BAŞKENT UNIVERSITY
HEALTH SCIENCES INSTITUTE
DEPARTMENT OF PHYSIOLOGY
PHYSIOLOGY MASTER'S PROGRAM WITH THESIS

THE CORRELATION BETWEEN SERUM VITAMIN D AND OOCYTE QUALITY, POTENTIAL OF FERTILIZATION AND EMBRYO DEVELOPMENT IN THE ASSISTED REPRODUCTIVE TECHNOLOGY (ART) CASES

PREPARED BY

ASMA BASHIR BEN ZAIR

MASTER'S THESIS

ANKARA - 2022

BAŞKENT UNIVERSITY
HEALTH SCIENCES INSTITUTE
DEPARTMENT OF PHYSIOLOGY
PHYSIOLOGY MASTER'S PROGRAM WITH THESIS

THE CORRELATION BETWEEN SERUM VITAMIN D AND OOCYTE QUALITY, POTENTIAL OF FERTILIZATION AND EMBRYO DEVELOPMENT IN THE ASSISTED REPRODUCTIVE TECHNOLOGY (ART) CASES

PREPARED BY
ASMA BASHIR BEN ZAIR

MASTER'S THESIS

THESIS SUPERVISOR
Prof. Dr. NAZAN DOLU

ANKARA - 2022

BAŞKENT UNIVERSITY
HEALTH SCIENCE INSTITUTE

This study, which was prepared by Asma Bashir Ben Zair within the framework of the Department of Physiology Master's Program was accepted as the Master's Thesis by the following jury.

Thesis Defense Date: … / … / ……..

Title of the
Thesis:……

Thesis Jury Members (Title, Name - Surname, Institution)	**Signature**
……	..……..
……	……
……	……
……	..……
……	……If Required
……	……... If Required

APPROVAL

……..

..…… Institute
Director Date: … / … / ……..

BAŞKENT UNIVERSITY HEALTH SCIENCES INSTITUTE MASTER'S THESIS STUDY ORIGINALITY REPORT

Date: 16/ 06 / 2022

Student Name: Surname: Asma Bashir Ben Zair.
Student Number: 21910604
Department: Physiology
Program: Physiology Master's program
Supervisor's Title /Name, Surname: Prof. Dr. Nazan Dolu
Title of Thesis: **The Correlation Between Serum Vitamin D And Oocyte Quality, Potential Of Fertilization And Embryo Development In The Assisted Reproductive Technology (Art) Cases**
According to the originality report, which was taken by the plagiarism detection program named Turnitin by the personal / thesis supervisor on 14/ 06/2022 on the 38-page part of my Master's / Doctoral thesis study mentioned above, which consists of Introduction, Main Sections and Conclusion Section, the similarity rate of my thesis is 17 %. Applied filters:

1. Excluding bibliography
2. Excluding quotations
3. Excluding text parts that contain less than five (5) words of overlap

I have examined the Baskent University Institute Thesis Study Originality Report Obtaining and Using Procedures and Principles. I hereby declare that my thesis work does not include any plagiarism, that I accept all kinds of legal responsibilities that may arise in case the contrary is determined and that the information given above is correct

Student Signature:…….

APPROVAL
Date: 16/ 06 / 2022

ACKNOWLEDGMENT

First and foremost, I'd like to express my gratitude to Almighty Allah for assisting me in completing the assignment set to me by my supervisor. Despite several challenges that developed during my thesis studies, I was able to complete my thesis with the help of my friends with whom I collaborated.

During this time, I'd want to express my gratitude to my supervisor, Prof. Dr. Nazan Dolu, because without his guidance, my thesis would not have been completed successfully. I'm grateful to him for her guidance and assistance during my studies, which enabled me to get positive results from my research. In additional to my supervisor, I would like to thank Prof. Dr. Mohamed Danfour and Dr. Nazlı Karimi for his consistent assistance.

Finally, I'd want to express my gratitude to my close family and friends for always encouraging and supporting my attempts to accomplish a good work. I'm hoping that all of our hard work will pay off for me and our other collaborators.

ÖZET

Asma Bashir Ben Zair, Yardımcı Üreme Teknolojisi (Art) Olgularında Serum D Vitamini İle Oosit Kalitesi, Fertilizasyon Potansiyeli Ve Embriyo Gelişimi Arasındaki İlişki, Başkent Üniversitesi Sağlik Bilimleri Enstitüsü Fizyoloji Anabilim Dalı, Fizyoloji Tezli Yüksek Lisans Programı Yüksek Lisans Tezi, 2022

D vitamini yetersizliği insidansının artmasının bir sonucu olarak D vitamini araştırmalarına daha fazla ilgi duyulmaktadır. D vitamini yetersizliği, bir dizi akut ve kronik bozuklukla ilişkilendirilmiştir. 25-hidroksi [25(OH)] D vitamininin insan üreme sistemi de dahil olmak üzere çeşitli sistemlere önemli etkileri olduğuna dair kanıtlar artmaktadır. D vitamini, adet döngüsün düzenlenmesi, endometriyal proliferasyon, foliküler gelişim, erken dismenorenin rahatlaması ve vajinal fibroidin azalmasına yardımcı olabilir. Çalışmalar, kanında D vitamini düzeyi yüksek olan kadınların gebe kalma olasılığının daha yüksek olduğunu göstermektedir. Bu çalışmanın amacı, tüp bebek hastalarında 25 (OH) D vitamini kan seviyeleri ile oosit kalitesi, *in vitro* bölünme başarısı ve embriyo kalitesi arasında bir ilişki olup olmadığını göstermektir. Ocak 2018 ile Aralık 2020 tarihleri arasında Libya, Misurata'daki bir sağlık merkezinde tüp bebek tedavisi gören bireyler geriye dönük olarak incelendi. Hastaların tedavi öncesi ve sonrası Luteinizan Hormon (LH) seviyeleri kaydedildi. Çalışmaya katılan tüm hastalara tedaviye başlandıktan sonra, iki ay boyunca günde 1.500 ile 2.000 IU (günde 3 sprey) dozunda D vitamini uygulandı. Tedaviden önce ve sonra LH seviyelerinde istatistiksel olarak anlamlı farklılık bulundu. Ancak D vitamini takviyesi ile oosit kalitesi, in vitro bölünme başarısı ve embriyo kalitesi arasında anlamlı farklılık gözlenmedi. Bu çalışmada, D vitamini takviyesinin kısır evliliklerdeki kadınlar üzerindeki etkisinin olmadığı gösterilmiştir. D vitamini takviyesinin invitro fertilizasyon başarısına yardımcı olup olmayacağını daha ayrıntılı görmek için ek çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: D vitamini, oosit kalitesi, bölünme başarısı, embriyo kalitesi, tüp bebek hastaları

Bu çalışma Başkent Üniversitesi Araştırma Komisyonu tarafından KA21/117 nolu proje ile desteklenmiştir.

ABSTRACT

Asma Bashir Ben Zair, The Correlation Between Serum Vitamin D and Oocyte Quality, Potential of Fertilization and Embryo Development in The Assisted Reproductive Technology (Art) Cases, Baskent University Institute of Health Sciences, Department of Physiology, Physiology Master's Thesis, 2022

There is increasing interest in vitamin D research as a result of the increased incidence of vitamin D insufficiency. Vitamin D deficiency has been associated with a number of acute and chronic disorders. There is increasing evidence that 25-hydroxy[25(OH)] vitamin D has important effects on a variety of systems, including the human reproductive system. Vitamin D can help regulate the menstrual cycle, endometrial proliferation, follicular development, relief of early dysmenorrhea, and decrease vaginal fibroid. Studies show that women with high levels of vitamin D in their blood are more likely to conceive. The aim of this study is to show whether there is a relationship between 25 (OH) vitamin D blood levels and oocyte quality, in vitro division success and embryo quality in IVF patients. Individuals who underwent IVF treatment at a health center in Misurata, Libya between January 2018 and December 2020 were retrospectively analyzed. Luteinizing Hormone (LH) levels of the patients were recorded before and after the treatment. All patients in the study were administered vitamin D at a dose of 1,500 to 2,000 IU (3 sprays per day) for two months after treatment was started. A statistically significant difference was found in LH levels before and after treatment. However, no significant difference was observed between vitamin D supplementation and oocyte quality, in vitro division success and embryo quality. In this study, it was shown that vitamin D supplementation did not affect women in infertile marriages. Additional studies are needed to see in more detail whether vitamin D supplementation will help in vitro fertilization success.

Keywords: Vitamin D, oocyte quality, cleavage success, embryo quality, IVF patients

This study was supported by the Başkent University Research Board with the KA21/117 project number.

TABLE OF CONTENT

LIST OF TABLES

page

LIST OF FIGURES

page

LIST OF SYMBOLS AND ABBREVIATIONS

25(OH)D	25-hydroxyvitamin D
AMH	anti-müllerian hormone
ART	assisted Reproductive Technologies
CLIA	chemiluminescent Immunoassay
CYP2R1	cytochrome P450 2R1
FDA	food and Drug Administration
FF	follicular Fluid
FSH	follicle-Stimulating Hormone
HCG	human Chorionic Gonadotropin
IVF	*in Vitro* Fertilization
LH	luteinizing Hormone
PCOS	polycystic Ovarian Syndrome
PCT	proximal Convolute Tubule
PVS	perivitelline Space
RXR	retinoid X Receptor
SHBG	sex Hormone Binding Globulin
UV	ultraviolet
VDBP	vitamin D-Binding Protein
VDRE	vitamin D Response Element
VDRs	vitamin D Receptors

1. INTRODUCTION

Vitamin D was once assumed to be just needed for bone homeostasis, but it has recently been "rediscovered" as a "multitasking vitamin" in addition to being a "bone vitamin." It has been connected to a variety of extraskeletal processes as well as system homeostasis. The scientific community is becoming more interested in vitamin D research as a result of its pleiotropic effects and the increasing rate of vitamin D deficiency. Vitamin D deficiency has been related to some acute and chronic illnesses, including musculoskeletal problems, type 1 and type 2 diabetes, hypogonadism, polycystic ovarian syndrome, neoplasm, autism, dementia, and cardiovascular disease. In light of these findings, vitamin D supplementation has been advocated as a possible treatment for a variety of illnesses. Vitamin D is produced predominantly through cutaneous synthesis as cholecalciferol (D3) and is available through diet and dietary supplements [1].

Calcium and phosphorus balance, as well as bone mineralization support, are all functions of vitamin D, and its shortage raises the danger of osteoarthritis, fractured bones, and muscular weakness. However, evidence is mounting that 25-hydroxyvitamin D [25(OH)D] [25(OH)D] has a variety of other important impacts, including those on the human reproductive system. Vitamin D may aid in the restoration of the menstrual cycle and endometrial proliferation, as well as follicular growth, the reduction of initial dysmenorrhea, and the reduction of vaginal fibroid [2].

Vitamin D receptors (VDRs) have already been discovered in the ovary epithelial cells, endometrial, fallopian tubes, placenta, and residual cells of female reproductive organs. Ovarian steroid synthesis could possibly be linked to vitamin D receptors. VDR expression rises throughout pregnancy. insufficient vitamin D values were also associated with gestational diabetes and preeclampsia in clinical studies [3].

The purpose of this research is to investigate if there's a correlation between blood levels of 25 (OH) D and oocyte quality, in vitro cleavage success, and embryo quality in IVF patients.

2. GENERAL KNOWLEDGE

2.1. Vitamin D

Vitamin D is a fat-soluble corticosteroid that improves calcium, magnesium, and phosphate absorption in the gut, among other things. Vitamin D3 (also known as cholecalciferol) and vitamin D2 are the most essential determinants in this group (ergocalciferol) in people [4].

Cholecalciferol production in the deeper surface of the epidermis via a chemical mechanism driven by sunlight exposure is the most natural source of the vitamin (particularly UV rays). Food and supplements are both good sources of cholecalciferol and ergocalciferol. Vitamin D levels are naturally high in only a few meals, such as fatty fish meat. Because sun exposure varies by demographic and there are dosage requirements, most dietary advice assumes that a person's vitamin D should be taken orally. Because of the potential for skin cancer, light exposure wasn't without risk. [5].

