Republic of Iraq

Ministry of Higher Education and Scientific Research

Karbala University / College of Veterinary Medicine

Physiology, Pharmacology and Biochemistry Department

Investigation the effect of Estrogen Alpha and Beta receptors with RANK, RANK-L and OPG pathway in the Osteoporosis induced by Ovariectomy in female Rabbits.

Thesis

Submitted to the Council of the College of Veterinary Medicine, university of Karbala in Partial Fulfillment of the Requirements for the Master Degree of Science in Veterinary Medicine / Physiology

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1447 A.H **2025 A.D**

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿قَالَ رَبِّ إِنِّى وَهَنَ ٱلْعَظْمُ مِنِّى وَٱشْتَعَلَ ٱلرَّأْسُ

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We certify that thesis entitled **(Investigation the effect of Estrogen Alpha and Beta receptors with RANK, RANK-L and OPG pathway in the Osteoporosis induced by Ovariectomy in female Rabbits)** has been prepared by **(Thekra Adel Assi Hamza)** under my supervision at the college of Veterinary Medicine, University of Karbala in partial fulfillment of the requirements for the Master of Degree in the Sciences of Veterinary Medicine in Veterinary Physiology, Pharmacology and Biochemistry.

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I hereby declare that this dissertation is my original work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at the University of Karbala or other institutions.

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Dedication

Praise be to Allah, first and last, and then to our Prophet Muhammad (May Allah bless him and his family).

To my great homeland, Iraq

I dedicate excerpts from my humble research to my Master, the awaited Imam Mahdi (may Allah hasten his reappearance).

My beloved father, you are the light that illuminates my path and the support that I continue to rely on in every situation. Thank you for all the sacrifices you have made for me. May you always be my pride and glory and the reason for my happiness. I love you without limits.

To my beloved mother, the soul of my life and the secret of my existence. Her constant prayers for my well-being and her heart full of love and mercy.

To my heart, my love, and my life partner. To my support in this journey. You gave me so much love, time, support, and did your best. You were the best husband and the best support... My husband.

To my beloved children and the apple of my eye, my sons Adam and Yamen, the source of my strength.

To my brothers and sisters, you are the shining moon, you are a blessing from heaven.

I extend my sincere thanks to everyone who supported me in the successful completion of my research

Thekra Adel Assi Hamza

Acknowledgments

First of all, I would like to thank **Allah**, the Most Merciful, who enabled me to accomplish this work. I extend my thanks to the Dean of the College of Veterinary Medicine / University of Karbala, represented by the Dean, **Assistant Professor Dr. Mohammed Asaad Saleh Al-Kaabi**, and the Vice Dean, **Prof. Dr. Ihab Ghazi Mahdi** for Postgraduate Studies and Scientific Research from the College of Veterinary Medicine, for allowing me and providing some facilities for this work. I would like to thank the Department Head, for her help and support. I would like to extend my sincere thanks and appreciation to my supervisors, **Prof. Dr. Wefak Jbori Al-Bazi** and **Prof. Dr. Rana Fadhil Mousa**, for their help, advice, and constructive criticism during the course of this work and writing the thesis, and for the great effort they made to achieve the best results. I would like to express my thanks to all the faculty members of the department. Finally, I would like to thank everyone who helped me in this study, and I apologize to those I forgot to mention.

Thekra Adel Assi Hamza

Summary

Estrogen hormones play a crucial role in bone metabolism and the maintenance of bone mineral density. The aim of the current study was to Investigation the effect of Estrogen Alpha and Beta receptors with RANK, RANK-L and OPG pathway in the Osteoporosis induced by Ovariectomy in female Rabbits.

Twenty rabbits four-month-old female New Zealand White rabbits (weighing 3–3.25 kg) were randomly divided into two groups (10 each): a control group and an ovariectomized (OVX) group. Six weeks after the experiment, the rabbits underwent femoral radiography. Blood samples were drawn for chemical analysis, including serum hormone levels estrogen, progesterone, luteinizing hormone (LH), Follicle-stimulating hormone (FSH), serum salt levels Calcium (Ca), and bone biomarkers RANK, RANKL and Osteoprotegrin (OPG), Cathepsin k. Femoral samples were also taken for histological examination and gene expression testing of estrogen receptor alpha and beta.

The results showed a decrease in estrogen and progesterone levels in the OVX group compared to the control group and a clear increase in levels, luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Calcium (Ca) level in rabbits with osteoporosis OVX compared to the control group, while the serum showed a clear increase in the levels RANK, RANKL, Cathepsin k in rabbits with osteoporosis, However, study showed that this group's OVX significantly decreased as compared to the control group. The OVX group's bone density significantly decreased, according to X-ray photography compared to the control group.

