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THE STUDY AND EVALUATION THE LEVELS OF AMH AND LIPIDS PROFILE AND THE ROLE IT IN EVALUATED THE LEVELS OF OVARY RESERVE IN WOMEN WITH INFERTILITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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HANAN KAREEM MOHAMMED AL-JAF
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By Hanan Kareem Mohammed AL-JAF

July 2022

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ABSTRACT

THE STUDY AND EVALUATION THE LEVELS OF AMH AND LIPIDS PROFILE AND THE ROLE IT IN EVALUATED THE LEVELS OF OVARY RESERVE IN WOMEN WITH INFERTILITY

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Master of Science in Chemistry
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Nowadays, there are many infertility problems and methods that should be accurate in estimating ovarian reserve in women, the aim of this study was (investigate the rol of AMH, lipids profile and some chemical tests in women and the rol it in Evaluation the levels of ovary reserve in women with infertility after comparing with controls). Our study supported previous studies in the importance of the AHS test in estimating ovarian stock. AMH/ FSH ratio test one of the recent evidence that we have examined from an analytical point of view, we are looking for the available evidence that looks precisely at predicting the excessive response of the ovaries to ovulation stimulation, and thus assessing the ovulation stage and the chance of conception. It seems that FSH and LH test have good discriminatory ability to know the ovum size in women, the study indicated that the closer the AMH/ FSH ratio is to 3, it is a good indicator of ovulation in women. However, the problem with many treatments remains one that affects the assessment of ovulation. As for the hemoglobin and platelet test, WBC test, FSB and HbA1c test, the results indicated that, uh, there are no statistically significant differences for our study. The age had significant statistically significant differences, as the study showed that the higher the age, the more problems increased, especially for women who delay marriage.

2022, 38 pages

Keywords: Ovary reserve, Lipids, AMH, Infertility, Biochemical tests

ÖZET

AMH VE LİPİD PROFİLİ DÜZEYLERİNİ İLE İNFERTİLİTESİ OLAN KADINLARDA YUMURTALIK REZERV DÜZEYLERİNİ DEĞERLENDİRMEDEKİ ROLÜNÜ İNCELENMESİ

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Kimya, Yüksek Lisans

Tez Danışmanı: Prof. Dr. Volkan EYÜPOĞLU

Temmuz 2022

Günümüzde kadınlarda yumurtalık rezervinin tahmininde doğru olması gereken pek çok infertilite sorunu ve yöntemi bulunmakta olup, bu çalışmanın amacı (AMH'nin, lipid profilinin ve bazı kimyasal testlerin kadınlarda rolünün ve düzeylerinin değerlendirilmesindeki rolünün araştırılması) idi. Kontrollerle karşılaştırıldığında kısırlığı olan kadınlarda yumurtalık rezervi). Çalışmamız, yumurtalık stokunun tahmininde AHS testinin önemi konusunda önceki çalışmaları desteklemiştir. AMH/FSH Oranı Test Testi Analitik bir bakış açısıyla incelediğimiz son kanıtlardan biri, tam olarak yumurtalıkların yumurtlama stimülasyonuna aşırı tepkisini tahmin etmeye ve böylece yumurtlama aşamasını değerlendirmeye bakan mevcut kanıtları arıyoruz. ve gebe kalma şansı. FSH ve LH testinin kadınlarda yumurta büyüklüğünü bilmek için iyi bir ayrım kabiliyetine sahip olduğu görülmektedir. Çalışma, AMH/FSH oranının 3'e ne kadar yakınsa, kadınlarda ovulasyonun iyi bir göstergesi olduğunu göstermiştir. Bununla birlikte, birçok tedaviyle ilgili sorun, yumurtlamanın değerlendirilmesini etkileyen sorun olmaya devam etmektedir. Hemoglobin ve trombosit testi, WBC testi, FSB ve HbA1c testine gelince, sonuçlar bizim çalışmamız için istatistiksel olarak anlamlı bir fark olmadığını gösterdi. Yaş, istatistiksel olarak anlamlı farklılıklara sahipti, çünkü çalışma, yaş arttıkça, özellikle evliliği geciktiren kadınlar için daha fazla sorunun arttığını göstermiştir.

2022, 38 sayfa

Anahtar Kelimeler: Yumurtalık rezervi, Lipitler, AMH, Kısırlık, Biyokimyasal testler

PREFACE AND ACKNOWLEDGEMENTS

I also want to express my gratitude to Prof. Dr. Volkan EYÜPOĞLU, my thesis advisorr, for his tolerance, advice, and empathy.

Hanan Kareem Mohammed AL-JAF
Çankırı-2022

CONTENTS

LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees Celsius
g	Gram
m2	Square meters
mg	Milligram
mL	Milliliters
ng	Nanogram
nm	Nanometer
µg	Microgram
µL	Microliter

LIST OF ABBREVIATIONS

AMH	Anti-mullerian hormone
ART	Assisted reproductive technology
CCCT	Clomiphene citrate challenge testing
CHD	Coronary heart disease
C-RP	C-reactive protein
FSH	Follicle stimulation hormone
GAST	Gonadotropin releasing hormone agonist stimulation test
GnRH	Gonadotropin releasing hormone
HDL	High-density lipoprotein
HDL	High-density lipoprotein
IVF	In vitro fertilization
LDL	Low-density lipoprotein
LH	Luteinizing hormone
ORT	Ovarian reserve test
PCOs	Polycystic ovarian syndrome
VLDL	Very low-density lipoprotein

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1. INTRODUCTION

Infertility can be caused by both men and women, or by a mixture of both. The women's type will receive the most of our attention in this investigation. For further information about male infertility, its causes, and kinds, the reader should consult other sources. A number of causes can contribute to male infertility. Varicocele, or swelling of the veins in the testes, can have an effect on sperm quality (Dağ and Dilbaz 2015, Melo *et al.* 2015).

A flawless ovarian reserve test that precisely measures a woman's reproductive potential does not exist yet. Because of the research's inconsistent findings, evaluating the usefulness of multiple ovarian reserve tests is challenging. Data from ART cycles may be used to build models that compare the effectiveness of various tests for predicting reproductive potential. None of the ovarian reserve tests can help you anticipate whether or not you'll get pregnant (Mallikarjuna and Rajeshwari 2015, Balen *et al.* 2016).

Infertility is caused by ovulation abnormalities, which occur when you ovulate infrequently or not at all. Around one out of every four infertile couples suffer from ovulation abnormalities. The term "ovarian reserve" refers to a laboratory assessment of how many eggs a woman has available at any particular time. A woman's reproductive capabilities is determined by the amount of eggs she has remaining in her ovaries. The greater the number of eggs in a woman's egg bank, the higher her chances of becoming pregnant; on the other side, a low ovarian reserve suggests a reduced chance of being pregnant. It's also crucial to remember that ovarian reserve testing can only tell you how many eggs you have remaining, not how excellent they are (Mallikarjuna and Rajeshwari 2015, Balen *et al.* 2016).

1.1 Aim of Study

The study's major goals are to (1) determine the levels of AMH and lipid profiles, and (2) examine the impact of AMH in determining the levels of ovarian reserve in infertile women.

2. LITERATURE REVIEW

2.1 Infertility

The infertility can be caused by the women, male or a combination of both of them. İn this study, we will devote most of it on the women type. The reader should refer to other references for more information about the male infertility and it causes and types. Each of these elements is required to conceive. Ovulation is the process through which the ovaries generate and release an egg. Your doctor can assist you in determining your menstrual cycles and confirming ovulation. Unless your spouse has a history of sickness or surgery, this isn't a concern for most couples. Simple tests can be performed by your doctor to determine the health of your partner's sperm (Dağ and Dilbaz 2015).

The inability to conceive is the most common sign of infertility. There may be no further symptoms at all. Infertile women may have irregular or nonexistent menstrual cycles. To get pregnant, all of the stages during ovulation and fertilization must be completed properly. Some disorders that cause infertility in couples are present from birth, while others emerge later in life. One or both spouses may be affected by infertility. There are occasions when no reason can be discovered (Melo *et al.* 2015, Hanson *et al.* 2017).

Men's sterility may be determined by a multitude of different circumstances. Aberrant sperm production or function may arise as a result of undescended testicles, genetic flaws, medical problems such as diabetes, or infections such as chlamydia, gonorrhea, mumps, or HIV. Varicocele, which is an enlargement of the veins in the testicles, may have an impact on the quality of sperm produced (Cabry *et al.* 2014). Sperm distribution difficulties may arise from a variety of factors, including sexual concerns like premature ejaculation; genetic illnesses like cystic fibrosis; anatomical abnormalities such a testicular obstruction; and damage or injury to the reproductive organs (Khizroeva *et al.* 2019).

Overexposure to specific environmental circumstances may be caused by pesticides and other chemicals, as well as radiation. Heat exposure may raise body temperature and disrupt sperm production. Cancer and its treatments have the potential to cause harm. Sperm production may be affected as a result of cancer therapy (Mallikarjuna and Rajeshwari 2015, Balen *et al.* 2016).

Infertility in women may be caused by a number of circumstances. Problems with egg release from the ovaries are known as ovarian ovulation disorders. Hormonal issues, such as polycystic ovarian syndrome, are one of them. Ovulation issues may also be caused by hyperprolactinemia, or having too much prolactin, the hormone that causes breast milk production. Excessively high (hyperthyroidism) or overly low (hypothyroidism) thyroid hormone levels may interrupt the menstrual cycle and cause infertility. Excessive physical activity, food disorders, and cancer are all probable causes (Menon *et al.* 2015, Hanson *et al.* 2017).

In addition to cervix abnormalities, lesions in the uterus and irregularities in the uterus's shape are also considered to be uterine deformities. Uterine fibroids are benign (noncancerous) tumors that either obstruct the urethra or inhibit a fertilized egg from implanting in the uterus, resulting in infertility. Uterine cysts are most often seen in women who are trying to conceive (Winkelman *et al.* 2016).

Inflammation of the fallopian tube is a primary cause of fallopian tube injury or obstruction (salpingitis). Endometriosis, adhesions in the pelvis, or a sexually transmitted infection are all possible causes of pelvic pain. Endometriosis is a condition in which endometrial tissue develops outside of the uterus, impairing the function of the ovaries, uterus, and fallopian tubes (Begum and Hasan 2014). Primary ovarian insufficiency occurs when the ovaries cease to function and menstruation ceases before the age of 40, a condition known as menopause (early menopause). Immune system problems, numerous genetic defects such as Turner syndrome or carriers of Fragile X syndrome, radiation or chemotherapy treatment, and other factors have all been linked to early menopause, albeit the exact reason is still unclear at this time (Morgante *et al.* 2018).

