30
Embryo GIII	R	0.123	-0.030	0.070
	P	0.510	0.872	0.708
	N	30	30	30

r: Pearson Correlation, P: P value, N: Number, (*) :significant at $p<0.05$

3.10.2.Correlation between study serum cytokines with BMI and clinical characteristics

There was no significant correlation between serum cytokines with BMI and clinical characteristics (Maturity rate, Fertilization rate, Embryo GI, Embryo GII and Embryo GIII) with ($P>0.05$), as in table (3-12).

Table (3.12): The correlation between serum cytokines with BMI and clinical characteristics regarding PCOS group

Variables		IL 5 S	IL 12 S	IL 18 S
BMI	R	0.063	0.133	0.099
	P	0.736	0.475	0.596
	N	30	30	30
Maturity rate	R	-0.080	0.190	0.253
	P	0.667	0.306	0.169
	N	30	30	30
Fertilization rate	R	-0.034	-0.125	0.207
	P	0.858	0.504	0.265
	N	30	30	30
Embryo GI	R	-0.158	-0.208	0.052
	P	0.396	0.261	0.780
	N	30	30	30
Embryo GII	R	0.007	-0.165	0.030
	P	0.972	0.376	0.874
	N	30	30	30
Embryo GIII	R	-0.173	0.123	-0.093
	P	0.351	0.509	0.618
	N	30	30	30

r: Pearson Correlation, P: P value, N: Number

3.11. Correlation of study cytokines in POR patients

3.11.1. Correlation between study follicular cytokines with BMI and clinical characteristics

There was no significant correlation between follicular cytokines with BMI and clinical characteristics (Maturity rate, Fertilization rate, Embryo GI, Embryo GII and Embryo GIII) with (P>0.05) except a negative correlation between IL-5 follicular fluid with embryo grade II (*P= 0.033*), and positive correlation between IL-18 follicular fluid with embryo grade II (*P= 0.021*), as in table (3-13).

Table (3.13): The correlation between follicular cytokines with BMI and clinical characteristics regarding POR group

Variables		IL 5 FF	IL 12 FF	IL 18 FF
BMI	R	0.147	0.162	0.223
	P	0.438	0.394	0.236
	N	30	30	30
Maturity rate	R	-0.246	-0.223	0.216
	P	0.190	0.236	0.252
	N	30	30	30
Fertilization rate	R	0.219	-0.036	-0.109
	P	0.246	0.850	0.566
	N	30	30	30
Embryo GI	R	0.010	0.071	0.151
	P	0.957	0.711	0.426
	N	30	30	30
Embryo GII	R	**-0.390**	0.048	**0.420**

	P	**0.033***	0.800	**0.021***
	N	**30**	30	**30**
Embryo GIII	R	0.020	-0.056	0.209
	P	0.918	0.767	0.268
	N	30	30	30

r: Pearson Correlation, P: P value, N: Number, (*) :significant at p<0.05

3.11.2. Correlation between study serum cytokines with BMI and clinical characteristics

There was no significant correlation between serum cytokines with BMI and clinical characteristics (Maturity rate, Fertilization rate, Embryo GI, Embryo GII and Embryo GIII) with (P>0.05), except a positive correlation between IL-5 serum level with maturity rate (*P= 0.044*), IL-12 serum level was negative correlation with fertilization rate (*P= 0.002*) and IL-18 serum level was positive correlation with embryo grade II (*P= 0.024*), as in table (3-14).

Table (3.14): The correlation between serum cytokines with BMI and clinical characteristics regarding POR group

Variables		IL 5 S	IL 12 S	IL 18 S
BMI	R	-0.044	0.037	0.264
	P	0.817	0.847	0.158
	N	30	30	30

Maturity rate	R	**0.371**	-0.289	0.040
	P	**0.044***	0.121	0.835
	N	**30**	30	30
Fertilization rate	R	0.163	**-0.532**	-0.129
	P	0.389	**0.002***	0.496
	N	30	**30**	30
Embryo GI	R	-0.047	0.083	0.317
	P	0.806	0.662	0.088
	N	30	30	30
Embryo GII	R	-0.069	-0.092	**0.412**
	P	0.718	0.630	**0.024***
	N	30	30	**30**
Embryo GIII	R	0.075	-0.143	0.253
	P	0.695	0.451	0.177
	N	30	30	30

r: Pearson Correlation, P: P value, N: Number**,** (*) :significant at p<0.05

3.12.1. Correlation between study serum and follicular cytokines of male factor

There was no significant correlation between serum cytokines and follicular cytokines (*P>0.05*), except between IL 5F and IL 5 S there is a positive correlation with (*P=0.002*); IL 12 F and IL 5 S there is a negative correlation with (*P=0.006*), IL 12 F and IL 12 S there is a positive correlation with (*P=0.049*) IL 12 F and IL 18 S there is a positive correlation with (*P=0.004*); IL 18 F and IL 18 S there is a positive correlation with (*P= 0.004*), as in table (3-15).

Table (3.15): The correlation between serum and follicular cytokines regarding male factor

Variables		IL 5 F (pg/ml)	IL 12 F (pg/ml)	IL 18 F (pg/ml)
IL-5 F (pg/ml)	R	1	-0.174	-0.166
	P		0.290	0.313
	N	40	40	40
IL-12 F (pg/ml)	R	-0.174	1	0.222
	P	0.290		0.175
	N	40	40	40
IL-18 F (pg/ml)	R	-0.166	0.222	1
	P	0.313	0.175	
	N	40	40	40
IL-5 S (pg/ml)	R	**0.474**	**-0.432**	-0.090
	P	**0.002**	**0.006**	0.587
	N	**40**	**40**	40
IL-12 S (pg/ml)	R	-0.187	**0.318**	0.254
	P	0.255	**0.049**	0.119
	N	40	**40**	40
IL-18 S (pg/ml)	R	-0.295	**0.449**	**0.449**
	P	0.068	**0.004**	**0.004**
	N	40	**40**	**40**

r: Pearson Correlation, P: *P value*, N: number

3.12.2. Correlation between study serum and follicular cytokines of PCOS

There was no significant correlation between serum cytokines and follicular cytokines (*P>0.05*), except between there is IL 5 F and IL 5 S a positive correlation with (*P=0.019*) and IL 18 S a negative correlation with (*P=0.026*); IL 18 F and IL 18 S there is a positive correlation with (*P=0.005*), as in table (3-16).

Table (3.16): The correlation between serum and follicular cytokines regarding PCOS

Variables		IL 5 F (pg/ml)	IL 12 F (pg/ml)	IL 18 F (pg/ml)
IL-5 F (pg/ml)	R	1	-0.072	-0.231
	P		0.698	0.210
	N	30	30	30
IL-12 F (pg/ml)	R	-0.072	1	0.235
	P	0.698		.202
	N	30	30	30
IL-18 F (pg/ml)	R	-0.231	0.235	1
	P	0.210	0.202	
	N	30	30	30
IL-5 S (pg/ml)	R	**0.419**	0.053	-0.196
	P	**0.019**	0.776	0.290
	N	**30**	30	30

IL-12 S (pg/ml)	R	0.297	-0.335	-0.348
	P	0.105	0.065	0.055
	N	30	30	30
IL-18 S (pg/ml)	R	**-0.400**	-0.163	**0.646**
	P	**0.026**	0.381	**0.005**
	N	**30**	30	**30**

r: Pearson Correlation, P: *P value*, N:Number

3.12.3. Correlation between study serum and follicular cytokines of POR

There was no significant correlation between serum cytokines and follicular cytokines (P>0.05); except IL 5 F and IL 18 F there is a negative correlation with (*P=0.002*) and IL 18 S there is a negative correlation with (*P=0.002*); IL 18 F and IL 18 S there is a positive correlation with (*P=0.005*), as in table (3-17).

