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Effect of Vitamin D Levels on Hormonal Profile in Infertile Women in Al-Anbar Governorate

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By

Eman Ali Gadaan

B.Sc -Department of Chemistry -Collage of Science-

University of Anbar -2014

Supervised by

Assist. Prof.

Dr. Hameed Hussein Ali

Biochemistry

Assist. Prof.

Dr. Rafal Mustafa Murshid

Gynecology and Infertility

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

يَٰٓأَيُّهَا ٱلَّذِينَ ءَامَنُوٓاْ إِذَا قِيلَ لَكُمۡ تَفَسَّحُواْ فِي ٱلۡمَجَٰلِسِ فَٱفۡسَحُواْ يَفۡسَحِ ٱللَّهُ لَكُمۡۖ وَإِذَا قِيلَ ٱنشُزُواْ فَٱنشُزُواْ يَرۡفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمۡ وَٱلَّذِينَ أُوتُواْ ٱلۡعِلۡمَ دَرَجَٰتٖۚ وَٱللَّهُ بِمَا تَعۡمَلُونَ خَبِيرٞ ﴿١١ المجادلة﴾

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Eman Ali Gadaan

Department of chemistry- College of Science

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Signature:
Name: Dr.Hameed Hussein Ali
The scientific title:Assist.Prof.
Titale:College of Science-Anbar of University
Data: / / 2021

Signature:
Name:Rafal Mustafa Murshed
The scientific title:Assist.Prof.
Titale:College of Science-Anbar of University
Data: / / 2021

Certification of the Head of the Department

In view of available recommendation, I forward this thesis for debate by the examination committee.

Head of the Department of Chemistry

Signature:

Name: **Dr. Ahmed Mishaal Mohammed**

The Scientific Title: Assist. Prof.

Title: Anbar University / College of Science

Date: / /2021

Dedication

To my companion, whose light illuminated my life ... Thanks to her, I reached this stage of success ... No matter how much I write, it will not fulfill your right, O most precious blessing that the Lord granted to me *Dear mother

To a spirit that was like heaven and a candle that illuminates my path, but it has departed from me, my God have mercy on her

*** My grandmother Nahda**

To those who provided me with encouragement and advice and was the source of my excellence

*** My sister INAS**

To the sweetness of my eyes, my children

*** (Sjaad and Zakaria)**

To my good friend who stood beside me

*** (Anwar Abd)**

To everyone who supported me in the most difficult circumstances *(Sanaa, Maysam, Asmaa)

EMAN

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Summary

Infertility is the inability to become pregnant after one year of intercourse without using contraceptives, and it is of two types (primary and secondary). Primary infertility: is the infertility that affects a woman since the beginning of her sexual life or marriage. Secondary infertility: It is infertility that affects a woman after giving birth to a child or a pregnancy that ended in a miscarriage or an ectopic pregnancy.

The aim of this study was to assess the prevalence of vitamin D deficiency among infertile women.

In this study, an interview was conducted to fill out the questionnaire, where the study was conducted on 101 women who were divided into two groups: the healthy group included 40 healthy women, and the infertile group included 61 women (30 women with type 1 infertility and 31 women with type 2 infertility) their ages ranged between 18- 45 years old) was collected from Al-Razi Hospital in Ramadi during the period from October 2020 to April 2021.

In conclusion, the levels of vitamin D in the group of infertile women of both types I and II were significantly lower compared to the fertile women (38.56 ± 1.48, 13.91 ± 1.07, 13.55 ± 1.21 ng/ml, respectively). In terms of age, we did not find a significant difference between the group of patients and healthy subjects, the mean BMI, increased significantly in the group of infertile patients compared to the healthy group, as well as the average waist-to-waist ratio, waist-to-hip and systolic blood pressure increased significantly in the group of infertile women compared to the healthy group.

Also, the levels of LH, PRL, and TT were significantly increased in the group of infertile women compared to the healthy ones, while an insignificant difference was observed in the level of TSH and FSH between the group of infertile and healthy women, while the level of AMH and E2 decreased significantly and significantly in the control group. Infertile women compared to fertile women. According to Pearson's analysis, a positive correlation was observed between vitamin D and AMH and E2, and a negative correlation was observed between vitamin D and each of: LH, PRL, TT, BMI, WHR, WWR, SBP, LH/FSH.

List of contents

List of Tables

List of Figures

List of Abbreviation's

Abbreviation	Details
AIA	Automated Immune Assay
AMH	Anti-Mullerian Hormone
BMI	Body Mass Index
CVD	Cardio Vascular Disease
DBP	Diastolic Blood pressure
DHT	Dihydrotestosterone
DHAE	Dehydroepiandrosterone
E_2	Estradiol
ELFA	Enzyme Linked Fluorescent
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
HCV	Hepatitis C Virus
HIV	Human immunodeficiency Virus
HyCosy	Hystero Contrast Sonography
IVF	In vitro fertilization
LH	Luteinizing Hormone
mRNA	Messenger Ribonucleic Acid
MRI	Magnetic Resonance Imaging
PCOS	Polycystic Ovary Syndrome
PRL	Prolactin
PTH	Parathyroid Hormone
SBP	Systolic Blood pressure
Sex Hs	Sex Hormones
SD	Standard Deviation
T_2DM	Type $_2$ Diabetes Mellitus
T-TEST	Total-testosterone
TSH	Thyroid Stimulating Hormone
VDD	Vitamin D Deficiency
VDR	Vitamin D Receptors
WHO	World Health Organization
WHR	Waist to Hip Ratio
WWR	Waist to Waist Ratio
7-DHC	7-Dihydro cholesterol

Chapter one

Introduction

and

Literature Review

Chapter One

1.1. Introduction

Infertility (a reproductive system condition) is defined by the World Health Organization (WHO) as “failure to obtain a clinical pregnancy following 12 months or more of frequent, unprotected intercourse", Southeast Countries in Asia and sub-Saharan Africa have the highest percentage prevalence of infertility [1] .

Worldwide infertility affects about 10-15% of couples that are trying to conceive. Infertility is a tragedy leads to physical, social and psychological danger and impotence in their lives. Infertility can be primary or secondary, according to the World Health Organization (WHO). Primary infertility is infertility that affects women who have never been pregnant. While, Secondary infertility is infertility that affects women who had at least one child but have failed to repeat [2].

Several factors can cause female infertility such as polycystic ovarian syndrome, premature ovarian failure, vaginal infections, endometriosis, fallopian tube obstruction, and congenital malformations and hormonal problems in affecting the uterus, and the causes of infertility can be the couple's age, profession, and socioeconomic status [3].

Infertility is one of the most important problems of the era, due to many factors. Among these factors, the effect of vitamin D (vit D) levels varies between 2% and 66.8%. Whereas, vitamin D deficiency can lead to infertility for both women and men , as well as in males it's been suggested that it has an impact on the number of sperm produced, at the same time, vit

D levels may have a role in promoting implantation in females, as vit D supplementation was found to improve IVF success rates [4] .

Vit D is a set of sterol hormones which have a function as a hormone and required for growing, conservation of bone tissue, calcium and phosphorous homeostasis [5].

Vit D can be performed in two ways: firstly, by intestinal absorption, and secondly, endogenously by a precursor of 7-hydroxyl cholesterol on the skin with adequate exposure to UV daytime [6]. Vit D is essential for the healthy functioning of the human body and there is evidence that it has a strong link to fertility issues in women [7]. Evidence suggests that vit D is associated with fertility problems in women ,it is associated with the function of the reproductive system in women and result of in vitro fertilization (IVF) [8].

Vit D plays a role in reproductive capability and several studies have found that women who are supplemented with vit D had a higher rate of pregnancy in IVF [9]. Ergocalciferol is one of two vitamin D precursors (vitamin D_2). Cholecalciferol is a kind of vitamin D that helps the body absorb calcium (vitamin D_3) [10] .

These two forms are delivered to the liver via binding to the vitamin D binding protein in human plasma. Both are hydroxylated to generate 25-hydoxy vitamin D, which is the most essential form of vit D [11] .

Presence of vitamin D receptors in Ovary (especially granulosa cells), uterus, placenta, testicle, hypothalamus, and pituitary gland, until now, and that 1,25-$(OH)_2D_3$ is the active metabolite of vit D that plays a major role in reproductive

physiology at the ovarian level, progesterone, estradiol, and estrogen production that are all stimulated by 1,25- (OH) $_2D_3$ at a rate of 13%, 9%, and 21%, respectively. As a result, there are various factors that influence vit D levels in the blood, such as age, Skin pigmentation, women's clothing, physical activity, obesity, sunlight (ultraviolet between 290-315 nm), and seasonal variations [12].

A study indicated that women with acceptable amounts of vit D in their serum were more likely to become pregnant than women with lower levels of vitamin D in their serum [13] . Vit D deficiency is one of the most common nutritional deficiency diseases worldwide. It is common among women with PCOS (about 67% -85%) and vit D affects the development of PCOS through genetic copying and hormonal modification, Influence on insulin metabolism and fertility regulation [14] .

One study showed a link between adequate vitamin D and successful fertility treatments for female monkeys with a history of infertility, and thus maintaining normal physiological levels of vitamin D is critical to increasing their normal reproductive capacity [15].

Vit D deficiency affects almost half of the world's population, with levels of vit D in the blood below 20 ng/ml. [16]. Vit D insufficiency is a worldwide health problem burden due to its high prevalence all over the world. East and South Asian countries have the highest rates of vit D insufficiency despite the abundance of sunlight, one study also showed that taking vit D supplements leads to improved menstrual regularity, restoration of ovulation, and a decrease Insulin resistance in PCOS patients [17].

Understanding the influence of vit D insufficiency on reproductive capability in women with infertility begins with a thorough examination of the vit D [18]. The rate of vit D deficiency among infertile women is a cause for concern, according to a lot of studies [19]. Vit D deficiency was shown to be considerably greater in infertility patients than controls in a research in Saudi Arabia (59.0 % versus 40.4 %) [20].

Literature review

1.2. Vitamin D

Vitamin D (vit D) is a fat-soluble vitamin and steroid hormone that is essential for calcium homeostasis and bone health, it has multi-directive roles in target tissues outside the skeleton, such as the immune system, cardiovascular and pancreatic endocrine cells, muscle and adipose tissue, and it regulates numerous genes (3 % of the human genome) involved in cell differentiation and cell cycle management [21].

The main source of vit D is daily exposure to sunlight, there are 2 types of vit D forms: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), both of these bind to the vitamin D-binding protein in human plasma and transport it to the liver, where it is hydroxylated to generate 25-hydroxyvitamin D, cholecalciferol is formed in Human skin from 7-dihydrocholesterol (7DHC) [22]. UV Radiation converts 7-DHC into provitamin D_3 and it is fast converted to D_3 [23]. Green plants, mushrooms, fish fat, and cod Liver oil are a rich source of ergocalciferol vit D that is provided either by ultraviolet rays to the skin or from a diet that is biologically inactive and requires hydroxylase in the liver and kidneys to produce its activity form-1,25 dihydroxy vitamin D or calcitriol [13].

Adolf Windaus received the Nobel Prize in Chemistry in 1928 for his researches on the structure of the sterols and their association with vitamins. He was the first person to receive a prize introducing vitamins, the vitamin was vit D [24] .

Vit D has a hormone-like function, taken either via endogenous synthesis by 7-dehydrocholesterol in the skin from direct daylight exposure or from food [25]. It is assigned as a hormone which acts through nuclear receptors (VDR) present in various organs such as the bone, kidneys, intestine, brain, immune system, and most different body parts [26]. In many studies, vitamin deficiency was noted with obesity [27]. When vit D is less than 20 ng/ml, it is called deficient, insufficient when 21-29 ng/ml, and full when more than 30 ng/ml [28].

1.2.1 Source of Vitamin D

The spontaneous synthesis of vit D by sunlight exposure, particularly UV radiation, is the primary source of vit D (290-315 nm) [29] .When the human skin is exposed to UV radiation, it produces vit D [30].Vitamin D_3 is abundant in fish and fish products, particularly salmon [31].

Cow's milk and plant-based milk alternatives, as well as many morning cereals, are fortified with vit D in the United States and other countries [32]. Moreover, vit D can be obtained additionally throughout the diet. However, there are just a few natural food sources – meat, eggs, fatty fish, and whole dairy products [33]. It is recommended to be exposed to the sun for a period of (10 - 20 min), depends on different factors like latitude, seasons, and skin pigmentation for all individuals [34].

Other factors that lead to insufficient sun exposure are lifestyle factors such as long working hours indoors [35]. A lot of factors that lead to vit D deficiency are low intake among foods high in vitamin D such as milk and fortified foods, the tendency to reduce the high diet

in fats, which in turn leads to a decrease in vit D intake, use of sunscreen, insufficiency in exposure to sunlight [36].

1.2.2. Types of Vitamin D

1) Vitamin D_1

Vit D1 is made up of an equal mix of ergocalciferol (vit D_2) and lumisterol, a steroid molecule formed when ergosterol is exposed to ultraviolet light. It is no longer considered a ‘type' of vit D because it is not a pure chemical [37].

2) Vitamin D_2 (Ergocalciferol)

Plants, fungi, and small invertebrates generate vit D_2 after being exposed to UV light, but it is not found in vertebrates. Vit D_2 has a double bond and a methyl group on its side chain, which distinguishes it from cholecalciferol. It is a "vegan" vit D that is typically added to bread, milk, and other food products to strengthen them. Some experts, however, question its bioavailability and effectiveness because it is not the form created by the human body and it has different by-products than vitamin D_3. Researches comparing the two forms and their effects on the body have been conducted [38].

3) Vitamin D_3 (Cholecalciferol)

When our skin is exposed to UV radiation, the vitamin cholecalciferol is created. The liver converts it to calcifediol, which is a prohormone, after it is generated. The kidneys then convert it to

calcitriol, Vitamin D's active form, which is a steroid hormone that boosts calcium absorption in the gut. Vit D_3 is synthesized and used in the food and supplement industries and it's usually made by irradiating lanolin (a fatty material found in sheep's wool) using ultraviolet light for this purpose [39].

4) Vitamin D_4 (22-dihydroergocalciferol)

When exposed to UV radiation, certain varieties of mushrooms such as portabella, oyster, and maitake mushrooms, produce this newly found vitamin because it is not found in humans and little is known about its functions or consequences [40].

5) Vitamin D_5 (Sit calciferol)

Sit calciferol is a pheromone and is related closely to vit D_3. It is being researched for its efficacy in preventing cancerous growth of cells vit D is thought to inhibit the growth of tumor and consequently prevent cancer [37].

1.2.3 Metabolism of Vitamin D

Vit D is a steroid hormone that controls the musculoskeletal system and is involved in a variety of biological functions via the vitamin D receptor (VDR) [41] . It exists in two main forms, D_2 (ergocalciferol) produced by ergosterol upon irradiation in plants and Fungi and D_3 (cholecalciferol) that are produced by 7-dihydrocholesterol on exposure to radiation in the skin [42] .Vit D is primarily carried in the bloodstream by the vit D binding protein (DBP), a serum glycoprotein released by the liver [23].

Vit D is physiologically inactive and in the liver is hydroxy bonded with 25 hydroxy vitamin D (25 (OH) D_3) because this isoform is the

primary metabolite of vit D and better reflects the quantity of vit D from all sources. [43]. Its half-life is just that few hours [44].

1, 25 $[OH]_2D$ ligand binds to vit D receptors (VDRs) with high affinity, increasing calcium and phosphorus absorption in the intestine. Vit D is also involved in bone formation, resorption, mineralization, and the maintenance of neuromuscular function and it also regulates bone metabolism by activating the VDRs found in osteoblasts, releasing biochemicals [45] . Vit D receptors (VDR) are located in nearly all human cells and tissues [46]. More than 3% of the human genome is regulated, including those genes that are vital to glucose metabolism [47].

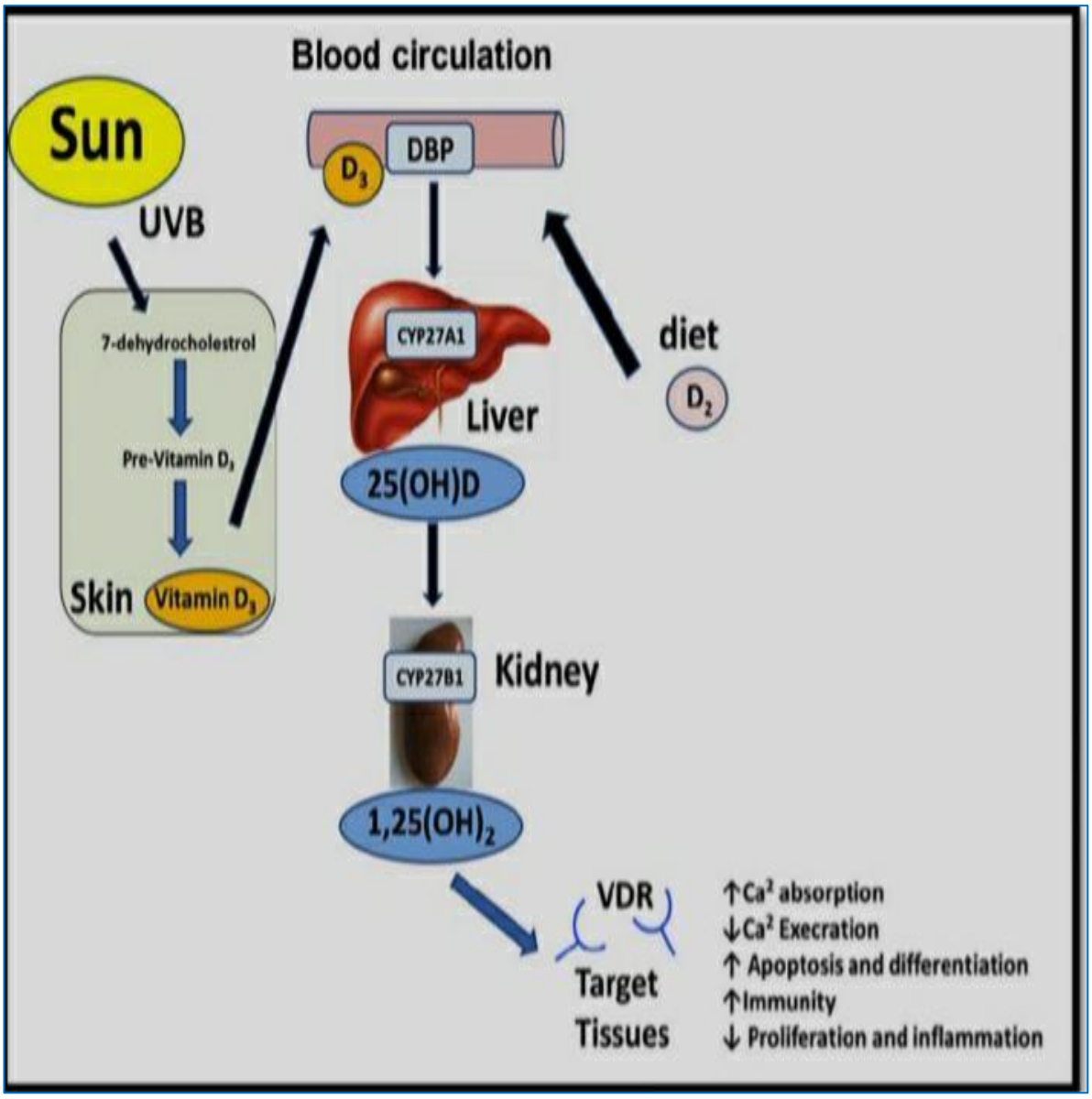


Figure (1.1) Vitamin D metabolism [48] .