Vitamin D acquired through food or skin production is physiologically inactive. The hydroxyprotease enzyme activates it in two stages, one of the primaries in the liver and the next in the kidneys. This is not precisely a vitamin because most mammals can create adequate levels of vitamin D if exposed to enough sunlight [6].

Cholecalciferol is metabolized to calcifediol (25-hydroxycholecalciferol) in the liver, and in the kidneys, ergocalciferol is turned to 25-hydroxyergocalciferol. The presence of these two vitamin D compounds (also known as 25-hydroxyvitamin D or 25(OH)D) in human plasma is used to measure vitamin D status [7]. Calcitriol (as well described as 1,25dihydroxycholecalciferol), the physiological source of vitamin D, is created by the kidney and some cells of the immune system. Calcitriol is a hormone that circulates in the bloodstream and helps with calcium and phosphate levels, as well as healthy growth and strong bones. Calcitriol has also been shown to improve cell formation, neuromuscular and immunological function, and inflammation decrease [8].

Vitamin D is an essential for calcium balance and metabolism. It was identified while looking for a poor diet in youngsters with rickets (a form of rickets in childhood). Vitamin D supplements are given to patients to prevent or treat osteomalacia and rickets. The evidence supporting additional health benefits of vitamin D supplementation in the wider public is mixed. Another meta-analysis indicated a modest reduction in senior death, while another found no strong evidence for supplementation to prevent particular diseases [9].

2.1.1. Numerous patterns (vitamers) of vitamin D

Vitamin D2 (ergocalciferol) and vitamin D3 are the main two kinds (cholecalciferol). Vitamin D without an index is referred to as calciferol and includes D2, D3, and both. Vitamin D2's chemical characterization was accomplished in 1931. In 1935, ultraviolet light from 7-dehydrocholesterol was utilized to study and confirm the chemical structure of vitamin D3. Even though a molecular name for vitamin D forms was developed in 1981, several names are still in use [10]. Vitamin D is available in several forms, all of which are secosteroids, or corticosteroids to one of the steroid ring's links disrupted. Vitamin D2 differs from vitamin D3 in that it contains a two-carbon bond and a methyl group at carbon 24.

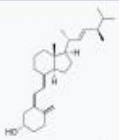
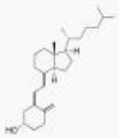
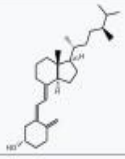
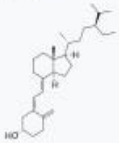
Name	Chemical composition	Structure
Vitamin D_1	Mixture of molecular compounds of ergocalciferol with lumisterol, 1:1	
Vitamin D_2	ergocalciferol (made from ergosterol)	
Vitamin D_3	cholecalciferol (made from 7-dehydrocholesterol in the skin).	
Vitamin D_4	22-dihydroergocalciferol	
Vitamin D_5	sitocalciferol (made from 7-dehydrositosterol)	

Figure 2. 1. Types of Vitamin D (Mostafizul Islam Ranzu , 2018)

2.1.2. Action mechanism

Vitamin D travels down and is transformed in the liver into the propeptide calcifediol. In the kidneys, circulating calcifediol can be transformed into calcitriol, the physiologically active form of vitamin D [11].

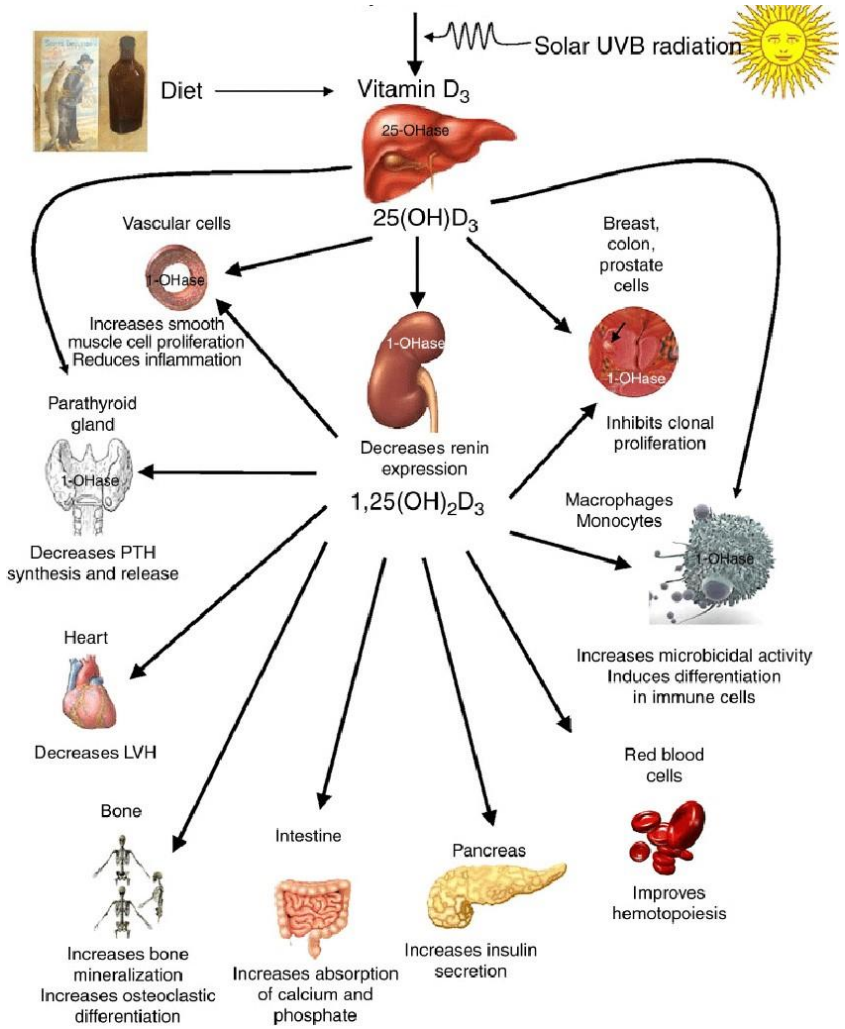


Figure 2. 2**. The Mechanism of Action of Vitamin D**. (Doç. Dr. INDRANİ KALKAN , İstanbul Aydın Üniversitesi)

Whether produced through the skin or consumed, vitamin D is hydrolyzed in the liver at position 25 (top right of the structure) to become 25-hydroxycholecalciferol (calcifediol or 25(OH)D. The microsomal enzyme vitamin D 25-hydroxylase, which is produced by the CYP2R1 gene sequence and produced by the liver, catalyzes this reaction. The result is subsequently released into the bloodstream, where it binds to a vitamin D-binding protein, a -globulin carrier protein [12].

Calcifediol is carried to the renal tubular of the kidneys, where it is turned to calcitriol (1,25dihydroxycholecalciferol, 1,25(OH)2D) via hydroxylation at the 1-position (bottom right of the molecule). The CYP27B1 human gene produces the enzyme 25-hydroxyvitamin D3alpha-hydroxylase, which catalyzes the hydrolysis of calcidiol to calcitriol. Parathyroid hormone, as well as low calcium or phosphate levels, increase the activity of CYP27B1 [13].

Calcitriol is carried in the bloodstream during the final transfer process in the kidney. Calcitriol circulates throughout the system via binding to vitamin D-binding protein, which can be found in the gut, kidneys, and bones. Calcitriol has been the greatest potent natural ligand for the vitamin D receptor, which is responsible for most of the physiological effects of vitamin D [14].

Calcitriol is produced by a range of cells, including immune system monocytes and macrophages, as well as the kidneys. Calcitriol, a cytokine produced by monocytes and macrophages, operates locally as a cytokine, changing physiological defenses toward bacterial invaders by enhancing the body's immune system [15].

To mediate its biological functions, the active vitamin D metabolite calcitriol binds to the vitamin D receptor (VDR), which is mostly found in target cell nuclei. When calcium absorption transportation protein in the gut bind to the VDR, the VDR acts as a transcription factor, affecting gene expression of calcium absorption transport proteins (such as TRPV6 and calbindin). VDRs are steroid/thyroid hormone receptors that are found in many systems, such as the brain, heart, skin, gonads, prostate, and breast [16]. VDR activation in gut, bone, kidney and parathyroid gland cells keeps calcium and phosphate levels in the blood (aided by parathyroid hormone and calcitonin) and bone content in check.

Some of vitamin D's most significant features include preserving the skeletal magnesium balancing act by trying to promote magnesium absorption in the intestine, trying to promote osteoclast activity by raising bone resorption counts, maintaining calcium and phosphate levels for bone growth, and enabling the parathyroid hormone to function helps to maintain serum calcium [17]. As a result, vitamin D changes the body's mineral metabolism, which may result in decreased bone mass and an increased risk of osteoporosis (osteoporosis) or broken bones. Vitamin D is indeed vital for bone formation due to its role as a powerful bone resorption stimulator [18].

The VDR regulates cell growth and development. VDRs are found in a range of white blood cells, especially macrophages and activated T and B cells, and vitamin D influences human health. Vitamin D stimulates tyrosine hydroxylase expression of genes in adrenal medulla cells in vitro and impacts neurotrophic factor, nitric oxide synthase, and glutathione synthesis in vitro [19]. All of these factors influence an athlete's performance [20].

2.1.3. The physiology and metabolism of vitamin D

Vitamin D is a hormone produced that is required by the body to control calcium and phosphate levels. Because the source of vitamin D is not purely dietary, the name "vitamin

D" is misleading. In response to UV radiation, vitamin D is mostly generated in the epidermis. Vitamin D is obtained in small amounts (5%) through food sources. In areas with limited sun radiation, external dietary supplements and fortified meals must be employed to meet the demands. The two types of vitamin D found in food are tree vitamin D2 (ergocalciferol) and mammal vitamin D3 (cholecalciferol) [21]. Dietary cholesterol produces provitamin D3 (7-dehydrocholesterol), the epidermis precursor of vitamin D. Short-wave UVB radiation converts the B-ring of 7-dehydrocholesterol producing previtamin D3 (cholecalciferol) or the inactive derivatives luminosterol and tachysterol. These two products aid in the regulation of vitamin D levels. Vitamin D is frequently found in the protein-bound form in the blood. The vitamin D binding protein binds to more than 80% of it (VDBP). Mixed-function P450 monooxidases hydroxylate it twice [22]. In the liver, mitochondria CYP27A1 or mitochondrial CYP2R1 regulate vitamin D hydrogenation to 25(oh)d. CYP27B1 converts 1,25-dihydroxyvitamin D3 (1,25 (OH) 2D3) to the biologically active state 1,25-dihydroxyvitamin D3 (1,25 (OH) 2D3) in the kidney's closer convolute tubule (PCT). The availability of 1,25 (OH) 2D3 in target tissue regulates the mitochondrial CYP24A1, which inhibits the activity circulation sources of vitamin D. Since then, these enzymes have been discovered in a variety of distant tissues, demonstrating that active vitamin D levels are controlled locally [23].

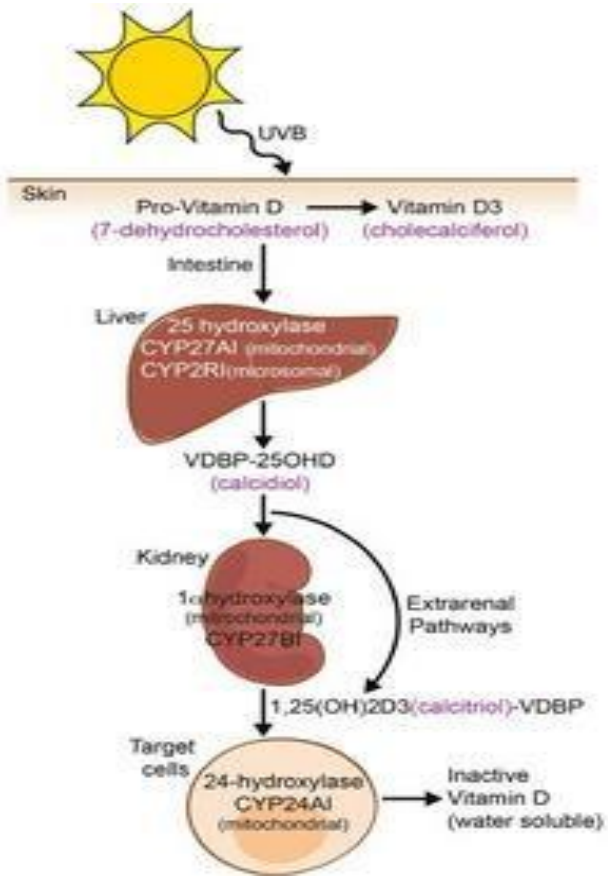


Figure 2. 3. The skin is the primary source of vitamin D [91].