Examination of gene expression of estrogen receptor alpha and beta in bones showed downregulation of gene expression of estrogen receptor alpha and upregulation of estrogen receptor beta in the OVX group compared to the control group. Histological examination of the femur of rabbits in the OVX group showed resorption with thinning of the bone plates and the presence of many osteoclasts compared to the control group.

Conclusion: The current study found that osteoporosis and the degradation of bone-forming tissues and cells are directly impacted by a decrease in estrogen hormone. Downregulation of estrogen receptor alpha gene expression means the loss of the most important pathway that maintains the balance between bone formation and bone breakdown, leading to weak bone structure and an increased risk of osteoporosis after a bilateral ovariectomy or menopause. This loss is partially compensated for by estrogen receptor beta, but its replacement is insufficient to replace the specific functions of estrogen receptor alpha.

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List of Abbreviations

Abbreviations	Meaning
AGE	**Advanced Glycation End Products**
AP-1	**Activator protein-1**
BM	**bone marrow**
BMD	**Bone mineral density**
BW	**Body Weight**
Ca	**Calcium**
CaSR	**Calcium-sensing receptors (CaSR)**

Cat K	**Cathepsin K**
CCD	**Cleidocranial Dysplasia**
D-gal	**D-galactose**
DXA	**Dual X-ray absorptiometry**
ECM	**Extracellular Matrix**
ER	**Estrogen receptors**
ERα and ERβ	**alpha and beta**
FAK	**focal adhesion kinase**
FSH	**Follicle stimulating hormone**
GAPDH	**Glyceraldehyde 3-phosphate dehydrogenase**
GPER1	**G Protein-Coupled Estrogen Receptor 1**
H&E	**hematoxylin and eosin**
HCD	**High cholesterol diet**
IGF-1	**Insulin growth factor 1**
IL-1	**Interleukin-1**
JNK	**Jun N-terminal kinase**
Kg	**Kilogram**
LH	**Luteinizing hormone**
m/s	**Meters per second**
MAPK	**Mitogen-activated protein kinase**
M-CSF	**Macrophage-colony stimulating factor**
MMPs	**Matrix metalloproteinases**

mRNA	**messenger RNA**
MSCs	**mesenchymal stromal cells**
NADPH	**Nicotinamide Adenine Dinucleotide Phosphate Hydrogen.**
NF-κB	**Nuclear factor-kappa B**
OC	**Osteoclast**
OP	**Osteoporosis**
OPG	**Osteoprotegrin**
OS	**Oxidative stress**
OVX	**ovariectomy group**
PGE2	**Prostaglandin E2**
PI3K	**activating phosphoinositide 3-kinase**
PTH	**Para thyroid Hormone**
RANK	**receptor activator of nuclear factor kappa-B**
RANKL	**Receptor Activator of Nuclear Factor kappa-B Ligand**
ROS	**Reactive oxygen species.**
RPM	**rotations per minute**
TGF- β	**Transforming Growth Factor Beta**
TNF-α	**Tumor necrosis factor-alpha**
TRAP	**Tartrate resistant acid phosphatase**
UDP	**Uridine diphosphate**
ΔΔCt	**Delta-Delta Ct**

Chapter one
Introduction

1. Introduction:

The body's highly specialized bone tissue serves as a mineral reservoir for calcium, protects vital organs, and provides structural support. It is made up of osteoblasts and osteocytes, which are support cells; osteoclasts, which are remodeling cells; osteoid cells, which are made of non-mineral collagen matrix and non-collagenous proteins; and inorganic mineral salts, like calcium, that are present in the matrix (**Briot** , ***2*015**; **Wawrzyniak and Balawender , 2022)**.

Low bone mass, a decline in the microarchitecture of bone tissue, an increase in bone fragility, and a vulnerability to fractures are the hallmarks of osteoporosis, a systemic skeletal disease (**Aibar-Almazán *et al*., 2022)**. Osteoporosis happens when bone cell function is out of equilibrium (**Cheng *et al*., 2022)**. Because of its consequences for public health, this disease has been dubbed "the silent epidemic of the 21st century." It is the most prevalent metabolic bone disease and is severe, chronic, progressive, and clinically silent (**Sobh *et al*., 2022**).