The polymorphism in the VDR genes in humans impacts vit D biological activity and is linked to the occurrence of numerous diseases and autoimmune disorders, including osteoporosis, cancer, cardiovascular disease, diabetes, multiple sclerosis, and Parkinson's disease [49].

1.2.4 Functions of Vitamin D in the body

Vit D is an important steroid hormone that is used in cell proliferation and plays a role in differentiation and apoptosis [50] .Vit D

has been demonstrated to improve implantation rates and clinical pregnancy rates in patients [51].

New studies has also shown that vitamin D_3 is linked to different types .Results such as rickets and osteomalacia are also a potential influencing factor in the pathogenesis of many non-structural, cancer, cardiovascular disease, immunological illnesses, and pregnancy complications, as well as the vit D receptor (VDR)is represented everywhere in all cells throughout the body [52].

Vit D insufficiency has been linked to heart disease, infection, autoimmune illness, cancer, obesity, osteoporosis, rheumatoid arthritis, inflammatory bowel disease, and type 1 diabetes, among other conditions [53] . Moreover, lower grade of vitamin D have also been linked to autoimmune thyroid disease [54].

Vit D effects on innate and adaptive immunity, as well as having an immunological effect on autoimmune diseases and cancers, according to recent data. Study has also indicated a link between vit D deficiency, autoimmune thyroid disorders, and thyroid cancer [55]. Vit D insufficiency has been linked to the development of musculoskeletal illnesses, type 1 and type 2 diabetes, male hypogonadism, cancer, autism, dementia, and cardiovascular disease in a number of animal and human studies and also associated with obesity, poor menstruation, hirsutism, and hyperandrogenism, according to in vitro studies [56].

Vit D has also been linked to male reproductive function, such as sperm quality, motility, and androgen status [57].

Males with severe vit D deficiency (10 ng/mL) have a reduced incidence of motile sperms [42].

Vit D is essential for brain health and neurological development, and its deficiency has been linked to schizophrenia [58]. It has a role in reducing the infection of Coronavirus disease (COVID-19), as it has already been shown to protect against difficult respiratory infections, we discovered a link between vit D levels and COVID-19 instances, especially deaths from this infection. The most vulnerable to infection with COVID-19 are the elderly, most of whom suffer from vitamin D deficiency [59] .It has a role in immunity [60] .In addition, vitamin D levels have been linked to skin conditions such as psoriasis [61] .

It has importance in bone physiology such as rickets, primary and secondary osteoporosis, as well as bone enlargement and muscle pain [62]. Also in diseases of the heart and blood vessels [63].

1.2.5 The relationship of Vitamin D to diseases

Low levels of vit D have been linked to insulin resistance, irregular ovulation and menstruation, reduced pregnancy success rates, hirsutism, hyperandrogenism, obesity, and greater risk factors for heart disease and vascular disease [64]. Numerous researchers have shown that vit D insufficiency causes a lot of skeletal disorders such as rickets and osteoporosis, as well as non-skeletal disorders, such as immune system disorders [65].

Vit D deficiency also leads to the development of various endocrine disorders Such as adrenal insufficiency [66]. Additionally ,it is possibly involved in the pathogenesis of some autoimmune disorders such as erythematous lupus, inflammatory bowel disease [67]. In addition, numerous studies have linked vitamin D insufficiency to infection,

obesity, and osteoporosis [68]. A study discovered a link between vitamin D insufficiency and autoimmune disorders [69].

Thyroid immunological disorders including Hashimoto's thyroiditis have also been linked to vitamin D deficiency in recent studies [70] .Vit D levels have been linked to reproductive success rates after ovulation induction [71]. Vit D insufficiency is more common in endometriosis patients [72]. Vit D insufficiency was linked to muscle weakness and performance in a large study of older persons living in the community [73].

Vit D deficiency is widespread in women with PCOS and infertility, and there is no link between vit D deficit and metabolic syndrome [17] . Vit D deficiency is common in inflammatory bowel illness [74] . Vit D insufficiency has been linked to an increased risk of cardiovascular disease and high blood pressure in numerous studies [75]. In addition, because vit D is an important ingredient for bone health, it has an impact on the risk of respiratory disorders, poor pregnancy outcomes, and chronic diseases later in life [76].

One study found a link between vitamin D deficiency and an increased risk of many infectious diseases such as sepsis, pneumonia, influenza , and the type of HIV and hepatitis C virus (HCV) [77].

1.3 Infertility and Vitamin D

The World Health Organization (WHO) has clinically defined infertility as a reproductive system disorder characterized by failure to obtain a clinical pregnancy after 12 months or more of having

regular, unprotected intercourse [78]. Vit D is an essential component of steroid formation, the ovaries, and the uterus [79]. Up to 85 percent of vitamin D deficiency appears in PCOS-affected women and this deficiency is also linked to a variety of symptoms such as menstrual defects and infertility [80].

According to recent study, vit D has a vital role in a woman's fertility by enhancing the synthesis of two main reproductive hormones, namely progesterone and estrogen [81]. According to approximately (10-25) percent of couples are concerned about infertility and dysfunction, affecting about (60-80) million couples worldwide. Female infertility can be caused by many causes including vitamin D deficiency [82] .

One study in women have indicated an association of vit D deficiency with PCOS, endometriosis, uterine fibroids, and premature ovaries failure [83] .Vit D receptors and metabolic enzymes, 1-α-hydroxylase and 24-hydroxylase are found in normal endometriosis as well as ectopic endometriosis in women with endometriosis [84] .

Vit D insufficiency has been linked to the formation of uterine fibroids, with researchers finding that adequate levels of 25 hydroxyvitamin D are linked to a lower chance of acquiring fibroids in both black and white people [85]. Women with higher levels of 25 (OH) D are less likely to develop endometriosis, according to several studies [86] . Miscarriage rates are linked to low vit D levels in the blood [87] . Also one study has found that women with vit D insufficiency had a lower clinical pregnancy rate than those with normal vit D levels [88].

1.4 Infertility

Infertility is defined as the inability to conceive after a year of unprotected sexual activity that affects up to 48.5 million people all over the world [89] .

According to a WHO task force on the diagnosis and treatment of infertility, the prevalence of infertility varies greatly depending on ethnic origin with an estimated one in every six couples having difficulty conceiving. A study of 8,500 infertile couples in developed nations found that female infertility accounts for 37% of infertility, while male and female factors together account for 35% of infertility causes, ovulation disorders (25 %), endometriosis (15 %), pelvic adhesions (12 %), the trumpet blockage (11 %), various ductal abnormalities (11 %), and hyperprolactinemia are the most prevalent causes of infertility in women (7%) [90] .

There are many studies on the role of psychological factors in the causes of infertility as it has a significant detrimental influence on both men and women's psychological and emotional well-being. Many studies have found that the inability to have a biological child is viewed as a personal tragedy and described as a stressful event for both

spouses [91]. Obesity, infertility, hair growth, and other issues are all linked to PCOS .Infertility and weight problems are an independent factor that aggravates infertility in females with PCOS, diminishes the effectiveness of infertility treatment, and raises the risk of miscarriage [92] .

Infertility is an emotional stress factor for a woman which negatively affects her mental health, family relationships, and quality of life. It is believed that the central mental cause of infertility in women is a violation of her femininity and the impossibility of pregnancy with the

presence of Psychosexual disorders [93] .The prevalence of infertility has been estimated among women of childbearing age trying to conceive of 25.0% and this percentage increases with age [94].

The reproductive age is a major factor affecting prognosis and treatment outcomes [95] .In addition to diseases such as T2DM, CVD, and cancer, there is also a strong association between obesity and infertility[96].

1.4.1 Type of infertility

1.4.1.1. Primary infertility:

Primary infertility is infertility that affects women who have never been pregnant [2].

1.4.1.2. Secondary infertility:

Secondary infertility is infertility that affects women who have had at least one child but have failed to repeat [2].

1.5. Diagnosis of infertility

The main causes of poor fertility in women are Ovulation defect, pelvic disorders, many factors of male infertility and female infertility. 15% of couples have more than one cause and in nearly 20% of couples the causes are unknown, this is classified as unexplained infertility [97]. Female infertility diagnostic work is a multimodal approach that identifies the organic causes of infertility and MRI is the most accurate imaging technique for diagnosing the main organic causes of infertility such as endometriosis, adenomyosis, leiomyoma, or congenital anomalies of the reproductive system. Fallopian tube obstruction is the most common cause of infertility that accounting for

25-40% of cases[98]. Diagnosis and treatment of female infertility takes place following steps:[99,100].

A-Early diagnosis by observing disturbances in the menstrual cycle, increasing hair on the body or face, and knowing the shape of the ovaries through ultrasound.

B-Laboratory analysis of ovarian and pituitary sex hormones for testosterone, FSH, luteinizing hormone (LH), and milk hormone PRL.

C - Most patients suffer from metabolic disorders, especially obesity, so losing excess weight, which helps in hormone regulation and leads to pregnancy, must be continued.

D - Surgical treatment of polycystic ovaries is used in case of failure in drug therapy.

Several studies have found that 80% of infertile women have excess androgens including hirsutism, acne, male pattern baldness, and elevated total testosterone. [101]. Impaired ovulation is a major cause of female infertility, as one study confirmed that biosensor can improve the standard diagnostic procedures used to determine ovulation impairment by monitoring temperature [102] . A study found a potential method for diagnosing tubal obstruction is TVS 4D - HyCoSy, which is a very useful technique in analyzing the potential causes of female infertility and is considered a safe, simple, non-invasive, reproducible, and cost-effective method [103] .

1.6. Hormone's profile

A hormone is a chemical messenger that originates from living cells (endocrine glands) that travels to the bloodstream through the

target tissue and controls special biochemical activities depending on the type of hormone secreted [101]. Hormones are one of the causes of ovulation failure, as hormonal infertility is the most common, as it is associated with complex hormone balance and treatment, and disorders in the hypothalamus or pituitary gland lead to hormonal infertility [104] . Hormones play a significant influence in human and mating behavior. The levels of ovarian hormones in women (mainly progesterone and estradiol) are not constant as they fluctuate throughout the menstrual cycle and hormone levels are influenced by many factors including genetics, developmental conditions, body size, shape, and lifestyle during adulthood [105].

Low calcium intake was independently associated with greater testosterone levels in the blood in women with PCOS [106]. Vitamin D influences estrogen biosynthesis by direct regulation of the aromatase gene and maintenance of extracellular calcium homeostasis. Vit D receptors play a crucial role in the production of the hormone estrogen in the ovaries [72].

Vit D is active in controlling many hormones such as anti-Muller hormones, follicle hormones, estradiol (a form of estrogen), and progesterone, all of which have a role in fertility [107]. In a study by Daniel Kohr in May 2018,he clarified the role of vitamin D with a combination of reproductive and endocrine outcomes such as estrogen concentration, androgen bioavailability, physical symptoms and menstruation [108].

1.6.1. Luteinizing Hormone (LH)

Luteinizing hormone (LH) is a glycoprotein secreted by reproductive cells in response to follicle-stimulating hormone

activation in the pituitary gland (anterior pituitary), LH belongs to a neural pathway formed by the pituitary gland and drives Leydig cells from the testes to generate testosterone. While in women, it prompts the ovaries to produce steroid hormones [109]. Furthermore, it aids in the regulation of the female menstrual cycle as well as the implantation and ovulation of eggs in the uterus [110].

The level of luteinizing hormone is vital not only in the ovulation process, but also in the maintenance of luteal function over the first two weeks and LH helps theca cells in the ovary that produce androgens and hormone precursors for estradiol synthesis [111].

The half-life of LH is 60 minutes since it is only needed for a short period of time throughout the reproductive cycle, just before ovulation[112].

1.6.2. Follicle Stimulating Hormone (FSH)

FSH is a glycoprotein released and produced by basophilic gonadotropin cells of the anterior pituitary gland that governs puberty maturation and reproductive processes in the human body as well as growth and development, FSH is identical to LH in terms of reproduction [113].

It's a glycoprotein made up of two polypeptide units (alpha and beta), with the alpha unit containing 92 amino acids and the beta unit containing 111 amino acids. Its biological role and immune properties depend on beta units, secreted by the anterior pituitary cells. It is measured at a specific time during a woman’s menstrual cycle, usually between the second and fourth days of menstruation, Its excretion during the menstrual cycle reaches its peak on day 14 of the cycle, and then begins to decline in the event of ovulation [114].

The process starts with pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus in the pituitary-portal venous system, where it regulates luteinizing hormone (LH) follicle activation synthesis FSH in the anterior pituitary gland with its release in rotation LH and FSH stimulate follicle growth, corpus luteum formation, ovulation, and coordinated secretion [115]. An increase in estrogen leads to an increase in the concentration of progesterone and thus slows down the secretion of FSH, as is the case for example during the luteal phase or in the stages of pregnancy [116] .

A high level of FSH suggests a decline in ovarian reserve, but a drop in the hormone's level implies problems with the pituitary gland or hypothalamus [117]. The ratio of FSH to LH is a measure for determining the condition of infertile women with PCOS and this ratio is greater than 2 according to the standard criteria for diagnosing the syndrome, and if this percentage increases will cause the ovulation process to stop[118].

The levels of steroid and sexual hormones in the blood are considered a single of most important factors that play a role in regulating FSH secretion through the negative and positive nutritional mechanism represented by the axial relationship between the hypothalamus (GnRH), the pituitary (LH, FSH) and the ovaries (estrogen and progesterone) [119].

1.6.3. Total Testosterone (TT)

It is one of the steroid hormones that belong to the group of androgens and is usually present in males and females and is excreted from the testicles in males and from the ovaries in females [120]. In men, testosterone is produced in large quantities by Leydig cells in the testicles and regulated by Sertoli cells [121].

The stromal cells secrete various small amounts of dihydrotestosterone (DHT) and dehydroepiandrosterone (DHAE) part of which is directly secreted into the plasma and the rest is converted within the ovary to estrogen or testosterone. The circulating androstenedione can also be converted to estrogen or testosterone in the additional glandular tissues [122].

Female testosterone has a biological role in folliculitis [123]. The cause of high testosterone in females is due to a tumor in the ovary or adrenal gland [124] .Also, the high testosterone hormone in females leads to irregularity of the menstrual cycle in women with polycystic ovaries and thus weak fertilization process at reproductive age [125].

Deficiency of 25-hydroxyvitamin was associated with a decrease men's testosterone levels, but there are conflicting reports of it being linked to sex hormones in women [126].

1.6.4. Prolactin (PRL)

Prolactin (PRL) is a polypeptide hormone with the high molecular weight ,lactotrophs are anterior pituitary gland cells that produce and secrete this hormone and this hormone act with estrogen on the growth of female sexual organs, especially the breast during the physiological activity stage [127].

High levels of PRL lead to gradual changes in luteal phase dysfunction, anovulation and menopause, and results in decreased production of progesterone lead to infertility [128]. Female patients with prolactinoma had lower vitamin D levels than normal women [129] . Prolactin (PRL) is a lactogenic hormone, along with estrogen and progesterone, begin to secrete milk and maintain the mammary glands. In addition to acting as a hormone, it acts as a growth and neurotransmitter factor and the presence of abnormally high levels of prolactin in the blood is known as hyperprolactinemia [130] .

Hyperprolactinemia is a prevalent endocrine condition in the hypothalamus - the pituitary gland, especially among females of childbearing age, affects about a third of women with infertility and hypothyroidism leads to hyperprolactinemia and enlarged pituitary gland ,both of which cause prolactinomas that limit the ability of hypothalamic dopamine to inhibit the production of prolactin and thus lead to ovulation disorders such as menopause, anovulation, insufficient luteal phase and galactorrhea [131].

Prolactin acts as a regulatory hormone for vit D which is a lactogen polypeptide the anterior pituitary gland secretes this hormone [132] . Sex hormones (SexHs) produced by the pituitary gland, such as follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL), are responsible for a variety of reproductive activities, including egg creation, sperm development, lactogens such as the

placenta, are a significant source of prolactin-like chemicals, PRLR with high affinity thus mimic PRL actions [116].

Prolactin is a multidirectional hormone with a number of endocrine functions, including modulation of gonadal function, maternal behavior, stress response, and anxiety and both PRL secretion and PRL-R receptor that are stimulated by estradiol (E_2) and signaling in the hypothalamus Notably, increased PRL is a major cause of infertility in both women and men and the etiology of hyperprolactinemia is different, including physiological, pathological, and genetic conditions, and is often associated with hypogonadal hypogonadism [133].

Prolactin stimulates the production of milk in women after the birth of the child. When prolactin levels rise, it inhibits two types of hormones necessary for ovulation: FSH and GnRH, and so does not stimulate the production of gametes and sexual stimulants, which means that women will not ovulate, and this lack of ovulation can also cause irregularity, menstrual cycles, and this will lead to infertility [104] .

1.6.5. Anti-Mullerian Hormone (AMH)

It is one among the most important in vital signs of reproduction, produced in granule cells and has a crucial role to perform in follicle formation [134]. Anti-Mullerian Hormone (AMH) is a bipolar non-associated glycoprotein that belongs to the beta-transforming growth factor family and is an excellent indicator of ovarian reserve. [135].

This study found that the serum level of anti- Mullerian Hormone (AMH) was lower in obese women than in non-obese adult women [136]. This hormone works primarily through vitamin D receptors (VDR), and studies in animals show that dietary VDD causes a 25%

reduction in fertility. [137]. The Arab population has the greatest of vitamin D deficiency in the world in the Middle East (90%) despite abundant sunlight and concealment of dress codes due to social and cultural / religious customs [138].

Additionally, low levels of (AMH) distinguish this population group [139] .Anti-Mullerian Hormone (AMH) level is the main indication for infertile women and when AMH level is elevated in serum of infertile women this means polycystic ovary syndrome (PCOS) [140].