The vitamin D prelude (7-dehydrocholesterol) is converted to cholecalciferol in this phase. In the intestines, a little amount of active vitamin D precursor is created. Vitamin D is converted into 25-hydroxyvitamin D by the mitochondrial CYP27A1 and microsomal CYP2R1 enzymes in the liver. Following the binding of vit D protein, it is 1-hydroxylated in the kidney by CYP27B1 (VDBP). 1,25 (OH) 2D3 activates CYP24A1, which converts vitamin D to its inactive form in target cells [24].

Vitamin D that is active attaches to VDBP and is delivered to the cell surface. When the VDBP reaches the target cell, vitamin D is generated, and 1,25 (OH) 2D3 attaches to the cytoplasmic vitamin D receptors (VDR). Vitamin D is delivered to the cell nucleus by VDR. To deal with a transcription factor, it experiences conformational. With the retinoid X receptor (RXR) in conjunction with other founders, including the DRIP complex, the active VDR creates a transcription factor unit that connects to the gene promoter's vit D response element (VDRE). As a result of this binding, genetic makeup is controlled. In addition to the genomic putative binding pocket that regulates gene transcription, the VDR protein has a second ligand-binding pocket that can promote faster non-genomic effects. Non-genomic effects include a fast rise in intracellular calcium channels, activation of phospholipase C, and calcium channel opening [25].

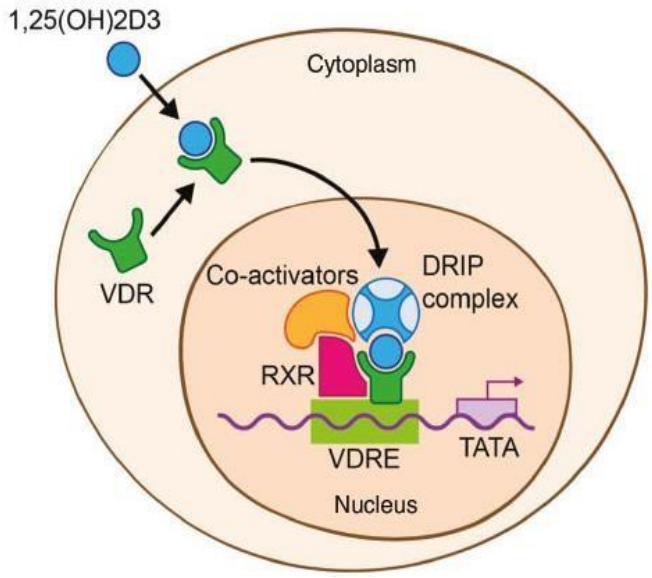


Figure 2. 4. Once within the target cell, 1,25(OH)2D3 interacts with the cytoplasmic vitamin D receptor (VDR) [91].

The 1,25(OH)2D3 that has been VDR-coupled is then transferred to the nucleus. It forms complexes with the founder such as the retinoid X receptor (RXR) and the DRIP complex.

This transcriptional unit regulates transcription by attaching to the vitamin D signaling route (VDRE) at the gene promoter [26].

2.1.4. Vitamin D insufficiency could be brought on by several variables

Vitamin D is clear as a serum the degree of 25 (OH) D between 21 and 29 ng/ml, whereas vitamin D deficiency is described as a serum point of 25 (OH) D less than 20 ng/ml. Vitamin D deficiency affects one-third of the number of people in the USA. By the conclusion of the winter, 42% of African American girls and women in the United States aged 15 to 49 had a 25 (OH) D level of less than 15 ng/ml. Ladies are taking vitamin D-containing prenatal vitamins and calcium supplements (400 IU/day) when pregnant or nursing and are nonetheless at risk of vitamin D deficiency. Decreased dietary intake and/or absorption: Celiac, short bowel syndrome, gastric band, inflammatory bowel, persistent pancreas failure, and cystic fibrosis are among the malabsorption disorders that can cause vitamin D deficiency. In the elderly, lower oral vitamin D consumption is more common [27].

Decreased sun exposure: Sunlight absorbs between 50 and 90 percent of vitamin D, with the rest coming from food. Vitamin D insufficiency can be avoided by spending 20 minutes a day in direct sunlight on more than 40% of the body's surface. With age, cutaneous vitamin D synthesis diminishes. People with dark skin have reduced cutaneous vitamin D synthesis. Reduced sun exposure in nursing homes or long-term hospital stays can also result in vitamin D insufficiency. Those who use sunscreen regularly have less effective sun exposure [28].

Endogenous synthesis has decreased: Persons with severe liver problems, such as fibrosis, may have a problem with 25-hydroxylation, resulting in a vitamin D deficit. Hyperparathyroidism, renal failure, or a deficiency of 1-alpha-hydroxylase can all produce problems with 1-alpha-25-hydroxylation [29].

Increased hepatic catabolism: Phenobarbital, carbamazepine, dexamethasone, nifedipine, spironolactone, clotrimazole, and rifampin are examples of medications that stimulate hepatic p450 enzymes, which promote vitamin D breakdown.

End organ resistance: Vitamin D resistance in the end organs can be found in inherited vitamin D-resistant rickets. The majority of people with vitamin D insufficiency have no symptoms. On the other hand, even a moderate chronic vitamin D can cause chronic hypocalcemia and hypothyroidism in the elderly, increasing the osteosarcoma danger, falls, and fractions. Secondary hyperparathyroidism signs include bone pain, muscle aches, myalgias, weariness, muscular jerking (fasciculations), and weakening in patients with chronic and severe vitamin D deficiency. Fragility fractures can be caused by chronic vitamin

D insufficiency, which leads to osteoporosis. Vitamin D deficiency in children can cause irritability, fatigue, developmental delays, bone deformities, and fractures [30].

2.1.5. Recommended serum levels

Guidelines for 25 (OH) D serum concentrations vary per agency and are based on a plethora of variables, including age. Give values for 25 (OH) D in ng/ml in general. Approximately 2.5 nmol / L is equivalent to 1 ng / mL [31].

For all outcomes, serum 25 (OH) D levels of around 30 ng/ml (75 nmol/L) appeared to be the best. According to another study, sportsmen should have vitamin D levels of 30 to 40 ng/ml (75 to 100 nmol/L). Several investigations have discovered differences in 25 (OH) D blood levels among ethnicities; studies suggest that these differences are caused by both inherited and environmental causes. Supplementation to meet these standards may result in detrimental vascular calcification [32]. Although the findings of the research looked at were mixed, the risk of heart disease rises when vitamin D standards in the blood fall below 8 to 24 ng/ml (20 to 60 nmol/l) [33]. For general health, a serum 25 (OH) D standard of 20 ng/mL (50 nmol/L) is recommended. To ensure that the prescribed consumption quantities satisfy the intended 25 (OH) D blood values in nearly most people, the recommended dietary consumption for vitamin D is chosen with a safety margin and "exceeds" the target serum value. The rules apply to everyone with dark skin or limited sun exposure because sun exposure is thought to have little effect on plasma 25 (OH) D levels. Vitamin D poisoning is quite uncommon. It's brought on by taking too much vitamin D and not getting enough sun rays. The toxicity limit for vitamin D has not been created; even so, a few studies suggest that the daily consumption level (UL) for ages 9 to 71 years (100 g / day), while other research findings suggest that maintained consumption of so much over 50,000 IU / day in healthy adults (1250 g) can cause obvious toxicity after many months and raise serum 25hydroxyvitamin D concentrations to 150 ng/ml and more [34]. People with these health conditions, such as congenital hypothyroidism, are much more sensitive to vitamin D and grow hyperparathyroidism due to rising vitamin D intake, whereas maternal hypercalcemia during pregnancy increases the fetus's sensitivity to vitamin D effects and can lead to a syndrome of intellectual disability and facial deformities [35]. Idiopathic infantile hypercalcemia is brought on by a mutation in the CYP24A1 gene, which reduces vitamin D breakdown. Infants with this mutation have a higher sensitivity to vitamin D and are more likely to develop hypercalcemia if they get too much of it. The sickness may endure far into adulthood [36]. In published cases of hypercalcemia toxicity, the vitamin D dose and

25hydroxy vitamin D standard involved a daily capacity of 40,000 IU (1,000 g) [37]. Before using any vitamin D supplement, pregnant or lactating women should visit their doctor. The Food and Drug Administration (FDA) has issued a warning to liquid vitamin D supplement producers to clearly and accurately identify the droppers provided with these products with 400 international units (1 IU equals 25 ng cholecalciferol or ergocalciferol in biology). Furthermore, the FDA recommended that the droppers hold a little greater than 400 IU for newborn products. The acceptable maximum bound (maximum tolerated quantity) for babies (birth to twelve months) is set at 25 g/day (1,000 IU). 1000 micrograms per day poisoned newborns in less than a month [38]. Calcitriol is naturally regulated by parathyroid glands, fibroblast growth hormone 23, mediators, calcium, and phosphate in a negative feedback cycle [39]. Hypercalcemia, which is produced by a vitamin D overdose, is an indication of vitamin D toxicity and can be recognized through increased urination and thirst. If left untreated, the hypercalcemia causes discomfort and organ damage by producing excessive calcium deposition in soft body tissues such as the kidneys, liver, and heart.[40].

The most typical symptom of a vitamin D excess is hypocalcemia, which involves anorexia, revulsion, and puking. Proteinuria, fluid retention, weariness, sleeplessness, anxiety, itchiness, and, in the worst-case scenario, kidney disease are all possible adverse effects. Side effects include albuminuria, urinary cylinders, azotemia, and tumor calcification (particularly in the kidneys). Vitamin D toxicity can cause intellectual retardation in young infants, as well as abnormal bone growth and formation, diarrhea, irritation, slimming, and severe sadness [41]. Vitamin D toxicity can be treated by discontinuing vitamin D medication or limiting calcium consumption. Acute kidney injury may be permanent. Vitamin D poisoning is rare as a result of long-term sun exposure. As the amounts of vitamin D precursors produced in the skin achieve an equilibrium, newly created vitamin D is eliminated [8].

2.2. Follicle Stimulating Hormone

To reproduce, FSH (follicle-stimulating hormone) is required. It acts on the outside of target tissue via a G-protein-coupled receptor to increase testicular and ovarian activity. At 2.9 A resolution (FSHRHB), the structure of a partially deglycosylated molecule of human FSH linked to its receptor's extracellular hormone-binding domain is described at 2.9 A resolution (FSHRHB). In a hand-clasp form, the hormone binds to an elongated, curved receptor. The complex's subsurface contact is massive (2,600 A 2) and has a high charge density [42].

2.3. Luteinizing Hormone

The anterior pituitary gland secretes luteinizing hormone (LH), which is involved in reproductive activities such as ovulation in females and androgen production in males. The endocrine system is critical in the regulation of the human reproductive cycle LH, along with the two other gonadotropin hormones, follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG), is necessary for the sexual and reproductive function to be controlled. LH plays a significant evolutionary role since it affects both male and female gonads (testes or ovaries). It is involved in several biological processes, including sex steroid production (for both sexes) and the important reproductive mechanism of ovulation in females [44].

2.4. Embryo quality

Embryo quality is a clinical phrase that refers to an attempt to measure the development potential of embryos in vitro. The estimation of embryo quality is used to choose the best embryos for transfer following in vitro cultivation. The quantity of good-quality embryos increases the pregnancy rate, implantation rate, and incidence of multiple pregnancies greatly. Embryo morphology is crucial in predicting embryonic advancement [45].