Large multinucleated cells called osteoclasts, which are generated from hematopoietic cells, are essential for bone resorption (**Elson *et al* ., 2022)**. Osteoblasts derive from mesenchymal stem cells (MSCs) that reside in the bone marrow. These cells are multipotent since they can become osteoblasts, adipocytes, or chondrocytes, according to specific factors present in the microenvironment (**Capulli *et al* .,2014)**. Osteoblasts are bone-forming cells that can synthesize the bone matrix, regulate mineralization, and eventually differentiate into bone cells or bone coating cells **(Donsante *et al* .,2021)**.

Bone homeostasis depends on maintaining a balance between osteoclastic bone resorption and osteoblastic bone production, However, osteoclasts play a crucial role in preserving bone homeostasis since they are the only cells that can resorb bone (**Omi & Mishina** , **2022)**.

The term "**estrogens**" refers to a group of female hormones that include estrone, estradiol, estriol, and estretrol. In both humans and rabbits, estrogens play a crucial role in preserving bone mineral density **(Ivory, 2021)**. Women who experience menopause have lower estrogen levels, lower bone mineral density, and a higher risk of fractures, especially those to the wrist, hip, and spine, Although the positive benefits of estrogen on bone have long been recognized, the molecular mechanism behind its function in bone cells is just now being uncovered **(Emmanuelle *et al*., 2021)**.

By reducing the rate of bone remodeling and preserving a focal balance between bone creation and resorption, estrogens shield the adult skeleton from bone loss. The effects of estrogen deprivation are the opposite **(Niwczyk *et al* .,2023)**. Studies on cells and biochemistry have strongly implied that estrogens' anti-remodeling actions stem from their capacity to limit osteoclast birth rates and reduce their lifespans (**Almeida ,2017)**.

The growth and activation of osteoclasts are regulated by three key proteins: osteoprotegrin (OPG), receptor activator of nuclear factor Kappa-B ligand (RANK-L), and receptor activator of nuclear factor kappa-B (RANK) (**Sadek *et al*., 2023)**. Furthermore, the cysteine protease cathepsin K, which breaks down the proteins that comprise the bone matrix, aids in osteoclast-mediated bone resorption (**Moon**, **2024)**. Cells of the osteoblast lineage, including matrix-embedded osteocytes, generate RANKL. Proteins break down membrane-bound RANKL to produce soluble RANKL. The majority of osteoblasts release OPG, which binds to RANKL to inhibit its activity and control osteoclastic bone resorption (**Qiu, 2022)**.

One of the strongest proteases in the lysosomal cysteine proteases family is cathepsin K cat k (**Lalmanach *et al*,.2020)**. The majority of cells have the endopeptidase, which participates in tissue self-digestion and cell autolysis (**Xie *et al*., 2023)**. It is created and released by osteoclasts, which are big,

multinucleated cells in bone and whose primary purpose is to mediate bone resorption **(Xu *et al* ., 2023)**. Activated osteoclasts release this substance to break down collagen and other matrix proteins during bone resorption **(Bordukalo-Nikšić *et al* ., 2022)**.The breakdown of collagen and other protein chemical linkages by cathepsin K causes the bone matrix to deteriorate (**Dai *et al*., 2020**).

Estrogens have physiological functions in almost all tissues in the body in both males and females (**Wend *et al* ., 2012)**. Estrogen receptors (ER) alpha and beta (ERα and ERβ) are influenced by estrogens, including the most prevalent estrogen 17β-estradiol (E2) **(Candelaria *et al*., 2013)**. Best investigated in the female reproductive system and breast cancer, ERα was discovered in the 1960s **(Gorski *et al*., 1968)** and cloned in 1986 **(Green *et al*., 1986)**. Less is known about ERβ, which was discovered and cloned in 1996 **(Kuiper *et al*., 1996)**. The DNA binding domain (95%) and ligand binding domain (60%) of ERα and ERβ are highly conserved, whereas the NH2-terminal regions, which include the transcriptional activation domain AF-1, are only 20% conserved **(Williams *et al*., 2008)**. The fact that ERα and ERβ oppose one another's functions in numerous tissues is starting to become evident **(Wu *et al* ., 2024)**. Overexpression of ERβ suppresses E2-mediated proliferation and gene expression in breast cancer cell lines, including T47D cells **(Williams *et al*., 2008)**.ERα and ERβ, however, play distinct roles in various tissues and environments **(Gan *et al* ., 2024)**.