Table (3.17): The correlation between serum and follicular cytokines regarding POR

Variables		IL 5 F (pg/ml)	IL 12 F (pg/ml)	IL 18 F (pg/ml)
IL-5 F (pg/ml)	R	1	0.236	**-0.540**
	P		0.210	**0.002**
	N	30	30	**30**
	R	0.236	1	0.022

IL-12 F (pg/ml)	P	0.210		0.907
	N	30	30	30
IL-18 F (pg/ml)	R	**-0.540**	0.022	1
	P	**0.002**	0.907	
	N	**30**	30	30
IL-5 S (pg/ml)	R	0.279	-0.017	-0.171
	P	0.136	0.928	0.365
	N	30	30	30
IL-12 S (pg/ml)	R	-0.235	0.009	0.202
	P	0.211	0.962	0.284
	N	30	30	30
IL-18 S (pg/ml)	R	**-0.546**	0.006	**0.762**
	P	**0.002**	0.975	**0.005**
	N	**30**	30	**30**

r: Pearson Correlation, P: *P value*, N: Number

3.13. Receiver Operating Characteristic Curves ROC

Assuming male factors as a control as female causes of infertility are not detected.

3.13.1. ROC measurement to study cytokines in PCOS cause

The largest area under the curve was for IL18 F (62.9%) but the *P value* was not significant (*P=0.064*). Other cytokines area under the curve were lower and no cytokines was able to detect PCOS as a cause of infertility.No sensitivity or specificity measurements were applied as there

was no significant ROC results were obtained, as in table (3-18) and figure (3-1).

Table (3-18): Area under the curve of cytokines in detecting PCOS cause in relation to male factor

Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
IL 5 F (pg/ml)	0.477	0.072	0.745	0.336	0.618
IL 12 F (pg/ml)	0.063	0.026	0.000	0.012	0.114
IL 18 F (pg/ml)	0.629	0.069	0.064	0.494	0.764
IL 5 S (pg/ml)	0.370	0.070	0.063	0.234	0.506
IL 12 S (pg/ml)	0.126	0.042	0.000	0.043	0.209
IL 18 S (pg/ml)	0.404	0.075	0.172	0.258	0.551

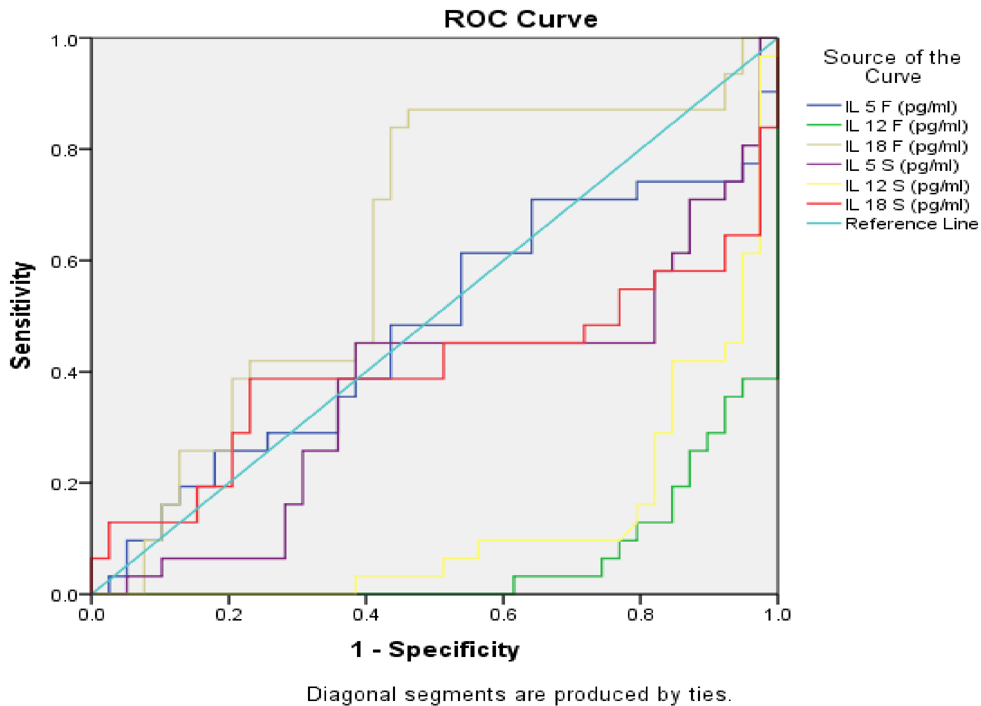


Figure (3-1): Receiver Operating Characteristic Curve of cytokines in detecting PCOS cause in relation to male factor

3.13.2. ROC measurement to study cytokines in POR cause

The largest area under the curve was for IL18 F (60.9%) but the P value was not significant (*P=0.124*). Other cytokines area under the curve were lower and no cytokines was able to detect poor ovarian reserve as a cause of infertility. No sensitivity or specificity measurements were applied as there was no significant ROC results were obtained, as in table (3-19) and figure (3-2).

Table (3-19): Area under the curve of cytokines in detecting POR cause in relation to male factor

Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound

IL 5 F (pg/ml)	0.507	0.075	0.923	0.361	0.653
IL 12 F (pg/ml)	0.100	0.038	0.000	0.025	0.175
IL 18 F (pg/ml)	0.609	0.070	0.124	0.472	0.745
IL 5 S (pg/ml)	0.426	0.074	0.292	0.281	0.570
IL 12 S (pg/ml)	0.152	0.049	0.000	0.057	0.247
IL 18 S (pg/ml)	0.458	0.073	0.553	0.315	0.602

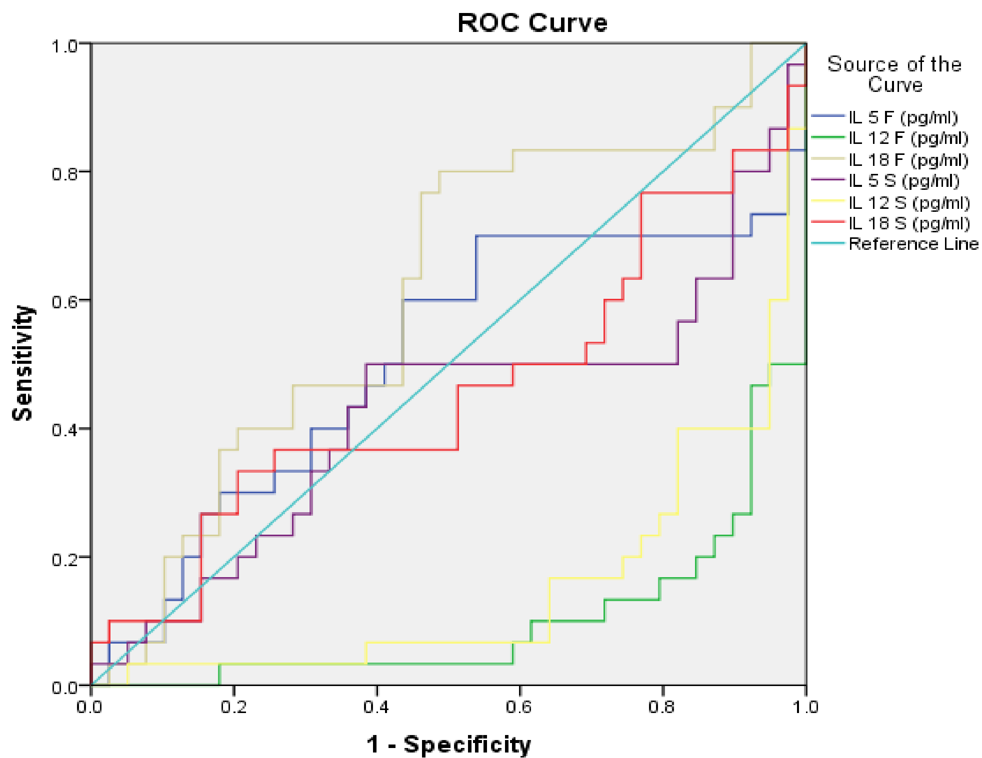


Figure (3-2): Receiver Operating Characteristic Curve of cytokines in detecting POR cause in relation to male factor

Chapter Four

Discussion

4. Discussion

4.1. Demographic characteristics among studied groups according to the infertility causes:

The age of patients ranged from (18-40) with means age (30.00), (29.97) and (31.50) among male factor, PCOS and POR patients respectively. There was no significant differences between the patient groups with (*P =0.439*) regarding the age. This results were consistent with other studies such as the study accomplished by Sarapik *et al.,* (2012) who found the mean age of male factor and PCOS were (32.6), (34.8) respectively and a study by Al-Musawy, Al-Saimary and Flaifil (2018) in which the mean age (27.7) which reported a non-statistically significant differences between the patients and control groups regarding age of PCOS group. Concerning the POR group the mean age of women in the present study was similar to the study conducted by Liu *et al.*, (2020) in which mean age was (32.00) with a non significant differences between the two groups (P > 0.05). While the study by Bouet *et al.*, (2020) in which the mean age was (36.68) and there was significantly lower in the control group compared with the POR group (*p< 0.001*).