The formation of pelvic adhesions, which are scar tissue bands that attach organs, may occur after a pelvic infection, appendicitis, endometriosis, or abdominal or pelvic surgery, among other things. Cancer patients are receiving treatment. Some conditions, most notably reproductive malignancies, have a significant influence on a woman's ability to reproduce. Both radiation and chemotherapy may have an effect on fertility (Lindsay and Vitrikas 2015, Barbieri 2019).

2.2 Assessment of Ovarian Reserve in Patients with Infertile

Unfortunately, at this time, there is no ideal ovarian reserve test that can precisely predict a woman's reproductive potential. It's difficult to assess the efficacy of various ovarian reserve tests because of the research' contradictory findings. Data from ART cycles may be used to create models for evaluating the efficacy of different tests for predicting reproductive potential. None of the ovarian reserve tests are appropriate for predicting pregnancy (Datta *et al.* 2016, Baheerati and Devi 2018).

Although aberrant hormonal parameters (basal or CC induced FSH and inhibin) have a good predictive value (specificity) for detecting reduced ovarian reserve, their sensitivity is limited. The CC test is more sensitive than FSH levels at rest. The most trustworthy ultrasonography measure is the antral follicle count. Ovarian reserve tests have only been utilized in a small number of studies to predict fertility prognosis in a general infertile population (Quintino-Moro *et al.* 2014).

The findings from these individuals will help researchers develop screening tools for asymptomatic instances of reduced fertility related to early ovarian aging in the general population. When compared to other ovarian reserve indicators, AMH offers a few benefits. It is the first marker to alter with age and has the least variability between and within cycles (Tanbo and Fedorcsak 2017).

2.3.1 Reproductive system in women

Many tasks are performed by the female reproductive system. The human embryo is subsequently carried to the uterus, which has grown owing to the usual hormones produced throughout the estrous period, where it is implanted and grows to full size. As soon as the fertilized egg is placed into the uterus, it may implant into the thicker uterine lining and continue to grow and develop. Unless implantation occurs, the uterine lining is lost as part of the menstrual flow. Female sex pheromones are also generated by the female reproductive system, and these hormones contribute in the continuance of the reproductive cycle in both men and women (Singh *et al.* 2016, Kahyaoglu Sut and Balkanli Kaplan 2015).

In the years after menopause, the female reproductive system progressively ceases to release the female hormones that are required for the reproductive cycle to function properly. Amenorrhea may become erratic at this time, and they may even come to an end. It is considered menopausal when a woman's menstrual periods have stopped after a year of not having them. The female reproductive anatomy is made up of both exterior and interior components that work together to form a whole (Hasanpoor-Azghdy *et al.* 2015). Women's outside reproductive structures (genitals) serve two important functions: they allow sperm to enter the body and they shield the inside genital organs from harmful microorganisms. Located on the outside of the female reproductive system, the Labia majora is one of the most essential external components. This organ helps to enclose and protect the other internal system on the exterior of the female reproductive system. When a child reaches puberty, hair begins to develop on the surface of the labia majora, which also includes perspiration and oil-secreting glands (Karaca and Unsal 2015). It is possible to purchase labia minora (little lips) in a number of different sizes and forms. In addition, they are placed immediately inside the labia majora (the opening to the lower half of the uterus) and urethra (the tube that connects the lower half of the uterus to the outside of the body) and surround the entrances to the vagina (the canal that connects the lower half of the uterus to the outside of the body) (the tube that carries urine from the bladder to the outside of the body). This skin is very sensitive, and it may become irritated and inflamed in a matter of minutes (Karaca and Unsal 2015).

The ovaries are little oval-shaped glands that create eggs on each side of the uterus. The ovaries are in charge of producing eggs as well as hormones. A developing child's corpus may easily extend to accommodate them. The fallopian tubes, which are narrow tubes connected to the upper region of the uterus, are where the ova (egg cells) travel from the ovaries to the uterus. In the majority of situations, a sperm fertilizes an egg in the fallopian tubes. The fertilized egg then makes its way to the uterus, where it attaches itself to the uterine lining (Shahraki *et al.* 2018).

There are several forms of infertility, however there are two main ones. The primary form, in which the woman did not become pregnant after a year. The secondary type, on the other hand, is when a person is re-enabled after at least one successful pregnancy. One of them is ovarian dysfunction. Ovulation abnormalities can be caused by difficulties with the hypothalamus or pituitary gland's control of reproductive hormones, or abnormalities with the ovaries. Female infertility is frequently linked to PCOS, insulin resistance, and obesity (Giviziez *et al.* 2016).

2.4 Ovarian

The female gonadsare the ovaries, which correspond to the testes in a man. A mesentery, or membrane fold, runs along the back layer of the uterus' broad ligament, holding each in place. In a woman who hasn't become pregnant, the almond-shaped ovary lies vertically against a dip on the lateral wall of the smaller pelvis called the ovarian fossa. The inner or medial surface of the fallopian tube bends downward, arching over the ovary (Bergh *et al.* 2016, Deyhoul *et al.* 2017).

Except at its hilum, where blood arteries and nerves enter the ovary and the mesentery is joined, the ovary's surface is smooth and covered with cubical cells. Under the surface, the ovary's substance is divided between an outer piece, the cortex, and an interior component, the medulla. The tunica albuginea is a thin connective tissue zone that appears immediately under the outer layer of the cortex's outer layer. The remainder of the cortex, which is linked by a thin network of fibers, is made up of stromal or framework cells, follicles, and corpora lutea (Arya and Dibb 2016). At birth and throughout childhood, they appear as many main or undeveloped ovarian follicles. Each includes a primitive ovum, or oocyte, which is covered by a single layer of flattened cells. A young girl's ovaries can contain up to 700,000 primary follicles. The majority of them decline before or after puberty (Poddar *et al.* 2014).

Each month, one or more follicles grow cyclically into mature follicles from the beginning of adolescence until menopause (except during pregnancy). The inner granulosa and the outer vascularized theca interna of the primary follicle thicken. LH, a hormone generated in the anterior lobe, helps FSH induce the release of estrogens from the growing, now fluid-filled follicle. A mature follicle ruptures in reaction to LH, releasing the egg into the peritoneal cavity and eventually the fallopian tube (Donnez *et al.* 2016). After four days, the corpus luteum becomes vascularized, and after nine days, it is fully grown. The gland produces the steroid hormone progesterone as well as certain estrogens. Luteinizing hormone is a hormone that simultaneously stimulates and maintains the function of the liver (Dağ and Dilbaz 2015, Melo *et al.* 2015). The main oocyte divides into a secondary oocyte and a first polar body as the ovarian follicle grows. The secondary oocyte, which has a diameter of 120 - 140 micrometers and is surrounded by the zona pellucida and the corona radiata, is discharged after ovulation in Figure 2.1 (Cabry *et al.* 2014, Khizroeva *et al.* 2019).

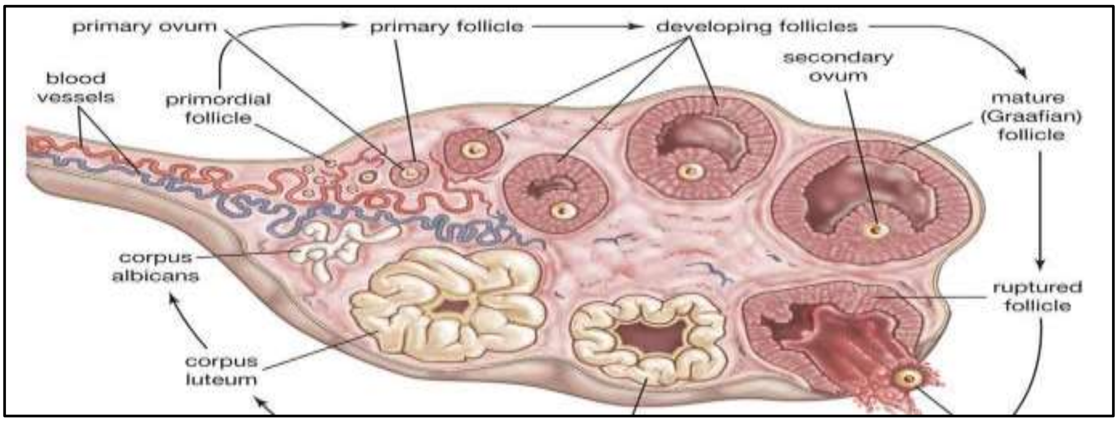

Figure 2.1 The stages of ovulation, which start with a latent primordial follicle that develops and matures before being discharged from the ovary and into the fallopian tube

2.5 Ovarian Reserve Tests

There are different types of test to check the ovarian reserve. In this research, we will discuss some of them, such as GnRH stimulation test (GAST), and Anti-Mullerian Hormone (AMH) in Figure 2.2.

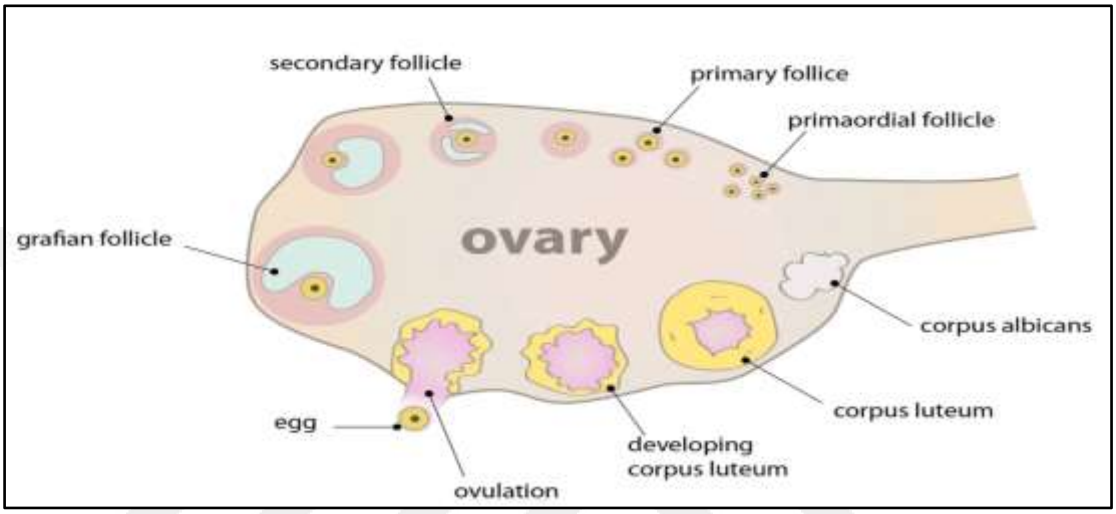

Figure 2.2 Sketch of ovarian reserve tests (Jayson *et al.* 2014)

2.5.1 Basal serum FSH level

For patients undergoing IVF, the baseline level of serum FSH is utilized as a screening test. A high day 3 baseline level of FSH is linked to a reduced pregnancy rate, according to research. Indeed, in order to maintain high clinic success rates, several Units have started employing this test to select individuals with a lesser possibility of pregnancy (Matulonis *et al.* 2016).