Various Techniques for Producing Animal Models of Premature Ovarian Failure these days, the most popular animal models of premature ovarian failure fall into one of the following categories: the models caused by chemotherapy drugs, radiotherapy, genetics, D-galactose, natural ovarian aging, physiological, with consecutive superovulation, oophorectomy, 4-vinylcyclohexene diepoxide, autoimmunity, and psychological stress

(Baseem *et al* ., 2024; Pouladvand *et al*., 2024). Our rabbit model is a valuable tool to study Osteoporosis (OP)because rabbits have much faster bone turnover than rodents or primates, and in contrast to rodents, they reach skeletal maturity soon after their sexual development is complete (**Gilsanz *et al* ., 1988)**.

Aim of study:

The present study aims to investigate the impact of bilateral ovariectomy in the rabbits for the depletion of estrogen levels on the following: -

1- Level measurement of Serum Calcium(Ca), progesterone, estrogen, LH, and FSH levels.

2-Bone biomarkers RANK, RANKL, OPG and Cathepsin-K.

3-Gene expression of estrogen alpha and beta receptors.

4-Detection of bone loosening X-ray.

5-Histopathological examination on the femur bone.

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Chapter Two

Literature Review

2. Literature Review

2.1 Bones:

According to (**Osterhoff *et al*., 2016)**. bones are calcified tissues made up of 5%–10% water, 50%–70% hydroxyapatite, and 20%–40% organic materials, such as type I collagen and 10% noncollagenous proteins that aid in bone mineralization. Bone is divided into two categories according to its porosity and unit microstructure. The dense bone tissue that envelops the medullary cavity, or bone marrow, is known as compact bone, cortical bone, or dense bone. Bone marrow has a component termed cancellous bone, which is often referred to as trabecular bone or spongy bone. The adult human skeleton is highly vascularized and made up of 20% cancellous bone and 80% compact bone **(Ullah *et al*., 2021)**.

The bones reach the highest bone mass in life, known as peak bone mass. After reaching peak bone mass, bone mass remains relatively stable until menopause, when female sex hormone levels decline and cause bone marrow density to decline over the next five or ten years (**Cheng *et al*., 2022**).

The sluggish phase of bone loss starts at about age 40 for cortical bone and 5–10 years earlier for trabecular bone. Compared to trabecular bone, cortical bone has a substantially lower surface to volume ratio (**Koh *et al* ., 2024**). In the long bones, greater porosity at the periosteal surface results in greater strength loss than increased porosity near the endocortical surface. Aging or illness leads the cortex to become more porous, increasing surface area but decreasing strength (**Isojima & Sims, 2021)**.

Because bending strength is proportional to the radius to the fourth power, slow periosteal expansion throughout life partially offsets this

strength loss. In the trabecular compartment, 20% of the volume is made up of bone, with the remaining space being made up of fat and marrow. Trabecular bone transfers mechanical loads from the articular surface to the cortical bone, and its hydraulic properties absorb shock (**Usgu & Usgu, 2023)**.

The material characteristics of the bone compartments are different: trabecular bone has more water and less calcium than cortical bone, has a large surface area exposed to blood flow and bone marrow, and has a higher turnover rate than cortical bone. In trabecular bone, resorption occurs along the surfaces of the bones, whereas in cortical bone, it occurs through the bone itself (**Abdallah *et al*., 2020)**.

2.2 Bone Cells:

2.2.1 Osteoblast:

Osteoblasts, as they are popularly known, are the cells that "build" bone. Osteoblasts produce and deposit collagen (type 1), proteoglycan, and organic bone matrix (osteoid) proteins, which mineralize in developing skeletons and throughout the ongoing process of bone remodeling that lasts a lifetime **(Henry & Bordoni, 2020)**. Pluripotent mesenchymal stem cells are the source of them. Through cell-cell contact, paracrine signaling, and cell-bone matrix interaction, osteoblasts also indirectly control the production of osteoclasts and bone remodeling **(Desai & Jayasuriya, 2020)**. To preserve the shape and structural integrity of bone, osteoblasts produce and secrete bone matrix. The development, remodeling, and repair of bones are encouraged by this process (**Khoswanto, 2023)**. In the bone tissue, osteoblasts primarily carry out two types of tasks. First, a number of proteins

that are necessary for the development of the bone structural matrix are released by osteoblasts **(Aslam Khan *et al* ., 2024)**. Second, osteoblasts play a role in controlling bone mineralization **(Khotib *et al* ., 2023)**. The following list includes a few of osteoblasts' main roles: Osteoblasts have unique hormone receptors for vitamin D, parathyroid hormone (PTH), and estrogen. Important substances that activate osteoclasts are secreted by osteoblasts, including the receptor activator of nuclear factor κ B (RANK) ligand and other related factors that interact with other cells **(van Driel & van Leeuwen, 2023)**.