2.5. Cleavage Rate

The rate of embryo development is recommended as a predictive marker for long-term viability, and it is more likely to be impacted by elements inherent to the oocyte and embryo rather than culturing procedures, female age, stimulation protocol, and sperm characteristics [46,47]. Within the first 25 hours following insemination, cleavage to the stage system is linked to a much larger number of clinical pregnancies. The number of blastomeres at the time of transfer also serves as a proxy measure of the embryo's developmental potential, as it is closely related to embryonic genome activation between the four-cell and eight-cell phases of preimplantation development [48].

2.5.1. The link between vitamin D levels and embryo development:

The information on a link between vitamin D levels and IVF success is still in the early stages of research. Based on these contradictory reports, as well as the fact that vitamin D deficiency doesn't correspond with ovarian stimulation character traits or quality indicators for embryos, Vitamin D insufficiency has been linked to an increase in IVF fertility rates via an endometrial-mediated action that can be detrimental [41]. When a coexistent euploid blastocyst is implanted, the vitamin D status does not affect the result [39]. Previous studies

on vitamin D and assisted reproductive technologies (ART) have revealed clinic fertility rates or live abortion rates . These investigations, on the other hand, were not designed to look into the direct relationship between intrafollicular vitamin D levels and the potential of individual egg cells to fertilize and form an embryo [38-40,48,49,50]. The researchers wanted to discover if there was a correlation between vitamin D status in individual follicles and the ability of egg cells to develop in this prospective investigation. As a result, each ovary's first cycle was harvested independently, and each follicle's functional fluids (FF) were assigned to its appropriate egg cell. Following that, we evaluated each egg cell's fertilization potential, embryonic development before implantation, implantation, and pregnancy [51]. Vitamin D is required for both biological systems and illness. The stimulation of the vitamin D receptor (VDR) can alter the expression of a vast number of genes both overt and covert (0.5-5 percent of the entire human genome). Vitamin D deficiency increases the risk of cancer, autoimmune diseases, infections, and pregnancy difficulties as a result [3].

It has been proposed that vitamin D plays a role in reproduction health and life expectancy. The ovary, endometrium, and endometrium all express VDR. Furthermore, vitamin D has been demonstrated to alter female fertility capacity in both people and animals. In vitro fertilization (IVF) success rates are greater in women with adequate vitamin D stores [7]. When a limit of 30 ng/ml was used, the positive effect was significantly more pronounced. Animal experiments and findings from human in vitro research strongly suggest a causal relationship, however careful studies are warranted to prove vitamin D's function in increasing pregnancy chances. Only one short Randomized controlled trials (RCT) has already been reported to yet, and no benefits have been demonstrated [9].

Studies into the mechanisms underlying vitamin D's beneficial effects could be useful and open up new research avenues. There isn't much strong evidence so far, but the picture isn't complete, particularly when it comes to interventional trials. Biological findings from RCTs, on the other hand, may shed light on the role of vitamin D in egg quality and endometrial receptivity [50]. For biomolecular analysis, egg cells cannot be decision-making related. Cells of the ovarian follicle's cumulus and granulose, on the other hand, may provide indirect information regarding the quality of the associated egg cells. It has been proposed that the transcriptomic profile of cumulonimbus complexes be used to determine which embryos have the best likelihood of implantation in the uterus. COX20, BMP15, and GDF9 expression have all been linked to the start of a pregnant or a healthy baby. Other markers of relevance include GREM1, HAS2, VCAN, and PTGS, however, there is less consistency

among research [52]. Endometrial samples, on the other hand, can be taken before the commencement of the IVF cycle and analyzed without interfering with the procedure. Early pregnancy endometrium and decidua both express VDR and may be potential vitamin D targets. According to a previous study [12], vitamin D influences the transcription of genes encoding in embryo responsiveness and interacts with local cytokine in female endometrial. According to a popular but not widely recognized theory, there was evolutionary pressure to obtain lighter skin to boost UV-B-induced vitamin D synthesis in the skin. The prevention of rickets and thus the avoidance of a pelvic constriction in an obstructed birth could be one explanation for the benefit of light skin. However, new research and reviews have highlighted the potential importance of vitamin D in maternal age [53].

2.6. Vitamin D and oocyte growth:

The growth of the antral follicles was linked to the maturation and expansion of the egg cells. More study is needed to investigate the activation of other VD3-activated target genes that may be involved in follicular development and egg maturation [54]. The current study suffers from the fact that the number of macaques used is quite small. Future studies on vitamin D metabolism and signal transmission in the ovary will require a larger sample size. In conclusion, these findings confirm and expand on prior research in determining vitamin D's significant influence and mechanisms of action on folliculogenesis. According to this study, vitamin D can affect ovarian function by regulating VDR and Within an ovary vitamin D enzyme expression. The effects of vitamin D on follicular viability, growth, and activity as endocrinology, paracrine, and autophagic factor are extremely context [55].

Vitamin D intra-ovarian action in vivo is influenced by follicular development stage, initial circulating vitamin D status, and prescription vitamin D intake. These results back up the theory that a person's overall health has an impact on these dynamical and homeostatic intraovarian systems. This knowledge can lead to the identification of vitamin D's involvement in sex hormones and insight into the potential mechanisms underlying vitamin D supplementation's reproductive benefits, such as in women with polycystic ovarian syndrome [56].

2.7. Quality of oocytes

Evaluating oocyte quality in human in vitro fertilization (IVF) is a growing area of interest for embryologists. In order to minimize embryonic overproduction and enhance the results of spermatozoon factors vary programs, spermatozoon selection and identification of the best

spermatozoon may be helpful. After spermatozoon extraction, follicular fluid (FF) is easily accessible and, in principle, makes for a good source of non-invasive chemical predictions of spermatozoon quality[57].

2.7.1. Vitamin D and oocyte quality:

The researchers looked into follicular life, development, steroid and anti-Müllerian hormone (AMH) synthesis, and egg maturation. In vivo and in vitro generated follicles, genes involved in vitamin D biosynthesis and signal transduction, gonadotropin signal transduction, the creation of steroids and paracrine hormones, and egg quality were investigated. In vivo and in vitro, mRNA coding for VDR, 25-hydroxylase, and 1hydroxylase was detected in pre-antral and antral follicles. In cultured follicles, 25hydroxylase activity was higher than in vivo follicles, and it increased even more after VD3 therapy. Antral follicles produced with VD3 displayed greater amounts of 1-hydroxylase in vitro. VDR expression was reduced in vitro created antral follicles when VD3 was not present during culture, VD3 supplementation, on the other hand, recovered it to a level similar to in vivo produced antral follicles. In granulosa cells and oocytes, VDR immunostaining was observed in the nucleus and cytoplasm. While VD3 therapy increased pre-antral follicle survival solely, VD3 supplementation improved antral follicle survival and growth by enhancing estradiol and AMH production as well as egg maturation. As a result, primate ovarian follicles express vitamin D synthesis and signaling systems. VD3 appears to have a role in stage-dependent follicular growth regulation, intrafollicular vitamin D generation, and direct signal transmission in the ovary [58]. VD3 impacts cell function via the vitamin D receptor (VDR). The brain, pituitary, ovary, fallopian tubes, uterus, and placenta all express VDR, and activation of VDR by circulating or locally produced VD3 may impact their function. To assess the serious influence of VD3 on procedures essential to the growth of follicular that deliver egg cells, fertilizer application, and resulting Embryogenesis improvement, ovarian parameters must be assessed in the appearance of physiological VD3 stages, particularly under non-pathological situations [59].

2.8. Vitamin D and fertility:

Several observational studies have demonstrated that plasma 25 (OH) D values are favorably connected to ovarian reserve indicators such as anti-Müllerian hormone (AMH), even though further study is needed in this field [60]. Some kinds of endometriosis have been linked with vitamin D insufficiency in observational studies, although not all. Several RCTs

on vitamin D supplementation in PCOS women have demonstrated inconsistently favorable effects on endocrine, metabolic, and reproductive aspects [61,62].

As a result, no definitive conclusion concerning the potential therapeutic effects of vitamin D on PCOS and its associated disorders can be formed at this time. In parallel to female fertility, there is growing recognition that vitamin D has a function in male fertility. Although the majority of RCTs found no role of vitamin D on testosterone status, vitamin D insufficiency has also been linked to low blood testosterone levels in observational studies. While descriptive studies have linked vitamin D deficiency to poor sperm quality, controlled trials have found that supplementing with vitamin D has a little effect [63]. Vitamin D appears to be becoming increasingly crucial in the reproductive health of women In vitro, 1,25-dihydroxyvitamin D3 (VD3), a physiologically active version of vitamin D, has been found to promote ovarian follicle survival and growth. As a result, we investigated the direct effects of VD3 on follicular growth throughout the preantral and antral stages, as well as whether the primate ovary possesses vitamin D receptors (VDRs) and vitamin D-producing enzymes. On secondary and antral follicles, PCR studies for VDR, vitamin D3 25hydroxylase, and 25-hydroxyvitamin D3-1-hydroxylase were performed. Immunoreactivity on ovarian sections was used to pinpoint the site of the VDR protein. During the preantral and antral phases, separate secondary follicles were produced underneath control and with VD3 supplementation [58].

2.9. Vitamin D's impact on ovarian activity:

Vitamin D's impact on ovarian function has largely been studied in the context of obstetric results, particularly in women seeking reproductive treatment who have the ovarian disease [55]. Data on the link between vitamin D concentrations in follicular fluid and pregnancy rates have been inconclusive thus far, with favorable correlations identified. Supplements of vitamin D, on the other hand, appear to be advantageous for women who are obese and have insulin resistance. Vitamin D administration enhanced follicle growth, dominant follicle creation, and pregnancy rates, and assisted in the resumption and maintenance of menstrual periods in women with polycystic ovarian syndrome [64]. In vitro, for example, VD3 supplementation increased macaque life, proliferation, and anti-Müllerian hormone (AMH) output, ruling out systemic side effects caused by climate changes, albeit egg competence was not examined [58].

Vitamin D synthesis can be shown in follicles in both in vitro and in vivo models. The effects of VD3 on gonadotropic hormone, steroids, peripheral immune factor synthesis, and egg

quality were also investigated. Vitamin D may have trophic effects on follicle life, size, and function in the ovary, including egg maturation, through endocrine, paracrine/autocrine, and stage-specific mechanisms. For the first time, investigations show that ovarian VD3 levels affect vitamin D synthesis and signaling components in follicles [58]. Because VDR is expressed in vivo follicles, VD3 could enter the circulatory system after being synthesized in the kidneys and liver and act directly on them to influence their development. During the development phase, VDR was predominantly found in the egg cells of follicles, particularly primordial and primary follicles. VDR was also detected in granulosa cells as the follicle grew bigger [58].

After concurrent supplementation with vitamin D-K calcium, individuals with vitamin D insufficiency and polycystic ovary syndrome showed a rise in overall antioxidant capacity in plasma [65]. In cultivated umbilical cord vein cell lines, VD3 treatment was also shown to lower caspase-3 activity. In living follicles, the mRNA for vitamin D3 25-hydroxylase (CYP2R1) and 25-hydroxyvitamin D3-1-hydroxylase (CYP27B1) is informed, showing that vitamin D3 synthesis takes place locally in the ovary. Although mRNA or protein expression of CYP2R1 and/or CYP27B1 has been established in the human ovary, neither species' rules during particular phases of follicular growth have been investigated [66; 67]. Vitamin D3 and 25-hydroxyvitamin D3 can act as substrates for VD3 production in the bleach, implying a paracrine and/or autocrine role in follicle growth regulation. Furthermore, the role of Vitamin d status on ovulation in vivo can vary depending upon the type of vitamin D used (cholecalciferol, calcifediol, or calcitriol), and the appearance and action of vitamin Dproducing organs such as the liver, kidneys, and ovaries. As a result, more research using follicular cultures is needed into the protein production and cell function of vitamin D biosynthetic protein, in addition to the signaling pathways and autocrine function of vitamin D [58].