It's also usual practice to keep an eye on the patient until his or her FSH and estrodiol levels drop below a specific threshold, signaling that the ovary will be more sensitive to stimulation. Repeated tests for day 3 basal FSH are performed on these individuals, and the treatment cycle will begin only if the basal FSH and estradiol levels are below particular cut-off thresholds (Coward *et al.* 2015, Stewart *et al.* 2019).

According to research, people with high basal FSH levels may have a decent pregnancy rate if the level recovers to normal. However, this hasn't been weighed against the likelihood of conceiving if basal FSH levels stay high. A high baseline FSH level, on the other hand, might indicate a lack of ovarian reserve. It has also been argued that if the FSH level is high on one or two times, this may represent the state within the ovary, and that waiting for the level to alter would have no significant impact on the result (Reid *et al.* 2017, Cortez *et al.* 2018).

2.5.2 Clomid challenge test (CCCT)

The Clomid challenge is a sort of ovarian reserve test (ORT) that is occasionally used before IVF (IVF). Clomiphene citrate challenge testing, or CCCT, is another name for it. Clomid challenge tests are designed to see whether your body will react well to fertility medicines and ovarian stimulation (Gambineri *et al.* 2019). Because IVF is costly - financially, emotionally, and physically - having this test done before you start treatment might help you prevent disappointment while also saving time and money. The Clomid challenge has been questioned in terms of its ability to assess ovarian function and subsequent IVF treatment results. While some studies have shown the CCCT to be a reliable predictor of IVF success, others have found it to be equivocal or less dependable than other ORTs (Lheureux *et al.* 2019, Patch *et al.* 2015).

However, the CCCT is simply one of numerous options for evaluating ovarian reserves, and not all medical specialists feel that it is the best choice. Here's why your doctor may or might not recommend a Clomid test, how it works, and what the findings signify. A bad Clomid Challenge Test, regardless of age, might suggest various things (Walker *et al.* 2015, Doubeni *et al.* 2016):

(i) In assisted reproductive technology cycles, there is a reduced response to injectable fertility medicines.
(ii) Retrieval of fewer eggs
(iii) IVF and intrauterine insemination have a lower pregnancy rate (IUI).
(iv) An increased risk of miscarriage.

(v) A higher chance of chromosomally defective embryos.

(vi) Women with a bad clomid challenge test result may not be eligible for IVF at certain fertility clinics.

2.5.3 GnRH stimulation test (GAST)

Another dynamic ovarian reserve test that may be conducted is the gonadotropin-releasing hormone agonist stimulation test (GAST). GAST is conducted. According to study, higher E2 levels are linked to higher conception rates. After the initial injection of Lupron, many blood tests are required to monitor E2 levels, and several more are required to complete the GAST treatment. This is not an often done technique due to the invasive nature of the test and the expensive cost of the GnRH-a injection (Momenimovahed *et al.* 2019, Wang *et al.* 2019).

The LH and FSH levels are measured following an intravenous injection of gonadotropin releasing hormone to determine the state of gonadotrophin release by the pituitary (GnRH). The patient may experience transitory thirst as a result of the GnRH side effect. Abdominal or stomach discomfort, flushing (for a short period of time), headaches, lightheadedness, and nausea are all possible but less typical side effects. Itching, redness, or swelling of the skin at the injection site, skin rash, breathing difficulties, and other hypersensitivity responses to GnRH are possible but uncommon. GnRH injection has been linked to pituitary apoplexy on rare occasions (Banerjee *et al.* 2017, Torre *et al.* 2018).

2.5.4 Anti-mullerian hormone (AMH)

This test determines the quantity of AMH present in the bloodstream of a patient. It is produced in both male and female reproductive tissues. Your age and gender impact the function of AMH and whether or not your levels are normal (Lee *et al.* 2019). While a newborn is still in the womb, AMH plays an important role in the development of his or her sex organs. The child will be born with the genes that will determine whether he or she will be a boy or a girl (Jacobs *et al.* 2016).

Women's AMH levels may provide information about their fertility and ability to conceive. The test might be used to detect menstrual irregularities or to monitor the health of women with particular types of ovarian cancer in Figure 2.3 (González-Martín *et al.* 2019).

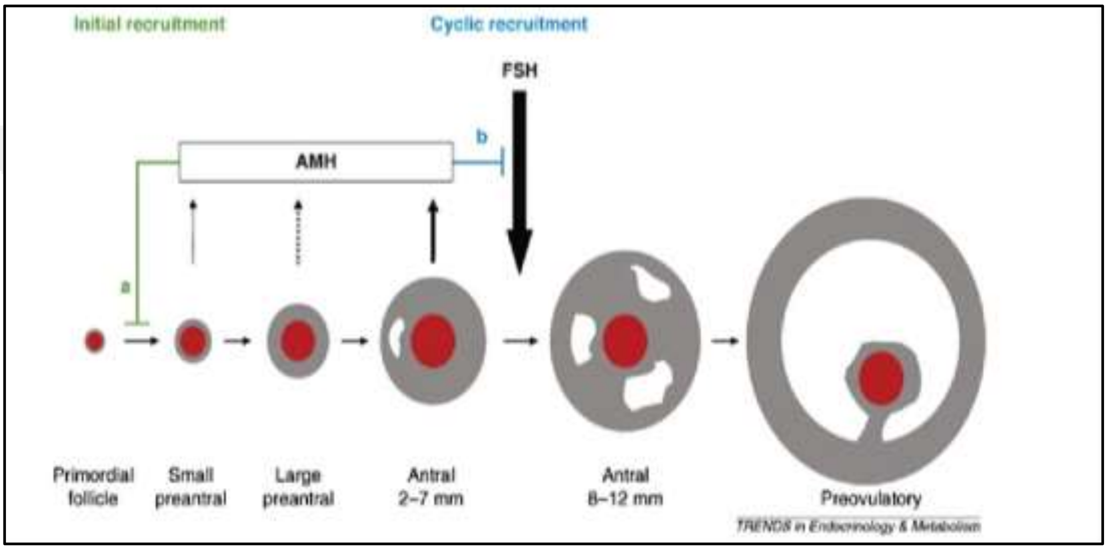

Figure 2.3 Anti-mullerian hormone (Webb and Jordan 2017)

2.6 Laboratory Assessment

The laboratory evaluation of the quantity of eggs a woman has accessible at any given moment is referred to as her "ovarian reserve." The more eggs in a woman's egg bank, the better her odds of getting pregnant; on the other hand, a low ovarian reserve indicates a lower possibility of getting pregnant. It's important to realize, too, that while ovarian reserve testing can tell you how many eggs you have left, it can't tell you how good they are (Schmid and Oehler 2014, Bowtell *et al.* 2015).

A woman's age is undoubtedly the most basic, as well as the most important, determinant in determining her reproductive capacity. However, age isn't the only factor to take into account. More thorough and sophisticated technology may be used to determine a woman's reproductive capacity (Giannouli and Stoyanova 2018). Passive testing methods do not need the measurement of hormone levels, but active testing methods must. Hormone stimulation, which is performed via injection, is employed in dynamic testing techniques. Examples of particular testing for both approaches are provided in the next section (Meinhold-Heerlein and Hauptmann 2014, Tal and Seifer 2017).

2.6.1 Lipids

A lipid is defined as a material that is insoluble in water but soluble in alcohol, ether, and chloroform, according to the description given by chemical textbooks. Lipids are essential for the proper functioning of living cells. Lipids make up the majority of the material that makes up plant and animal cells, in addition to carbohydrates and proteins (La Vecchia 2017, Kroeger and Drapkin 2017).

Cholesterol and triglycerides are two types of lipids. Lipids are easily available in the body for storage. These molecules supply energy to cells and are an important part of the cell's structure. Fatty acids, neutral fats, waxes, and steroids, among other things, make up lipides (like cortisone). Compound lipids (lipids that have formed a complex with another type of chemical component) include lipoproteins, glycolipids, and phospholipids (La Marca and Sunkara 2014, Odunsi 2017).

2.6.2 Folicle stimulation hormone FSH

The hormone folicle stimulating hormone (FSH) stimulates the ovaries to generate mature eggs. When a woman's egg production declines as she matures, the pituitary gland senses a drop in estrogen levels. The pituitary gland is told to release more FSH by the brain so that the ovaries can produce more eggs and estrogen. High FSH levels indicate that the body is striving to produce follicles. It is not necessary for younger women who have a strong egg production (Eisenhauer 2017, Van Driel *et al.* 2018).

Taking luteinizing hormone (LH) levels is also crucial in ovarian reserve evaluation, but FSH levels may be a stronger predictor of ovarian reserve. FSH levels grow more quickly than LH levels in a woman as she approaches menopause. It can be advantageous to combine FSH and LH testing and calculate the FSH: LH ratio from the findings (Cox and Liu 2014, Barani *et al.* 2021).

2.6.3 Prolactin

The level of the hormone prolactin in your blood is determined by a prolactin (PRL) test, the hormone is produced by your pituitary gland, which is located directly behind your brain. Prolactin levels rise when a woman is pregnant or has recently given birth, allowing her to make breast milk in Figure 2.4 (Barani *et al.* 2021, Amanvermez and Tosun 2016).

It could be a prolactinoma, which is a kind of pituitary tumor, if the prolactin levels are higher than usual. The tumor causes the gland to release too much prolactin. Excess prolactin can lead non-breastfeeding men and women to produce breast milk. Too much prolactin in women can potentially cause menstrual problems and infertility (the inability to get pregnant). In males, it may induce a reduction in sex drive as well as erectile problems (ED). ED refers to the inability to produce or maintain an erection, often known as impotence (Kinose *et al.* 2014, Amanvermez and Tosun 2016).