A regulatory protein secreted by osteoblasts has also a role in controlling the kidneys' excretion of phosphate **(Agoro & White , 2023)**. Following the completion of the formation of new bone, some osteoblasts will develop into osteocytes, while the remaining ones will encircle the bone matrix **(Krasnova *et al* ., 2023)**. If left behind, some of them will either develop into lining cells or remain on the surface of the new bone. Calcium is transported by lining cells both inside and outside of the bone (**Shapiro & Landis ,2024)**.

2.2.2 Osteocyte:

A specific type of cell present in bone tissue is called an osteocyte. The mineralized bone matrix is maintained by these cells (**Győri, 2024)**. Bone-forming cells called osteoblasts give rise to osteocytes. Osteoblasts develop into osteocytes after becoming embedded in the bone matrix (**Šromová *et al* ., 2023**). Osteocytes are linked to one another by microscopic channels known as canalicul, and their lengthy, branch-like processes create a network inside the bone **(Schurman *et al*., 2021)**. They can interact and share waste materials and nutrients thanks to this network (**Yang *et al* ., 2023**).

By detecting mechanical pressures and reacting to variations in bone density, osteocytes play a critical role in controlling bone remodeling (**Uda, 2022)**. By monitoring and regulating the bone matrix, osteocytes are essential for preserving bone health and function (**Robling *et al*., 2020**). They also play a vital role in the growth and upkeep of bone tissue, and their imbalance or malfunction can exacerbate diseases like osteoporosis **(Metzger *et al*., 2019**). A reduction in bone mass and density is the hallmark of osteoporosis, which makes bones more brittle and increases the risk of fractures (**Alcorta-Sevillano *et al* ., 2020**).

There may be a net loss of bone mass in osteoporosis when the activity of osteoclasts, which break down bone, exceeds that of osteoblasts, which create new bone. This process is also aided by osteocytes, which are key regulators of bone remodeling **(Banoriya *et al*., 2025**). Osteoporosis may occur as a result of modifications to the osteocytes' communication network, changes in how they react to mechanical loading, and disturbances in the signaling pathways they participate in (**Chen *et al*., 2024**).

Reduced mechanical loading, such as inactivity, can result in a decrease in bone density because osteocytes are sensitive to mechanical stressors (**Kushchayeva *et al*., 2022)**. Hormonal fluctuations can also impact osteocyte function and lead to bone loss, particularly in postmenopausal women (**McNamara, 2021**). To effectively manage and prevent osteoporosis, treatments and interventions must take into account the role of osteocytes (**Pathak *et al*., 2020**). Addressing osteoporosis requires strategies that support overall bone health, encourage appropriate osteocyte activity, and preserve a balance between bone creation and resorption **(Chen *et al*., 2024**). As seen in **Figure 2.1.**