In addition, the study showed that male factor patients had a body mass index mean values (BMI) was (28.82) and (26.80) of PCOS while the BMI of POR with mean value (28.69). There was no significant differences in BMI between patients groups with (*P =0.474*). The results of current study were related with other studies such as the study by Spanou *et al.*, (2018) and Martinez *et al.*, (2019) whose reported the BMI means were (23.4), (23.6) of male factor and PCOS respectively with no significant association was observed (P > 0.05). Also, a study by Mehta *et al.*, (2013) in which BMI mean of male factor was (23.97) with no significant result and the study by Kudsy, Alhalabi and Al-Quobaili, (2016) in which BMI mean was (27.19) in PCOS group with no significant difference (*P>0.05*).

Various studies have shown the prevalence of obesity among PCOS, ranging from 42% to 62.5%. Sangabathula and Varaganti., (2017) reported BMI mean as (34.3). At the same time, Joshi, Yonzon and Tandukar, (2017) and a study of Alakananda, Prasad and Goel, (2015) reported that BMI was (27.4) and (25.5), respectively. There is wide variability in the prevalence of overweight (BMI 25 to 30 kg/m2) and obese (BMI >30 kg/m2) women in PCOS populations across different countries. The proportion of women with PCOS who are overweight but not obese ranges from 10% in Italy to 37% in Kuwait. The highest prevalence of obesity is reported in studies conducted in the United States and Australia, with 61% to 76% of women with PCOS considered obese (Glueck *et al.,* 2005; Ching, Burke and Stuckey, 2007; Rawdhah, AL Hasnawi and Hadi, 2023).

The BMI mean of women with the POR group in the present study was (28.69). This finding is related with the study by Eskew, *et al.*, (2022) in which the BMI mean was (27.2) with no significant differences between groups (P > 0.05). On the other hand the study that conducted by Vural, Vural and Çakıroğlu, (2015) clarified that obesity rather than overweight in POR adversely affects IVF outcomes due to hormonal disturbances.

About duration of infertility, the mean values of male factor, PCOS and POR in present study were (9.19), (8.00) and (9.20) respectively with a non significant differences (*P= 0.532*). It is consistent with other studies such as the study by Ali, Al-Murshidi and Al-Jarrah (2018). In which the mean of infertility duration was (7.51) with no significant result. Also, the study by Swadi, Edan and Al-Dulaimi (2023) in which mean of infertility duration was (5) with no significant difference. The mean of infertility duration of women with the PCO group in the present study was incompatible with the study by Agarwal and Trolice, (2019) who found the mean of infertility duration was (2.9). Whereas, the mean of infertility duration of women with the POR group in the present study was (9.20)

which is similar to the study by Liu *et al.,* (2020) in which mean of infertility duration was (9.3) with no significant differences (P > 0.05). While in the study by Vural; Vural and Çakıroğlu (2015) in which the mean of infertility duration was (7.6) with highly significantly association (*P =0.01*) .The study by Zhang *et al.,*(2022) whose reported that women with infertility exceeding (4.8) years had decreased incidence of IVF success rate.

Regarding the types of infertility, there was no significant differences about infertility types between patient groups with (*P =0.684*) in current study . Several studies such as the study by Swadi, Edan and Al-Dulaimi (2023) whose reported that primary infertility count was 37 (74%) and secondary infertility was 13 (38%) of male factor infertility. The study by Ali, Al-Murshidi and Al-Jarrah (2018) whose found the primary infertility was (34) and secondary infertility was (11) with no significant difference. In addition, the study by Sarapik, *et al*., (2012) who demonstrated that primary count was (42) of male factor and count of secondary was (25), while, in PCOS the primary count was (6) and secondary count was (2). These results are logical results where the majority of patients with secondary infertility already had children so that they may direct attend for fertility management by assisted reproductive techniques for many reasons like it is cost and relating low success rate and it is need for anesthesia .

4.2. Mean Hormonal levels in studied groups:

The mean of follicle stimulating hormone (FSH) in male factor cases was (5.63) and in PCOS group was (5.72), while in POR group was (7.42). However, POR was significantly different from male and PCOS causes *(P=0.005*). FSH is a neuroendocrine hormone secreted from the anterior pituitary that plays an essential role in female reproduction (Padmanabhan and Cardoso, 2020). This heterodimeric glycoprotein is produced from neurons within the anterior pituitary and secreted to affect ovarian follicles. In turn, FSH acts on follicular granulosa cells to induce the production and

secretion of the key steroid hormone estrogen, which stimulates the growth and maturation of ovarian follicles and subsequently improves the quality and recovery rate of oocytes (Bosch *et al.,* 2016; Abbara, Clarke and Dhillo, 2018). Therefore, at the level of the pituitary-ovarian axis, consecutive FSH treatment to induce hyperovulation is widely used as the gold standard protocol for most assisted reproductive technologies (ARTs). (Fauser, 2017; Schoolcraft and Meseguer, 2017)

The mean of follicle stimulating hormone (FSH) in male factor and PCO were no significant, it is consistent with other studies such as the study conducted by Anwary, Alfazzaman and Begum, (2010) in which fifty subfertile women suffering from PCOS were recruited for evaluation in which serum FSH mean was (6.10) and a study by Sarapik, *et al*., (2012) who reported the mean of FSH in male factor was (5.6), in PCOS group was (5.7), with no significant result, the levels of FSH was normal in these groups. Also, a study by Mehde and Resan, (2014) who found the FSH mean in PCOS cases was (5.03) with *(P*=0.045) and the study by Jain, Malik and Prakash, (2022) whose found the mean of FSH was (5.7) in PCOS cases. The determinations of FSH are characterized by many difficulties. One obvious problem is the inconvenience of the required blood draw on day 2 or 3 of menses. The second issue of concern is the degree of cycle-to-cycle fluctuation in baseline FSH levels (Hehenkamp *et al*., 2006).The mean of follicle stimulating hormone (FSH) in POR group was (7.42). POR was significantly different from male and PCOS causes ($P=0.005$). Rebar, (2007) revealed that high levels of FSH are necessary for POR diagnosis. Present study result was consistent with other studies such as the study by Barbakadze *et al*., (2015), who reported the mean of FSH were (8.96) in group (<35years) and (11.23) in group (35-40 years). There was a positive correlation between age and FSH, with a highly significant ($P<0.0001$) positive correlation with FSH level. A study conducting by Zhang *et al*.,

(2021) found that mean of FSH in POR was 10.5. this result due to almost depletion of ovarian reserve which in turn resulted in defect negative feedback mechanism letting FSH secretion high.