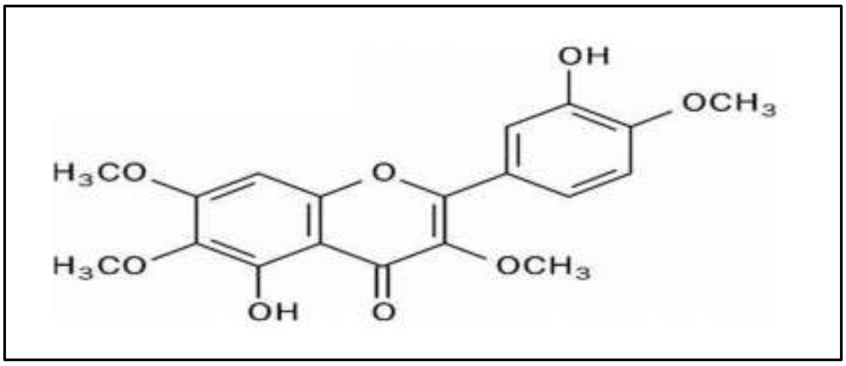

Figure 2.4 The Prolactin chemical components (Kinose *et al.* 2014)

3. MATERIALS AND METHOD

3.1 Materials

3.1.1 Equipment and apparatus

Table 3.1 shows the materials, equipment, and apparatuses used in vitro during the current study.

Table 3.1 The materials, equipment, and apparatuses

No	Material	Company	Origin
1	Centrifuge	Hettich	Germany
2	Micropipettes	Slamed	U.S.A
3	Tips (blue, yellow)	AFCO	Jordan
4	Automatic Elisa Reader	Human	Germany
5	Gel tube	AFCO	Jordan
6	Plain Tubes	AFCO	Jordan
7	ATELLIKA CH-930	SIEMENS	Germany
8	Incubator	Pasteur	France
9	Centrifuge tube	Kokusan	Japan
10	Water path	Memmert	Germany
11	CPC	Mindrey	China

3.1.2 Tests and kits

tjose were used in this study are presented in Table 3.2 with the Lot number.

Table 3.2 chemical and kits

Kits	Lot N
AMH	10995124
FSH	10995580
LH	10995635
Prolactin	10995656
C-RP	211465
FBS	211478
HbA1c	110224

3.1.3 Patients groups

In this study, 120 subjects (Control group 45 healthy women, Pregnancy 45 women, and Infertility 30 women) with varying degrees of disease activity. Which was matched for age, sex and body mass index will be evaluated. The study was conducted in Baghdad Governorate / (Al-Imameen Al-Kazimin Teaching Hospital).

3.2 Materil and Kits

3.2.1 FSH

In the next section, we provide the findings of comparison testing on the Dimension Vista System for lithium heparin, sodium heparin, and EDTA plasma samples compared to serum samples. Testing was based on CLSI/NCCLS EP9-A2 protocol. Linear regression analysis showed excellent agreement among the specimen types, it is illustrated in Figure 3.1.

3.2.2 LH

Testing was based on CLSI/NCCLS EP9-A2 protocol. Linear regression analysis showed excellent agreement among the specimen types.

3.3 Statistics

The SPSS 23 program was used to conduct statistical operations and the use of the ANOVA One Way test at P = 0.05.

4. RESULTS AND DISCUSSION

4.1 Ovarian Reserves in Patients Analysis

In recent years, much attention has been paid to the ovarian reserves in patients with infertility. Numerous prior studies have shown that low AMH increases the risk of infertility in women. Our studied included 135 people, their ages between 22- 46 year for analysis. Control group 45 people (Group AC), while Group BP involved 45 women with pregnant, while Group CI involved 45 women with infertilities.

4.2 Age

In this study, results showed the mean Age of the group BP, CI and total was (35.1 ± 5.23; 38.1 ± 6.85; 35.0 ± 6.54 year) respectively, there were different between means but there is not clinical Significant when compared to the AC group (controls group, 31.8 ± 6.44), as shown in Table 4.1 and Figure 4.1. The results of the age-related association test with AMH test indicated r= 0.432* at $P < 0.05$.

Table 4.1 Age in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	31.8 ± 6.44	23.00	41.00	0.095
Group BP	45	35.1 ± 5.23	25.00	42.00	0.095
Group CI	45	38.1 ± 6.85	29.00	48.00	0.095
Total	135	35.0 ± 6.54	23.00	48.00	0.095
a = Mean significant difference between Group (A) with Group (B). b = Mean no significant difference between Group (A) with Group (C).					

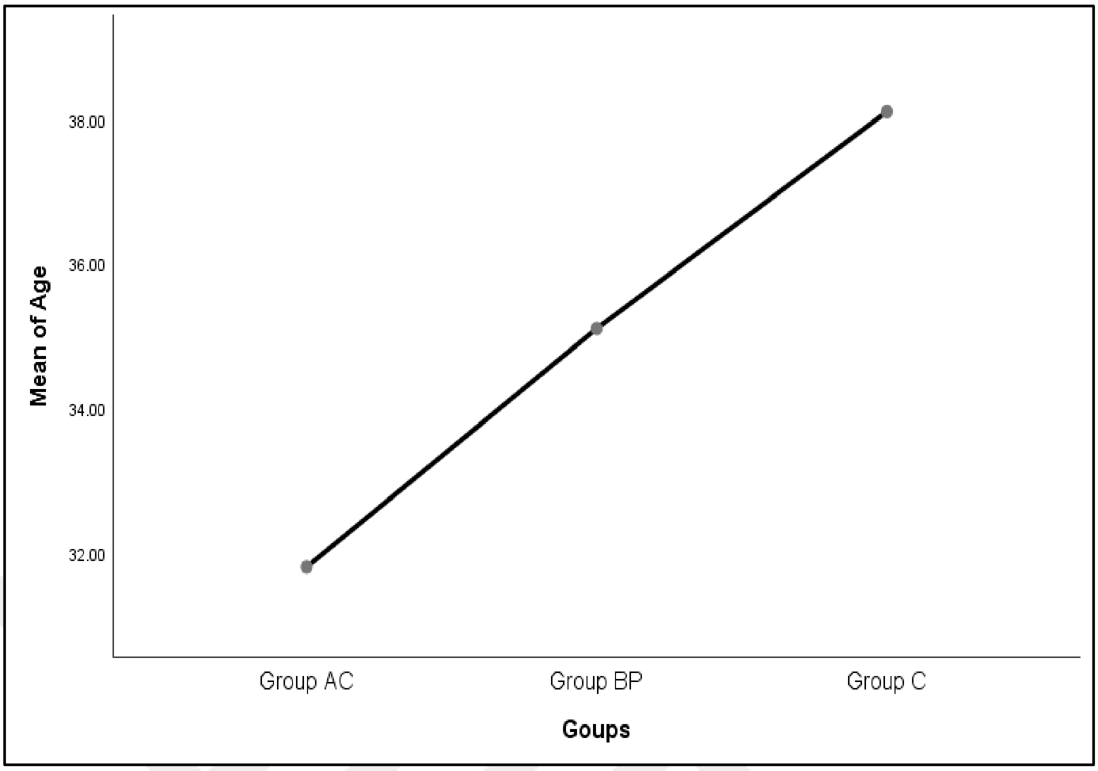

Figure 4.1 The mean age of the groups

4.3 Weight

In same study, results showed the mean weight of the group BP, CI and total was (69.6 ± 6.89; 78.1 ± 10.5; 78.1 ± 10.5 year) respectively, there were different between means but there is clinical Significant when compared to the AC group (controls group, 65.1 ± 6.11), as shown in Table 4.2 and Figure 4.2. The results of the weight-related association test with AMH test indicated r= 0.428* at $P < 0.05$.

Table 4.2 Weight (kg) in Patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	65.1 ± 6.11	57.00	78.00	0.004
Group BP	45	69.6 ± 6.89	58.00	81.00	0.004
Group CI	45	78.1 ± 10.5	59.00	91.00	0.004
Total	135	78.1 ± 10.5	57.00	91.00	0.004

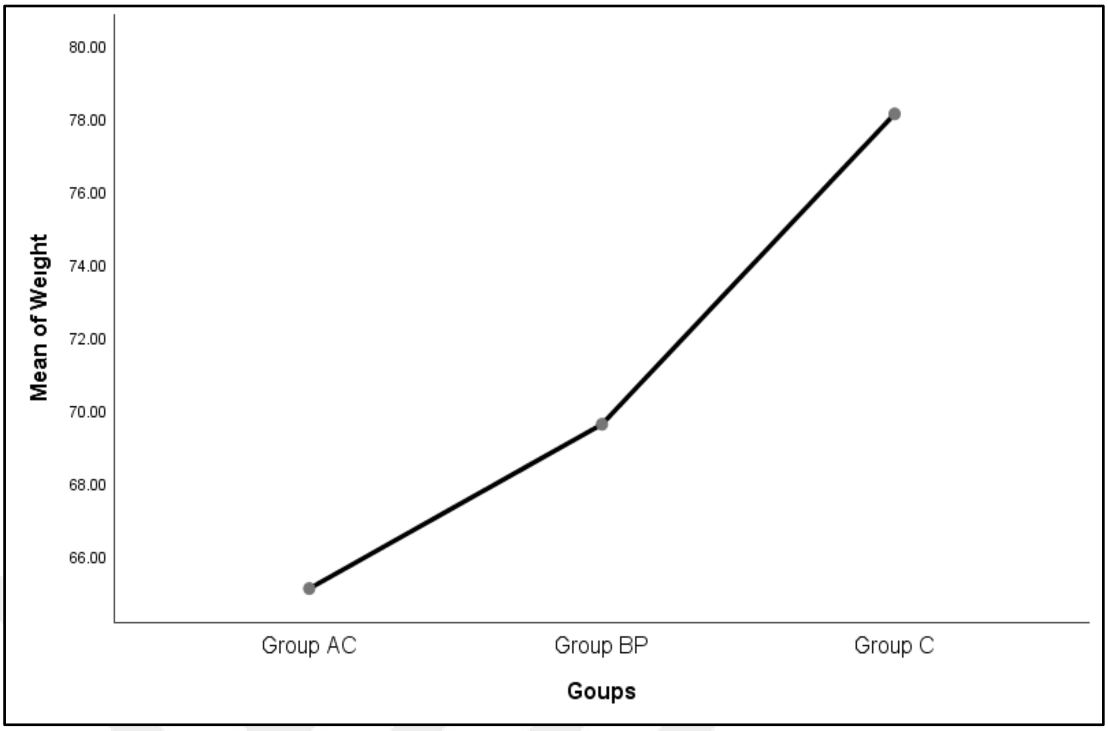

Figure 4.2 The different between weight mean groups

4.4 AMH (ng/mL)

For AMH, results showed the mean AMH of the group BP, CI and total was (1.51 ± 0.97; 2.15 ± 1.36; 4.82 ± 4.52) respectively, there were high different between means there is clinical significant when compared to the AC group (controls group, 10.8 ± 1.76), as shown in Table 4.3 and Figure 4.3.