AMH is a gonadotropin hormone that is also released in circulation. It modifies the follicle response to stimulating (FSH) and with a deficiency of this hormone, ovarian follicles are rapidly depleted. Vit D regulates the levels of this hormone in vitro, either directly through a stimulator or indirectly by regulating the number of granule cells and AMH signaling in ovarian follicle cultures [141].

1.6.6. Estradiol (E_2)

Estradiol (E_2) is a natural estrogen hormone produced by humans and secreted from the female ovaries or male testicles. It is involved in a variety of physiological processes with a focus on reproduction and sexual function, and is used by doctors to diagnose sexually transmitted infections [142].

The ovary is a female reproductive organ that is made up of granule cells and theca granule cells surrounding the oocytes .It makes female steroid hormones such as progesterone and estrogen .Estrogen is a female sex hormone that is important for the development and regulation of the reproductive system as well as secondary sexual characteristics .Estrogen comes in three different forms in women: estrone (E_1), estradiol (E_2), and estriol (E_3), Estetrol (E_4). Estradiol

steroid is the most strong and widespread type of endogenous estrogen as it regulates the menstrual cycle, controls growth from the endometrium during the first part of the cycle, and participates in the process of development of secondary sexual characteristics and is associated with many venereal diseases, including miscarriage, endometriosis, ovarian cancer and breast cancer [143].

E_2 is vital in the growth of female reproductive tissues such as the mammary glands, uterus and vagina during pregnancy, the menstrual cycle, which is important in the development of female sexual features such as breasts, enlargement of the hips, the female pattern of fat distribution in women, pregnancy and puberty [144].

The naturally occurring estrogens are characterized by being lipids containing 18 carbon atoms, an aromatic ring and a hydroxyl group on carbon atom No. 3, in addition to a ketone group on carbon atom No. 17 [145].

It also stimulates the secretion of mucous fluids from the glands in the cervical region with the change in the nature of the chemical composition of these fluids to suit the activity of the sperm and also helps to expand the cervical muscles [146]. Also it also works to prepare the uterus to receive the process of implantation of the embryo by speeding up the absorption of water and salts and activating the process of protein formation [147]. One study looked at the impact of vit D on E_2 synthesis in granule cells and discovered that vit D boosted E_2 production considerably [143].

Also, lack of FSH may prevent the necessary stimulation of the aromatase enzyme activity within the granulocytes estrogen-synthesized enzyme and thus reduce the conversion of androgens to estradiol [148] . Vit D_3 in human ovarian tissue stimulates estradiol

production by 9%, progesterone production by 13% and estrone production by 21% [81].

1.6.7. Thyroid Stimulating Hormone (TSH)

Thyroid hormone (TSH) is a glycoprotein hormone that governs the thyroid glands endocrine function and it is produced by thyrotropin cells in the anterior pituitary gland [149]. Menstrual abnormalities, reproductive issues, pregnancy challenges, and the intricate control of ovarian function are all common side effects of hypothyroidism [90].

When compared to fertility controls, women with unexplained infertility had greater levels of TSH [150] . A result of one study indicated that treatment with cholecalciferol improved thyroid function, as a significant decrease in TSH levels was observed in people after taking high monthly doses of cholecalciferol [151] . In addition, a recent study found a link between low vitamin D levels and thyroid function parameters, with vit D levels being lower in thyroid patients compared to healthy people [65].

Many researchers have found relationship of vit D with an increased group of hormones, the pituitary axis, and the immune regulatory cycle. It was observed that patients with vitamin deficiency had higher levels of TSH compared to control those with adequate or ideal levels of vit D[152].TSH is the primary hormone for diagnosing thyroid and dysfunction and for guiding the treatment of thyroid disease [153].

1.7. Aims of the Study

The aim of the study is to assess the prevalence of vitamin D deficiency among infertile women.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1 Subject

This study was conducted in Al-Razi Hospital in Anbar Governorate - Ramadi during the period from October 2020 to April 2021.

2.1.1 The Healthy Control Group

The group of healthy women consists of 40 women with good reproductive health between the ages of 18-45 years (34.08,30.93,31.23) and they were chosen based on the following: [154]

1. Menstrual cycle that is regular (26 to 30 days).

2. Age (18 - 45 years old).

3. There has been no previous history of endocrine disorders.

4. They did not use any medications for chronic diseases such as hypertension, diabetes, or oral contraceptives.

2.1.2 The Patients Group

The study included 61(30 women with type 1 infertility, 31 women with type 2 infertility) infertile women in Al-Anbar Governorate. The questionnaire form was filled out for the patients as shown in Appendix (1).

2.2 Materials

2.2.1 Equipment and Apparatuses

The sources of Equipment's and apparatuses used in this study are presented in Table (2-1).

Table (2-1): Equipment's and apparatuses that used in the study

Equipment's and Apparatuses	Country
Centrifuge	Japan
Deep Freeze	Sanyo
Eppendorf Tube	China
Micropipette 10-100 ul	Japan
Micropipette 100-1000 ul	Japan
Syringe 5ml	China
VIDAS (bioMérieux)	France
Yellow, blue tips	Germany

2.2.2 Materials that are used

All materials used in this study are explained in Table (2-2)

Table (2-2): Materials that are used

Kit	Sources	Country
AMH	VIDAS	France
E_2	VIDAS	France
FSH	VIDAS	France
LH	VIDAS	France
PRL	VIDAS	France
TSH	VIDAS	France
T-Test	VIDAS	France
Vit D	VIDAS	France

2.3 Methods

2.3.1 The Collection of Blood Sample

Blood samples were collected during the experimental phase (Day 2, 3) of the menstrual cycle of each woman from both groups (patients and Health Control). From ten in the morning until four in the afternoon 5ml of venous blood samples were collected and left for coagulation at a temperature of 23-27 ° C for 30 minutes. The serum was isolated by centrifugation at 5000 x g for 15 minutes. The serum was separated into Eppendorf tubes and stored at -20 ° C in the freezer until it was used for hormonal assays.

2.3.2 Body Mass Index

Regular scale was used to determine adult height / weight characteristics and is an indicator of individual obesity. It is widely used to improve many health problems and is widely used in determining public health policy. [155]

$$BMI = mass\ (kg) / height\ (m^2)$$

Table (2-3): The parameter of Body Mass Index

Clinical state	BMI
Under weight	15-19.9
Normal weight	20-24.9
Over weight	25-29.9
Obese	30-39.9
High Obese	>40

2.4 Laboratory Methods

2.5 Hormonal Tests:

With AMH, E_2, LH, FSH, TSH, T-Test, Prolactin and Vit D the hormonal analysis was performed with automatic immunoassay (AIA) of the

VIDAS automatic analyzer (bioMérieuxux) France.

2.6. Assay principle[156]

VIDAS ELFA technology was used in a hormonal assay for the quantitative evaluation of human serum (also known as an enzyme-linked fluorescence assay). The solid phase vessel (SPR) performs the function of a solid phase vessel. Assay reagents are ready-to-use in pre-prepared strips of sealed reagents. All testing phases were done via machine automatically. Many times, the reaction medium was cycled in and out of the SPR. The one-step enzyme includes FSH concept and LH quantification with immunoassay technology with final fluorescent ELFA detection.

The sample was obtained and delivered to a well containing alkaline phosphatase, a hormonal antigen (conjugate there is a competition between the antigen present in the sample and the antigen present in the well), called antigen covered inside the unique anti-hormone antibody RS.SPR. The sandwich process: a sample was transferred to the well containing the body, the hormonal antagonist called alkaline phosphatase (conjugated) for an anti-sandwich system. In and out of the SPR, the sample conjugate mixture was rotated. The antigen binds to the antibody coated with SPR and a "sandwich" forms the union. During the washing steps, the non-sticky components were removed.

The substrate (4-methyl-phosphate umbelliferyl) was cycled during the final step: In SPR and out of it. The hydrolysis of this conjugated enzyme is catalyzed and a fluorescent layer of a fluorescent material (4-methylumbiliferon) was setted 450 nm.

figure (2-1): Vidas Biomerieux

In the sample method to compete, the fluorescence intensity is inversely proportional to the concentration of antigen contained in the sample. In the sandwich method, the intensity of fluorescence is proportional to the amount of antigen contained in the sample. Effects were determined automatically by the tool in terms of results. The memory calibration curve was processed and then printed.

2.7 Assay procedure [156]

1- The required reagents were taken out of the refrigerator and placed allow it to reach room temperature for 30 minutes.

2- One FSH strip and one FSH-SPR from the kit were used for each sample, (control or calibrator) to be tested. The storage bag is then closed Required SPRs have been removed.

3- FSH is selected on the device test code. It must be calibrator determined by (S1), and tested. If it is necessary to test the control, it must be determined by C1.

4- Samples, calibrators and/or the controller were mixed using a vortex-type mixer.

5. 200 µl of the sample, titrated or control was drawn into the sample well.

6- Strips and VIDAS SPRs were inserted at the indicated positions the screen.

7- The inspection process has begun as directed by the operator's manual.

8- After completing the scan, the SPRs and strips from around the device. All test steps were performed automatically by the method. In approximately 40 minutes, the test was complete. The same procedure was performed on LH, Prolactin, TSH, AMH, FSH, E_2, T-TEST, Vit D. The subjects' hormonal assay values were compared with the normal range values:

❖ Normal Value

Table (2-4): Normal value for Hormone

Hormones	Normal value
AMH	4-6.8 ng/ml
E2	18-147 µg/ml
FSH	3.9-12 mlU/ml
LH	1.5-8 mlU/ml
TSH	0.25-5 µLu/ml
T-Test	0.09-0.7 ng/ml
PRL	5-25 µg/ml
Vit D	30-70 ng/ml

2.8 Vit D KIT Contents: LOT10089358650, REF 30463

Control VITD 1 x 1.5 mL (liquid)	C1	Ready to use. Vitamin D (25-(OH)) was diluted in serum + preservative The confidence interval in ng/mL is indicated by MLE data ("C1 control dose value range")
Calibrator VITD 1 x 2.5 mL (liquid)	S1	Vitamin D (25-(OH)) was diluted in serum + preservative titrator or standard concentration in ng/mL ("titrator dose value (S1)") and confidence interval in "relative fluorescence value" titrated (S1) Radio Frequency Range (RFV)" is indicated in the MLE data.

❖ Description of the Vit D Strip:

Wells	Reagents
1	Lots of samples were taken
2	TRIS, NaCl + anti-vitamin D antibody coupled with alkaline phosphatase + human origin stabilizer+ preservative (300 ml).
3	TRIS, NaCl + dissociation agent + surfactant + methanol

	(600 ml) Pre-treatment solution
4-5-6	Empty well
7-8-9	TRIS, NaCl + preservative + surfactant (600ml) wash buffer
10	-4Methyl-umbelliferyl phosphate (0.6 mmol/l) + diethanolamine (DEA) (0.62 mol/L or 6.6 percent, pH 9.2) + 1 g/L sodium amide (300 ml)

2.8.1 LH Kit Contents: LOT 1008311150, REF 30406-01

LH Control (lyophilized) (1 x 3 ml)	C_1	The LH kit was used to measure LH levels and the procedure was performed according to the manual, briefly as follows: 3 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes. MLE results represent confidence interval in mIU/mL (milli-international units per milliliter).("Control dose value range C1")
LH Calibrator (lyophilized) (3 x 2 ml)	S_1	The LH kit was used to measure LH levels and the process was performed according to the manual, briefly as follows: 2 ml of distilled water was added, mixed well and the mixture was left for 5-10 minutes. S_1 includes phosphate buffer (0.05 mol/L, pH 7.4), human LH and protein stabilizers. Concentration in mIU/mL (2nd IS 80/552) ("titrant dose value (S1)") and confidence interval for "relative fluorescence value" ("titrator range (S_1) RFV") are both shown in the MLE data.
LH Dilution buffer (liquid) (1 x 3 ml)	R1	This product is ready to use. It consists of 1 g/L of sodium amide and phosphate solution (0.05 mol/L, pH 7.4) with protein and chemical stabilizers.

❖ Description of the LH Reagent Strip

Wells	Reagents
1	Sample well.
2-3-4-5	Empty w ells
6	Anti-LH antibodies conjugated to alkaline phosphatase (calf intestine) with 1 g/L sodium amide (400 ml) in a mouse monoclonal antibody
7-8	1g/L sodium amide (600 ml) and sodium phosphate (0.05 mol/l, pH 7.4) with chemical stabilizers
	diethanolamine (1.1 mol/l or 11.5 percent, pH 9.8) with 1 g/L

9	sodium amide (600 ml) as a wash buffer
10	Substrate: 4-methylumbelliferyl phosphate (0.6 mmol/l) + 1 g/L sodium amide (300 ml) + diethanolamine (DEA) (0.62 mol/l or 6.6 percent, pH 9.2)

2.8.2 FSH Kit Contents: LOT 1008418160, REF 30407-01

FSH Control (lyophilized) (1 x 3 ml)	C1	The FSH kit was used to measure FSH levels and the procedure was performed according to the manual, briefly as follows: 3 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes. The confidence interval in mIU/mL (milli-international units per milliliter) is indicated by MLE data ("Control C1 Dose Value Range").
FSH Calibrator (lyophilized) (3 x 2 ml)	S1	The FSH kit was used to measure FSH levels and the procedure was performed according to the manual, briefly as follows: 2 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes. After reconstitution, the product is stable for 14 days at 2-8°C or until the expiration date on the kit at - 25° 6°C. There are five freeze/thaw cycles available. Human FSH and preservatives in bovine serum. The concentration in mIU/mL (2nd IRP 78/549) ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range") are indicated in MLE data.
FSH Diluent (liquid) (1 x 3 ml)	R1	Ready-to-use. 1 g/L sodium amide in bovine serum

❖ **Description of the (FSH) Reagent Strip:**

Wells	Reagents
1	Lots of samples were taken
2-3-4-5	Empty wells
6	Anti-FSH antibodies conjugated to alkaline phosphatase using 1g/L sodium amide (400 ml) in a mouse monoclonal antibody.
7-8	1 g/L sodium amide (600 ml) and sodium phosphate (0.01 mol/l, pH 7.4) with chemical stabilizers
9	Diethanolamine (1.1 mol/l or 11.5 percent, pH 9.8) with 1 g/L sodium amide (600 ml) as a wash buffer
10	Reading Cuvette with substrate: 4-Methylumbelliferyl phosphate (0.6 mmol/l) + 1 g/L sodium amide (300 ml) + diethanolamine (DEA) (0.62 mol/l or 6.6 percent, pH 9.2)

2.8.3 TT Kit Contents: LOT 1008358650, REF 414320

TES_2 Control 1 x 1 mL (lyophilized)	C1	TheTES2 kit was used to measure TES2 levels and the procedure was performed according to the manual, briefly as follows: 1 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes.The control is stable for 4 weeks at 2-8°C or until the expiration date of the kit when stored at -25°C after reconstitution. There are a total of five freeze/thaw cycles available. Preservatives + human* serum + testosterone. The confidence interval in ng/mL is indicated by MLE data ("Control C1 Dose Value Range")
TES_2 Calibrator 1 x 2 mL (lyophilized)	S1	The TES_2 kit was used to measure TES_2 levels and the procedure was performed according to the manual, briefly as follows: 2 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes. The calibrator is stable for 4 weeks at 2-8°C or until the expiration date of the kit when stored at 6-25 °C after reconstitution. There are a total of five freeze/thaw cycles available. Preservatives +

		human serum + testosterone the "Calibrator (S1) Dose Value" and the "Calibrator (S1) Dose Value" in the MLE data reflect the concentration in ng/mL and the "Calibrator (S1) Dose Value" in the "Relative Fluorescence Value ("Calibrator (S1) RFV Range") confidence interval

❖ Description of the TT Reagent Strip

Wells	Reagents
1	Sample well.
2	Phosphate buffer + bovine albumin + anti-testosterone antibody labeled with alkaline phosphatase + preservative (300 ml)
3	Phosphate buffer + bovine albumin + dissociation agent + preservative (600 ml) is the pre-treatment solution
4-5-6	Empty wells 7 – 8 - 9 Wash buffer: Tris-HCL
7-8-9	Wash buffer (600 ml): Tris + surfactant + preservative
10	-4Methyl-umbelliferyl-phosphate (0.6 mmol/L) + diethanolamine (DEA) (0.62 mol/L or 6.6 percent) reading cuvette (300 ml) pH 9.2 + 1 g/L sodium amide

2.8.4 PRL Kit Contents: LOT 1008294300, REF 30410-01

PRL Control (lyophilized) (1 x 3 ml)	C1	The PRL kit was used to measure PRL levels and the procedure was performed according to the manual, briefly as follows: 3 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes. It is stable for 24 hours at 2-8°C or until the expiration date on the kit at6 6-25°C. There are five freeze/thaw cycles available. Prolactin and 0.02 percent bromide in human serum. The confidence interval in ng/mL (“Control

		C1 Dose Value Range") is indicated by MLE data
PRL Calibrator (lyophilized) (3 x 2 ml)	S1	The PRL kit was used to measure PRL levels and the procedure was performed according to the manual, briefly as follows: 3 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes., it is stable for 24 hours at 2-8°C or until the expiration date on the kit at -25°C. There are five freeze/thaw cycles available. Prolactin and 0.02 percent bromide in human serum. The calibration interval in "Relative Fluorescence Value" ("Calibrator (S1) RFV Range") and the concentration in ng/mL ("Calibrator (S1) Dose Value") are shown in MLE data
PRL Diluent (liquid) (1 x 3 ml)	R1	Ready-to-use. 1 g/L sodium amide in human free prolactin serum

❖ Description of the Prolactin (PRL) Reagent Strip:

Wells	Reagents	
1	Sample well	
2-3-4-5	Empty wells	
6	Conjugate	Mouse monoclonal anti-prolactin antibodies conjugated to alkaline phosphatase with 1 g/L sodium amide (400 µl).
7-8	Wash buffer	0.01g/L sodium amide (600 ml) with DEA* (1.1 mol/l or 11.5 percent, pH 9.8)
9	Wash buffer	0.01g/L sodium amide (600 ml) with DEA* (1.1 mol/l or 11.5 percent, pH 9.8)
10	Reading Cuvette with Substrate	4Methyl-umbelliferyl-phosphate (0.6 mmol/l) + diethanolamine (DEA) (0.62 mol/l or 6.6 percent, pH 9.2) + 1 g/l sodium amide (300 ml) reading cuvette with substrate

2.8.5AMH Kit contents: LOT 1008418160, REF 417011

http://www.biomerieux.com/techlib

AMH Positive control (lyophilized) 1 x 2 mL	C1	The AMH kit was used to measure AMH levels and the procedure was performed according to the manual, briefly as follows: 2 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes. The control is stable for 7 days at 2-8°C or until the expiration date of the kit when stored at -25°C after reconstitution. There are a total of eight freeze/thaw cycles available. Preservative + human serum. The appropriate range in ng/mL is determined using MLE data ("Control C1 Dose Value Range").
AMH Calibrator (lyophilized) 2 x 2 mL	S1	The AMH kit was used to measure AMH levels and the procedure was performed according to the manual, briefly as follows: 2 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes. The calibrator is stable for 7 days at 2-8°C or until the expiration date of the kit when stored at 6-25 °C after reconstitution. There are four freeze/thaw cycles available. Human serum + preservative + protein stabilizer the calibrator concentration in ng/mL ("Calibrator (S1) Dose Value") and the allowable range in "Relative Fluorescence Value" (Calibrator (S1) RFV Range) are both indicated in MLE data.
AMH Sample Diluent (liquid) 1 x 4.1 mL	R1	Ready-to-use. Only used to dilute samples that are outside of the measurement range. Preservatives Plus protein and chemical stabilizers

❖ **Description of the AMH strip**

Well	Reagents
1	Lots of samples were taken
2-3-4-5	Empty wells.
6	AMH antibody with alkaline phosphatase labeling + preservative (400 ml)
7-8-9	Preservative wash buffer (600 ml).
10	4-Methyl-umbelliferyl-phosphate (0.6 mmol/L) + diethanolamine (DEA) (0.62 mol/L or 6.6 percent) reading cuvette (300 ml) pH 9.2 + 1 g/L sodium amide

2.8.6 E_2 Kit Contents: LOT 1008005350, REF 30431

E_2 II control 1 x 3 mL (liquid)	C1	This product is ready to use. 1 g/L sodium amide + human serum* + 17 -estradiol the confidence interval in pg/mL ("Control C1 Dose Value Range") is indicated by MLE data.
E_2 II calibrator 2 x 4 mL (liquid)	S1	This product is ready to use. 1 g/L sodium amide + human serum* + 17 -estradiol the calibration interval in "Relative Fluorescence Value" ("Calibrator (S1) RFV Range") and the concentration in pg/mL ("Calibrator (S1) Dose Value") are shown in MLE data.