In vitamin D-depleted settings, VDR adherence to its particular reaction sites was discovered, but it presence of sufficient VD3 treatment, was connected to enhanced VDR occupation and gene function in which was before mice bearing [68]. Thus, the number of VDR binding sites that are accessible in the absence or presence of VD3 can be linked to variations in follicle VDR transcription throughout the culture. Additionally, beneath controlled circumstances, follicular CYP2R1 production rose from the pre-antral to the antral stage, which was stronger than that of in vivo generated antral follicles [58]. It's possible that the absence of VD3 in cultivated follicles spurred local biosynthesis, resulting in active VD3. Stronger CYP2R1 expression in VD3-treated follicles might imply a larger requirement for

VD3 as a result of increased VDR-mediated effects, which current VD3 supplementation at low dosage cannot provide. As a result, various aspects must be addressed while examining the influence of vitamin D on ovary functioning in vivo, including the patient's baseline circumstances, as well as the dose and duration of vitamin D treatment [58]. Before receiving more vitamin D treatment, vitamin D-deficient individuals may experience an initial vitamin D injection to ensure adequate VDR transcription and to balance circulatory vitamin D availability and localized vitamin D synthesis.VD3 medication at the current dose had no influence on follicular mRNA levels or AMH medial concentrations during the preantral stage. In vitro generated antral follicles, however, VD3 therapy increased medial AMH levels during the antral stage but did not affect AMH mRNA levels. Because the pre-antral follicle diameters in the control and VD3-treated groups were comparable, but the reticulocyte size and shape in the VD3-treated follicles were larger, the increased AMH concentrations in the media can be attributed to the rising follicular cells cell numbers in vitro established antral follicles as a result of VD3 treatment. VD3 medication increased the number of steroids in the media generated by the much bigger antral follicles, e.g. B. E2, without changing the methylation status of steroidogenic in the follicles, e.g. B. CYP19A1 [58].

3. MATERIAL AND METHOD

3.1. Subjects

The data was gathered from the medical records of patients (females aged 35 years and over) who received IVF therapy between (2018-2020). A total of 100 Intracytoplasmic sperm injection ICSI cycles performed at the IVF and Genetic Center Center, Alamal Hospital Misurata-Libya. The second and third ICSI trials for some individuals were considered for analysis to prevent bias in the measurement of the fertilization rate. All of the patients' medical records revealed a full record of the IVF procedure, including a detailed record of the eggs' evolution in the hospital and vitamin D treatment before the procedure. Patients who did not have 25(OH)D serum level tests; imperfect charts that did not have a report that contains all the character traits of fertilizer application and embryo segments, as well as the evolvement during the IVF procedure; infertility caused by an adult element or when the semen was using has been of inadequate quality for successful fertilization were all excluded before ICSI, digital imaging of the ovarian soon before sperm insertion was used to examine the quality of all accessible mature oocytes. Individual oocytes were placed in an ICSI dish in a single-numbered drop, allowing for long-term monitoring of fertilization and embryo development. This scoring system used in this study was published by Lazzaroni Tealdi (10).

3.2 Preparation of cultural dishes:

We produce culture dishes for the wash of cumulus corona oocyte complexes (CCOCs), culture dishes for oocyte denudation, and culture dishes for cultivating the denuded oocytes or injected oocytes on the last day of the oocyte retrieval operation. At the same time, we constructed injection plates for the ICSI method and incubated all of the culture and injection dishes for 24 hours at 37°C in a 5% CO2 incubator.

We placed some universal-IVF media into a Petri dish to prepare the culture plates for transfer and to wash the cumulus corona oocyte complexes (CCOCs) following their identification under the stereo microscope.

To prepare the culture plates for the oocyte wash technique (oocyte denudation), we produce two droplets of Synvitro Hyadase and five droplets of universal IVF medium, each with 50ul of medium on the bottom of the Petri dish and a layer of liquid paraffin oil on top. To prepare the culture plates for the denuded oocytes and injected oocytes, we produce three droplets of universal -IVF medium, each with 50ul of medium on the bottom of the Petri dish and a layer of liquid paraffin oil on top.

To make the injection dishes, we use a pipette (10ul) to make six small droplets of Universal -IVF medium and arrange them in parallel groups (2 x 3), then use a pipette (10ul) to make one large droplet of PVP medium over these droplets in the center, which is then covered by a layer of liquid paraffin oil.

Following the fabrication of these culture dishes, we incubated them in the incubator for 24 hours at 37°C and 5% CO2.

3.3 Preparation of Pasteur pipettes made of glass:

We utilized glass Pasteur pipettes to produce these pipettes, and we made them according to the following method: Hold the pipette at both ends.

 Over a mild heat, roll a region about 2.5cm below the tapering part of the pipette. - When the glass begins to melt, swiftly pull the pipette in both directions to separate, then gently and quickly break the pipette at an acceptable place. The tip must have a smooth break with no harsh or uneven edges. Under an inverted microscope, inspect the tip of each pipette to confirm that it has an exact diameter and smooth, clean edges.

3.4. The number of oocytes

The greatest cumulative live birth rate CLBR was seen when approximately 25 oocytes were extracted in women between the ages of 18 and 35, approximately 9 oocytes in women over the age of 45, and continued to increase beyond 30 oocytes in women between the ages of 36 and 44 [69].

3.5. Quality of Oocytes:

Morphological, cytoplasm [70], perivitelline space (PVS) [71], zona pellucida (ZP) [72], polar body (PB)[73], and oocyte size [74] were employed to describe oocyte quality. Each was assigned a score of -1 (worst), 0 (average), or +1 (best), yielding an average total oocyte score (TOS). Only ICSI cycles and patients who had embryo transplantation on day 5 (blastocyst) were studied.

3.6. Oocyte preparation:

The follicular aspirates were collected into warm 14ml falcon tubes containing some synvitro flush media and incubated at 37°C during the oocyte retrieval. The circulating nurse passed the follicular aspirates to the embryologist, who emptied the contents of the tubes into sterile

shallow Petri plates and viewed the cumulus corona oocyte complexes under the stereo microscope (CCOCs). When the follicular aspirates contain blood during identification of the CCOCs, we first transfer and wash the CCOCs in another Petri dish containing a universal -IVF medium, because the blood is toxic to the oocyte, then we transfer them to a petri dish that contains the CCOCs found in clear follicular aspirates, and incubated at 370° + 5 percent CO2 in a sterile glass Pasteur pipette. The length of the incubation time is determined by the timing of the oocyte wash operation. This operation was carried out by (Elder & Dale, 2000). Elder and Dale employed one droplet (100 ul) of Hyaluronidase solution in their approach, whereas, in our investigation, we split the (100ul) droplet into two droplets (50 ul). We rinsed the CCOCs with two synvitro Hyadase droplets containing 80IU/ml hyaluronidase and aspirated them repeatedly for one minute with a hand-drawn Pasteur pipette. Repeated aspiration via mouth-controlled polished glass Pasteur pipettes with varied diameters through five drops of universal -IVF medium improved the enzymatic removal mechanically. The integrity and maturity of the denuded oocytes were assessed. Before ICSI, all oocytes were incubated for one hour at 370° + 5% CO2 in Petri plates containing three droplets of universal -IVF medium.

3.7. ICSI procedure:

After incubating the denuded oocytes for one hour at 37° + 5% CO2, the injection culture plates and oocyte dishes are removed from the incubator and the ICSI method is conducted. The oocytes were put in each surrounding droplet after adding roughly (2-5 ul) sperm solution to the border of the PVP droplet. A single spermatozoon with seemingly normal morphology were picked under the inverted microscope, trapped by striking its tail, and aspirated tail first in the injection pipette. The sperm-containing pipette was then transferred from the PVP droplet to one of the surrounding oocyte-containing droplets. The oocyte was rotated to identify the first polar body at 6 or 12 o'clock on the holding pipette, which was maintained in place by moderate suction. To break the oolemma, the injection pipette was gently pushed into the ooplasm, sucking the membrane into the pipette before releasing the sperm and injecting the sperm slowly into the egg with little media. The injection pipette was gently removed after that, and the oocyte was gently freed from the holding pipette. For each oocyte, the process was repeated. The injected oocytes were then washed in universal-IVF medium and cultivated on culture dishes containing three droplets of universal IVF media, each with a different composition (50ul).

3.8. Assessment of the fertilization and embryo quality:

We evaluated the injected oocytes using an inverted microscope at x 200 magnification the next day of the oocyte injection (24 hours later) to determine fertilization. Normal fertilization was defined as the presence of two pronuclei (2PN) and two polar bodies (2PB). We assessed the embryo cleavage after another 24 hours. When we saw the unambiguous division in the cells at different phases of embryo cleavage, we regarded the cleavage to have occurred. However, when the oocyte was still at the 2PN stage, we concluded that the cleavage had not occurred. The size of the blastomeres and the proportion of cytoplasmic fragmentation were used to determine embryo quality. The cleaved embryos were divided into four groups based on this criterion. Grade-1: an embryo with equal blastomere size and no cytoplasmic fragments; grade-2: an embryo with equal blastomere size and 10% cytoplasmic fragments; grade 3, the percentage of cytoplasmic fragmentation was 20%; grade 4, the percentage of cytoplasmic fragmentation was 30%.

3.9. Vitamin stimulation

All women received the same Vit D stimulation protocol by D-Sorp Forte. Treatment started on the first day with doses of 1,500 to 2,000 IU daily(1-5 spraysdaily), this dose was given for two month.

3.10. Measurement of 25(OH)D

The most frequent circulation form of vitamin D is 25(OH)d D, which stays constant all through the menstruation [75] Serum 25(OH)D percentage is thought to be the greatest measure of vitamin D status because of its consistency [76, 53]. All patients' li-heparin serum and follicular fluid samples were collected on the day of spermatozoon retrieval and remained frozen at 80°C until the analysis was performed. In this study, DiaSorin Inc was used for the measurement of 25(OH)D (Stillwater, MN, USA, chemiluminescent immunoassay (CLIA) LIAISON® 25 OH vitamin D TOTAL Assay to determine the 25(OH)D degree (REF 310600). The quantitation limit for the test was 4.0 ng/ml. Six samples were collected and two sets of LIAISON 25 OH calciferol TOTAL controls were tested over 20 days using the CLSI technique EP05-A2 [76]. The total accuracy relative standard deviations for serum showed 12.6–10.8% (7.9–112.1 ng/ml) and for the kit, standards were 9.7–9.5% (18.0–61.8 ng/ml). The Institute of Medicine (IOM) and the Endocrine Society have published clinical practice guidelines [77, 78]. The level of vitamin D in the follicular fluid has yet to be measured.

3.11 Statistical analysis:

There will be a digital dataset developed (SPSS v. 19, IBM). To determine statistically significant differences by Wilcoxon test that depending on on related sample test. Descriptive statistics are used to present the clinical outcome data (mean SEM). To see if there is any dependency or statistical link between variables, correlation analysis will be utilized. The level of significance was defined as a P-value of less than 0.05.

4. RESULT

A sample of 100 individuals was enlisted, with 45 (45%) having an unclear kind of infertility, 40 (40%) having primary infertility (Primary infertility is the phrase used to describe a situation when a couple has tried unprotected sexual activity for at least a year without success. Numerous mental as well as physical factors might contribute to infertility), and 15 (15%) having secondary infertility (The inability to conceive or carry a child to term after previously giving birth is referred to as secondary infertility) (Figure 4.1).

The highest percentage had primary infertility type was 14% in the age group 35-37 by mean (35.78 ± 0.21). While the lowest percentage had primary infertility type was 2% in the age group more than 47 years by mean (47.50 ± 0.50).

On other hand, the highest percentage had secondary infertility types was 7% in the age group 35-37 by mean (35.86 ± 0.22). While the lowest percentage had secondary infertility type was 2% in the age group 41-43 by mean (42.00 ± 1.00) (Table 4.1).