About the mean of luteinizing hormone (LH) in male factor group was (3.43) and PCOS group was (6.27), while in POR group was (7.01). However, Male factor cause was significantly different from PCOS and POR causes with (*P=0.006*). Several studies by Liu *et al*., (2019) and Ye *et al*., (2009) whose found the mean of LH was (3.2) and (3.8). respectively with no significant result (*P =0.20*). Regarding the PCOS group mean was higher than male factor this consistent with many studies such as the study by Lisi *et al.,* (2005), who found the endocrinological disorder which is linked to hypersecretion of LH and ovulatory dysfunction is attributed to increased levels of LH . And the study by Jain, Malik and Prakash, (2022) revealed that mean of LH in PCO was (6.95). While, current study disagree with the study conducted by Rawdhah, AL Hasnawi and Hadi (2023), whose reported the Mean of (LH) in PCOS cases was (5.46) and in healthy control was (4.53). However, the association was non significant (*P*=0.429) between PCOS patients and the control. A study by Anwary, Alfazzaman and Begum (2010) reported that serum LH mean in PCOS was (15.02). Another study by Mehde and Resan, (2014) in which LH in PCOS cases with mean (12.68) and control had mean (6.96) with *(P*=0.0008). POR patients result agree with many studies such as the study by Broekmans *et al.,* (2014) who found that previous model for the prediction of ovarian response in antagonist cycles showed high levels of LH could predict the ovarian response .And the study by Tsakos *et al*., (2014) was found that basal LH levels to be good predictors for ovarian stimulation. While, a study by Liu *et al*., (2020) reported that mean of LH in poor ovarian reserve was 5.64 and control was 5.63 with, no significant result (*P=927*). In addition, study by Zhang *et al*., (2021) found that mean of LH in POR was (4.9).There was no significant correlation

between LH serum levels and the other variables, but optimum effects were observed in patients with LH ≥ 8 . Increasing level of LH in POR patients may be due to poor negative feedback mechanism due to depletion of the follicles which minimize the hormones secreted by their granulose cells .

About the mean of prolactin hormone in male factor was (18.79), in PCOS cases was (31.29) and in POR was (16.74). However, PCOS cause was significantly different from male and POR causes with (*P=0.005*). It is consistent with other studies such as the study by Begum, (2009) who found higher level of prolactin in PCOS of a group of Bangladeshi women and a study by Mehde and Resan, (2014) whose found a highly significant increase in serum prolactin (*P=0.001*) in PCOS cause. Also, a study conducted by Islam, Pathan and Ahmed, *et al*., (2015), in Bangladeshi study, observed hyperprolactinemia in 18.6% of cases of PCOS. But this study incompatible with the study by Rawdhah, AL Hasnawi and Hadi, (2023) whose demonstrated the mean of prolactin in PCOS cases was (25.24) and in healthy control was (20.93) with non significant result (*P=0.111*). And different from a study by Nahar *et al*., (2017) in which the mean serum prolactin level was found to be (315.15) and a study by Anwary, Alfazzaman and Begum, (2010), in which serum prolactin was normal in 43 (86%) and raised in 7 (14%) women of mean (23.52) with no significant result in PCOS patients. About the mean of prolactin in POR , it is consistent with other studies such as the study by Liu *et al*., (2020) who reported the mean of Prolactin level in cases was (13.91) and control was (15.38) with no significant association between groups (*P =095*).

Regarding Estradiol hormone (E2) at cycle day two, the mean in male factor was (48.07), in PCOS cases was (51.97) and in POR was (33.59) However, POR was significantly different from male and PCOS causes with (*P=0.008*). It is consistent with other studies such as the study by Zhang *et al*., (2019) who found the mean of Estradiol hormone in non PCO cases was

(53.8) and in the PCOS cases was (51.97) with no significant result. Concerning Estradiol hormone (E2) at cycle day two in POR patients, several studies conducted by Liu *et al.*, (2020) and Zhang *et al.*, (2021) whose revealed that mean of Estradiol hormone were (30.10) and (34.8) respectively with result.

Regarding the anti mullerian hormone (AMH) mean in male factor was (3.28), in PCOS group was (4.13) and in POR was (0.87). However, Male factor was significantly different from PCOS and POR. Also, PCOS was significantly different from POR (*P=0.005)*. It is consistent with other studies such as the study by Jain, Malik and Prakash, (2022) who found the mean of AMH was (7.04) in PCOS patients. Another study accomplished by Liu *et al.*, (2020) revealed the mean of AMH of POR cases was (0.58) and control was (2.56) with significant between groups (*P=0.001*). And the study by Zhang *et al.,* (2021) found the mean of AMH in POR was (0.6) associated with age of female, the advanced age group showed lower AMH was significantly different between young and old patients with POR patients. On the other hand the study by Barbakadze *et al.*, (2015) Reported the mean of AMH was (2.5) in group (<35years) and (1.1) in group (35-40 years) AMH values reflected age-specific changes better than other indicators.

About the mean of Estradiol hormone (E2) at day of HCG injection in male factor was (2262.58), in PCOS cases was (1951.83) and in POR was (1282.20) However, POR was significantly different from male and PCOS causes with (*P=0.005*). The mean of Estradiol hormone (E2) at day of HCG injection in male factor was non significant . It is similar to the study by Zhang *et al.*, (2019) who found the mean of E2 level on HCG of non PCOS patients was (3911.0) with (*P= 0.001*), while the mean of this hormone in PCOS cases was (1951.83). In present study the mean of Estradiol hormone at day of HCG injection in POR was significantly different from male and

PCOS causes with (*P=0.005*). It is consistent with other studies such as the study conducted by Kavrut , Kahraman and Kumru, (2022) who revealed that mean of E2 on HCG day was (684.66) in POR cases.

4.3. Clinical characteristics of different infertility groups

Present study reported that the mean of Intracytoplasmic sperm injection (ICSI) attempt in male factor cases was (0.41) and in PCOS group was (0.35), while in POR group was (0.77). However, no significant difference between groups (*P=0.060*).

About the mean of Total follicle number in male factor cases was (12.33) and in PCOS group was (13.65), while in POR group was (4.43). However, POR was significantly different from male and PCOS causes (*P=0.005*). In male factor and PCO was good number of follicles because stimulation by antagonist protocol and good timing of the administration of HCG. A study conducted by Swadi, Edan and AL-Dulaimi (2023) whose reported the median of total oocytes was (12) associated with good stimulation of E2, AMH and FSH . While in POR patients , current study results are logic because in POR patients , there is a low level of AMH and decrease number of Follicles and this result was associated with a study by Dai *et al*., (2020) who demonstrated low number of oocytes retrieval associated with low AMH.

Regarding the mean of Maturity rate in male factor cases was (84.37) and in PCOS group was (76.74), while in POR group was (77.35). However, no significant difference was detected (*P=0.109*). This result was inconsistent with a study of Ms, *et al*., (2008) who found a significant result with (*P=0.006*) between maturity rate and PCOS cause.

The mean of Fertilization rate in male factor cases was (80.74) and in PCOS group was (79.52), while in POR group was (83.70). However, no significant difference was detected (*P=0.623*). These results confirm the importance of ICSI treatment in ensuring a high fertilization rate regardless

of the causes of fertility. The present study result compatible with a study accomplished by Xu, *et al*, (2022) who found the fertilization rate of infertile patients was (88.02). ICSI is effective and improve fertilization rate of oocytes .

About the mean of Embryo Grade l (GI) and Grade ll (GII), POR was significantly different from male and PCOS causes with (*P= 0.005*) and (*P =0.003*). Current study result agreed with study by Sarapik *et al.,* (2012) who reported good quality embryos (embryo Grade I and ll) of male factor was (3.8), PCO was (2.9) and POR was (1.0). POR patients was significantly different from other patients groups.

Regarding the mean of Embryo Grade III (Glll), PCOS was significantly different from POR and male factor causes with (*P=0.030*). A study conducted by Lin, *et al*., (2013) revealed to that decrease of AMH was correlated with bad quality of embryos (Embryo Grade III) .

The mean of Transferred embryo in male factor cases was (3.69) and in PCOS group was (3.39), while in POR group was (3.07). However, POR was significantly different from male causes (*P= 0.015*). Regarding POR in present study, the low number of Oocytes and their bad quality result in a decrease number of embryos that suitable for transfer. This result compatible with a study result of Opsahl, *et al*., (2001) who demonstrated the associated with number and quality of oocytes and embryos. And marked discrepancies in the size of follicles could be related to differences in follicles sensitivity to FSH and un-satisfactory maturation. This phenomenon potentially causes a decrease in the number of viable oocytes and embryos.