Table 4.3 AMH in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	3.89 ± 0.76	2.64	6.65	0.027
Group BP	45	1.51 ± 0.97	0.59	3.77	0.027
Group CI	45	8.15 ± 1.36	0.65	9.75	0.027
Total	135	4.51 ± 1.03	3.44	6.72	0.027

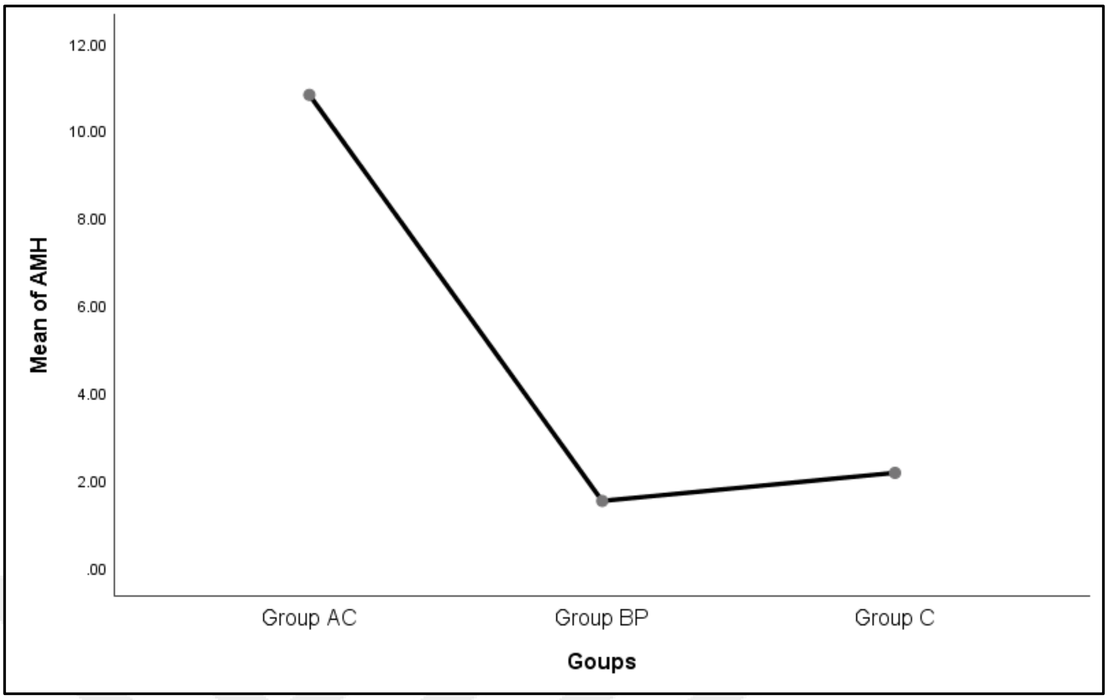

Figure 4.3 The different between AMH mean groups

4.5 FSH (mLu/mL)

The mean of FSH (%) was has a significant difference between group BP, CI and total (0.79 ± 0.21; 0.98 ± 1.01; 2.44 ± 2.48, respectively) as compare to the AC groups (5.56 ± 1.60). The results of the FSH-related association test with AMH test indicated r = 0.893** at P <0.05.

Table 4.4 FSH in Patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	5.56 ± 0.10	2.85	8.29	0.001
Group BP	45	0.79 ± 0.09	0.47	1.07	0.001
Group CI	45	0.98 ± 0.05	0.08	3.65	0.001
Total	135	2.44 ± 0.08	0.08	8.29	0.001

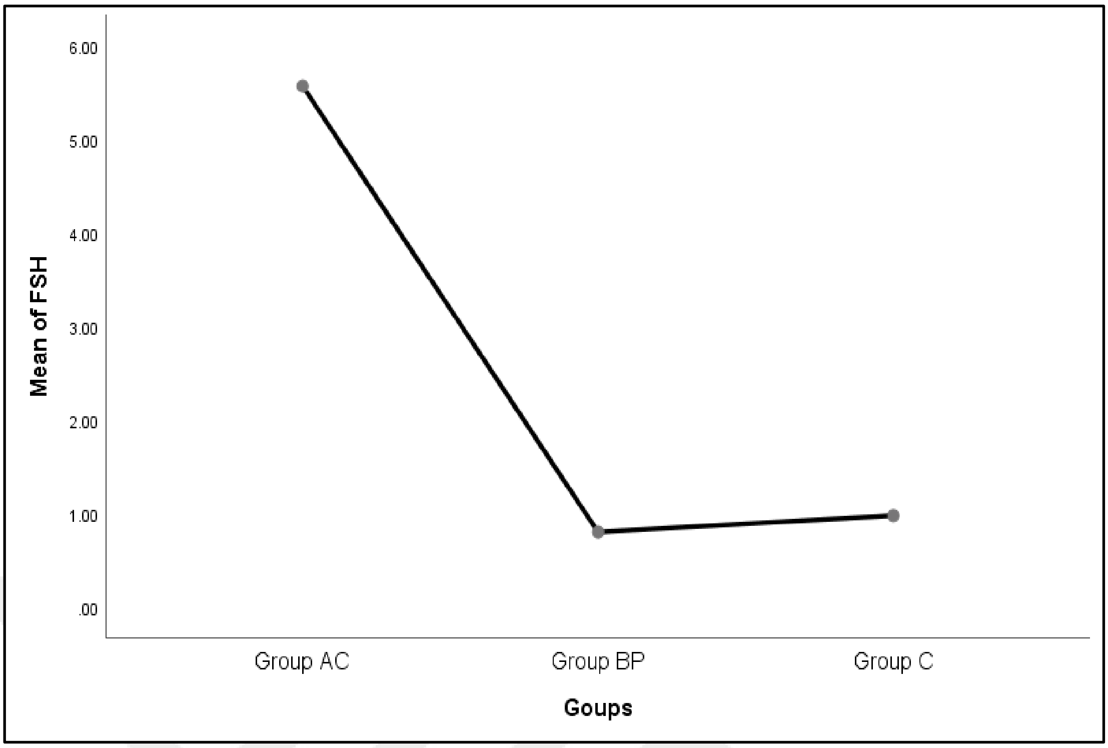

Figure 4.4 The different between FSH mean groups

4.6 LH (mLu/mL)

Also, the mean of LH (mg/dL) was has a significant difference between group BP, CI and total (4.29 ± 1.56; 3.28 ± 1.46; 4.78 ± 1.49, respectively) as compare to AC groups (6.75 ± 1.43), as shown in Table 4.5 and Figure 4.5. The results of the LH-related association test with AMH test indicated r = 0.017 at P <0.05.

Table 4.5 LH in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	6.75 ± 1.43	3.38	7.85	0.035
Group BP	45	4.29 ± 1.56	3.65	8.74	0.035
Group CI	45	3.28 ± 1.46	3.69	7.86	0.035
Total	135	4.78 ± 1.49	3.38	8.74	0.035

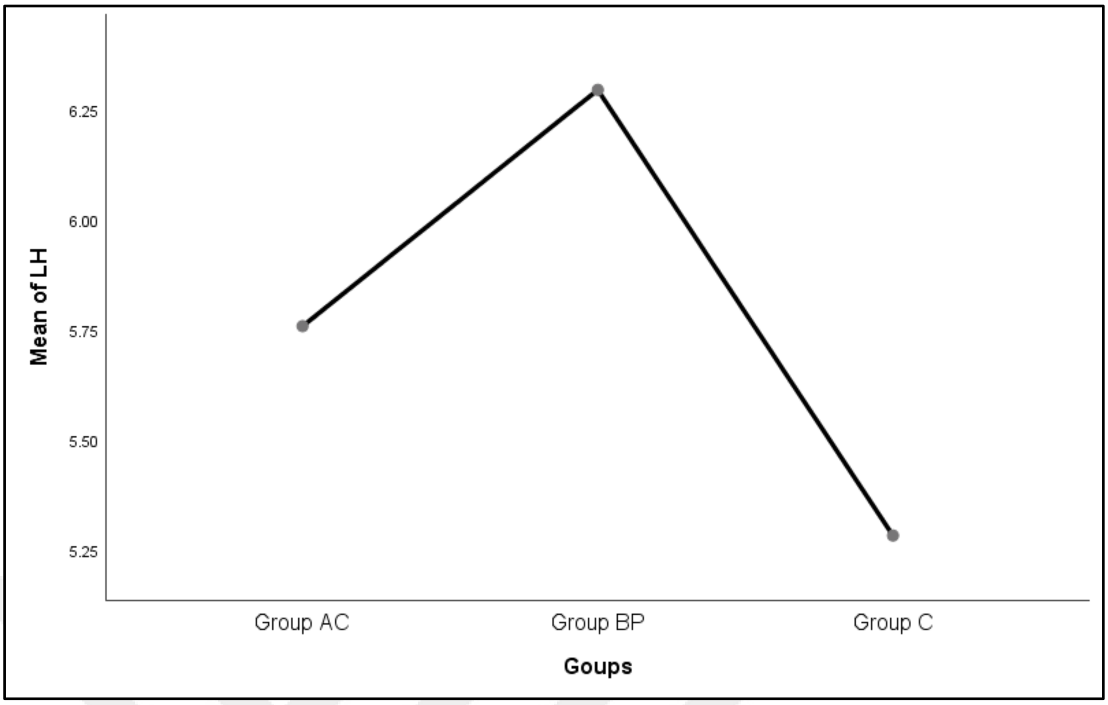

Figure 4.5 The different between LH mean groups

4.7 Prolactin (ng/mL)

The mean prolactin concentration (mg/dL) in group BP was (98.4 9.48), which was significantly higher than the mean prolactin concentration in group AC (15.9 2.70). As indicated in Table 4.6 and Figure 4.6, there was no statistically significant difference between the CI and AC groups (14.2 2.91) as compared to the AC group (15.9 2.70). The results of the prolactin-related association test with AMH test indicated r = 0.505* at P <0.05.