❖ **Description of the E_2 II strip**

Wells	Reagents
1	Sample well.
2-3-4	Wells that are dry.
5	0.9 g/L sodium amide (400 ml) + alkaline phosphatase-labeled estradiol derivative
6	Wells that are dry.
7-8	Tris-NaCl (0.05 mol/L) pH 9 + 1 g/L sodium amide (600 ml) wash buffer

9	Diethanolamine (DEA) (1.1 mol/L, or 11.5 percent, pH 9.8) + 1 g/L sodium amide (600 ml) is the wash buffer.
10	4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine (0.62 mol/L, or 6.6 percent, pH 9.2) + 1 g/L sodium amide (300 ml) Cuvette with substrate

2.8.7 TSH Kit contents: LOT 1008418160, REF 30400-01

TSH Control lyophilized) (1 x 3 ml)	C_1	The TSH kit was used to measure TSH levels and the procedure was performed according to the manual, briefly as follows: 1 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes. After reconstitution, the product is stable for 14 days at 2-8°C or until the kit expiration date at 6-25°C. There are five freeze/thaw cycles available. Human serum* containing human TSH as well as preservatives. The confidence interval in lU/mL is indicated by MLE data ("Control C1 Dose Value Range")
TSH Calibrator lyophilized (1 x 2 ml)	S_1	The TSH kit was used to measure TSH levels and the procedure was performed according to the manual, briefly as follows: 3 mL of distilled water was added, mixed well and the mixture was left for 5-10 minutes. After reconstitution, the product is stable for 14 days at 2-8°C or until the kit expiration date at 6-25°C. There are five freeze/thaw cycles available. Human TSH and preservatives in calf serum. The concentration in IU/mL ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range") are indicated in MLE data
TSH diluent (liquid) (1 x 3 ml)	R1	Ready-to-use. 1 g/L sodium amide in calf serum

❖ **Description of the TSH Reagent Strip:**

Wells	Reagents
1	Sample well
2-3-4-5	Empty wells
6	Anti-TSH antibodies conjugated to alkaline phosphatase using 1 g/L sodium amide (400 ml) in a mouse monoclonal antibody.
7-8	1 g/L sodium amide (600 ml) and sodium phosphate (0.01 mol/l, pH 7.4) with chemical stabilizers
9	Diethanolamine (DEA) (1.1 mol/l or 11.5 percent, pH 9.8) with 1 g/L sodium amide (600 l) as a wash buffer
10	4-Methyl-umbelliferyl phosphate (0.6 mmol/l) + diethanolamine (DEA) (0.62 mol/l or 6.6 percent, pH 9.2) + 1 g/l sodium amide (300 ml) = reading cuvette with substrate.

2.8 Statistical Analysis

SPSS version V23 was used to conduct statistical analyses, arithmetic mean ± standard error (SE) and confidence limits were calculated. The comparison between the coefficients was done by the one-way ANOVA test. The correlation coefficients between variables with vit D, Pearson correlation analysis was also used to calculate the differences, which were declared statistically significant at $P \leq 0.01$.

Chapter three

Results and Discussion

3. Results and Discussion

3.1 General Description of the Study Samples

Our study included 101 samples of women, divided into two groups, a group of 61 infertile women (30 women with the first type of infertility and 31 women suffering from the second type of infertility) and the second group is the group of 40 fertile women any other disease affecting the variants was excluded in this study.

3.2 Anthropometric Analysis

In terms of average age, there were no statistically significant changes between the healthy controls and the first type group, and the second type group of infertility (34.08±1.05,30.93±1.36, 31.23±1.10 years respectively) at a probability level ($P \leq 0.01$) as shown in Figure (3.1) and in (Table 1) appendix 2.

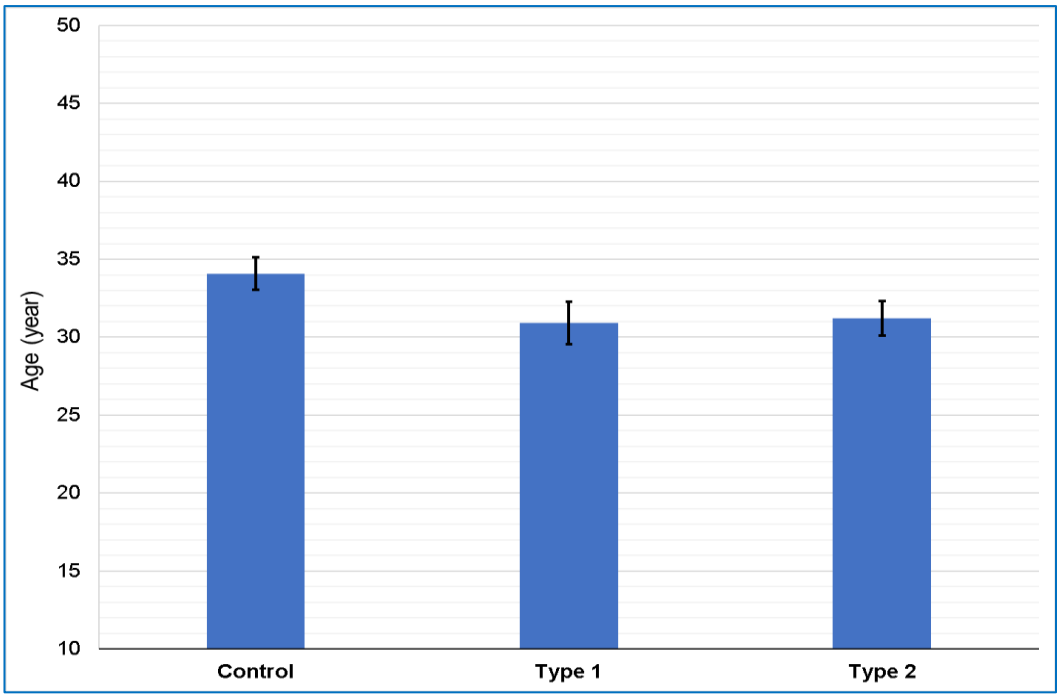


Figure (3-1): Mean of Age in Patients and HCs

Our results shown in Figures (3-2), (3-3), (3-4) indicate that the group of infertile women of the first and second types suffer from an increase compared with the control group in the ratio of the waist to the hip (49.60±1.003 , 47.42± 0.936,42.63±1.05 cm respectively) and the ratio of the waist to the waist(46.17±0.929 , 44.84±0.896,40.10±0.576 cm respectively)and an increase in BMI(30.99±0.83 , 31.01±0.84,25.00±0.42 kg/m2 respectively) who were Weights at the normal level our findings are consistent with that of Ollila et al., Who found a higher average BMI in obesity and overweight. Significantly greater in patients compared to healthy people [165] .

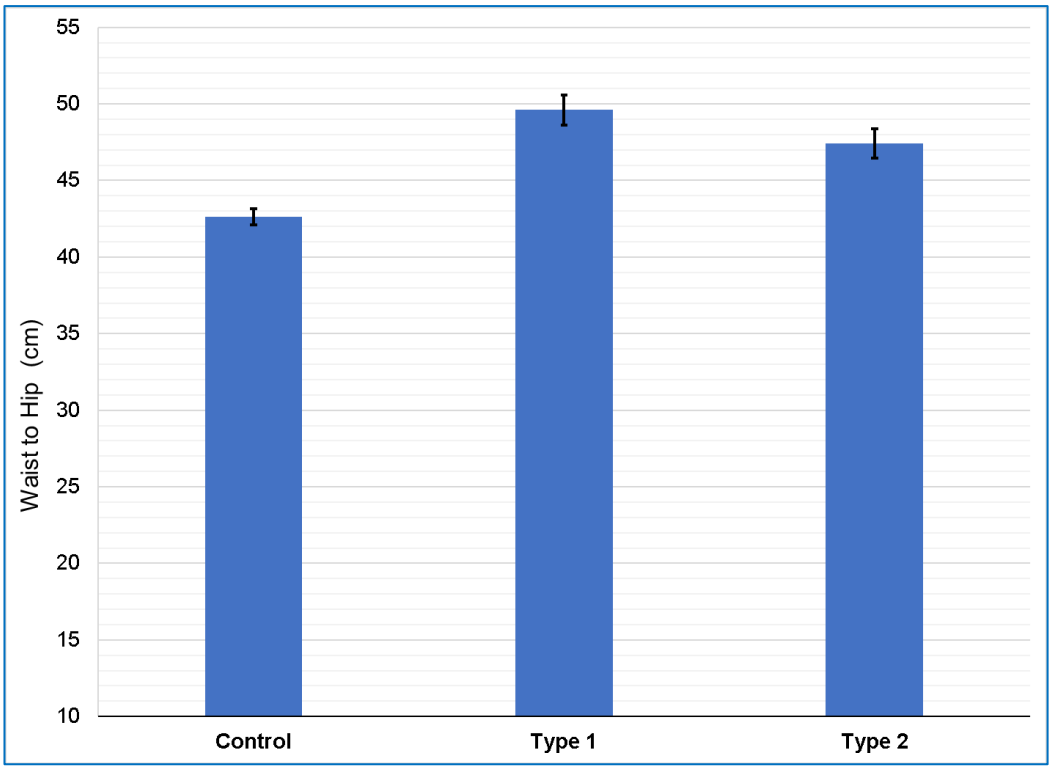


Figure (3-2): Mean of Waist to Hip in Patients and HCs

Obesity has many negative effects on female fertility, as obese women suffer from poor ovulation due to a defect in the regulation of the ovarian axis - the pituitary gland, and obese women show poor results with the use of *in vitro* fertilization, where obesity affects the

egg and the fetus before implantation [166] .The extra weight of the female leads to abnormal levels of hormones that affect fertility and ovulation that lead to polycystic ovarian disease and inhibit pregnancy [167].

Our study results are in agreement with many studies [168,169,170] . Obesity affects the functions of the ovaries and the endometrium and produces a state of estrogen and androgen excess that causes infertility by obstructing ovulation and reducing the receptivity of the endometrium [171].

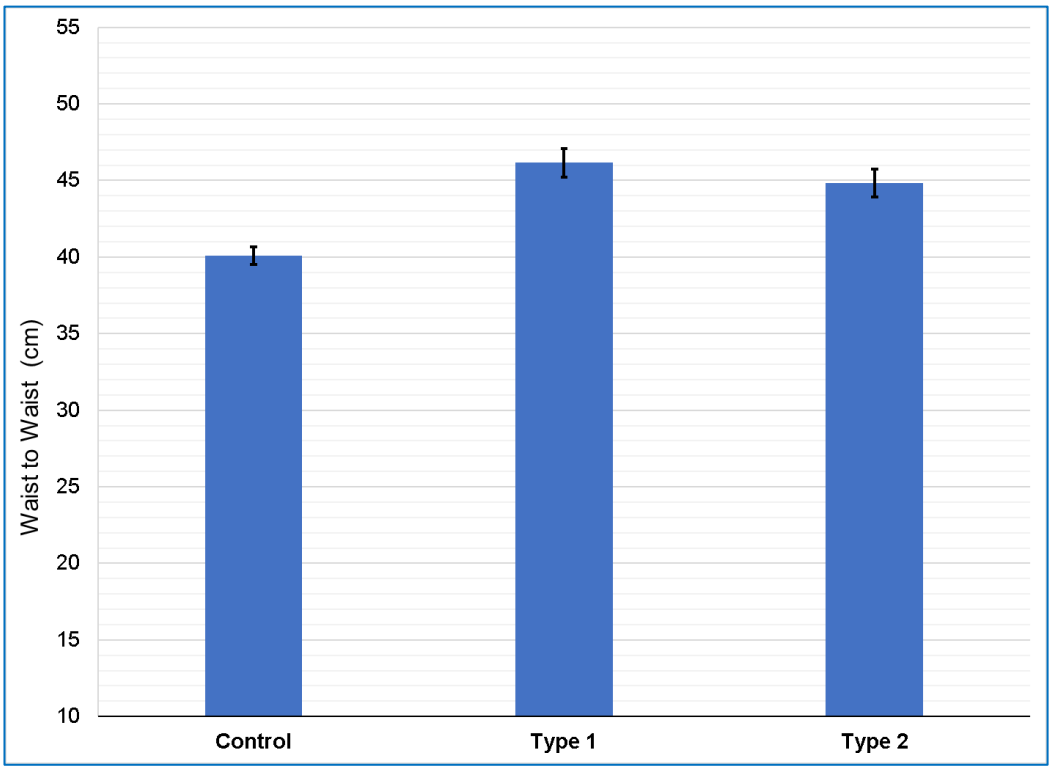


Figure (3-3): Mean of Waist to Waist in Patients and HCs

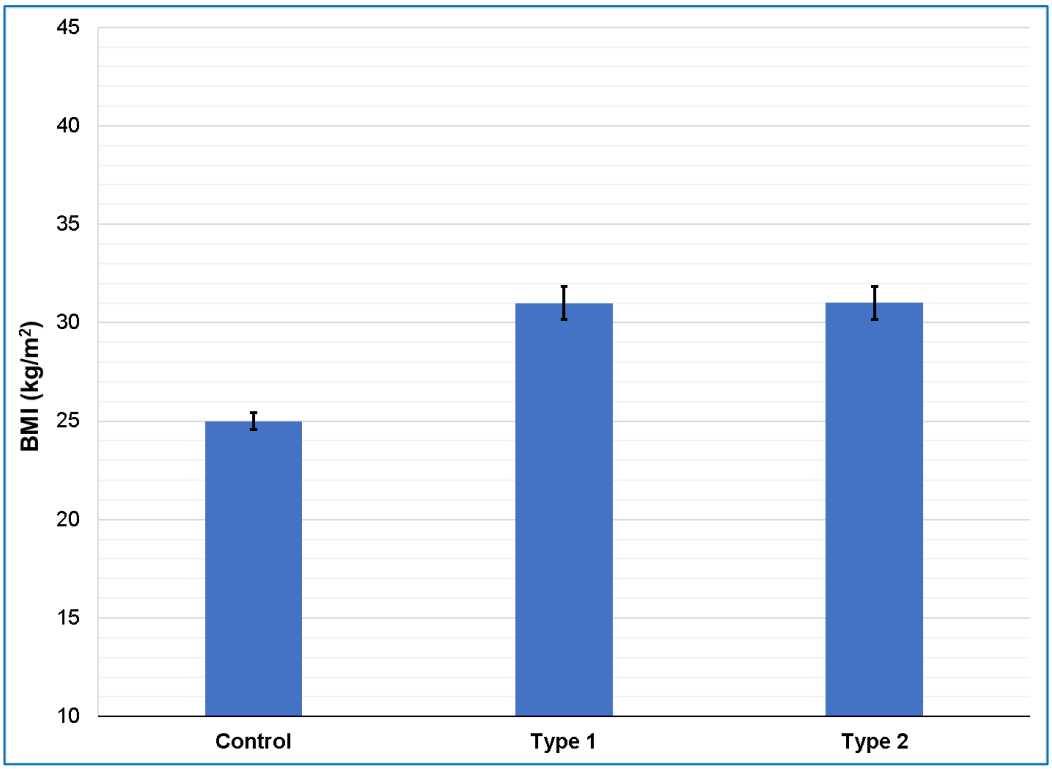


Figure (3-4): Mean of BMI in Patients and HCs

One of the studies also showed the bad effect of obesity on the rates of embryo transfer, pregnancy rates, the duration of ovarian stimulation and fertilization,the reason for impaired fertility in obese women is also due to the partial or complete absence in the frequency of ovulation [172].

Our study does not agree with the research of Baetge *et al* and Deng *et al*, as they showed no difference in BMI between the patient group and the healthy group [173,174] .

While our study is in agreement with many studies that observed an increase in BMI and waist-to-hip ratio in patients compared to healthy women[175,176,177].