On the other hand, concerning the count of Beta-Human Chorionic Gonadotropins (B-HCG) test, there were no significant difference between groups with (*P=0.439*). In present study the pregnancy rate not depended on quality and number of embryos but also associated with others factor effect on implantation rate. In this study no significant between B-HCG test and

causes of infertility groups, this is proof that treatment for ICSI is the best option for pregnancy. This result disagree with study by Ashrafi, *et al.,* (2013) who evaluated the relation between ICSI outcome and different causes of infertility and reported the different success rate of ICSI in various causes of infertility.

4.4. Cytokine levels among studied groups

Regarding the median of IL-5 levels in the follicular fluid and serum. There were no significant difference between groups with (*P= 0.629*) and (*P=0.169*) respectively. This study agreed with the study by Terenina *et al.,* (2017), who suggested that IL-5 acts as one of the upstream key regulators of Porcine ovarian follicular atresia. And the study by Niu *et al*., (2017) who demonstrated a non significant correlation between FF IL-5 of patients with or without PCO syndrome undergoing IVF. Also the study by Alhilali *et al.,* (2019), who observed that the elevated FF IL-5 levels was associated with poor oocyte quality. In addition, Bouet *et al.,* (2020) reported that IL-5 in FF was no significant result in POR patients.

About the median of IL-12 levels in the follicular fluid and serum. The male factor was significantly different from PCOS and POR causes with (*P=0.005*). IL- 12 has a role in follicular fluid and correlation of their concentration with follicular and embryo development during fertility procedures. A study conducted by Lédée *et al*., (2008), who reported the significant elevation of IL-12 in highly fragmented embryos on Day 2, suggests that it has a deleterious role.While the study by Sarapik *et al.,* (2012), who noted that positive association between the concentration of follicular IL-12 and the number of fertilized oocytes and the proportion of good-quality embryos, when the data were adjusted for age, cause of infertility, and follicular size. And study by Piccinni, *et al.,* (2021) who found that IL- 12 has a role in increased follicle diameter during Puncture. Also the study by Adamczak, *et al*., (2021), who noted about the role of IL-18 as well

as IL-12 are thought to be of particular importance in the context of embryo implantation.

On the other hand, about the median of IL-18 level in the follicular fluid and serum. There were no significant difference between groups with (*P=0.117*) and (*P=0.342*) respectively. IL-18 is important in implantation and successful IVF. The over expression of IL-18 may lead to endometrial cytotoxicity by recruiting the uterus natural killer (uNK) cells, but this process is controlled by a number of mediators that under physiological conditions, allowing for proper embryo implantation and angiogenesis.

Some studies consistent with present study such as the study by sarapik *et al.*, (2012), who demonstrated the median of IL- 18 in FF of Male factor infertility was (311.0), in PCO was (463.4) with no significant association between groups. While another studies disagree with current study such as the study by Chen *et al.*, (2015), who found that IL-18 levels were significantly higher in serum than in follicular fluid. And IL-18 levels in follicular fluid also increased with a rising BMI, the correlation between IL-18 levels in serum and the ovarian stimulation response was not statistically significant (*P= 0.116*), whereas the correlation between IL-18 levels in follicular fluid and the ovarian stimulation response was significant (*P= 0.003*). Also, study by Al-Musawy, Al-Saimary and Flaifil. (2018), who reported that level of IL18 was highly statistically significant in PCOS comparing to normal women, and these high levels were related to PCOS independent on the presence of obesity or hyperandrogenism, and there is a positive correlation between IL18 and negative correlation between these markers and obesity and hyperandrogenism. In addition, the study by Ibrahim and Al-saffar, (2018) Whose demonstrated IL-18 in serum of PCO patients and control were highly significant. And there is a significant difference of IL-18 level between PCOS patients and BMI according to the

normal weight, overweight and obese PCOS women. The differences of these results may be due to lifestyle in different populations.

4.5. Demographic characteristics according to the result of B HCG (pregnancy result)

Regarding the mean of age, body max index and duration of infertility in pregnant and non pregnant women, there were no significant difference between B HCG result groups with (*P=0.542, P= 0.753 and P=0.264*) respectively, as clarified in table(3-5).

Concerning the age, this study different with many studies such as the study by Ahmed *et al*., (2015) who found the mean of age of non pregnant women was (33.5) while, the mean of age of pregnant women was (29.8) with highly significant result (P=*0.001*). Also studies by Zahir *et al.*, (2020) and Chen *et al.,* (2022) whose mentioned that age of the female was the main predictor of pregnancy but its significant effect in decreasing pregnancy rate appear if age was increased more than 35. While this study agreed with the study by Swadi, Edan and Al-Dulaimi, (2023) whose demonstrated the age mean of non pregnant women was (31.80) while the age mean of pregnant women was (30.86) with no significant result (*P=0.61*).

About BMI, current study consistent with the study by Ahmed *et al.,* (2015) who found the BMI of non-pregnant women was (28.8) and pregnant women was (28.6) non significant result (*P=0.825*). Also the study by Swadi, Edan and Al-Dulaimi, (2023) revealed to the BMI of non-pregnant women was (28.23) and pregnant women was (26.50) with no significant result (*P=0.24*). While, the mean of infertility duration in this study compatible with study accomplished by Swadi, Edan and Al-Dulaimi, (2023) whose reported the mean of infertility duration of non pregnant women was (8.30) while pregnant women was (9.06)with no significant result (*P=0.63*).

About the primary and secondary types of infertility in pregnant and non pregnant women, there were no significant relation to the B HCG results with (*P=0.894*).There is no significant influence of infertility type on pregnancy rate and that consistent with some studies such as a study of Metello, Tomás, and Ferreira . (2019) and Swadi, Edan and Al-Dulaimi, (2023) whose reported the primary and secondary types of infertility in pregnant women were (9) and (6) respectively, whereas, in non pregnant women were (28) and (7) respectively with no significant result (*P= 0.13*) .

Concerning the infertility causes in pregnant and non pregnant women, there were no significant association to the B HCG results with (*P=0.439*).This results consistent with the study by Swadi, Edan and Al-Dulaimi, (2023) whose reported there is no significant influence of infertility causes on pregnancy rate, this can be attributed to the role of ICSI in overcoming almost all causes of subfertility.

4.6. Hormonal study according to the result of B HCG (pregnancy result)

According to the Mean differences of FSH, LH, Prolactin, E2 day 2, AMH and E2 HCG hormones in pregnant and non pregnant women, there were no significant differences between B HCG result groups with (*P=0.086, P=0.924, P=0.318, P=0.422, P=0. 560 and P=0.767*) respectively, as demonstrated in table (3-6). This finding consistent with many studies such as the study by Bjercke *et al*., (2005); Bedaiwy *et al.,* (2007); Al-Ghazali and Al-Jarrah, (2013); Ashrafi, *et al.,* (2013); Cicek, Kahyaoglu and Kahyaoglu, (2015) and Ali ,Al-Murshidi and Al-Jarrah, (2018) whose mentioned the same results of current study.

4.7. About the clinical characteristics according to the result of B HCG (pregnancy result)

Current study found in pregnant and non pregnant women, there were no significant difference between groups with (*P=0.201, P=0.332, P=0.509,*

P=0.189, P=0.619, P=0.252 and P= 0.316) respectively regarding the clinical characteristics, while concerning the transferred embryo in pregnant and non pregnant women, there were a significant difference with (*P=0.013*) between B HCG groups, as clarified in table (3-7).