Table 4.6 Prolactin in patients and HCs

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	15.9 ± 2.70	11.60	19.40	0.022
Group BP	45	98.4 ± 9.48	88.40	113.00	0.022
Group CI	45	14.2 ± 2.91	9.48	18.90	0.122
Total	135	85.6 ± 5.30	36.4	50.1	

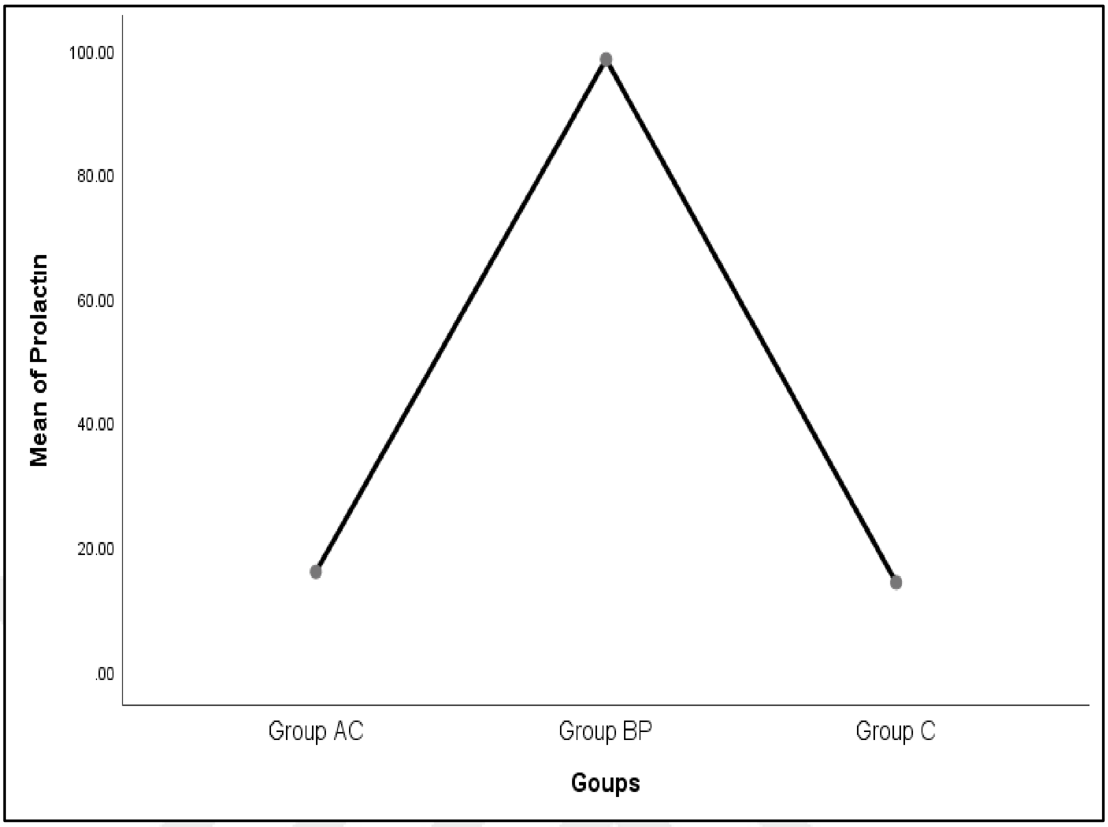

Figure 4.6 The different between Prolactin mean groups

4.8 Hb, Platelet and WBC

As well as the mean of Hb, platelet and WBC in group BP was (10.9 ± 1.43; 257.2 ± 45.3; 9.963 ± 2.633, respectively) which showed a significant difference when compared to group AC (12.1± 1.56; 211.5 ± 36.5; 8.985 ± 2.115, respectively) and also, the mean of Hb, Platelet and WBC in CI group (12.4 ± 1.54; 269.3 ± 46.7; 12.748 ± 3.107, respectively) showed a non-significant difference when compared to AC group (12.1± 1.56; 211.5 ± 36.5; 8.985 ± 2.115, respectively) as shown in the Table 4.7, Table 4.8, Table 4.9, Figure 4.7, Figure 4.8, and Figure 4.9. The results of the Hb, platelet and WBC-related association test with AMH test indicated r = 0.173; 0.122; 0.537 respectively at $P < 0.05$.

Table 4.7 Hb in patients and controls group

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	12.1± 1.56	10.40	14.70	0.090
Group BP	45	10.9 ± 1.43	8.99	13.47	0.090
Group CI	45	12.4 ± 1.54	10.40	14.80	0.090
Total	135	11.8 ± 1.59	8.99	14.80	0.090

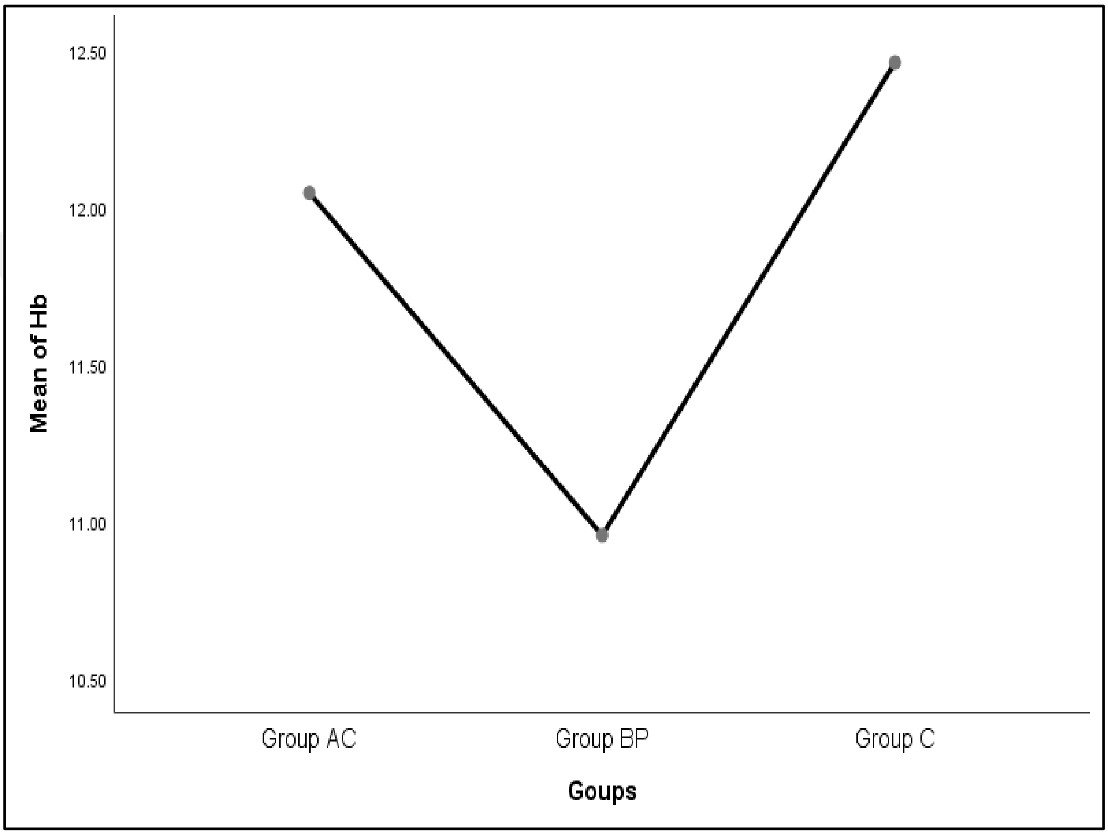

Figure 4.7 The different between Hb mean groups

Table 4.8 Platelet in patients and controls group

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	211.5 ± 36.5	158.00	274.00	0.014
Group BP	45	257.2 ± 45.3	175.00	309.00	0.014
Group CI	45	269.3 ± 46.7	147.00	319.00	0.014
Total	135	246.0 ± 48.7	147.00	319.00	0.014

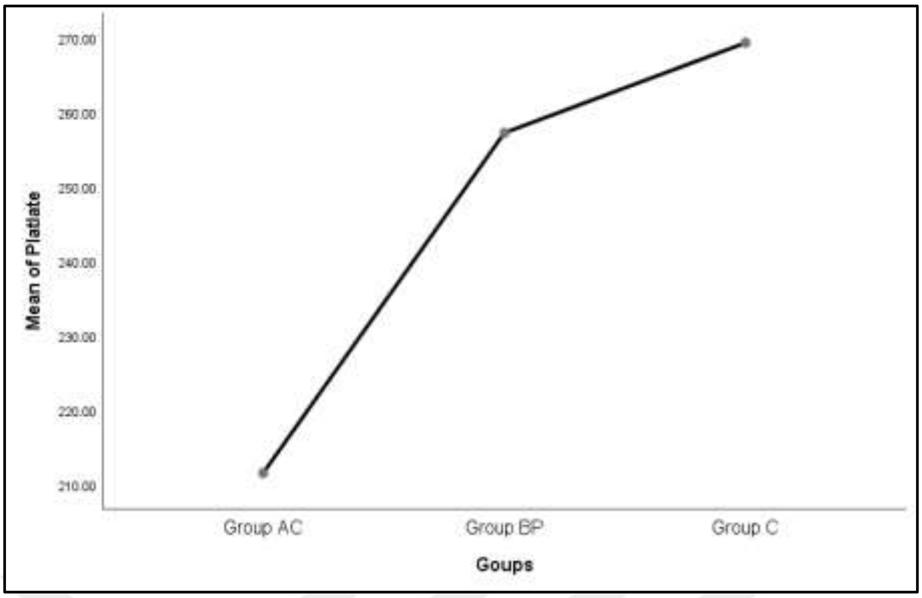

Figure 4.8 The different between platelet mean groups

Table 4.9 WBC in patients and controls group

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	8.985 ± 2.115	4.687	11.975	0.490
Group BP	45	9.963 ± 2.633	5.975	13.960	0.490
Group CI	45	12.748 ± 3.107	4.743	11.4509	0.490
Total	135	10.565 ± 5.236	4687	11.4509	0.490

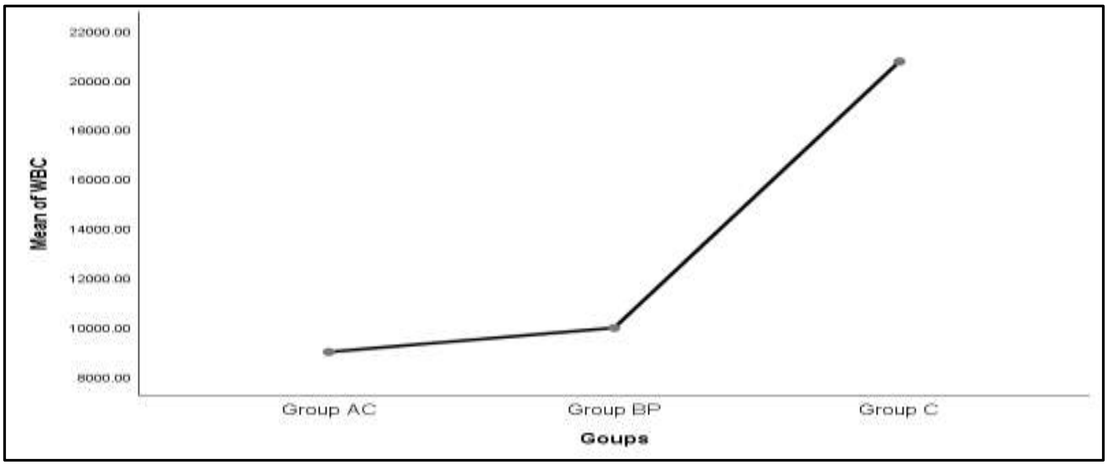

Figure 4.9 The different between WBC mean groups

4.9 C-RP (mg/L) and FSH/LH

As well as the mean of C-RP (mg/dL) in group BP was (0.70 ± 0.27) which showed a non-significant difference with group AC (0.73 ± 0.20) and CI group (0.82 ± 0.39) showed a non-significant difference when compared to AC group (0.73 ± 0.20). In same our study as well as the mean of FSH/ LH (mg/dL) in group BP was (0.13 ± 0.04) which showed a non-significant difference with group AC (0.98 ± 0.01) and CI group (0.22 ± 0.08) showed a non-significant difference when compared to AC group (0.98 ± 0.01). The findings of the C-RP-related association test with AMH test indicated r = 0.135; 0.821respectively at P < 0.05.