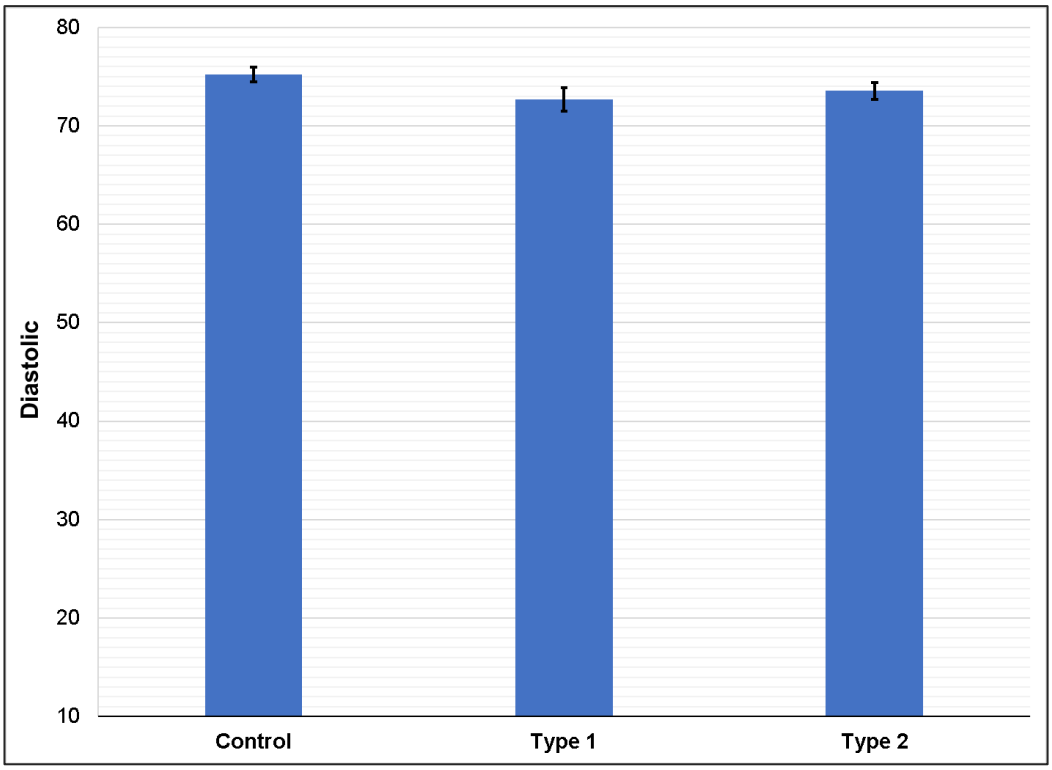


Figure (3-5): Mean of Diastolic in Patients and HCs

Our study found that no statistically significant differences existed in diastolic pressure (DBP) (75.23±0.76,72.67±1.17, 73.55±0.87 mmHg respectively), while differences were statistically significant in systolic pressure between women with type 1 and type 2 infertility compared to healthy women (124.67±1.71, 128.39±1.68, 116.28±0.94 respectively).

Our results are in agreement with the results of a study by Azevedo *et al*. and Conway *et al*. They found a significant increase in systolic blood pressure in infertile women compared to healthy women.[178,179].

In contrast, other studies found no differences in systolic and diastolic pressure between the infertile and intact female group [180] .

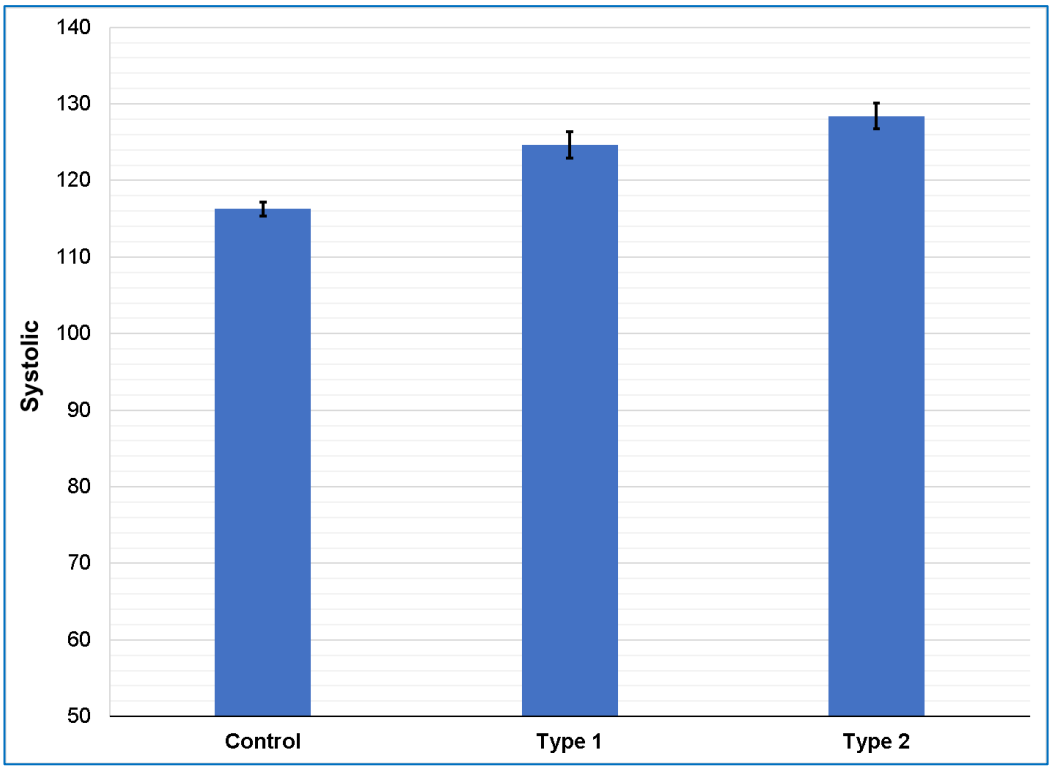


Figure (3-6): Mean of Systolic in Patients and HCs

3.3 Vitamin D

Our results showed a decrease in vit D in infertile women in both types I and II compared with fertile women, since the normal value of vit D ranges from 30 to 70 ng / ml.

Average rate of the group of infertile women I and II is (13.91±1.06), (13.55±1.21) respectively, and the average fertile women is (38.56±1.48). It showed a big difference as shown in Figure (3-7) and Table (1) appendix 2.

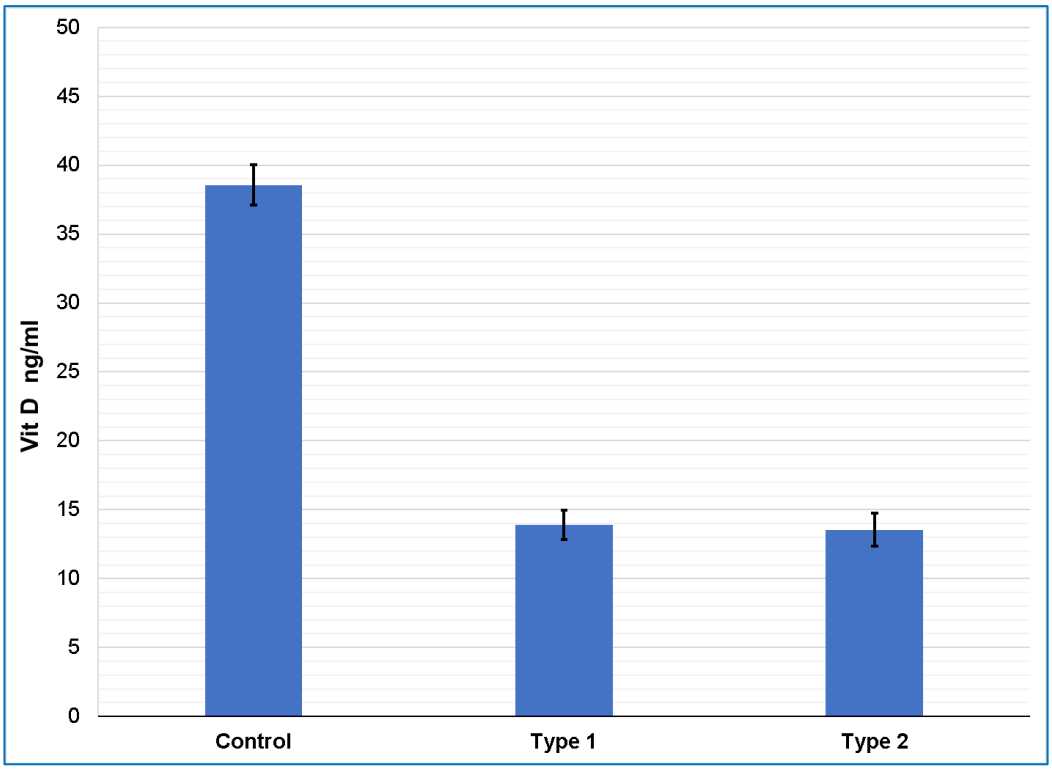


Figure (3-7): Mean for Vitamin D in Patients and HCs

There are many reasons for low vit D levels, such as irregular bowel function and poor absorption of vit D [157]. Lack of exposure to sunlight and geographical location, or to the lack of eating meals rich in vitamin D such as milk, egg and cheese [158]. Our study is in agreement with Abodunrin O.N. who found in his study a decrease vit D levels in infertile women than in fertile women [18].

Our study is also in agreement with Al-Assadi *et al*(2018). study that was conducted in the city of Basra which included 106 infertile women and 104 fertile women. Infertile women's vit D levels were lower than fertile women's [12]. Our study also is in agreement with [19,20,137].

A study was conducted in the of Baghdad city by Abdul-Rasheed in which they reported vit D deficiency in the blood among infertile [159]. Our study also is in agreement with a study conducted in Iran [160] .

In another study they came to the same findings as ours and the main role of vitamin D insufficiency in causing skin pigmentation problems[161] . Vit D insufficiency was shown to be more common among infertile women with PCOS in a study conducted in southern India [17]. Vitamin D insufficiency has also been linked to an increased risk of miscarriage in numerous studies [87,162].

One of the studies also confirmed the great vitamin D's advantages in the success of in vitro fertilization, the prevention of endometriosis, and the improvement of metabolic functions [163] .

A study was also conducted in Jordan Hospital aimed at investigating the vitamin D's impact on androgen levels and hirsutism in women with PCOS and overweight, they showed that a weekly vitamin D dosage of 50,000 IU used as a treatment improved fertility and reproductive health through lower levels of the total testosterone hormone, thyroid hormone, androgen index and degree of hirsutism [164] .

3.4 Luteinizing Hormone (LH)

Our statistical results showed that LH hormone levels increased significantly among infertile women in both types I and II compared to the healthy group (10.67±2.09, 7.12±0.55, 3.81±0.22 mlU/ml respectively), at a probability level ($P \leq 0.01$) as demonstrated in Figure (3-6), Table (1), appendix 2**.**

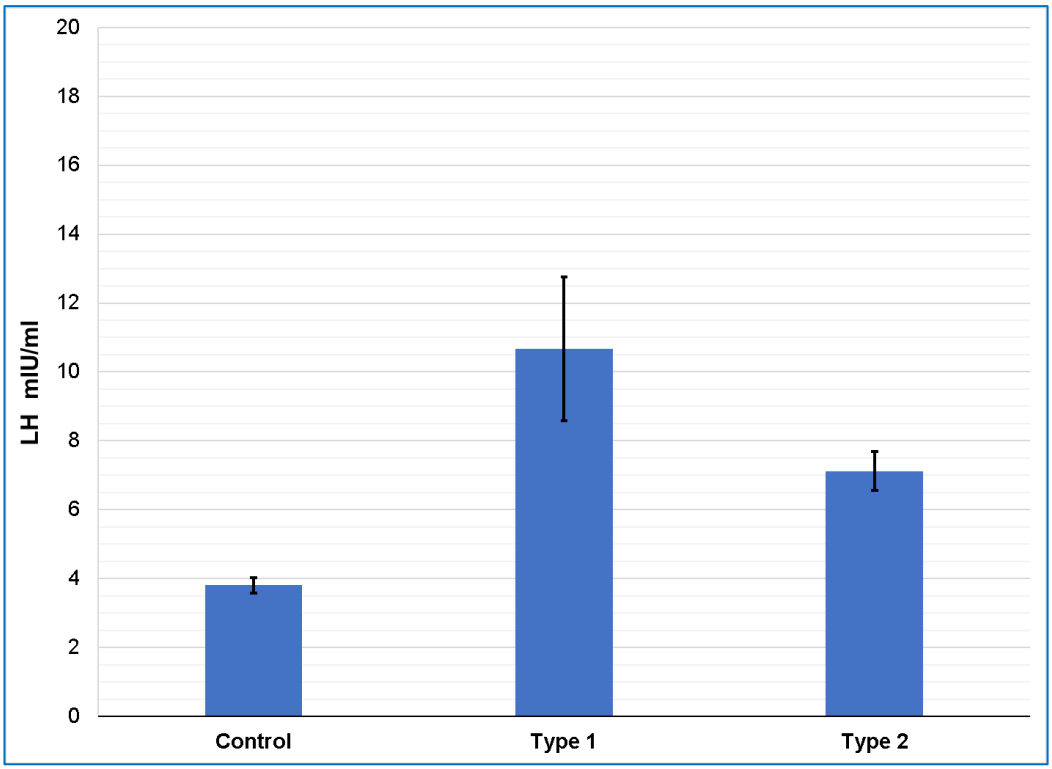


Figure (3-8): Mean of LH in Patients and HCs

Our findings were in line with a study by Nath *et al* (2019). Their study showed an increase in the level of LH in infertile women due to an increase in the production of ovarian androgen [181].

Our study results are in agreement with the Kumar *et al* study conducted in India on women with PCOS, as it showed higher levels of the LH hormone affected women compared to healthy ones [182].

Our results are consistent with Prasad *et al*, as they found high levels of LH and prolactin in infertile women compared to fertility and this increase is considered one of the most common reasons of female infertility [183] . High levels of the hormone LH have an effect on the ration of eggs and a cause of low fertility and frequent miscarriages [184] .High levels of LH were also observed in infertile women due to a change in physiological factors in a study by Elsamahi [185] .

3.5 Follicle stimulating hormone (FSH)

It was found through the results of the study and the statistical results that there are no statistically significant differences in the level of the FSH hormone between infertile women in both types I and II compared to fertile women (6.19±0.23,10.12±2.68 , 6.87±1.19 respectively), at a probability level P ≤ 0.163 as demonstrated in Figure (3-9) and Table(1) ,appendix 2.

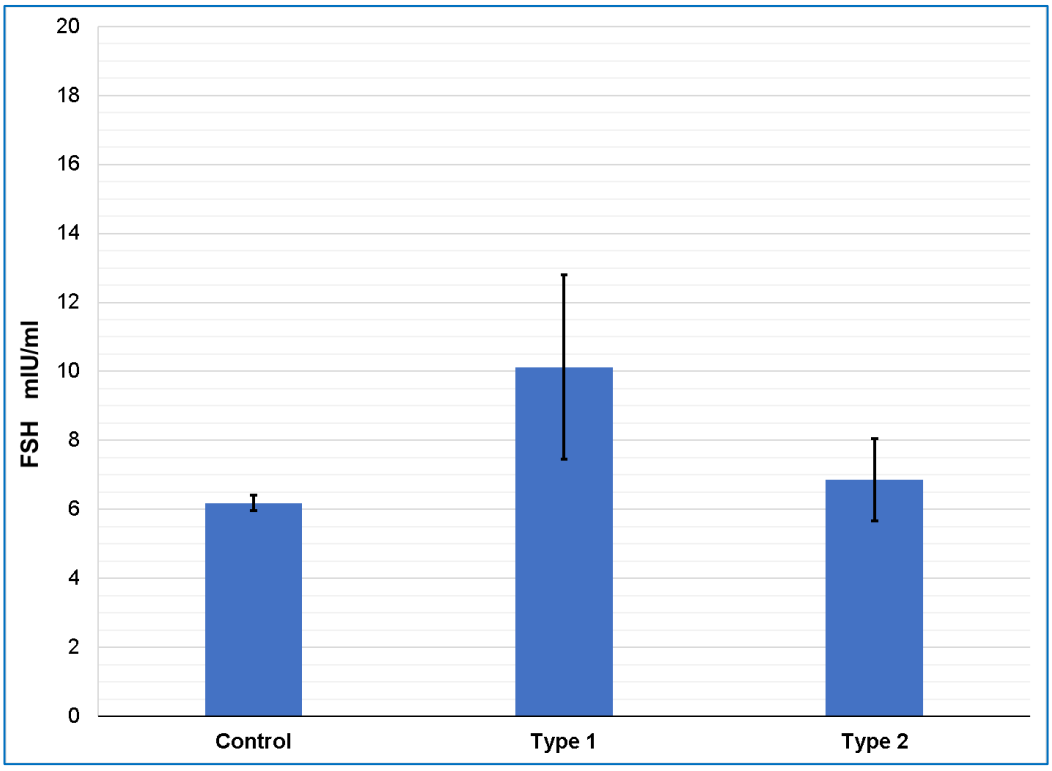


Figure (3-9): Mean of FSH in Patients and HCs

Our study was consistent with the study of Ilgaz *et al*, as their study showed that there were no differences that were statistically significant between the infertility group and controls [186] . Our results are in agreement with a study conducted in Baghdad by Mohammed ,M.I which showed through its study that there were no differences that were statistically significant in the level of the FSH hormone [187] .

Our results are in agreement with Al-Musawi *et al*., (2018) [188] .Also with A.M Nasser *et al*(2021)[189].

Our results are inconsistent with Prasad et al, their study found higher levels of FSH in infertile women compared to fertility[183].

The results of the study differed with Shore *et al* [190] .Catteau Jonard *et al*, who found lower FSH infertile women's levels compared to fertile women's [191]. FSH is one of the hormones that controls egg growth and improvement, and its decrease in the blood can be caused by excessive levels of prolactin, which can cause the ovulation cycle to be stopped by suppressing FSH secretion [189].

3.6 LH/FSH Ratio

Current results showed considerable increase in the LH / FSH ratio among infertile women in both types I and II compared to the healthy group(1.41±0.19, 1.36±0.16 vs 0.61±0.03 respectively) , at a probability level (P≤ 0.01), as demonstrated in Figure(3-10) and Table (1) appendix2.

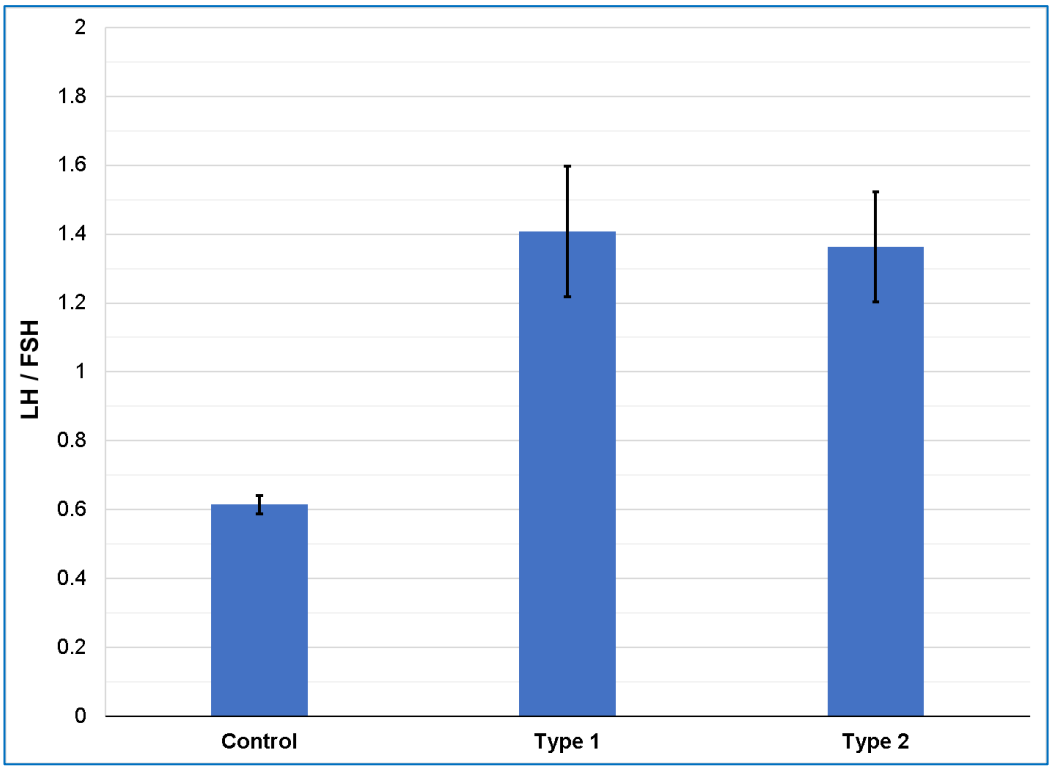


Figure (3-10): Mean of LH/FSH in Patients and HCs

Our data are in agreement with many studies that showed an increase in the ratio of the LH hormone to the FSH hormone. This percentage is higher in patients with infertility and this ratio is one of the criteria for diagnosis [192].