The present study agreed with the study conducted by Al -Ghazali and Al-Jarrah, (2013) whose revealed to the mean of embryos was higher in pregnant women with significant result. Also, the study by Ali, Al-Murshidi and Al-Jarrah, (2018) whose demonstrated that ICSI outcome is similar in pregnant compared with non-pregnant patients. In addition, the follicles, maturity rate, embryo GI ,embryo GII, and embryo GIII were no significant results while transferred embryos was significant, but this study inconsistent with current study regarding fertilization rate was significantly higher in pregnant than non-pregnant women. The chance of pregnancy increases along with number of embryo transferred.

4.8. Regarding the Cytokines according to the result of B HCG (pregnancy result)

Regarding the median of IL-5 in follicular fluid and serum levels in pregnant and non pregnant women, there were a significant difference between B HCG groups with (*P=0.005*). This study disagreed with the study accomplished by Niu *et al.,* (2017) who found a significant correlation between FF IL-5 from the largest follicle with top-quality embryo and the potential development of embryo and ICSI outcome in a cohort of patients with or without PCO syndrome undergoing IVF . Also, this result compatible with study by Alhilali *et al.,* (2019) who reported that elevated IL-5 level in follicular fluid was associated with poor oocyte quality and asserted that IL-5 could be a negative predictor to the pregnancy outcome in stimulated ICSI cycles.

About the median of IL-12 in follicular fluid and serum levels in pregnant and non pregnant women, there were no significant difference

between B HCG groups with (*P=0.953) (P=0.674)* respectively. This study disagree with many studies such as the study by Bedaiwy *et al.,* (2007) who revealed to the concentrations of IL-12 in FF were significantly lower in pregnant compared with non-pregnant women with (*P = 0.0002*). Higher concentrations of IL-12 is associated with a negative outcome in IVF treatment. Interleukins-12 is one of biological markers that appear to affect IVF outcome. Also, the study by Lédée *et al.*, (2008) who reported the significant elevation of IL-12 in highly fragmented embryos on Day 2, suggests that it has a deleterious role. In addition, the elevated IL-12 levels in pooled FF is associated with a poor pregnancy rate .While the study that conducted by Sarapik *et al.,* (2012) who reported that higher concentration of IL-12 correlated with the successful fertilization of the ovum and development of an embryo.

Concerning the median of IL-18 in follicular fluid and serum of pregnant and non pregnant women, there was a significant difference between B HCG groups with (*P=0.005*) .This study consistent with the study by Günther *et al*., (2016) who found that IL-18 have a significant result with (*P= 0.0001*), the presence of IL-18 in follicular fluid suggests ovarian secretion..

4.9. Correlation of serum and FF cytokines level with BMI and clinical characteristics in male factor, PCOS and POR patients.

Regarding male factor in present study, the correlation between follicular and serum cytokines with BMI and clinical characteristics were no significant results with (*P>0.05*), except a positive correlation between IL-18 serum level with fertilization rate (*P= 0.033*) as in table (3-9) and (3-10). While in PCOS cases, the correlation between study follicular cytokines with BMI and clinical characteristics were no significant with (*P>0.05*) except a negative correlation between IL-5 follicular fluid with fertilization rate (*P= 0.020*), and a positive correlation between IL12 follicular fluid with embryo

grade II (*P= 0.014*) as in table (3-11). The result of present study inconsistent with with a study of Coskun and colleagues (1998) whose reported lower FF IL-12 level in preovulatory than in immature follicles, whereas Gazvani *et al.,* (2000) demonstrated a marked association between the presence of IL-12 in the FF and the incidence of fertilization failure. There were a negative correlation between fertilization rate and IL-12 and suggested this cytokine as a predictor of negative outcome of IVF–embryo transfer procedure. In addition, current study compatible with a study of Gallinelli *et al.,* (2003) who reported unable to find a significant correlation between fertilization rate and FF IL-12 concentration in PCOS patients.

Furthermore, concerning the correlation between study serum cytokines with BMI and clinical characteristics were no significant result in PCOS women with (*P>0.05*), as in table (3-12). This disagree with the study by Yang *et al.,* (2011) who demonstrated serum IL-18 concentration had positive correlation with BMI, it seemed that obesity may accelerate the increase of IL18 serum level and also suggested that IL-18 might be produced by adipose tissue. And the study by Günther *et al*., (2016) who demonstrated the correlation between IL-18 level in serum and BMI was statistically significant. Also the study by Jasim *et al.,* (2020) reported the correlation of IL-18 level with BMI of PCOS had positive correlation proposing that IL-18 may be originated by the adipose tissue. Hence, it is also probable that higher mean serum IL-18 level found in obese PCOS women were only a result of the main visceral deposition of fat associated with the PCO syndrome, rather than having any pathogenic role. In addition, the study by Benson *et al*., (2008) revealed that IL-18 serum level was increased in PCOS patients and correlated with obesity.

About POR cases, correlation between study follicular cytokines with BMI and clinical characteristics were no significant result with (*P>0.05*) except a negative correlation between IL-5 follicular fluid with embryo grade

II (*P= 0.033*), and positive correlation between IL-18 follicular fluid with embryo grade II (*P= 0.021*). While, Correlation between study serum cytokines with BMI and clinical characteristics were no significant result with (*P>0.05*), except a positive correlation between IL-5 serum level with maturity rate (*P= 0.044*), IL-12 serum level was negative correlation with fertilization rate (*P= 0.002*) and IL-18 serum level was positive correlation with embryo grade II (*P= 0.024*), as in table (3-13) and (3-14). The study by Liu *et al*., (2020) reported that Improved oocyte quality has been linked with increased levels of IL-12 and IL-18 as well as decreased levels of IL-12 have also been positively correlated with oocyte quality. While IL 5 in FF and serum levels were no correlated with BMI, maturity and quality of embryos. The differences of these results may be due to lifestyle .

4.10. Correlation between study serum and follicular cytokines of male factor, PCOS and POR cases

About male factor there were no significant correlation between serum cytokines and follicular cytokines (*P>0.05*), except between IL-5 F with IL-5 S (*P=0.002*); IL-12 F with IL-5 S (*P=0.006*), IL-12 S (*P=0.049*) and IL-18 S (*P=0.004*); IL-18 F with IL-18 S (*P= 0.004*). While, correlation between study serum and follicular cytokines of PCOS were no significant result with (*P>0.05*), except between IL-5 F with IL-5 S (*P=0.019*) and IL-18 S (*P=0.026*); IL-18 F with IL-18S (*P=0.005*), as in table (3-15) and (3-16). This result disagree with the study by Zhang *et al,* (2020) who reported the elevated FF IL-18 level was no correlated with the serum IL-18 level.

In addition, current study reported in table (3-17), the correlation between serum and follicular cytokines of POR women were no significant result with (*P>0.05*), except IL-5 F with IL-18 F (*P=0.002*) and IL-18 S (*P=0.002*); IL-18 F with IL-5 F (*P=0.002*) and IL-18 S (*P=0.005*). The differences of these results may be due to selection of the growing follicles.

4.11. ROC measurement to study cytokines in PCOS and POR causes

The cytokines area under the curve were lower and no cytokines were able to detect PCOS and poor ovarian reserve as a cause of infertility. No sensitivity or specificity measurements were applied as there was no significant ROC results were obtained.

The result of present study incompatible with the study conducted by Liu *et al*., (2021) who reported that the content of IL-18 in the follicular supernatant was higher in PCOS patients compared with control with ($P = 0.021$). IL-18 was analyzed by ROC analysis and obtained AUC of 0.711. These data indicate that the content of IL-18 in the follicular fluid of PCOS patients is abnormally increased, which may underpin the pathogenesis of this disorder.

In addition, a study of Samawi *et al.,* (2023) who revealed according to the Receiver Operating Characteristic analysis, IL-12 concentration is a good marker for predicting infertility, with a predictive cut-off value of 2510.50 U/ml, an AUC of 0.98, specificity of 95%, and sensitivity of 95%. This variety may be attributable to the difference in sample group or differences in study geographic location and also lifestyle .

Conclusions and Recommendations

Conclusions:

1. The hormonal level of FSH was higher. While , E2, AMH and E2 HCG were lows in POR were significantly different from male and PCOS women, whereas, Prolactin hormone in PCOS female was higher level compared with from male factor and POR cases.