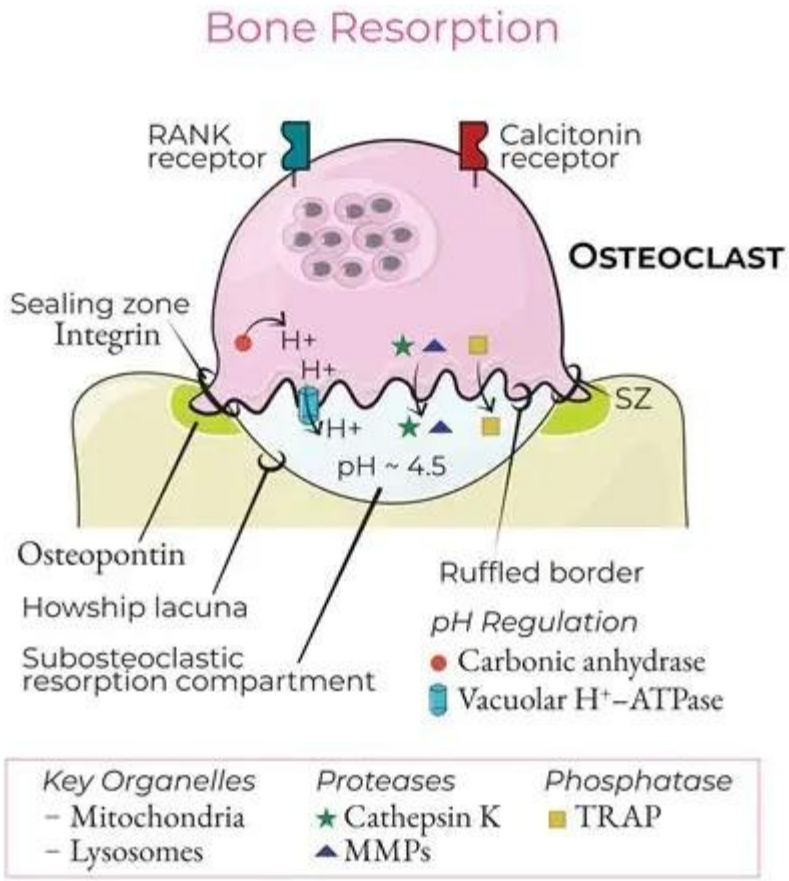


Figure (2.1) : Bone Resorption

2.2.3 Osteoclast:

The multinucleated, tissue-specific, highly specialized macrophage called an osteoclast is in charge of bone resorption (**Sun *et al.*, 2021**). At or close to the bone surface, osteoclasts develop from monocyte precursors **(Iwamoto & Kawakami ,2022)**.

The active osteoclast resorbs bone by creating an acidic environment that allows H+ ions to be carried by proton pumps and the lytic enzymes cathepsin K and tartrate resistant acid phosphatase (TRAP) to be released

into the resorption compartment (**Anwar *et al* ., 2023)**. This breaks down hydroxyapatite and permits the bone matrix proteins to be broken down by enzymes. The osteoclasts subsequently phagocytose and expel the proteins (**Saxena *et al* ., 2021**).

Three molecules: receptor activator of nuclear factor kB ligand (RANKL) and its receptor RANK, and the decoy receptor osteoprotegerin (OPG), form the RANKL/RANK/OPG pathway. which plays a critical role in the differentiation of osteoclasts from its haematopoietic progenitors (**Brodetska *et al* ., 2020**).

2.3 Osteoporosis :

Bone health of the elderly is a major global health concern, since about 1 in 3 women and 1 in 5 men suffer from bone loss and fractures, often called osteoporosis, in old age. Bone health is a complex issue affected by multiple hormones and minerals (**Elbossaty ,2017)** .

Bone mass or bone density can be used to measure osteoporosis, a disease that affects the bones and is characterized by decreased bone mass, deterioration of bone structure, increased bone fragility, and an increased risk of fracture (**Aibar-Almazán *et al*., 2022)**. Patients with osteoporosis experience a reduction in their quality of life and regular everyday activities due to the increased risk of fractures in places like the wrist and hip vertebrae (**Fitzgerald *et al* .,2024)**. Significant morbidity and mortality may eventually result from these issues **(Fink *et al* .,2023)**.

Because of the gradual loss of bone that usually happens over the years and without any symptoms until the bone becomes so brittle that a catastrophic fracture occurs, this metabolic bone issue is also known as "the

silent thief" **(Sale *et al*., 2010)**. The most prevalent age-related condition is osteoporosis (**Lippuner *et al*., 2005; Abdallah *et al*., 2020**). Consequently, bones often grow more brittle and prone to breaking (**Piñar-Gutierrez *et al*., 2022**).

It is considered as primary osteoporosis in post-menopausal women and in the elderly without an obvious disease, while secondary osteoporosis is a consequence of medications or diseases (**Grygorieva *et al*., 2023**). Physicians possess knowledge of the association between osteoporosis and advanced age, postmenopausal status, and secondary causes, such as chronic diseases and lifestyle issues, that contribute to osteoporosis **(Aibar-Almazán *et al* ., 2022**) .

2.3.1 Causes of Osteoporosis:

A number of known factors has been linked to an imbalance in osteoclasts and osteoblasts, such as deficiencies in estrogen and testosterone (**Cheng *et al* ., 2022)**, hyperthyroidism malabsorption **(Du *et al* ., 2021**), smoking and increased alcohol intake (**Khiyali *et al* .,2024**), unbalanced diets and disordered eating (**Sachdeva *et al* .,2024**). Low calorie intake and excessive exercise (**Ratajczak *et al* ., 2021**), calcium and vitamin D deficiency **,** women with type I diabetes (**Abdallah *et al*., 2020**), immobilization due to lifestyle factors, certain conditions like juvenile or pregnancy-related osteoporosis (**Galanis *et al* .,2023)**, and prior medical disorders like corticosteroid intake (**El-Haroun *et al*., 2020**).