The findings of our research matched those of Kumar *et al*. Infertile patients had a higher level of LH/FSH than healthy patients, according to his research, and a larger number of LH / FSH was considered an important criterion for the clinical diagnosis of PCOS [182]. The increase in the ratio of LH / FSH is due to the continuous rise in the hormones LH and the decrease of the FSH hormone, thus reducing the estrogen secretion necessary to nourish the ovaries [189].

3.7 Total testosterone hormone (TT)

There was a substantial increase in the level of the total testosterone hormone among infertile women in both the first and second types compared to the healthy group (0.71±0.08,1.03±0.12 , 0.33±0.03 ng/ml respectively), at a probability level $P \leq 0.01$ and its increase in the second type of infertility was higher than the first type of infertility, as shown in Figure (3-11) and Table (1) appendix 2.

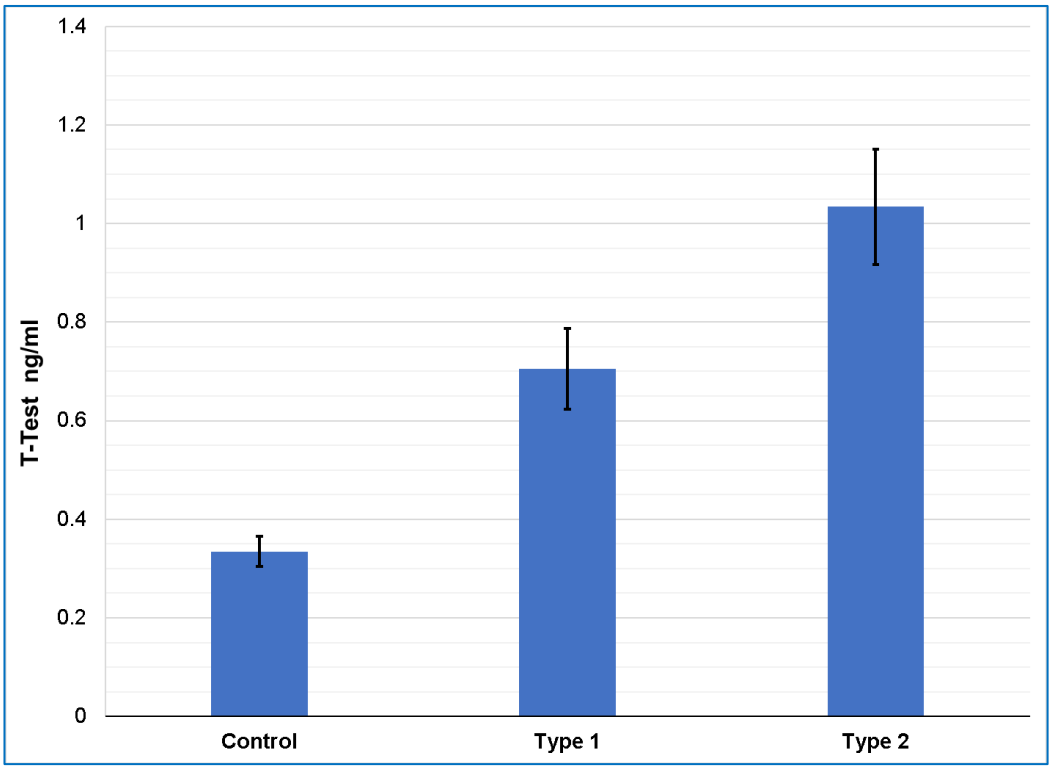


Figure (3-11): Mean of (TT) in Patients and HCs

Current data was consistent with many studies that showed an increase in total testosterone in infertile patients with PCOS compared to controls [193,194,195]. Our results are in agreement with another study that showed that testosterone levels were much higher in infertile women in both types I and II compared to fertile women. In addition the high percentage of the sex hormone (testosterone) causes menstrual disturbance and irregular ovulation, where menstruation

comes every two or three months and is often accompanied by the appearance of pimples on the face and increased on the face, hair growth and abdomen and chest.[196]

The reason for the high testosterone hormone in the bloodstream is either due to the fact that the outer layer or the tissues inside the ovaries are thick in infertile women, where the dense pulp of the ovary contains theca cells that produce testosterone more frequently compared to the normal theca cells, or the reason for the rise is due to the low level of β- Globulin, where a large part of testosterone remains unbound to any protein and thus its concentration in the blood increases. It is worth noting that 8% of testosterone binds to β-globulin, 19% binds to albumin and only 1% is not bound to any type of binding protein [104] .

3.8 Prolactin Hormone (PRL)

The findings revealed that there was a considerable difference increase in the level of prolactin hormone between infertile women in both types compared to the healthy group (24.63±2.50, 19.90±1.33 , 10.11±0.44 µg/ml respectively) at a probability level $P \leq 0.01$ and as shown in Figure (3-12) and Table (1) in the appendix 2.

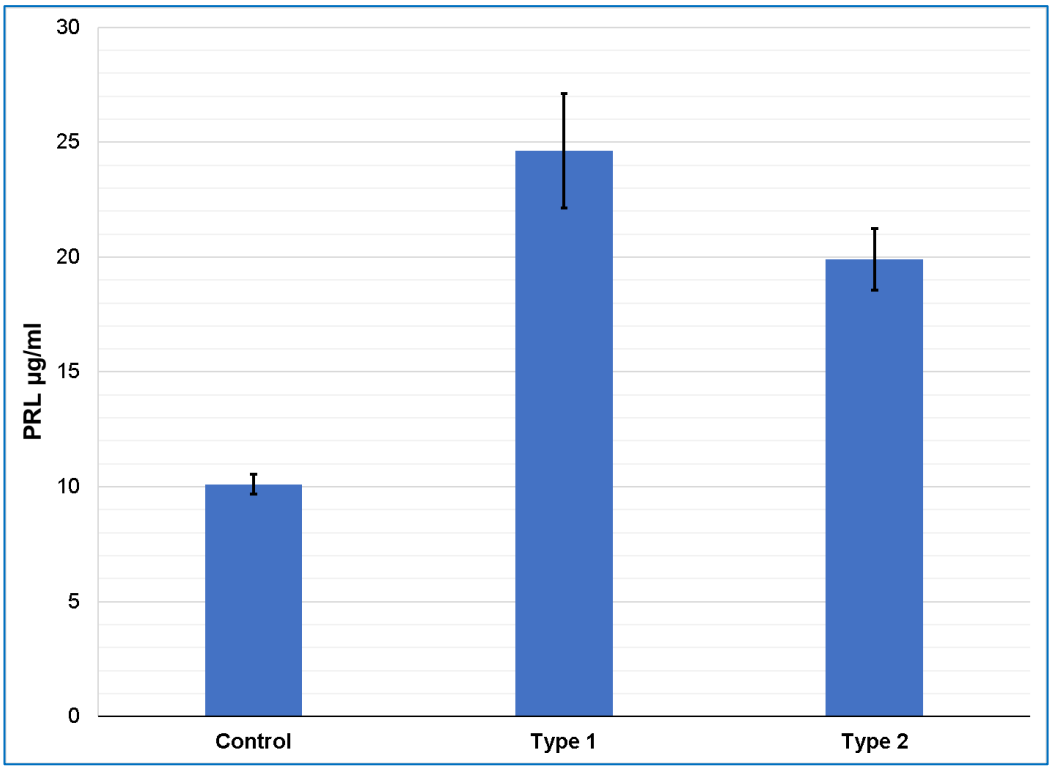


Figure (3-12): Mean of (PRL) in Patients and HCs

Present results agree with Marbut *et al* which showed an increase in the level of the hormone prolactin among infertile patients with PCOS compared to healthy subjects [193].

The results of our study were also agreement with the AL-Hashimy et al study, whose statistical results revealed a considerable rise in the level of the hormone prolactin in women with infertility compared to fertility and there are many factors that lead to an increase in it, such as pituitary tumors or cardiovascular disease. This increase can lead to luteal dysfunction, anovulation and menopause due to the effect of prolactin on the secretion of the GnRH hormone [104].

The results of our study were also in agreement with a study conducted at the University of Baghdad. This study found that the level of the hormone prolactin increased in women with infertility compared to healthy people, and that hyperprolactinemia affects a

third of women with infertility, it is a common problem in reproductive dysfunction [130].

Many studies have also shown raise in the level of the hormone prolactin in the blood of infertile women compared to healthy subjects, and this increase is considered one of the main hormonal factors that contribute to infertility in women[189 ,197,198,199,].

3.9 Anti-Mullerian Hormone (AMH)

Our findings revealed that the level of AMH at the likelihood level decreased significantly $P \leq 0.01$ in women with infertility in both types I and II compared to fertile women (2.83±0.35 ,2.88±0.41vs 4.67±0.17 ng/ml respectively), as shown in Figure (3-13) and Table (1), appendix 2.

Our results are in agreement with Kadhim,H.H study, showed in its study a decrease in the level of the hormone AMH in infertile women compared to fertile women, as well as a decrease in this hormone with age low AMH concentration in the blood indicates a decrease in ovarian reserve, resulting in a low response to stimulation in IVF in most women with PCOS [200] .

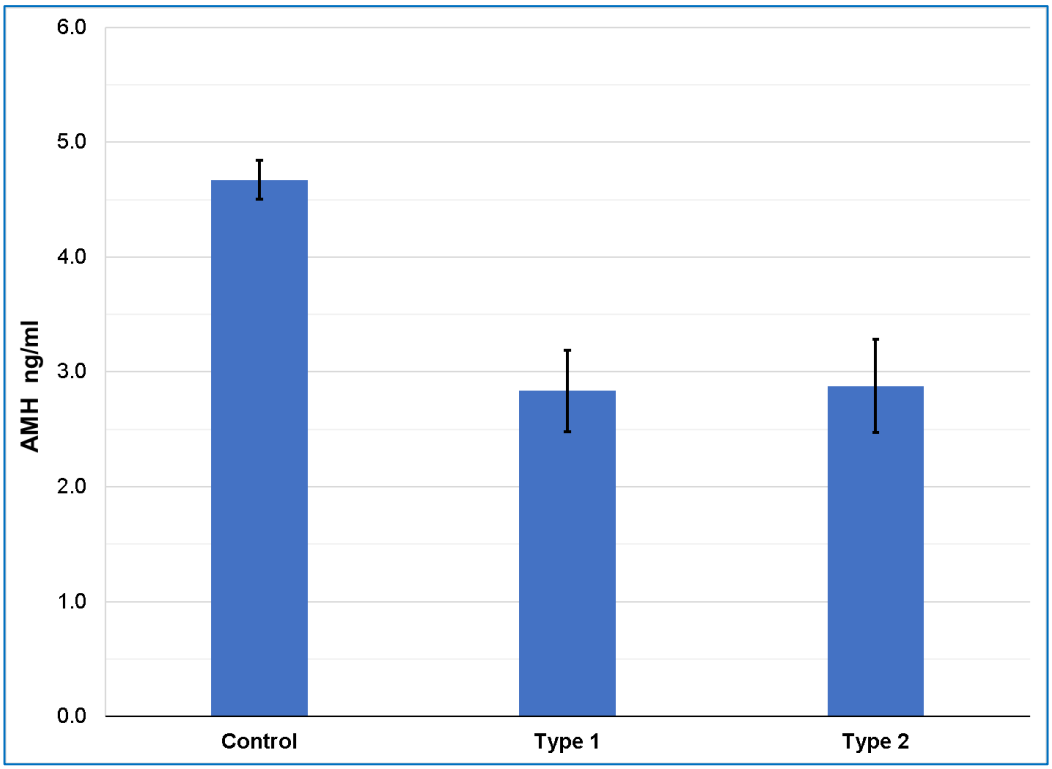


Figure (3-13): Mean of (AMH) in Patients and HCs

According to one study, the low level of the hormone AMH in the blood of infertile women is due to high psychological pressure that may affect the ovarian reserve [201] .

Our results are in agreement with another study that found reduction in the level of the AMH hormone in women with infertility and showed that the reason for the decrease is due to the effect of this hormone by weak thyroid function, as they found an inverse relationship between AMH, which is a vital indicator of ovarian function, inversely with the hormone TSH [202] .

Medications can also lower the level of the hormone AMH in infertile women, according to a study that indicated that taking metformin can considerably lower the level of the hormone AMH in infertile women. [203]. A study conducted on infertile Korean women

showed that the low level of the hormone AMH in them is due to the harmful effect of air pollution on women's fertility [204] .

3.10 Estradiol Hormone(E_2)

There was a significant reduction in the level of the estradiol hormone(E_2) among infertile women in both types I and II compared to the healthy group (33.69±3.60, 29.89±2.99,45.06±2.95 µg/ml respectively) with a probability level (P ≤0.02) as demonstrated in Figure (3-14) and Table (1) in the appendix 2.

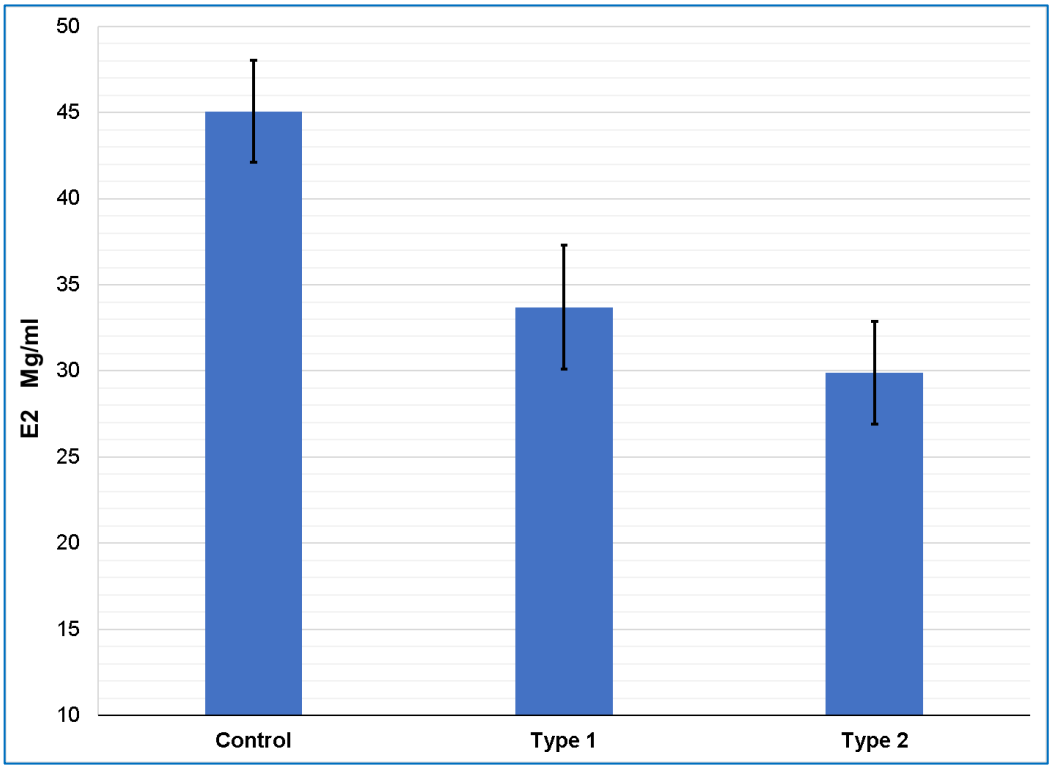


Figure (3-14): Mean of (E2) in Patients and HCs

Our study is in agreement with Al-Hashemi *et al*, they showed that there were significant differences in the E_2 hormone in infertile women compared to healthy women [205]. Anovulation interruption

associated with low estradiol secretion due to the peripheral transfer outside the glands and a minimal progesterone production [206].

Our study is also consistent with another study, where estradiol levels were lower in infertile women of both types I and II compared to fertile women, where estrogen controls uterine thickness and egg maturation during menstruation or pregnancy, and reduces bacterial infection and the pH of the vagina s it reduces estrogen levels and thus affects sexual desire in women and controls the production of milk from the breast [196].

3.11 Thyroid stimulating hormone (TSH)

Our study showed that TSH hormone levels did not differ in any statistically meaningful ways for infertile women in both the first and second types compared to the statistic group (2.35±0.33, 2.24±0.22, 1.90±0.20 µlU/ml respectively) at the importance level ($P \leq 0.381$) as shown in Figure (3-15) and Table (1) in the appendix 2.