Table 4.10 C-RP in patients and controls group

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	0.73 ± 0.20	0.29	1.05	0.664
Group BP	45	0.70 ± 0.27	0.21	1.06	0.664
Group CI	45	0.82 ± 0.39	0.47	1.86	0.664
Total	135	0.75 ± 0.29	0.21	1.86	0.664

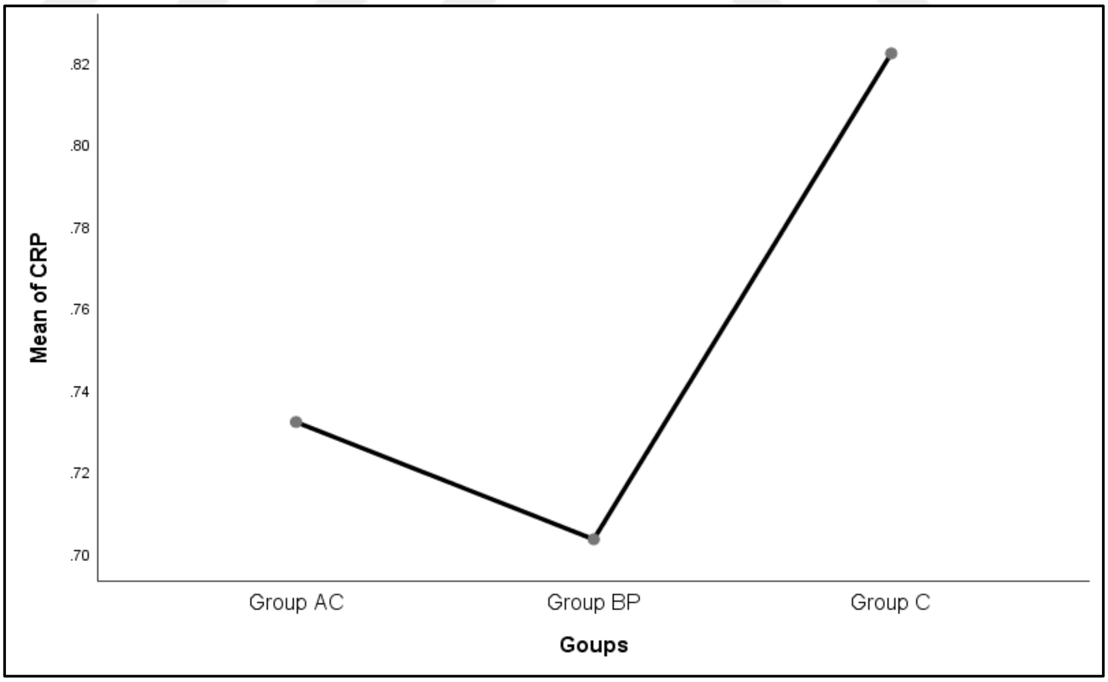

Figure 4.10 The different between C-RP mean groups

Table 4.11 FSH/ LH in patients and controls group

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	0.98 ± 0.01	0.52	1.23	0.013
Group BP	45	0.13 ± 0.04	0.07	0.19	0.013
Group CI	45	0.22 ± 0.08	0.02	0.97	0.013
Total	135	0.44 ± 0.04	0.02	1.23	0.013

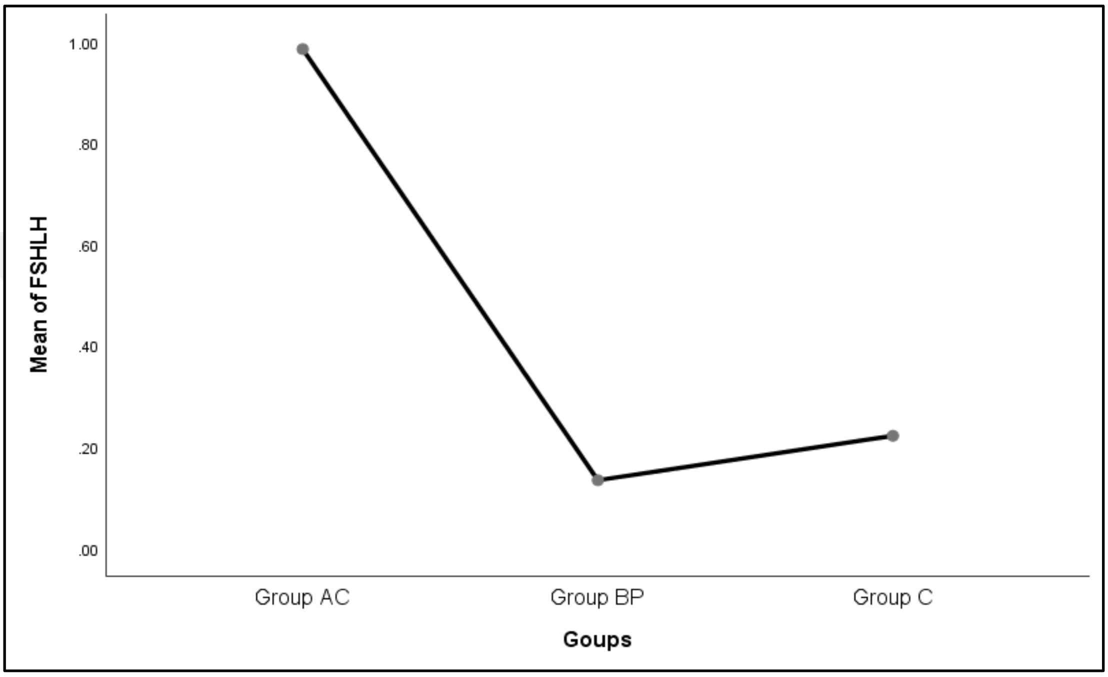

Figure 4.11 The different between FSH/LH mean groups

4.10 FBS (mg/dL)

Also the mean of FBS (mg/dL) was has a non-significant difference between group AC (91.0 ± 14.4) as compare with BP and CI groups (102.6 ± 15.6; 111.0 ± 17.1, respectively), as shown in Table 4.12 and Figure 4.12. The results of the FBS-related association test with AMH test indicated r= 0.410* 7 respectively at P <0.05.

Table 4.12 FBS in patients and controls group

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	91.0 ± 14.4	75.00	119.00	0.059
Group BP	45	102.6 ± 15.6	84.00	137.00	0.059
Group CI	45	111.0 ± 17.1	85.00	138.00	0.059
Total	135	101.5 ± 17.3	75.00	138.00	

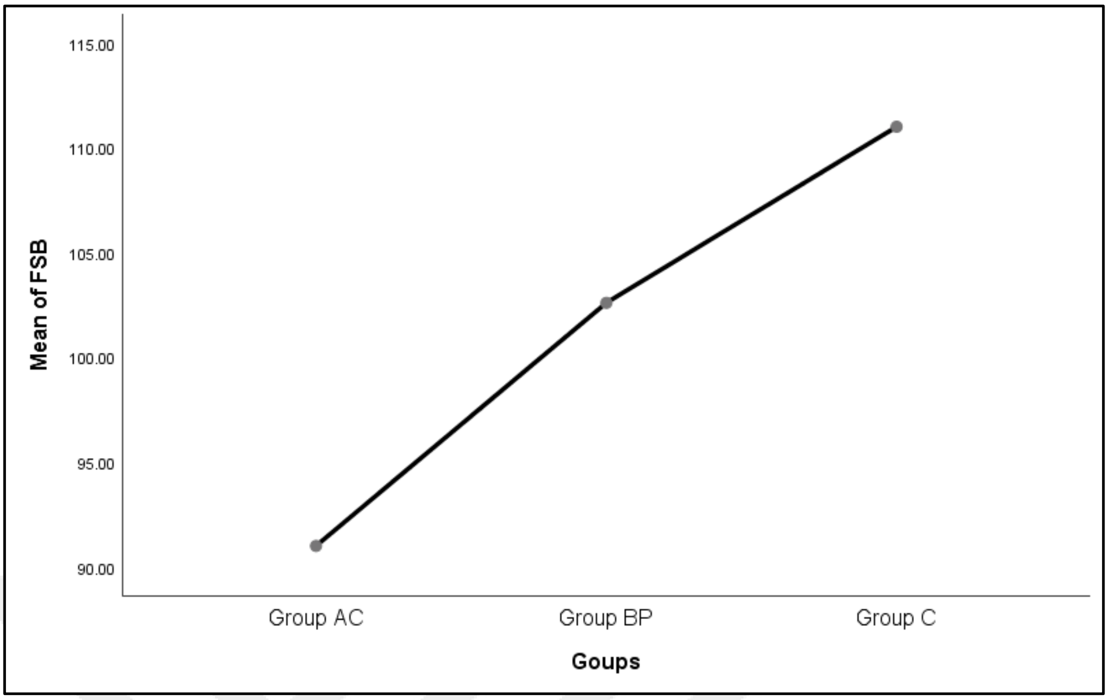

Figure 4.12 The different between FBS mean groups

4.11 HbA1c

As well as the mean of HbA1c (%) in group AC was (4.67 ± 1.01), which showed a non-significant difference with group BP (4.71 ± 1.24) at P value < 0.05 and non- significant difference with group CI (5.06 ± 1.19) at P value < 0.05 as presented in the Table 4.13 and Figure 4.13. The findings of the HbA1c-related association test with AMH test indicated r= 0.161 respectively at P <0.05.