2. The total follicle number, Embryo Grade l (GI), Embryo Grade ll (GII) and ET of POR female decreased compared with male factor and PCOS cases, while, Embryo Grade III (Glll) of PCOS female was increased compared with male factor and POR patients.

3. Follicular fluid and serum levels of IL-12 in male factor cases was higher than from PCOS and POR female.

4. There were no significant difference between patients groups regarding follicular fluid and serum levels of IL-5 and IL-18.

5. In male factor patients, there were a positive correlation between IL- 18 serum level and fertilization rate, while, a positive correlation between IL12 level in follicular fluid with embryo grade II.

6. In PCOS patients, there were a negative correlation between serum cytokines and clinical characteristics (Maturity rate, Fertilization rate, Embryo GI, Embryo GII and Embryo GIII)

7. In POR women , there were a positive correlation between follicular fluid and serum levels of IL-18 with embryo grade II and a positive correlation between IL-5 serum level with maturity rate.

8. No sensitivity and specificity measurement of ROC analysis in PCOS and POR cases .

Recommendations

1. Further studies with a larger samples size for each group of infertility causes are needed to better understand the impact of immunological markers.
2. Other studies are required to evaluate the association of immune markers with others hormones such as progesterone and estrogen or clinical characteristics such as antral follicle count (AFC).
3. Studying the role of immunological markers with other infertility causes such as endometriosis.
4. Studying the role of these immunological markers in diseases of the female reproductive system other than infertility.
5. The effect of other cytokines on ICSI outcome need to be studied

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Appendix

Appendix I: Male factor ,PCOS and POR patients questionnaires

Pt. serial no.	
Pt. phone no.	
Pt. name	
Age	
BMI	
Type of infertility	
Factors(male or female)	
Cause	
Duration	

Hormonal analysis at cycle day 2 (CD2)

1- FSH	
2- LH	
3- PROLACTIN	
4- A.M.H	
5- E2	

Stimulation protocol: Antagonist -

Ovulation trigger: date and time of ECG-

Serum estradiol hormone (E2) at time of HCG-

Table of oocyte at day of retrieval:

Total no. of oocyte retrieved	GV	Ml	Mll	Oocyte (Mll) quality	Total no. of injected (Mll)

Table of fertilization rate at day1 :

No. Of oocytes successfully fertilization	No. Of oocytes that failed fertilization

Semen analysis of male:

Normal: Mild *Oligoasthenoteratozoospermia* (OAT):

Day2 or 3 after oocytes retrieval:

Total no. of embryos	Embryos quality (I,II,III)	Total no. of embryos transferred	Date of embryos transfer