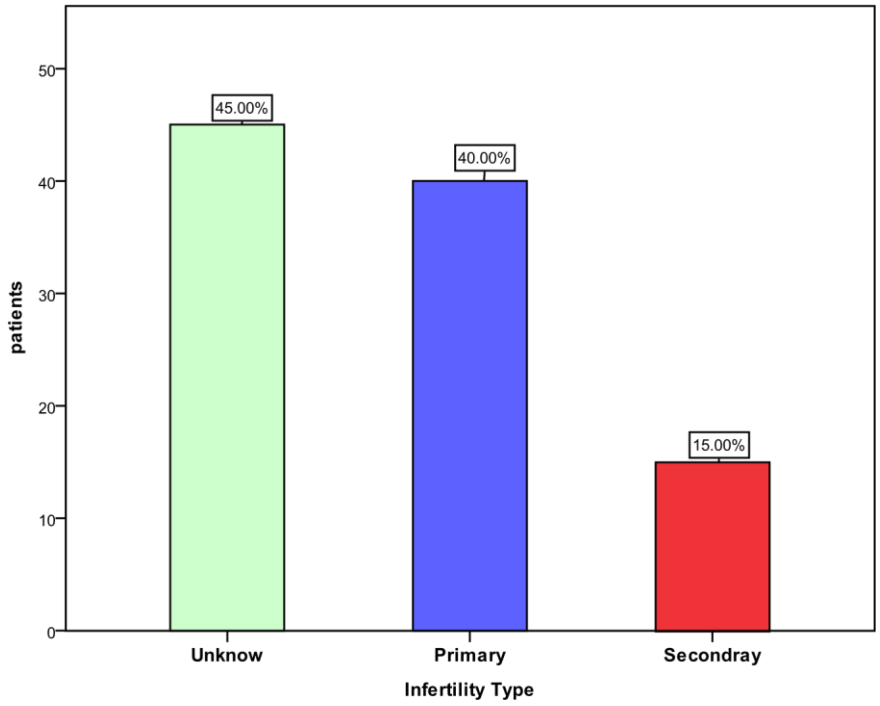


Figure 4. 1 Type of infertility

4.1 Patient characteristics at baseline.

Table 4. 1. Patient characteristics at baseline

Age Groups	Infertility Type					
	Unknow ı		Primary		Secondary	
	M ±S.D	%	M ±S.D	%	M ±S.D	%
35-37	35.54 ± 0.24	11	35.78 ± 0.21	14%	35.86 ± 0.22	7
38-40	39.18 ± 0.26	11	38.77 ± 0.22	9%	38.67 ± 0.67	3
41-43	42.00 ± 0.33	8	42.00 ± 0.23	9%	42.00 ± 1.00	2
44-46	44.75 ± 0.18	12	45.00 ± 0.37	6%	44.00 ± 0.00	3
>46	49.33 ± 2.33	3	47.50 ± 0.50	2%	-	0

M:Mean S.D : Stander Division % : Percentages

4.2 Other characteristics were compared to vitamin D levels before and after therapy.

Table 4.2 displays the summary statistics of the 100 patients, and it can be shown that the levels of Luteinizing Hormone before and after treatment were significantly different. There was a significant positive relationship between them as well ($r = 0.522$, $p = 0.000$). The other factors, on the other hand, demonstrated no significant change.

Table 4.2. Other characteristics were compared to vitamin D before and after treatment

Variables	Before treatment			After treatment			Value of P
	M±S.D	Min	Max	M±S.D	Min	Max	
FSH	8.16 ± 4.77	1.70	23.3	8.69 ± 9.32	2.37	90.10	0.660
LH	7.39 ± 22.74	1.10	230.0	7.24 ± 10.90	0.07	69.0	0.020
ES	120.15 ± 106.04	2.50	625.0	144.98 ± 153.11	1.20	708.0	0.210
Oocyte Number	5.84 ± 6.016	0.0	28.0	5.57 ± 5.388	0	23	0.221
Oocyte Quality	12.72 ± 24.401	0.0	100.0	10.59 ± 20.02	0	100	0.708
Fertilization rate	17.04 ± 29.769	0.0	100.0	18.54 ± 29.20	0	100	0.527
Cleavage rate	32.50 ± 46.804	0.0	100.0	38.50 ± 48.12	0	100	0.202
Embryo quality	29.91 ± 44.724	0.0	100.0	37.00 ± 47.47	0	100	0.153

5. DISCUSSION

Traditional roles of vitamin D involve calcium and phosphorus balancing, and also bone mineralization support. The skin produces vitamin D, a steroid hormone when exposed to the elements. Nevertheless, new research suggests that 25-hydroxyvitamin D [25(OH)D] has a variety of other critical effects, including on human reproductive systems [28]. Low exposure to light and/or sunscreen use, black skin tone, overweight, a lack of state control for vitamin D supplementation of food, and cultural clothing practices can all contribute to vitamin D deficiency in this group of infertile women [77].

The focus of this research was to see how vitamin D affected oocyte number, oocyte quality, fertilization rate, cleavage rate, embryo quality, FSH, LH, and Es, as well as investigate correlations among both vitamin D and oocyte number, oocyte quality, fertilization rate, cleavage rate, embryo quality, FSH, LH, and Es.

Our findings revealed that there were significant variations in Luteinizing Hormone levels before and after therapy, as well as a significant positive connection between them. However, there is no meaningful change in the other characteristics when vitamin D is administered. There was no connection between 25-OH vitamin D levels and the number of oocytes in this investigation. The data suggests that vitamin D can increase implantation on its own without affecting the number or quality of oocytes [79]. In comparable studies, Anifandis, Ozkan, and Rudick revealed a forthright link between vitamin D levels and IVF success [40, 49,80]. Because investigations have shown that vitamin D deficiency or a malfunction in its receptor can alter follicle and oocyte growth and gonad function, notably in estradiol synthesis, high levels of vitamin D may improve pregnancy outcomes by modulating these items [5, 40,81, 82]. Our data, however, reveal that vitamin D does not influence oocyte quality. Vitamin D levels haven't been connected to the number or quality of oocytes and embryos in previous studies [48, 83]. This shows that vitamin D's effect on implantation rate is most likely owing to its binding to endometrial receptors. According to Ozkan et al., vitamin D may increase endometrial receptivity and the likelihood of conception by binding to its receptor in the endometrium [40]. One of the suggested mechanisms of the vitamin D-receptor complex is overexpression of the HOXA10 gene, which plays a critical role in implantation [84]. Vitamin D levels in a substantial quantity of unique follicles, as well as the findings of their related oocytes, were revealed to have a strong and unfavorable link with oocyte quality in a recent study. Performance depends on such findings, we recommend that levels of vitamin D in the intrafollicular area can be used to predict oocyte quality. Oocyte quality was strongly

and inversely correlated to the quantity of 25(OH)D in local follicular fluid [85]. In a recent study on an oocyte donor population, Fabris et al. found no causative link or analytical connection in both overall 25(OH)D and bioavailable 25(OH)D values and ovulatory reserve, reaction to ovulation induction, fertilized ovum quality in egg donation, or ongoing conception rate after a new embryo transfer in oocyte recipients.

The clinical aspects of the 3051 participants in the research were based on their 25(OH)D state. Overall T, DHT, FSH, and SHBG concentrations were not varied meaningfully among 25(OH)D classifications, even though free T was reduced ($P<0.001$), E2 ($P=0.04$), and LH ($P=<0.02$) were greater in the insufficient and subpar categories. After adjusting for age, Center, and other factors (all $P>0.05$), there were no independent correlations with 25(OH)D and either of the HPT axis hormones or SHBG [86].

Serum 25-hydroxyvitamin D (25(OH)D) levels in 180 PCOS girls and 150 non-PCOS girls were 75 nmol/L in the research. For 24 weeks, individuals were administered either 20,000 IU of VD3/week (119 PCOS, 99 non-PCOS girls) or a placebo (61 PCOS, 51 non-PCOS women). AMH, follicle-stimulating hormonal (FSH), luteinizing hormone (LH), estradiol, dehydroepiandrosterone sulfate, and androstenedione were all measured as outcomes. In PCOS girls, there was a significant therapeutic impact on FSH and the LH/FSH ratio, but not in non-PCOS ladies. VD treatment for 24 weeks reduced FSH and the LH/FSH ratio in PCOS women but had no influence on AMH values [87].

The relationship between blood Vit D levels and IVF/ICSI results was investigated in this study. From Nov 2017 and July 2019, researchers from IVFMD, My Duc Hospital, and IVFMDPN, My Duc Phu Nhuan Hospital in Ho Chi Minh City, Vietnam, conducted a comprehensive retrospective observational analysis. Low vitamin D levels in the blood (25(OH)D) in Vietnamese individuals between the ages of 18 and 40. Individuals were split into four groups based on their 25(OH) D stages: 10 ng/mL, 10 to 20 ng/mL, 20 to 30 ng/ml, and 30 ng/mL, according to the data. As per results, the number of recovered oocytes, embryos, clinically childbirth, implantation, and miscarriage rates did not differ substantially between subgroups [88].

The method by which vitamin D influences fertility is not well understood. Among the hypothesized mechanisms are its effects on ovarian steroidogenesis and pregnancy. Vitamin D's effect in ART results has been investigated in a variety of modern research. Garbedian et al. explored the role of vitamin D levels on IVF clinical pregnancy rates. They discovered that ladies with a suitable level of serum 25-(OH) vitamin D (>75nmol/l, equivalent to >30 ng/ml) had a greater rate of diagnostic pregnancy, but because they did not assess embryo

quality, they were unable to determine whether endometrial or embryo quality influenced implementation and clinical total fertility rate [89].

According to Rudick et al. vitamin D level is connected to IVF outcome in non-Hispanic white women in an ethnically diverse setting. As time goes forward, with reduced levels of vitamin D, pregnancy rates decreased. However, the favorable benefit of adequate vitamin D levels was not evident in Asians, and in fact, vitamin D deficiency has been linked to IVF failure. When the race was taken into consideration, the link between vitamin D and being clinically pregnant was clinically meaningful. In this investigation, vitamin D level was found to be independent of ovarian stimulation parameters or embryo quality in this investigation, showing that vitamin D's influence is most likely conveyed through the endometrium [90]. Higher 25-(OH) D levels were found to have a negative impact on embryo quality by Anifandis et al. Those with sufficient follicular fluid vitamin D had worse embryo quality and were less likely to accomplish clinically pregnant in their study as compared to women with insufficient or deficient follicular fluid vitamin D [49]. Several studies have shown that women with high blood vitamin D levels had a higher probability of becoming pregnant [91], but the benefit of 25(OH)D in ladies from childless couples is still to be completely examined. As a result, it's worth looking into if vitamin D will help with IVF success. D-Sorp Forte supplementation dosage followed the same vit D stimulation regimen. The treatment began on the first day with a dose of 1,500 to 2,000 IU daily (1-5 sprays daily), which was given for two months.

Some of the flaws in our research should be highlighted. To begin with, a non-noteworthy outcome will not always mean that prior to IVF, vitamin D supplementation should be stopped, since a more regular and consistent dose (such as 2.000 IU daily) may be more effective, the 25(oh)d period leading up to IVF can be concerning, especially as we allowed for considerable variance in vitamin D dose and cycle start (2–8 weeks). Third, we will encourage some women to refrain from consuming vitamin D, but we will not ask them to forego sun exposure for the duration of the IVF cycle. Even if the two study arms had a similar prevalence of this illness, this possible confounder may dilute the potential advantages of the examined vitamin D treatment. From the other side, we picked practical research in which the confounder merely simulated real-life situations.

6. CONCLUSION

Vitamin D was formerly thought to be a vitamin required for bone equilibrium, but it has lately been "rediscovered" as a "multitasking vitamin" in addition to being a "bone vitamin." Vitamin D has been linked to a variety of extra-skeletal processes as well as the homeostasis of numerous systems, which explains why. Because of its pleiotropic effects and the increased frequency of vitamin D deficiency in the general public, scientists have become more interested in vitamin D research. Vitamin D deficiency has been associated to musculoskeletal disorders, two types of diabetes, male hypothyroidism, polycystic ovary syndrome, cancer, autism, dementia, and heart disease, among other acute and chronic diseases. This thesis has the potential to add to the research on the connection between 25 (OH) D blood levels in IVF patients and oocyte quality, in vitro cleavage success, and embryo quality.

It can be seen that there were substantial differences in Luteinizing Hormone levels before and after treatment. There was also a substantial positive association between them. However, there was no statistically significant change in FSH, Es, oocyte number, oocyte quality, fertilization rate, cleavage rate, and embryo quality.

Although various studies have revealed that ladies with high levels of serum vitamin D have an upper chance of conception, vitamin D supplementation's influence on ladies from infertility marriages has yet to be completely investigated. As a result, more research into whether vitamin D supplementation can improve IVF outcomes is necessary.