Because bone loss is asymptomatic, osteoporosis is typically not identified until a patient has a fracture (**Ayers *et al* ., 2023**).Consequently, the following are typical signs of the illness: Common risk factors for

osteoporosis include back pain from vertebral compression, height loss, spinal deformity (kyphosis with a loss of 4-8 inches in height), fractures of the vertebrae (approximately one per year in the early stages of the disease), hips, wrists, and other bones (**Feng *et al* .,2024**), gender, ethnicity, diet (low calcium and vitamin D intake), hyperthyroidism, Cushing's disease, anorexia nervosa, lifestyle (sedentary, cigarette smoking, excessive alcohol consumption, use of certain medications (glucocorticoids and some anticonvulsants), obesity, and disuse/microgravity conditions (space flight, bed rest, paralysis) **(Foroutan, 2024)** .

2.3.2 Diagnosis of Osteoporosis:

Bone loss usually occurs gradually and painlessly; fracturing a bone can be the initial indication of osteoporosis (**Jo'rayevna, 2023**). However, osteoporosis can be identified even before a bone breaks by obtaining a bone density test, which can also identify if bone density is below normal. Osteopenia is the term for bone loss that has not progressed to the point where osteoporosis has been diagnosed. Dual X-ray absorptiometry is one of the most widely used tests for osteoporosis (DXA or DEXA) (**Nazia Fathima *et al* .,2022)**.

The most recent technique used to measure bone mass or bone mineral density(BMD), has better reproducibility, shorter scan time and is capable of measuring the density of the entire skeleton, It uses two x-ray beams with different levels of energy, After eliminating soft tissue absorption, the absorption of each beam by bone is used to calculate the bone mineral density, It is a portable device that is easy to use and convenient **(Lorente-Ramos *et al* ., 2011; Nazia Fathima *et al* ., 2022)**.

2.4 Process of Bone Remodeling:

In general, bone remodeling occurs in five stages: During the activation phase, when bone remodeling is initiated by a local mechanical or hormonal stimulation, osteocytes are believed to recognize these signals and translate them into a biological response in bone **(Katsimbri, 2017; Fang *et al*., 2022)**. As seen in **Figure (2.2).**

During the activation phase, a new remodeling cycle starts when osteoclastogenesis is stimulated by systemic (like vitamin D, calcium, PTH, estrogen, androgen, and glucocorticoids), local (like TGF-β, macrophage colony-stimulating factor (M-CSF), and receptor activator of NF-κB ligand (RANKL) regulators (**Anwar *et al*., 2023)**.

The resorption phase, during which mature osteoclasts break down both organic and mineral matrix by secreting matrix metalloproteinases (MMPs). Underneath the canopy cells, the How ship's resorption lacunae form during this period (**Ono *et al*., 2018; Li *et al* ., 2021)** .

Reversal phase, during which osteoblasts are guided to the resorption site and mature osteoclasts will undergo apoptosis **(Li, *et al* ., 2023**). Local chemicals like TGF-β will be released during this phase, drawing osteoblasts to start the production of new bone **(Dang *et al* ., 2024)**.

The formation phase, which typically lasts four to six months, is when osteoblasts take over the bone remodeling process (**Uenaka *et al* ., 2022**). Numerous systemic and local regulators, including Wnt, Sclerostin, and PTH, will cause osteoblastogenesis in bone during this period (**Marini *et al* ., 2023**).Until the full compensation for bone resorption is

obtained, the organic bone matrix (osteoid), which is made up of various proteins such type I collagen, begins to deposit.

Termination phase: The formation phase will come to an end when the same amount of bone matrix has been reabsorbed and created. During this stage, osteoblasts will either undergo apoptosis or develop into new osteocytes and mineralize bone **(Katsimbri, 2017; Chang & Liu ,2022)**.