Serum B-HCG Result: Positive or negative

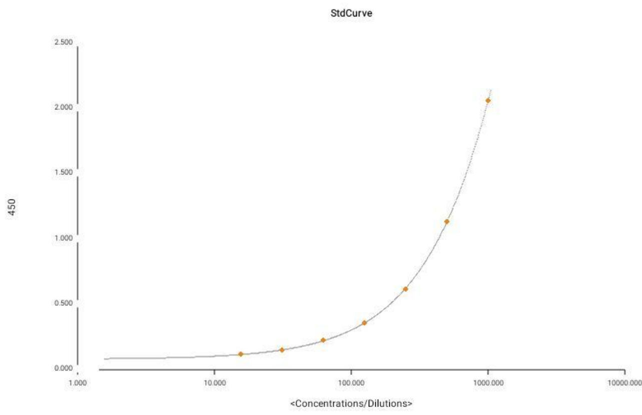
Appendix II: ELISA instruments

a **b**

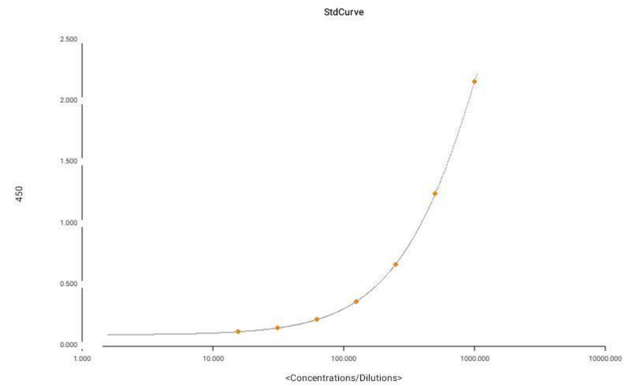
c .ELISA kits

Appendix III : ELISA curve for IL-5 ,IL-12 and IL-18 in Follicular

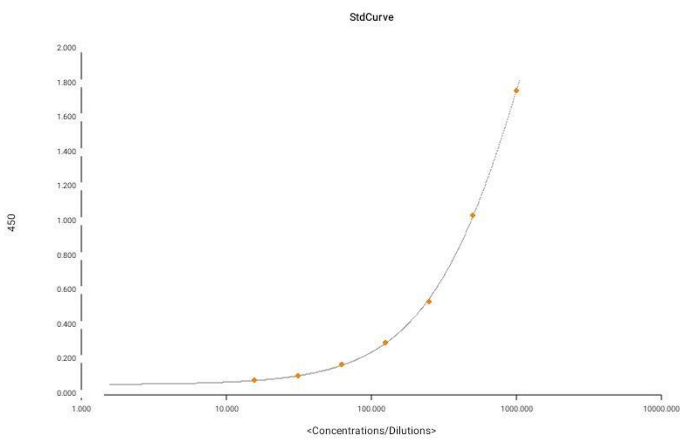
fluid (FF) and serum of patients



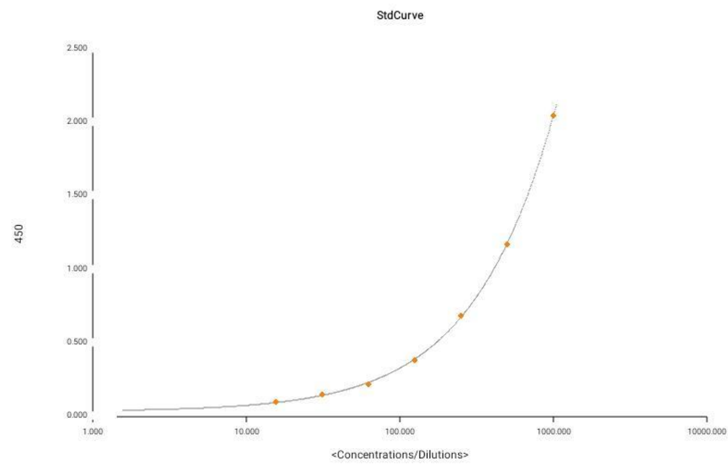
IL-5 in FF



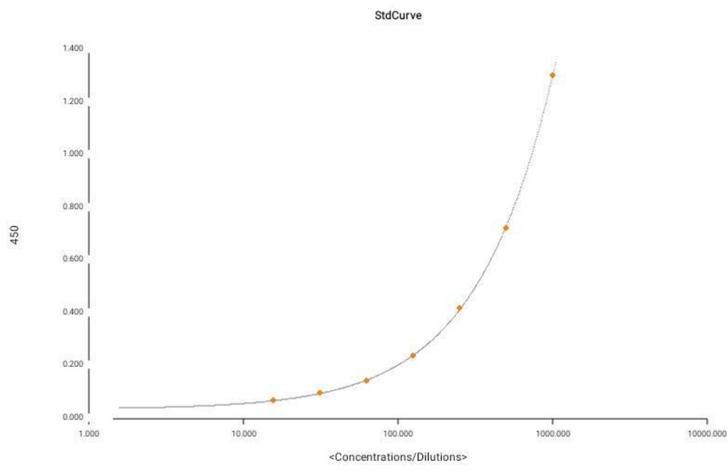
IL-5 in serum



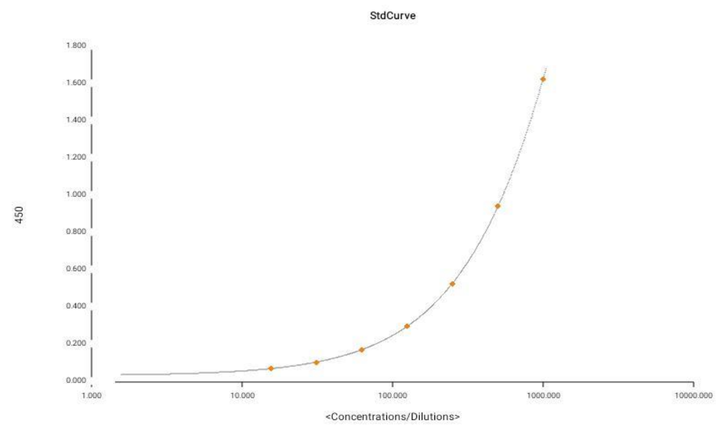
IL-12 in FF



IL-12 in serum



IL-18 in FF



IL-18 in serum

الخلاصة

العقم هو عدم القدرة على تحقيق الحمل بعد وقت مناسب من الجماع دون اتخاذ تدابير لمنع الحمل. تعد اضطرابات الإباضة أحد العوامل الأنثوية التي تسبب العقم، بما في ذلك تكيس المبايض وضعف احتياطي المبيض. ينتج نسيج المبيض بالإضافة إلى الخلايا المناعية السايتوكينات. توصف وظيفة السيتوكين في المبيض بأنها تعزز عمليات نمو الجريبات وتكوين الستيرويد وتجنيد وتنشيط الكريات البيضاء اللازمة للإباضة وإعادة تشكيل الأنسجة أثناء الإباضة واللوتين والتحلل الأصفر. هدفت الدراسة الحالية إلى تقييم الإنترلوكين (5)، الإنترلوكين (12) و الإنترلوكين (18) في السائل الجريبي والمصل ودرست العلاقة بين هذه العلامات ونتائج حقن الحيوانات المنوية داخل السايتوبلازم .

هذه الدراسة عبارة عن مقطع عرضي وتنقسم موضوعات هذه الدراسة إلى ثلاث مجموعات عامل الذكور (40) و تكيس المبايض (30) و ضعف المبايض (30) المسجلين في مدينة الصدر التعليمية الطبية ومستشفى الكفيل التخصصي ومركز الأمير الدولي للاخصاب واطفال الانابيب خلال الفترة من تشرين الثاني ٢٠٢٢ إلى نيسان 2023. تم جمع عينات الدم في اليوم الثاني من الدورة والكشف عن مستوى الهرمون المنبه للجريب، والهرمون اللوتيني، وهرمون البرولاكتين، وهرمون الاستراديول، وهرمون مخزون البيوض وهرمون الاستراديول عند حقن HCG بواسطة MINI VIDS والمصل والسائل الجريبي الذي تم جمعه في يوم بقايا البويضات والكشف عن مستويات الإنترلوكينات 5 ، 12و 18 بواسطة تقنية الأليزا.

أظهرت نتيجة هذه الدراسة أن هرمون المنشط للحويصلة و الاستراديول و هرمون مخزون البيوض وهرمون استراديول في يوم حقن HCG في ضعف احتياطي المبيض كانوا مختلفين بشكل كبير عن حالات العامل الذكري ومتلازمة تكيس المبايض مع ($P= 0.005$، $P= 0.008$ ، $P= 0.005$،$P= 0.005$) على التوالي. كان هرمون اللوتيني في العامل الذكري مختلفًا بشكل كبير عن مرضى متلازمة تكيس المبايض وضعف احتياطي المبيض مع ($P = 0.006$). بينما كان سبب البرولاكتين في متلازمة تكيس المبايض مختلفًا بشكل كبير عن العامل الذكري وضعف احتياطي المبيض مع ($P=.0.005$). بالإضافة إلى ذلك، كان إجمالي عدد البصيلات ودرجة الجنين I(GI) ودرجة الجنين II(GII) لنساء ضعف احتياطي المبيض مختلفين بشكل كبير عن حالات عامل الذكور ومتلازمة تكيس المبايض مع ($P=0.005$،$P=0.005$ ، $P=0.003$) على التوالي. في حين أن

الجنين من الدرجة GIII (Glll) لنساء متلازمة تكيس المبايض كان مختلفًا بشكل كبير عن مرضى عامل الذكور وضعف احتياطي المبيض مع ($P= 0.030$)، كان الجنين المنقول في ضعف احتياطي المبيض مختلفًا بشكل كبير عن مرضى عامل الذكور ومتلازمة تكيس المبايض مع ($P= 0.015$).

فيما يتعلق بمستويات السايتوكينات (IL-5 و IL-12 و IL-18) في المصل والسائل الجريبي، لم يكن هناك فرق كبير بين المجموعات باستثناء مستوى IL-12 في السائل الجريبي ومصل عامل الذكور كان مختلفًا بشكل كبير عن مرضى متلازمة تكيس المبايض وضعف احتياطي المبيض الذين يعانون من ($P= 0.005$). أيضًا، حول متوسط نقل الجنين في النساء غير الحوامل، كان هناك فرق كبير بين مجموعات B.HCG مع ($P=0.013$). من ناحية اخرى، كان لمستويات IL-5 في السائل الجريبي والمصل فرقًا كبيرًا بين مجموعات B. HCG مع ($P = 0.005$). بالإضافة إلى ذلك، كانت مستويات IL- 18 في السائل الجريبي والمصل فرقًا كبيرًا بين مجموعات B. HCG مع ($P = 0.005$).

علاوة على ذلك، في هذه الدراسة كان هناك ارتباط إيجابي بين مستوى مصل IL- 18 ومعدل الإخصاب في حالات العامل الذكري مع ($P= 0.033$). بينما في نساء متلازمة تكيس المبايض، كان هناك ارتباط سلبي بين IL-5 في السائل الجريبي ومعدل الإخصاب مع ($P= 0.020$) وعلاقة إيجابية بين IL12 في السائل الجريبي والجنين من الدرجة II مع ($P= 0.014$). فيما يتعلق بـضعف احتياطي المبيض كان هناك ارتباط سلبي بين IL-5 في السائل الجريبي ودرجة الجنين II مع ($P= 0.033$) والارتباط الإيجابي بين IL-18 في السائل الجريبي ودرجة الجنين IIمع ($P= 0.021$)، أيضًا، الارتباط الإيجابي بين مستوى مصل IL-5 مع معدل النضج مع ($P=0.044$). حول مستوى مصل IL-12، كان هناك ارتباط سلبي مع معدل الإخصاب مع ($P= 0.002$)، بينما كان مستوى مصل IL-18 مرتبطًا إيجابيًا مع الجنين من الدرجة II مع ($P= 0.024$).

أظهرت نتائج هذه الدراسة عدم وجود فرق معنوي بين المجموعات فيما يتعلق بمستويات السيتوكينات في السائل الجريبي والمصل باستثناء ارتفاع مستوى IL-12 في السائل الجريبي والمصل لمرضى العامل الذكور مقارنة بمرضى متلازمة تكيس المبايض و POR. لذلك ، قد لا يكون لهذه العلامات دور مهم في نتائج حقن الحيوانات المنوية داخل السايتوبلازم (الحقن المجهري).

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب

فرع الأحياء المجهرية

دراسة الحركات المناعية 12,5 و 18 لدى الاناث العقيمات : الارتباط مع مستوى هرمونات الخصوبة ، نضوج البيوض و تطورالجنين في دورة أطفال الأنابيب والحقن المجهري

رسالة

مقدمة الى مجلس كلية الطب/جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في الاحياء المجهرية الطبية/الاحياء المجهرية

من قبل الطالبة

ضحى قاسم نعمه فحامه

كلية العلوم–جامعة الكوفة / بكالوريوس علوم حياة

2015

بأشراف

أ.د عبير ظاهر ناجي الحسناوي

أ.م.د علي ابراهيم رحيم

2023 م **1445 هـ**