REFERENCES

1. Muscogiuri G. Introduction to Vitamin D: Current evidence and future directions. European Journal of Clinical Nutrition. 2020 Nov;74(11):1491-2.
2. Rosen CJ. Vitamin D insufficiency. New England Journal of Medicine. 2011 Jan 20;364(3):248-54.
3. Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. Molecular aspects of medicine. 2008 Dec 1;29(6):361-8.
4. Luk J, Torrealday S, Neal Perry G, Pal L. Relevance of vitamin D in reproduction. Human reproduction. 2012 Oct 1;27(10):3015-27.
5. Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinology. 2000 Apr 1;141(4):1317-24.
6. Lerchbaum E, Rabe T. Vitamin D and female fertility. Current Opinion in Obstetrics and Gynecology. 2014 Jun 1;26(3):145-50.
7. Zhao J, Huang X, Xu B, Yan Y, Zhang Q, Li Y. Whether vitamin D was associated with clinical outcome after IVF/ICSI: a systematic review and meta-analysis. Reproductive Biology and Endocrinology. 2018 Dec;16(1):1-7.
8. Paffoni A, Ferrari S, Vigano P, Pagliardini L, Papaleo E, Candiani M, Tirelli A, Fedele L, Somigliana E. Vitamin D deficiency and infertility: insights from in vitro fertilization cycles. The Journal of Clinical Endocrinology & Metabolism. 2014 Nov 1;99(11):E2372-6.
9. Aflatoonian A, Arabjahvani F, Eftekhar M, Sayadi M. Effect of vitamin D insufficiency treatment on fertility outcomes in frozen-thawed embryo transfer cycles: A randomized clinical trial. Iranian journal of reproductive medicine. 2014 Sep;12(9):595.
10. Chang HM, Qiao J, Leung PC. Oocyte–somatic cell interactions in the human ovary—novel role of bone morphogenetic proteins and growth differentiation factors. Human reproduction update. 2017 Jan 1;23(1):1-8.
11. Uyar A, Torrealday S, Seli E. Cumulus and granulosa cell markers of oocyte and embryo quality. Fertility and sterility. 2013 Mar 15;99(4):979-97.
12. Vigano P, Lattuada D, Mangioni S, Ermellino L, Vignali M, Caporizzo E, PaninaBordignon P, Besozzi M, Di Blasio AM. Cycling and early pregnant endometrium

as a site of regulated expression of the vitamin D system. Journal of Molecular Endocrinology. 2006 Jun 1;36(3):415-24.

13. Faulisi S, Reschini M, Borroni R, Paffoni A, Busnelli A, Somigliana E. Clinical value of basal serum progesterone prior to initiate ovarian hyper-stimulation with GnRH antagonists: a retrospective cohort study. Gynecologic and Obstetric Investigation. 2017;82(2):175-80.
14. Papaleo E, Pagliardini L, Vanni VS, Delprato D, Rubino P, Candiani M, Viganò P. A direct healthcare cost analysis of the cryopreserved versus fresh transfer policy at the blastocyst stage. Reproductive biomedicine online. 2017 Jan 1;34(1):19-26.
15. Balaban B, Brison D, Calderon G, Catt J, Conaghan J, Cowan L, Ebner T, Gardner D, Hardarson T, Lundin K. Alpha scientists in reproductive medicine and ESHRE special interest group of embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011 Jun;26(6):1270-83.
16. Ferrero H, Delgado-Rosas F, Garcia-Pascual CM, Monterde M, Zimmermann RC, Simon C, Pellicer A, Gomez R. Efficiency and purity provided by the existing methods for the isolation of luteinized granulosa cells: a comparative study. Human reproduction. 2012 Jun 1;27(6):1781-9.
17. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. Nature biotechnology. 2016 May;34(5):525-7.
18. Pagliardini L, Vigano P, Molgora M, Persico P, Salonia A, Vailati SH, Paffoni A, Somigliana E, Papaleo E, Candiani M. High prevalence of vitamin D deficiency in infertile women referring for assisted reproduction. Nutrients. 2015 Dec;7(12):9972-84.
19. Czernichow S, Fan T, Nocea G, Sen SS. Calcium and vitamin D intake by postmenopausal women with osteoporosis in France. Current medical research and opinion. 2010 Jul 1;26(7):1667-74.
20. 20 Naeem Z. Vitamin d deficiency- an ignored epidemic. Int J Health Sci (Qassim). 2010 Jan;4(1):V-VI.
21. Thomas MK. Lloyd-J1s DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, Vamvakas EC, Dick IM, Prince RL, Finkelstein JS. Hypovitaminosis D in medical inpatients. N Engl J Med. 1998;338:777-83.
22. Gröber U, Kisters K. Influence of drugs on vitamin D and calcium metabolism. Dermatoendocrinol. 2012 Apr 01;4(2):158-66.

23. Cipriani C, Romagnoli E, Scillitani A, Chiodini I, Clerico R, Carnevale V, Mascia ML, Battista C, Viti R, Pileri M, Eller-Vainicher C. Effect of a single oral dose of 600,000 IU of cholecalciferol on serum calciotropic hormones in young subjects with vitamin D deficiency: a prospective intervention study. The Journal of Clinical Endocrinology & Metabolism. 2010 Oct 1;95(10):4771-7.
24. Vieth R. Vitamin D and cancer mini-symposium: the risk of additional vitamin D. Annals of epidemiology. 2009 Jul 1;19(7):441-5.
25. Teh WT, McBain J, Rogers P. What is the contribution of embryo-endometrial asynchrony to implantation failure?. Journal of assisted reproduction and genetics. 2016 Nov;33(11):1419-30.
26. Roth DE, Leung M, Mesfin E, Qamar H, Watterworth J, Papp E. Vitamin D supplementation during pregnancy: state of the evidence from a systematic review of randomised trials. Bmj. 2017 Nov 29;359.
27. Holick MF. Vitamin D deficiency. New England journal of medicine. 2007 Jul 19;357(3):266-81.
28. Lucas RM, Ponsonby AL, Pasco JA, Morley R. Future health implications of prenatal and early-life vitamin D status. Nutrition reviews. 2008 Dec 1;66(12):710-20.
29. Wehr E, Pieber TR, Obermayer-Pietsch B. Effect of vitamin D3 treatment on glucose metabolism and menstrual frequency in polycystic ovary syndrome women: a pilot study. Journal of endocrinological investigation. 2011 Nov;34(10):757-63.
30. Asadi M, Matin N, Frootan M, Mohamadpour J, Qorbani M, Tanha FD. Vitamin D improves endometrial thickness in PCOS women who need intrauterine insemination: a randomized double-blind placebo-controlled trial. Archives of gynecology and obstetrics. 2014 Apr;289(4):865-70.
31. Ott J, Wattar L, Kurz C, Seemann R, Huber JC, Mayerhofer K, Vytiska-Binstorfer E. Parameters for calcium metabolism in women with polycystic ovary syndrome who undergo clomiphene citrate stimulation: a prospective cohort study. European journal of endocrinology. 2012 May 1;166(5):897.
32. Lasco A, Catalano A, Benvenga S. Improvement of primary dysmenorrhea caused by a single oral dose of vitamin D: results of a randomized, double-blind, placebocontrolled study. Archives of internal medicine. 2012 Feb 27;172(4):366-7.
33. Wise LA, Ruiz-Narváez EA, Haddad SA, Rosenberg L, Palmer JR. Polymorphisms in vitamin D–related genes and risk of uterine leiomyomata. Fertility and sterility. 2014 Aug 1;102(2):503-10.

34. Wojtusik J, Johnson PA. Vitamin D regulates anti-Mullerian hormone expression in granulosa cells of the hen. Biology of reproduction. 2012 Mar 1;86(3):91-1.
35. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. The Journal of Clinical Endocrinology & Metabolism. 2007 Sep 1;92(9):3517-22.
36. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. Bmj. 2013 Mar 26;346.
37. Polyzos NP, Anckaert E, Guzman L, Schiettecatte J, Van Landuyt L, Camus M, Smitz J, Tournaye H. Vitamin D deficiency and pregnancy rates in women undergoing single embryo, blastocyst stage, transfer (SET) for IVF/ICSI. Human reproduction. 2014 Sep 1;29(9):2032-40.
38. Firouzabadi RD, Rahmani E, Rahsepar M, Firouzabadi MM. Value of follicular fluid vitamin D in predicting the pregnancy rate in an IVF program. Archives of gynecology and obstetrics. 2014 Jan;289(1):201-6.
39. Franasiak JM, Molinaro TA, Dubell EK, Scott KL, Ruiz AR, Forman EJ, Werner MD, Hong KH, Scott Jr RT. Vitamin D levels do not affect IVF outcomes following the transfer of euploid blastocysts. American journal of obstetrics and gynecology. 2015 Mar 1;212(3):315-e1.
40. Ozkan S, Jindal S, Greenseid K, Shu J, Zeitlian G, Hickmon C, Pal L. Replete vitamin D stores predict reproductive success following in vitro fertilization. Fertility and sterility. 2010 Sep 1;94(4):1314-9.
41. Rudick B, Ingles S, Chung K, Stanczyk F, Paulson R, Bendikson K. Characterizing the influence of vitamin D levels on IVF outcomes. Human reproduction. 2012 Nov 1;27(11):3321-7.
42. Fan QR, Hendrickson WA. Structure of human follicle-stimulating hormone in complex with its receptor. Nature. 2005 Jan;433(7023):269-77.
43. Belanger J, Tremblay C, Davis A, Arnocky S. Luteinizing hormone. Encyclopedia of Evolutionary Psychological Science. 2019:1-8.
44. Staessen C, Camus M, Bollen N, Devroey P, Van Steirteghem AC. The relationship between embryo quality and the occurrence of multiple pregnancies. Fertility and Sterility. 1992 Mar 1;57(3):626-30.

45. Cummins JM, Breen TM, Harrison KL, Shaw JM, Wilson LM, Hennessey JF. A formula for scoring human embryo growth rates in vitro fertilization: its value in predicting pregnancy and in comparison with visual estimates of embryo quality. Journal of In Vitro Fertilization and Embryo Transfer. 1986 Oct;3(5):284-95.
46. Sakkas D, Shoukir Y, Chardonnens D, Bianchi PG, Campana A. Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability. Human Reproduction (Oxford, England). 1998 Jan 1;13(1):182-7.
47. Shoukir Y, Campana A, Farley T, Sakkas D. Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability. Human Reproduction (Oxford, England). 1997 Jul 1;12(7):1531-6.
48. Aleyasin A, Hosseini MA, Mahdavi A, Safdarian L, Fallahi P, Mohajeri MR, Abbasi M, Esfahani F. Predictive value of the level of vitamin D in follicular fluid on the outcome of assisted reproductive technology. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2011 Nov 1;159(1):132-7.
49. Anifandis GM, Dafopoulos K, Messini CI, Chalvatzas N, Liakos N, Pournaras S, Messinis IE. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. Reproductive Biology and Endocrinology. 2010 Dec;8(1):1-5.
50. Farzadi L, Bidgoli HK, Ghojazadeh M, Bahrami Z, Fattahi A, Latifi Z, Shahnazi V, Nouri M. Correlation between follicular fluid 25-OH vitamin D and assisted reproductive outcomes. Iranian journal of reproductive medicine. 2015 Jun;13(6):361.
51. Ciepiela P, Dulęba AJ, Kowaleczko E, Chełstowski K, Kurzawa R. Vitamin D as a follicular marker of human oocyte quality and a serum marker of in vitro fertilization outcome. Journal of assisted reproduction and genetics. 2018 Jul;35(7):1265-76.
52. Chang HM, Qiao J, Leung PC. Oocyte–somatic cell interactions in the human ovary—novel role of bone morphogenetic proteins and growth differentiation factors. Human reproduction update. 2017 Jan 1;23(1):1-8.
53. Pacis MM, Fortin CN, Zarek SM, Mumford SL, Segars JH. Vitamin D and assisted reproduction: should vitamin D be routinely screened and repleted prior to ART? A systematic review. Journal of assisted reproduction and genetics. 2015 Mar;32(3):323-35.