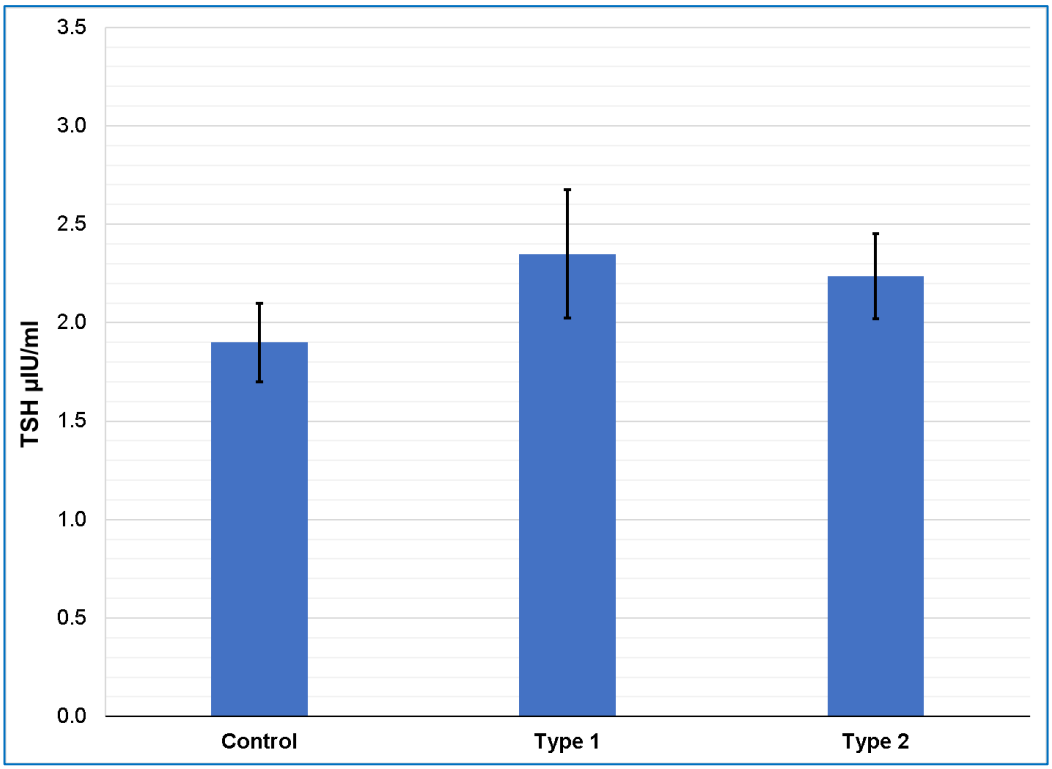


Figure (3-15): Mean of (TSH) in Patients and HCs

Current study is in agreement with Al-Hashemi *et al*, where he showed in his study that no significant differences were found in the hormone TSH in infertile women compared to fertile women[104 ,205] .

Our study is in different with that of Nasser *et al*., it was noted that the level of TSH hormone increase in women with infertility compared to fertility, where thyroid hormones directly affect the ovaries, so one of the causes of female infertility was an over or underactive thyroid gland [189] .

3.12 Correlation between Vitamin D with Studied Parameters

Correlation measures the extent of the relationship between two or more variables and that those relationships differ in terms of their

strength, as well as determining the correlation between the variables through the correlation coefficient (r), which ranges between 0 and 1 or -1, where a value close to 1 indicates a relatively strong correlation and a value close to zero indicates a relatively weak correlation through the statistical results .The correlation coefficient was found to study the correlation relationship between the level of vitamin D and the level of hormones and other variables.

The association between the level of vit D and the parameters used in this study showed there is no link between vit D levels, age, and diastolic blood pressure Table (3-1), Figure (3-16 A, E). While it showed moderate to strong negative association mostly with BMI, WHR, WWR, Systolic blood pressure, as shown in Table (3-1) and in Figure (3.16 B, C, D, and F, respectively). This link was found to be statistically significant at ($P \leq 0.01$).

($r=-0.536$, $r=-0.495$, $r=-0.473$, $r=-0.444$ at $P \leq 0.01$, respectively).

Table (3-1): Correlation between Vitamin D with Some Variables and Hormones in all Patients of Infertility Women's

Vitamin D	R	P-Value
Age (years)	**0.057**	**0.097**
BMI(Kg/m^2)	**-0.536****	**0.01**
WHR cm	**-0.495****	**0.01**
WWR cm	**-0.473****	**0.01**
DBP (mm Hg)	**0.095**	**0.01**
SBP (mm Hg)	**-0.444****	**0.01**
LH mlU/ml	**-0.347****	**0.01**
FSH mlU/ml	**0.095**	**0.163**

LH/FSH	**-3.99****	**0.01**
T-Test ng/ml	**-0.451****	**0.01**
PRL µg/ml	**-0.486****	**0.01**
AMH ng/ml	**0.398****	**0.01**
E2 µg/ml	**0.270***	**0.02**
TSH µLU/ml	**-0.085**	**0.381**

****Correlation is Significant at the 0.01 level(2-tailed)**

***Correlation is Significant at the 0.05 level(2-tailed)**

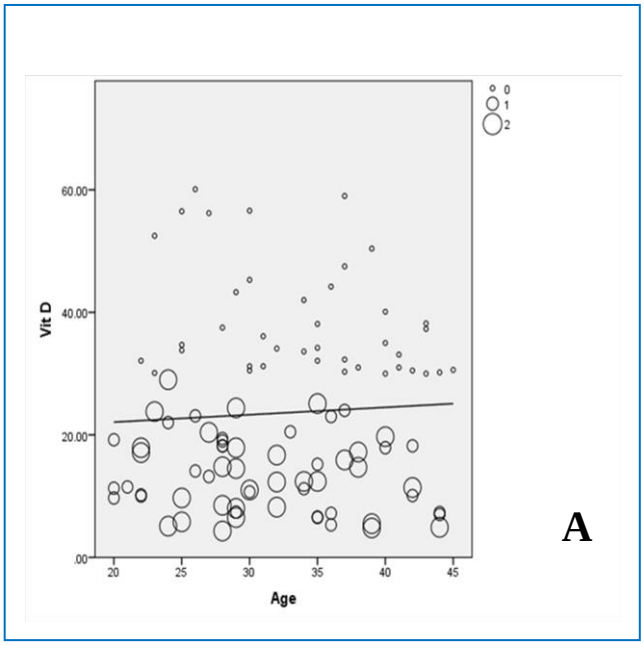
Our results of our study showed moderate negative correlation between vit D and BMI, WHR, WWR, as shown in Table (3-1) and in Figure (3-16). Our results are in agreement with many studies that showed that the level of vitamin D has an inverse relationship with the mass index body, waist, and hip proportions .This may be due to the fact that there is a greater storage of the lipid-soluble vitamin D in lipophilic fats, in obese women compared to women who are not obese or may increase your time spent indoors with less exposure to sunlight[19, 161 , 207,208.]

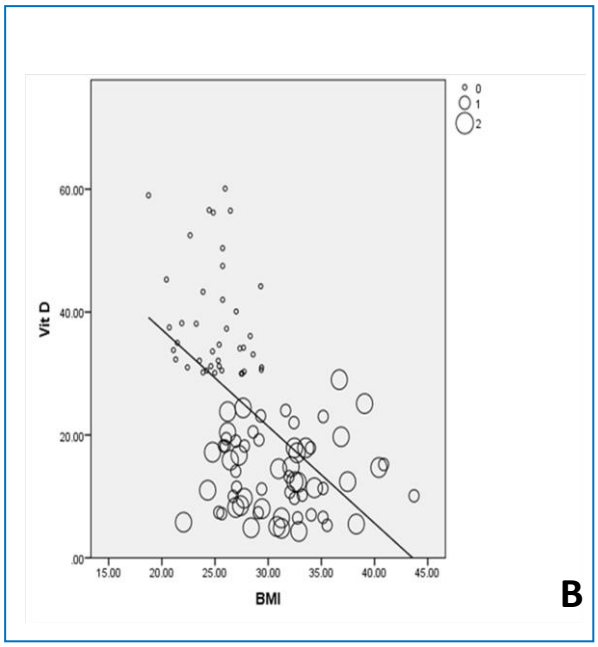
There is an inverse link between vit D levels and metabolic risk factors such as body fat percentage, waist-to-hip , blood pressure, and total testosterone [209 .]

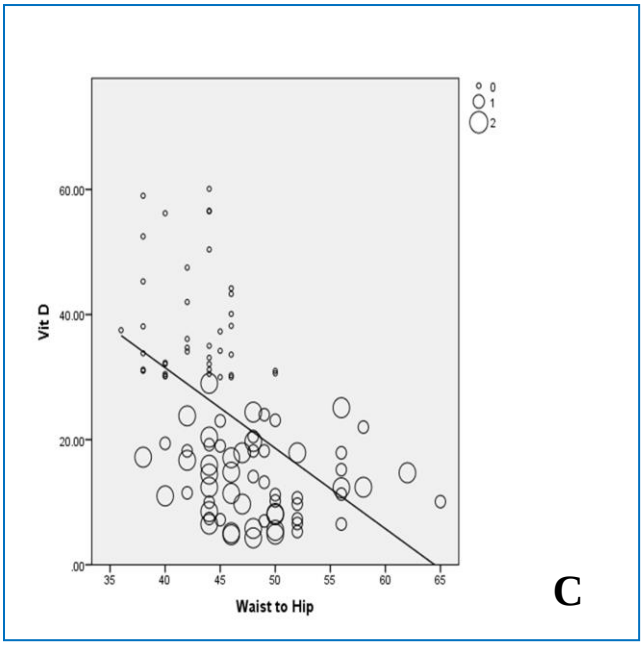
A study also discovered a link between vitamin D insufficiency and waist circumferences greater than 80 cm [17] .

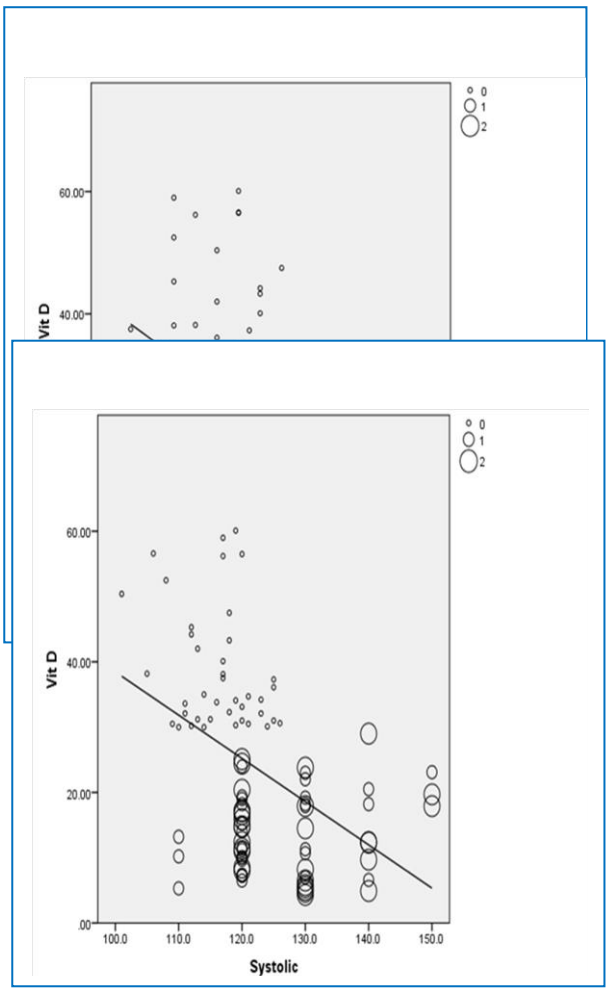
Our findings revealed a considerable negative relationship between vitamin D and systolic blood pressure (r = -0.444), as shown in Table (3-1) and in Figure (3-16 F).

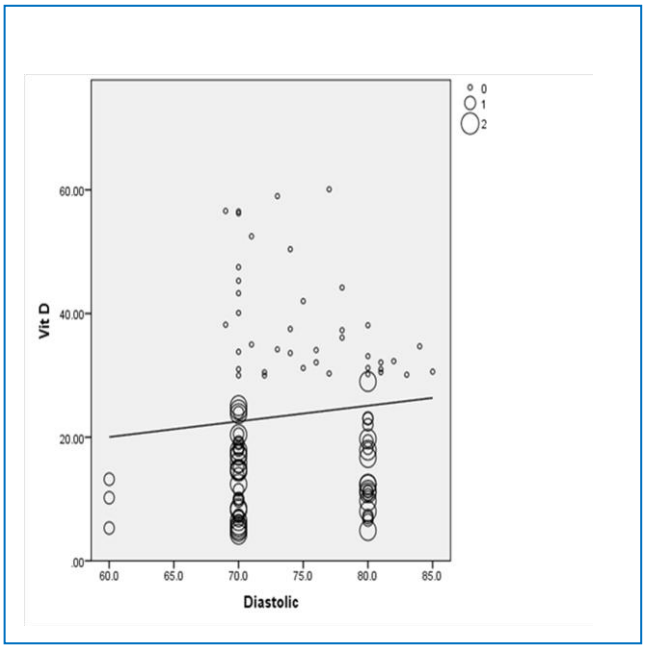
Vit D deficiency is associated with the risk of high blood pressure 210.











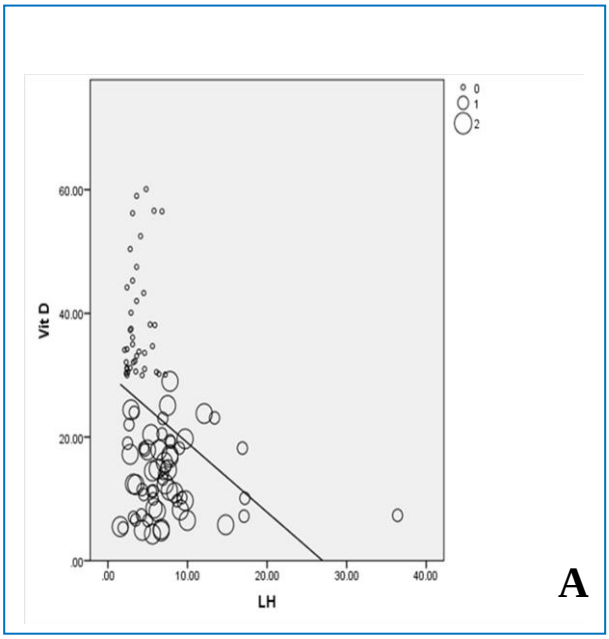
E

Figure (3-16): Correlation between Vitamin D with (A)Age, (B) BMI (C)WHR, (D) WWR, (E) Diastolic, (F) Systolic.

Our results also showed that there no statistically meaningful link between vitamin D and FSH hormone as shown in Table (3-1) and in Figure (3-17B).

While discovered a substantial negative with moderate correlation link between Vitamin D levels and LH, LH / FSH, and TT (r = -0.347, r = -0.399, r = -0.451 at p-value-0.01 respectively) as shown in Table (3-1), Figures (3-17 A, C and D respectively). Our findings are consistent with those of another study and it found a negative relationship between vit D and total testosterone in infertile women, as it was (r = -0.375).[211]

The results of our study indicated that vitamin D affects infertility and the level of hormones that are the main regulators of reproduction, as vitamin D receptors have in the uterus, oviduct, placenta, and ovaries [212].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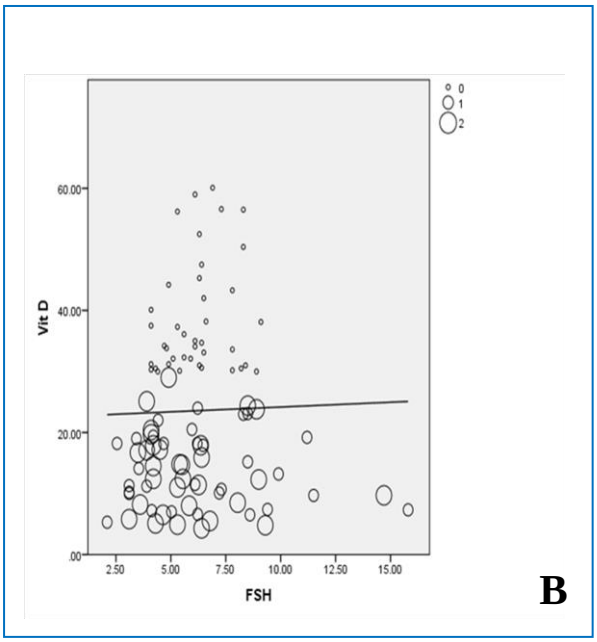
A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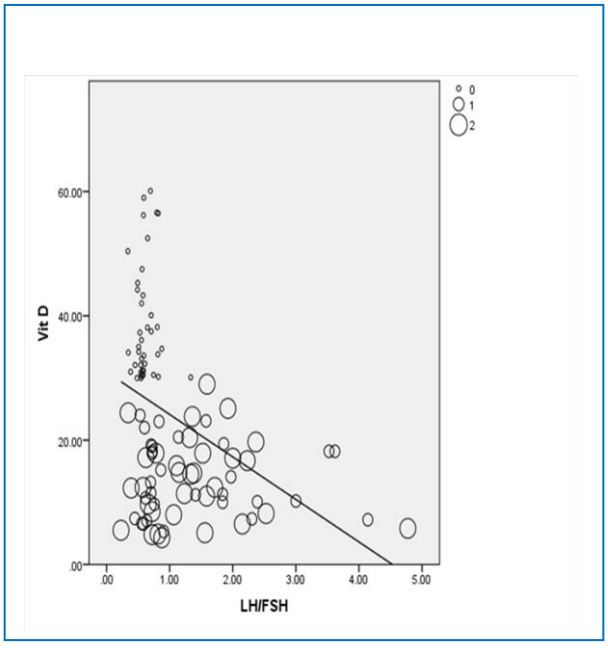
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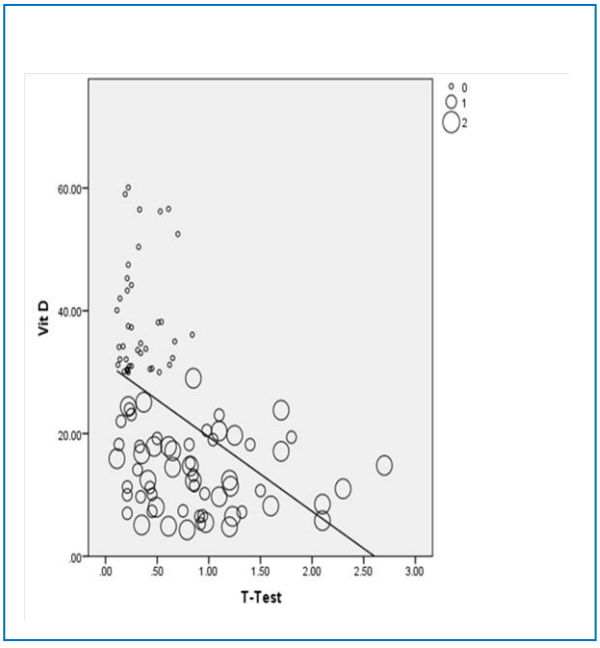
LH/FSH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T-Test

It was also shown by the results of the study that there is moderate negative association between vitamin D and the hormone prolactin, where (r = -0.486) as shown in Table (3-1), Figure (3-18A). The results of our study is in agreement with the study of Abodnaga *et al* [129].

While no association between vitamin D and the TSH hormone (r = -0.085), as shown in Table (1-3) and in Figure (3-18 D).

Our also discovered that there is moderate positive association between vitamin D levels and the AMH, as well as a weak to moderate positive correlation between vitamin D levels and the hormone E_2 (r = 0.398, r = 0.270 respectively) as shown in Table (3-1) and in figure (3-18 B and C, respectively).