Table 4.13 HbA1c in patients and controls group

Groups	No	mean ± SD	Minimum	Maximum	P value
Group AC	45	4.67 ±1.01	3.04	6.01	0.714
Group BP	45	4.71 ± 1.24	2.83	6.72	0.714
Group CI	45	5.06 ± 1.19	3.10	6.28	0.714
Total	135	4.82 ± 1.12	2.83	6.72	0.714

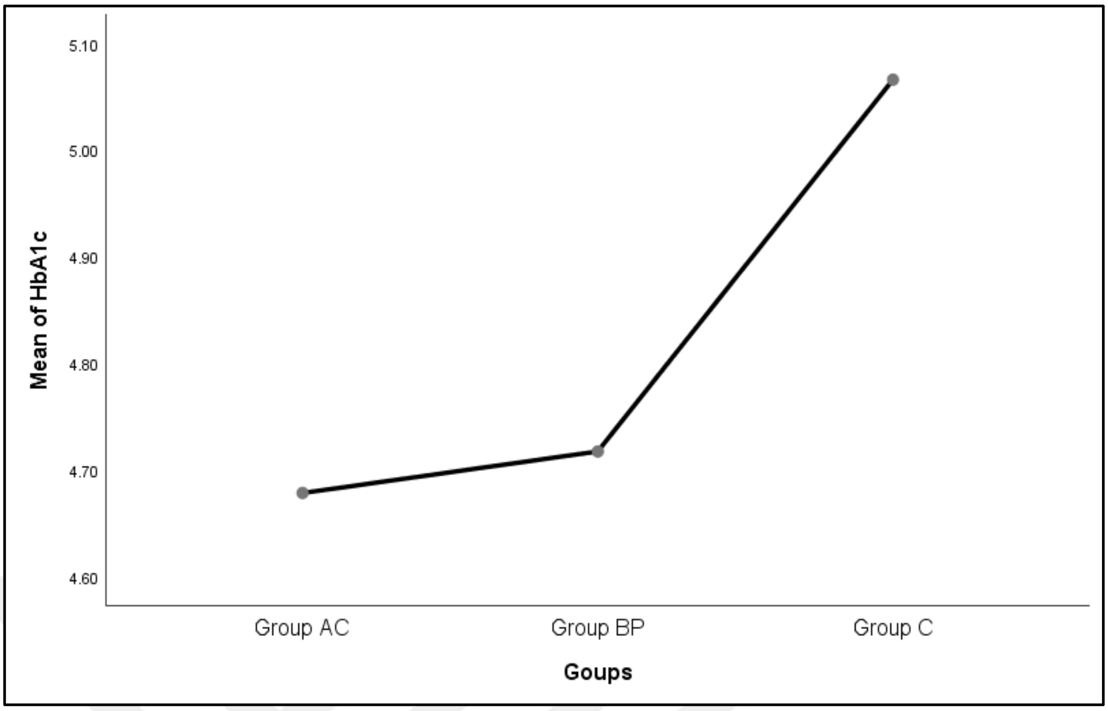

Figure 4.13 Shows the different between HbA1c mean groups

4.12 AMH/ FSH

As demonstrated in Table 4.14 and Figure 4.14, the average AMH/FSH in collective AC was (26.2 ± 7.65), a substantial difference from the mean AMH/FSH in unit BP (32.8 ± 13.6; 29.9 ± 11.9) and the mean AMH/FSH in group CI (32.8 ± 13.6; 29.9 ± 11.9), respectively. The results of the AMH/ FSH-related association test with AMH test indicated r= 0.057 at P <0.05.

Table 4.14 AMH/FSH in patients and controls group

Groups	No	mean ± SD	Minimum	Maximum	P value
Group A	45	1.79 ± 0.77	1.26	6.48	0.040
Group B	45	1.91 ± 0.12	0.61	3.56	0.040
Group C	45	2.58 ± 0.63	0.18	9.73	0.040
Total	135	2.09 ± 0.50	0.18	6.25	

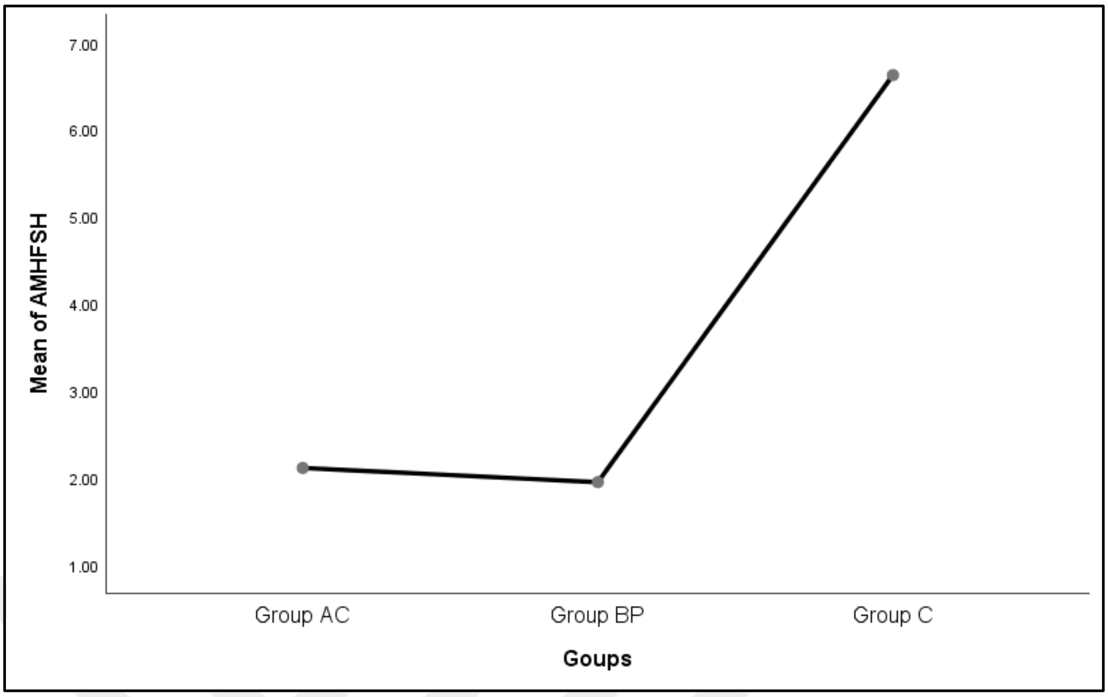

Figure 4.14 The different between AMH/FSH mean groups

4.13 Correlation between AMH and Some Biochemical Test

Table 4.15 Correlation between AMH and Some Variables in all Case

	AMH	
Variables	r	P-Value
AMH - Age (years)	0.432*	< 0.05
AMH - Weight (kg)	0.428*	< 0.05
AMH - FSH (kg/m²)	0.839**	< 0.05
AMH - LH (mg/dL)	0.017	N.S
AMH - Prolactin (%)	0.505**	0.05 >
AMH - Hb (mg/dL)	0.173	N.S
AMH - WBC (mg/dL)	0.022	N.S
AMH - Platelet (mg/dL)	0.537	N.S
AMH - C-RP (mg/dL)	0.015	N.S
AMH - FSH/LH (%)	0.821**	< 0.05
AMH - FBS (mg/dL)	0.410*	< 0.05
AMH - HbA1c (%)	0.161	N.S
AMH - AMH/FSH (mg/dL)	0.057	N.S
No asterisk : (P > 0.05);** highly significant at (P < 0.01);* Statistically significant at (P < 0.05); N.S. : non-significant		

5. CONCLUSIONS AND RECOMMENDATION

The AMH/FSH ratio test is one of the recent evidence that we have studied from an analytical point of view, in which we search for the available evidence that accurately looks at predicting the excessive response of the ovaries to stimulating ovulation, and thus assessing the stage of ovulation and the chance of pregnancy. It seems that the FSH and LH test have a good discriminatory ability to know the size of the egg in women, and also through our study, the FSH/LH test can lead to predicting the rate of ovulation, but this idea still needs more studies. Furthermore, both AMH and FSH have clinical value, with an increased test rate during the ovulation period. At relatively medium levels, where our study indicated that the closer the AMH/FSH ratio is to then 3, it is a good indicator of ovulation in women. But the problem of many treatments remains one of the problems that affect the assessment of ovulation. As for the Hb, platelet, WBC, FSB and HbA1c test, the results indicated that, uh, there are no significant statistically significant differences for our study. The age had significant statistically significant differences, as the study indicated that the older the age, the more problems, especially for women who delay marriage. AMH is a hormone that is only generated in the gonads and is involved in the control of follicular growth and development in the female reproductive system. AMH is generated in the ovary by the granulosa cells of early developing follicles, and it seems to be capable of inhibiting the onset of primordial follicle development as well as FSH-induced follicle growth in certain cases. AMH is highly expressed throughout the course of folliculogenesis, from the primary follicular stage to the antral stage. As a result, blood levels of AMH may be a good indicator of both the amount and the quality of the ovarian follicle pool throughout pregnancy (La Marca and Volpe 2006), this study agreed with our study. Intention AMH has a central role in the regulation in the stimulation of folliculogenesis and has an impact on the pace at which atresia develops. It is regarded as a reliable indicator of ovarian reserve. AMH levels are known to be associated with the number of retrieved oocytes, the number of antral follicles, pregnancy rates, and birth rates. The function of AMH as an effective predictive factor in assessing the likelihood of pregnancy has been extensively addressed in the scientific literature over the last few decades. Specifically, the purpose of this research is to assess the impact of age and AMH levels on the success rates of IVF/ICSI treatments. The sample group we studied was one of the biggest ever evaluated in a single research on this issue, and we were able to find out a lot about them (Gomez *et al.* 2016). The findings indicate that age is associated with clinical pregnancy, the number of oocytes, and the number of fresh and frozen embryos (all p 0.001). There was no link between AMH and FSH and clinical or chemical pregnancy in the entire population or age subgroups, with the exception of a substantial correlation between AFC and clinical pregnancy in the 33-37 year old group. In the 20-32 age range, AMH was shown to be linked with the number of oocytes and the number of fresh and frozen embryos. AMH was shown to be associated with both fresh and frozen embryos in this age range. In the age range 33-37 years, the hormones AMH, AFC, and FSH were shown to be linked with the number of oocytes and the number of fresh embryos. In the 38-43 age range, AMH was shown to be linked with the number of oocytes and the number of fresh embryos (Tehraninezhad *et al.* 2016). Many studies have indicated what we have come to, and research in this field is still ongoing, as the problem of estimating ovarian stock is considered one of the most important problems facing female infertility doctors.

5.1 Recommendation

1. Increasing the number of samples in the study, as well as increasing the number of study groups.
2. Emphasis on studying the effect of the AMH/FSH ratio, taking into account the type of treatments.
3. Studying the type of infertility in women and not being distracted by the study in order to be more focused.
4. Adding an E2 test in future studies in order to expand the study.

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