54. Anifandis GM, Dafopoulos K, Messini CI, Chalvatzas N, Liakos N, Pournaras S, Messinis IE. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. Reproductive Biology and Endocrinology. 2010 Dec;8(1):1-5.
55. Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertility and sterility. 2014 Aug 1;102(2):460-8.
56. Lv SS, Wang JY, Wang XQ, Wang Y, Xu Y. Serum vitamin D status and in vitro fertilization outcomes: a systematic review and meta-analysis. Archives of gynecology and obstetrics. 2016 Jun;293(6):1339-45.
57. Revelli A, Piane LD, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reproductive biology and endocrinology. 2009 Dec;7(1):1-3.
58. Xu J, Lawson MS, Xu F, Du Y, Tkachenko OY, Bishop CV, Pejovic-Nezhat L, Seifer DB, Hennebold JD. Vitamin D3 regulates follicular development and intrafollicular vitamin D biosynthesis and signaling in the primate ovary. Frontiers in Physiology. 2018:1600.
59. Pérez-López FR, Pasupuleti V, Mezones-Holguin E, Benites-Zapata VA, Thota P, Deshpande A, Hernandez AV. Effect of vitamin D supplementation during pregnancy on maternal and neonatal outcomes: a systematic review and metaanalysis of randomized controlled trials. Fertility and sterility. 2015 May 1;103(5):1278-88.
60. Moridi I, Chen A, Tal O, Tal R. The Association between Vitamin D and AntiMüllerian Hormone: A systematic review and meta-analysis. Nutrients. 2020 Jun;12(6):1567.
61. Pasquali R, Gambineri A. A comprehensive approach in diagnosing the polycystic ovary syndrome. Women's Health. 2015 Jul;11(4):501-12.
62. Dunaif A, Book CB. Insulin resistance in the polycystic ovary syndrome. Clinical research in diabetes and obesity. 1997:249-74.
63. Trummer C, Pilz S, Schwetz V, Obermayer-Pietsch B, Lerchbaum E. Vitamin D, PCOS and androgens in men: a systematic review. Endocrine Connections. 2018 Mar 1;7(3):R95-113.
64. Thys-Jacobs S, Donovan D, Papadopoulos A, Sarrel P, Bilezikian JP. Vitamin D and calcium dysregulation in the polycystic ovarian syndrome. Steroids. 1999 Jun 1;64(6):430-5.
65. Razavi M, Jamilian M, Karamali M, Bahmani F, Aghadavod E, Asemi Z. The effects of vitamin DK-calcium co-supplementation on endocrine, inflammation, and

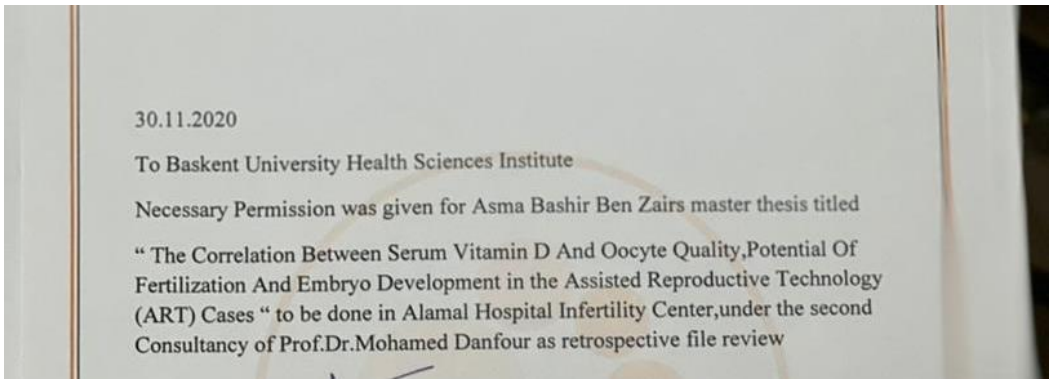
oxidative stress biomarkers in vitamin D-deficient women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Hormone and Metabolic Research. 2016 Jul;48(07):446-51.

66. Bièche I, Narjoz C, Asselah T, Vacher S, Marcellin P, Lidereau R, Beaune P, de Waziers I. Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP) 1, CYP2 and CYP3 families in 22 different human tissues. Pharmacogenetics and genomics. 2007 Sep 1;17(9):731-42.
67. Fischer D, Thome M, Becker S, Cordes T, Diedrich K, Friedrich M, Thill M. 25-Hydroxyvitamin D3 1α-hydroxylase splice variants in benign and malignant ovarian cell lines and tissue. Anticancer research. 2009 Sep 1;29(9):3627-33.
68. DeLuca GC, Kimball SM, Kolasinski J, Ramagopalan SV, Ebers GC. the role of vitamin D in nervous system health and disease. Neuropathology and applied neurobiology. 2013 Aug;39(5):458-84.
69. Law YJ, Zhang N, Venetis CA, Chambers GM, Harris K. The number of oocytes associated with maximum cumulative live birth rates per aspiration depends on female age: a population study of 221 221 treatment cycles. Human Reproduction. 2019 Sep 29;34(9):1778-87.
70. Ebner T, Shebl O, Moser M, Sommergruber M, Tews G. Developmental fate of ovoid oocytes. Human Reproduction. 2008 Jan 1;23(1):62-6.
71. Farhi J, Nahum H, Weissman A, Zahalka N, Glezerman M, Levran D. Coarse granulation in the perivitelline space and IVF-ICSI outcome. Journal of Assisted Reproduction and Genetics. 2002 Dec;19(12):545-9.
72. Balakier H, Sojecki A, Motamedi G, Bashar S, Mandel R, Librach C. Is the zona pellucida thickness of human embryos influenced by women's age and hormonal levels?. Fertility and sterility. 2012 Jul 1;98(1):77-83.
73. Ciotti PM, Notarangelo L, Morselli-Labate AM, Felletti V, Porcu E, Venturoli S. First polar body morphology before ICSI is not related to embryo quality or pregnancy rate. Human Reproduction. 2004 Oct 1;19(10):2334-9.
74. Balakier H, Bouman D, Sojecki A, Librach C, Squire JA. Morphological and cytogenetic analysis of human giant oocytes and giant embryos. Human Reproduction. 2002 Sep 1;17(9):2394-401.
75. Johnson LE, DeLuca HF. Vitamin D receptor null mutant mice fed high levels of calcium are fertile. The Journal of nutrition. 2001 Jun 1;131(6):1787-91.

76. Le Goff C, Cavalier E, Souberbielle JC, González-Antuña A, Delvin E. Measurement of circulating 25-hydroxyvitamin D: a historical review. Practical Laboratory Medicine. 2015 Aug 1;2:1-4.
77. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of clinical endocrinology & metabolism. 2011 Jul 1;96(7):1911-30.
78. Rosen CJ, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Manson JE. IOM committee members respond to Endocrine Society vitamin D guideline. The Journal of Clinical Endocrinology & Metabolism. 2012 Apr 1;97(4):1146-52.
79. Parikh G, Varadinova M, Suwandhi P, Araki T, Rosenwaks Z, Poretsky L, SetoYoung D. Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP-1) production in human ovarian cells. Hormone and metabolic research. 2010 Sep;42(10):754-7.
80. Rudick B, Ingles SA, Stanczyk F, Chung K, Paulson R, Bendikson K. Characterizing the role of vitamin D levels on IVF outcomes: stimulation, embryo, or endometrium?. Fertility and Sterility. 2010 Sep 1;94(4):S72.
81. Bodnar LM, Krohn MA, Simhan HN. Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy. The Journal of nutrition. 2009 Jun 1;139(6):1157-61.
82. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Arioka K, Sato H, Uchiyama Y, Masushige S. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nature genetics. 1997 Aug;16(4):391-6.
83. Rudick B, Ingles SA, Stanczyk F, Chung K, Paulson R, Bendikson K. The role of vitamin D levels on IVF outcomes in donor-recipient cycles. Fertility and Sterility. 2011 Mar 15;95(4):S8.
84. Lerchbaum E, Obermayer-Pietsch B. Vitamin D and fertility: a systematic review. Eur J Endocrinol. 2012 May 1;166(5):765-78.
85. Farzadi L, Bidgoli HK, Ghojazadeh M, Bahrami Z, Fattahi A, Latifi Z, Shahnazi V, Nouri M. Correlation between follicular fluid 25-OH vitamin D and assisted reproductive outcomes. Iranian journal of reproductive medicine. 2015 Jun;13(6):361.

86. Lee DM, Tajar A, Pye SR, Boonen S, Vanderschueren D, Bouillon R, O'Neill TW, Bartfai G, Casanueva FF, Finn JD, Forti G. Association of hypogonadism with vitamin D status: the European Male Ageing Study. European journal of endocrinology. 2012 Jan 1;166(1):77-85.
87. Lerchbaum E, Theiler-Schwetz V, Kollmann M, Wölfler M, Pilz S, ObermayerPietsch B, Trummer C. Effects of vitamin D supplementation on surrogate markers of fertility in pcos women: A randomized controlled trial. Nutrients. 2021 Feb;13(2):547.
88. Ha AN, Pham TD, Vuong LN. Association between Vitamin D Levels and Fertility Outcomes in Patients Undergoing IVF/ICSI. Fertility & Reproduction. 2020 Sep 10;2(03):85-92.
89. Garbedian K, Boggild M, Moody J, Liu KE. Effect of vitamin D status on clinical pregnancy rates following in vitro fertilization. Canadian Medical Association Open Access Journal. 2013 May 16;1(2):E77-82.
90. Rudick BJ, Ingles SA, Chung K, Stanczyk FZ, Paulson RJ, Bendikson KA. Influence of vitamin D levels on in vitro fertilization outcomes in donor-recipient cycles. Fertility and sterility. 2014 Feb 1;101(2):447-52.
91. Nandi A, Sinha N, Ong E, Sonmez H, Poretsky L. Is there a role for vitamin D in human reproduction?. Hormone molecular biology and clinical investigation. 2016 Jan 1;25(1):15-28.

APPENDICES



30.11.2020

To Baskent University Health Sciences Institute

Necessary Permission was given for Asma Bashir Ben Zairs master thesis titled

" The Correlation Between Serum Vitamin D And Oocyte Quality,Potential Of Fertilization And Embryo Development in the Assisted Reproductive Technology (ART) Cases " to be done in Alamal Hospital Infertility Center,under the second Consultancy of Prof.Dr.Mohamed Danfour as retrospective file review

APPENDIX 1 PROJECT APPROVAL

Evrak Tarih ve Sayısı: 30.03.2021-22879

1993

BAŞKENT ÜNİVERSİTESİ

Tıp ve Sağlık Bilimleri Araştırma Kurulu

30.03.2021

Sayı : E-94603339-604.01.02-22879
Konu : Proje Onayı

Sağlık Bilimleri Enstitüsü Müdürlüğüne

Fizyoloji Anabilim Dalında görev yapmakta olan Prof. Dr. Nazan Dolu'nun danışmanlığında Sağlık Bilimleri Enstitüsü / Fizyoloji Tezli Yüksek Lisans Programı öğrencisi Asma Bashir Salem Ben Zair'in sorumluluğunda yürütülecek olan KA21/117 nolu "The correlation between serum vitamin D and oocyte quality, potential of fertilizationand embryo development in the Assisted Reproductive Technology (ART) cases" başlıklı araştırma projesi Kurulumuz tarafından uygun bulunmuştur. Projenin başlama tarihi ile çalışmanın sunulduğu kongre ve yayınlandığı dergi konusunda Kurulumuza bilgi verilmesini rica ederim.

Not: Çalışma bildiri ve/veya makale haline geldiğinde "Gereç ve Yöntem" bölümüne aşağıdaki ifadelerden uygun olanının eklenmesi gerekmektedir.

— Bu çalışma Başkent Üniversitesi Tıp ve Sağlık Bilimleri Araştırma Kurulu ve Etik Kurulu tarafından onaylanmış (Proje no:…) ve Başkent Üniversitesi Araştırma Fonunca desteklenmiştir.

— This study was approved by Baskent University Institutional Review Board and Ethics Committee (Project no:…) and supported by Baskent University Research Fund.

Prof. Dr. Hakan ÖZKARDEŞ
Kurul Başkanı

Dağıtım:
Sağlık Bilimleri Enstitüsü Müdürlüğüne
Fizyoloji Anabilim Dalına