Our study is in agreement with previous study one that showed a significantly increased AMH with increased vitamin D levels [200] .

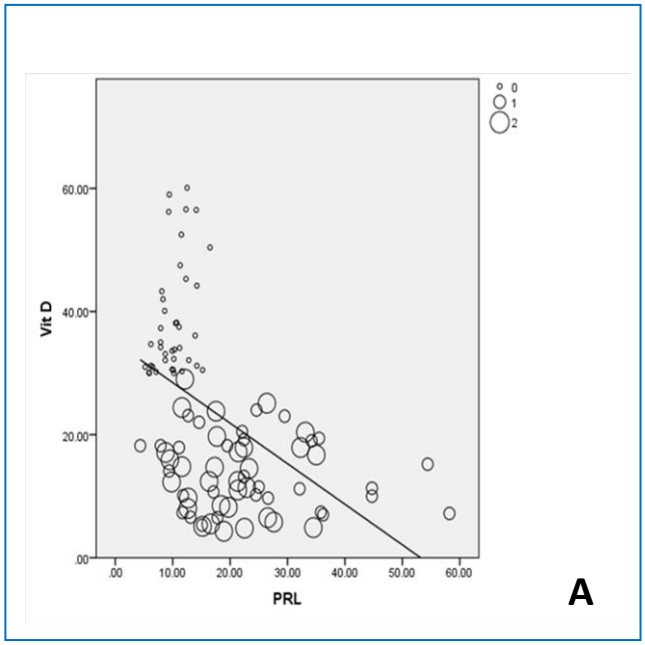
The results of our study also agreement with the study of Zahra Naderi, et al, that showed a positive correlation between vitamin D and AMH, as with an increase in the level of vit D. The level of AMH, which is produced from granular cells, increases and is considered the most reliable description of ovarian reserve, giving vitamin D helps reduce the rapid loss of ovarian reserve [213] .Our study also is in agreement with another studies [214,215].

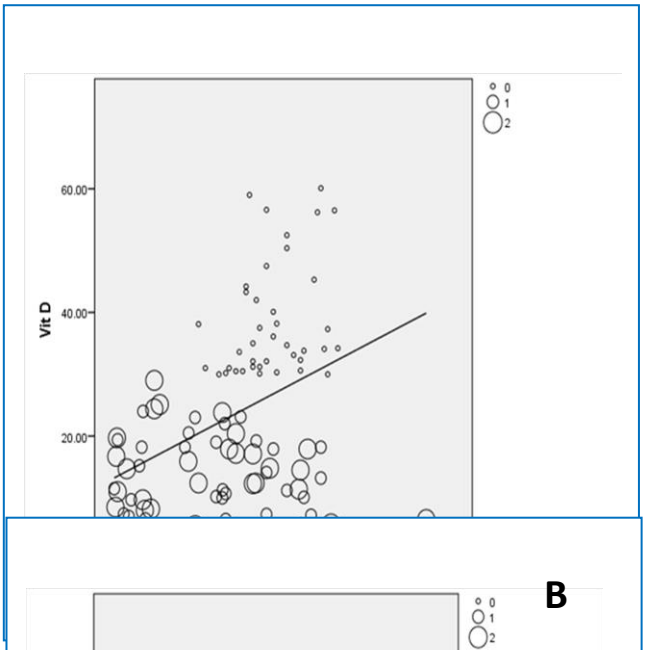
Another study conducted Abdul-Rasheed *et al* as an example a positive association of vitamin D with the level of AMH (r = 0.86) in women with infertility. This outcome is explained by the fact that vitamin D has a biological role in female reproduction [159] .

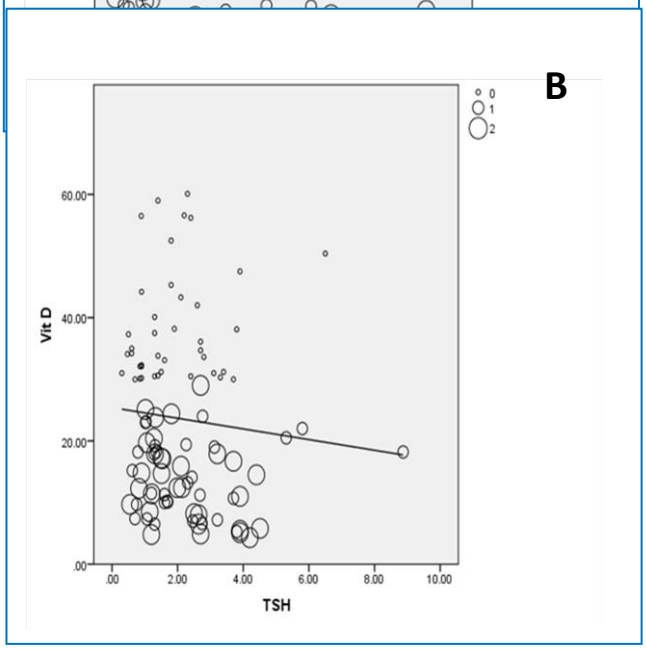
Vitamin D stimulates the production of estradiol by 9%, the production of progesterone by 13%, and estrone by 21% [81] .

Low levels of vitamin D have a deleterious influence on ovarian reserve as shown by a study done by found a favorable link between vitamin D and AMH [216] .

One study showed an association of vitamin D with AMH mRNA in human granulocytes [217] . One of the studies conducted on female mice discovered that vitamin D treatment had a positive impact on hormonal and structural changes, as they found a reduction in the levels of testosterone, LH, FSH, and LH / FSH in the group of mice with PCOS that underwent vitamin D treatment compared to the PCOS group [218] .







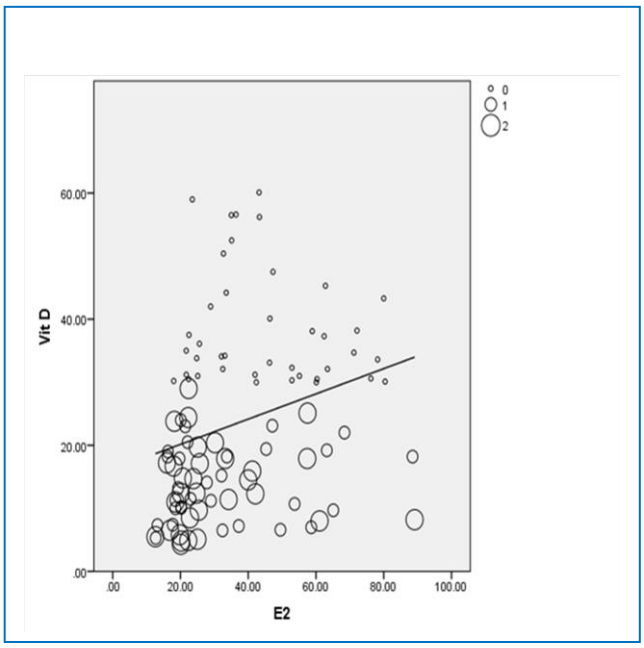


Figure (3-18): Correlation between Vitam
(C) E2, (D) TS

3.13 Conclusions

From our result, we conclude that:

1- The results of our study showed that vitamin D among infertile women compare

2- There were imbalances in horm level of LH, PRL, LH/FSH and testosterone and a decrease in the level of AMH and E2 were observed in infertile women compared to fertile women in the reproductive period.

3- It turns out that there is a relationship between vitamin D and most hormones, as its deficiency leads to a disturbance in the hormonal balance causing infertility. A positive correlation was found between vitamin D, AMH and E2, and a strong negative correlation was found between vitamin D and PRL, LH, TT, LH/ FSH, body mass index, waist to waist ratio, waist to hip ratio and systolic blood pressure.

4- There was an increase in body mass index and systolic blood pressure in infertile women compared to fertile women of childbearing age.

5- Increasing awareness among women of childbearing age by following a balanced diet with adequate exposure to sunlight,

taking vitamin D supplements, and exercising to avoid obesity, which has a major impact on causing infertility.

3.14 Recommendations

Based on the results we reached in our study, which showed the prevalence of vitamin D deficiency among infertile women compared to fertility and its relationship to hormones that cause infertility in women.

Our recommendation:

1- That vitamin D be part of the secondary evaluation of infertility and its use as a treatment for infertility.
2- Conducting more studies by collecting larger samples to clarify the effect of vitamin D on female infertility.
3- Studying genetic changes and identifying the genes responsible for infertility in women and studying the prevalence of vitamin D receptors FOKI, BsmI, TaqI, ApaI and their impact on infertility.

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Appendices

Appendix1: Questionnaires in Infertility patients in women

Questionnaire Form	
Fil No.	
Sample No.	
Code No.	
Data	2020 / /
Name	
Address	Rural ( ) - Urban( )
Age(years)	
Weight	KG( )
High	cm ( )
No. of children	( )
Smoking	( )
Alcohol use	( )
Waist to Hip	cm( )
Waist to waist	cm ( )
Medical History	) Years (
Age of Getting Disease	) Years(
Treatment	
Blood pressure	Systolic( ) Diastolic ( )
Hypertension drugs	
Menstrual State	Regular-R ( ) - Irregular-1( )
Ethnicity	
Dietary Vit D intake	
Educational state	
Type of infertility	Primary( ) cause: Secondary( ) cause:
High Sugar diet	

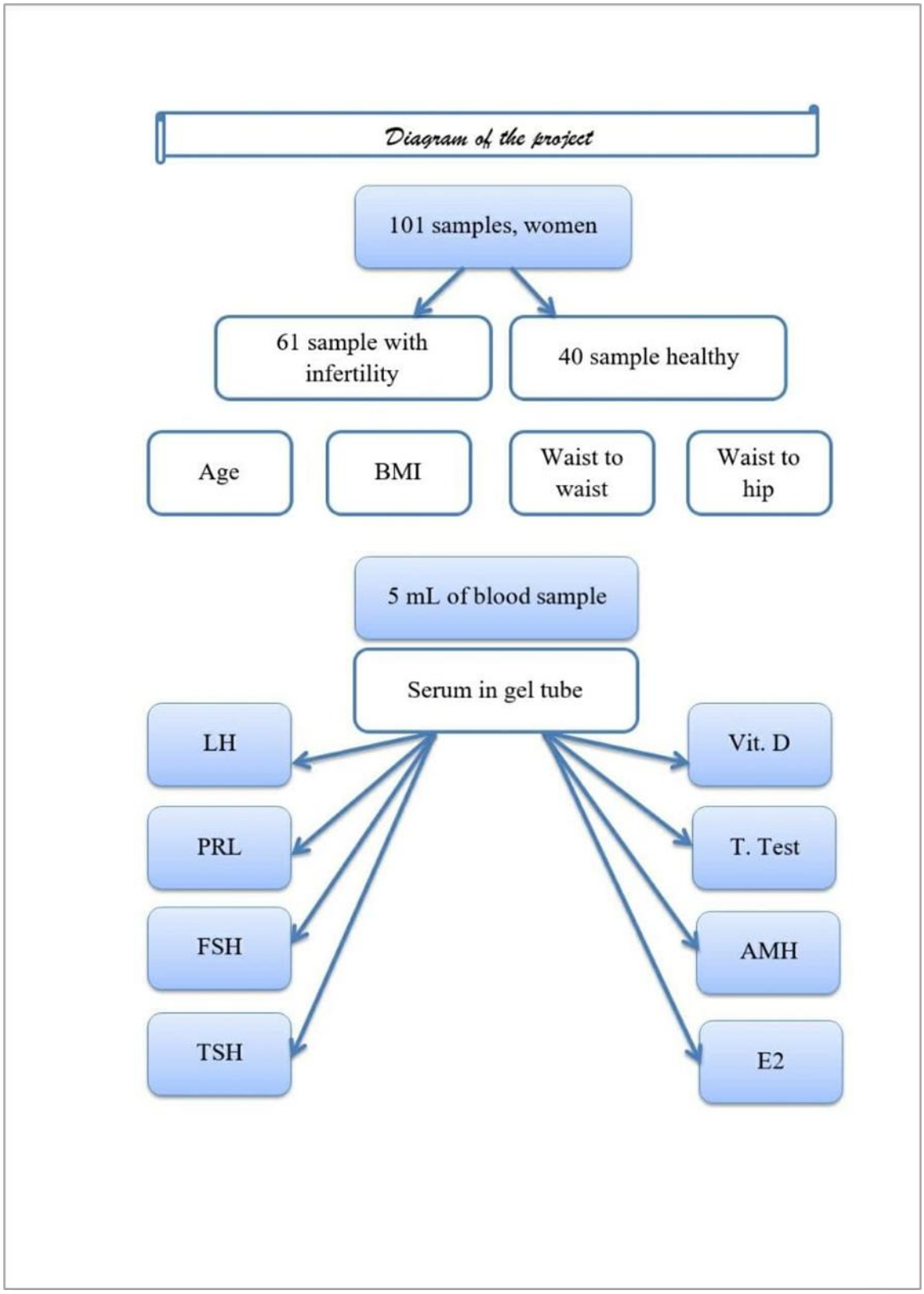
Diagram of the project
101 samples, women
61 sample with infertility
40 sample healthy
Age
BMI
Waist to waist
Waist to hip
5 mL of blood sample
Serum in gel tube
LH
PRL
FSH
TSH
Vit. D
T. Test
AMH
E2

Biochemical Test

Test	Result
AMH	Ng/ml
E2	µg/ml
FSH	mlU/ml
LH	mlU/ml
PRL	µg/ml
TSH	µLU/ml
T-Test	ng/ml
Vit D	ng/ml

Appendix 2: Table (1) Variables and Hormones in patient and Healthy

Variable	Groups	Mean ±St. Error	P-Value
Age (years)	C	34.08±1.05	0.097
	1	30.93±1.36	
	2	31.23±1.10	
Waist to Hip WHR (cm)	C	42.63±0.543	0.01
	1	49.60±1.003	
	2	47.42±0.936	
Waist to Waist WWR (cm)	C	40.10±0.576	0.01
	1	46.17±0.929	
	2	44.84±0.896	
BMI	C	25.00±0.42	0.01

	1	30.99±0.83	
	2	31.01±0.84	
Diastolic	C	75.23±0.76	0.128
DBP (mmHg)	1	72.67±1.17	
	2	73.55±0.87	
Systolic	C	116.28±0.94	0.01
SBP (mmHg)	1	124.67±1.71	
	2	128.39±1.68	
Vit D	C	38.56±1.48	0.01
ng/ml	1	13.91±1.07	
	2	13.55±1.21	
LH	C	3.81±0.22	0.01
(mlU/ml)	1	10.67±2.09	
	2	7.12±0.55	
FSH	C	6.19±0.23	0.163
MlU/ml	1	10.12±2.68	
	2	6.87±1.19	
LH/FSH	C	0.61±0.03	0.01
	1	1.41±0.19	
	2	1.36±0.16	
PRL	C	10.11±0.44	0.01
µg/ml	1	24.63±2.50	
	2	19.90±1.33	
TSH	C	1.90±0.20	0.381
µLU/ml	1	2.35±0.33	
	2	2.24±0.22	
AMH	C	4.67±0.17	0.01
ng/ml	1	2.83±0.35	

	2	2.88±0.41	
E2 µg/ml	C 1 2	45.06±2.95 33.69±3.60 29.89±2.99	0.02
T-Test ng/ml	C 1 2	0.33±0.03 0.17±0.08 1.03±0.12	0.01

C-Control, 1-Type one of infertility, 2-Type tow of infertility

الخلاصة

العقم هو عدم القدرة على الحمل بعد عام واحد من الجماع دون استخدام وسائل منع الحمل، وهو على نوعين (اولي وثانوي) فالعقم الاولي: هو العقم الذي يصيب المرأة منذ بداية حياتها الجنسية او زواجها، اما العقم الثانوي: فهو العقم الذي يصيب المرآة بعد إنجاب طفل او حمل انتهى بإجهاض او حمل خارج الرحم.

كان الهدف من هذه الدراسة هو تقييم مدى انتشار نقص فيتامينD بين النساء المصابات بالعقم.

في هذه الدراسة تم اجراء مقابلة لملئ الاستبيان حيث أجريت الدراسة على 101امراة قسمت الى مجموعتين: مجموعة الاصحاء تضمنت 40امراة سليمة، ومجموعة المصابات بالعقم تضمنت 61 امرأة (30امراة مصابة بالنوع الأول من العقم و31 امرأة مصابه بالنوع الثاني من العقم) أعمارهم تتراوح (18-45 سنة) جمعت من مستشفى الرازي في مدينة الرمادي خلال الفترة من تشرين الأول 2020 الى نيسان 2021.

في الختام كانت مستويات فيتامين د في مجموعة النساء المصابات بالعقم في كلا النوعين الأول والثاني اقل بشكل كبير مقارنة بالنساء الخصبات (38.56±1.48,13.91±1.07,13.55±1.21 نانوغرام /مليلتر على التوالي).

من ناحية العمر لم نجد فرق معنوي بين مجموعة المرضى والاصحاء، متوسطBMI,ازداد معنويا في مجموعة المرضى المصابين بالعقم مقارنة بمجموعة الاصحاء كذلك متوسط نسبة الخصر الى الخصر، الخصر الى الورك وضغط الدم الانقباضي ازداد معنويا في مجموعة النساء المصابات بالعقم مقارنة بالسليمات.

كما ان مستويات كل من هرمون LH،PRL ،TT ارتفعت بشكل ملحوظ في مجموعة النساء المصابات بالعقم مقارنة بالسليمات في حين لوحظ فرق غير معنوي في مستوى هرمون TSHوFSH بين مجموعة النساء المصابات بالعقم والسليمات في حين انخفض مستوى هرمون ال AMH وE_2 انخفاضا معنويا ملحوظا في مجموعة النساء المصابات بالعقم مقارنة بالنساء الخصبات.

طبقا الى تحليل بيرسون، لوحظ ارتباط موجب بين فيتامين D وهرمون الAMH وE_2 كما لوحظ وجود ارتباط سالب بين فيتامين D وكل من: LH،PRL ،TT ،BMI ، WHR،WWR، SBP، LH/FSH .

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
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كليه العلوم
قسم علوم الكيمياء

تأثير مستويات فيتامين د على مرتسم الهرمونات عند النساء المصابات بالعقم في محافظة الانبار

رسالة مقدمة

الى مجلس كلية العلوم، جامعة الانبار كجزء من متطلبات نيل شهادة الماجستير في علوم الكيمياء

من قبل الطالبة

ايمان علي جدعان

بكالوريوس علوم الكيمياء/كلية العلوم/جامعة الانبار/ 2014

بأشراف

الأستاذ المساعد الدكتور

حميد حسين علي

كيمياء حياتية

الأستاذ المساعد الدكتور

رفل مصطفى مرشد

اختصاص نسائية وعقم وتوليد

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