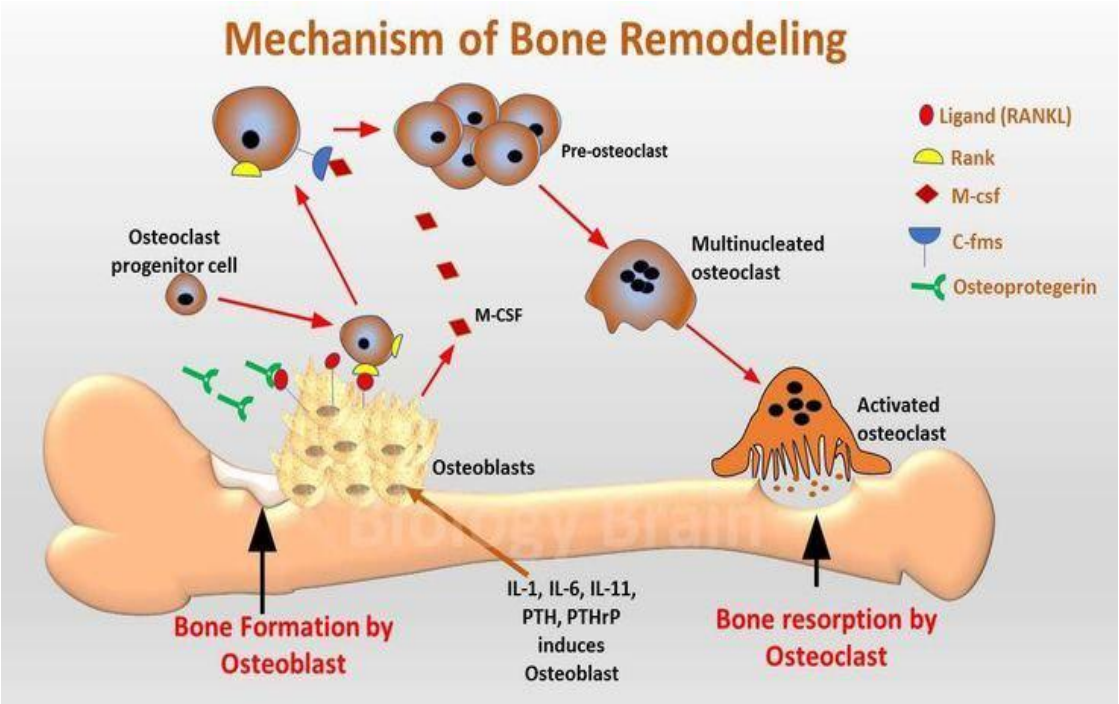


Figure (2.2) : Mechanism of bone remodeling (Chang & Liu ,2022)

2.5. Estrogens: Definition:

A class of female hormones known as "estrogens" includes estrone, estradiol, estriol, and estretrol. In terms of chemistry, estrogens are members of the steroid family of chemical molecules. Therefore, 17 carbon-carbon bonds grouped in four fused rings—three cyclohexane rings and one cyclopentane ring—make up their fundamental structure. The four estrogens are referred to as C18 steroids since they all have 18 carbons (C18H24O2).

They are made up of a phenolic hydroxyl and one benzene ring (**Ivory, 2021 ; Georgin *et al* ., 2024)**. As seen in **Figure (2.3).**

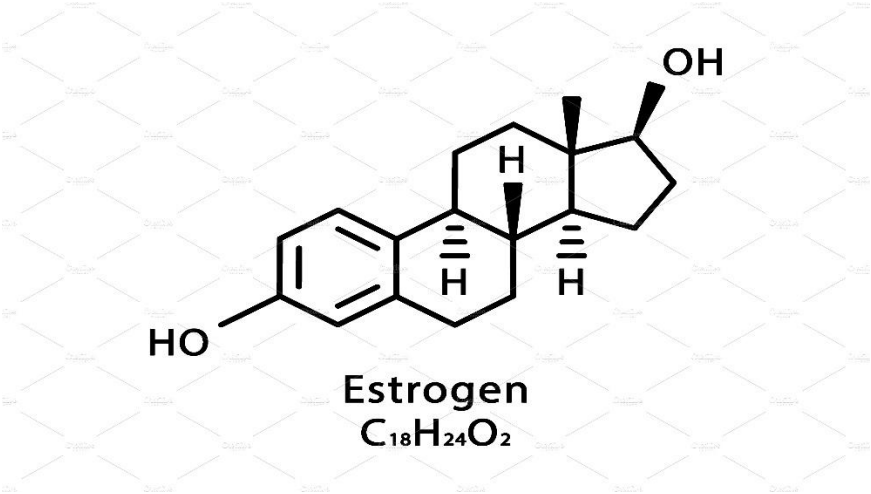


Figure (2.3) : Estrogen molecular structure (Georgin *et al* ., 2024)

2.5.1. Estrogen Biosynthesis :

Dietary cholesterol, more especially low-density lipoprotein (LDL) cholesterol, is the primary substrate for the production of steroid hormones (**Upadhyay,2023)**. In the gonads, adrenal cortex, and adipose tissue, cholesterol undergoes a process known as steroidogenesis, which results in the production of the 21-carbon (pregnanes, progestogens), 19-carbon (androstanes), and 18-carbon (estranes) steroid hormones (**Kater *et al* ., 2022**). The granulosa cells in the ovaries are the primary location for the manufacture of estrogen (**Storbeck *et al* ., 2019)**. As seen in **Figure (2.4) .**

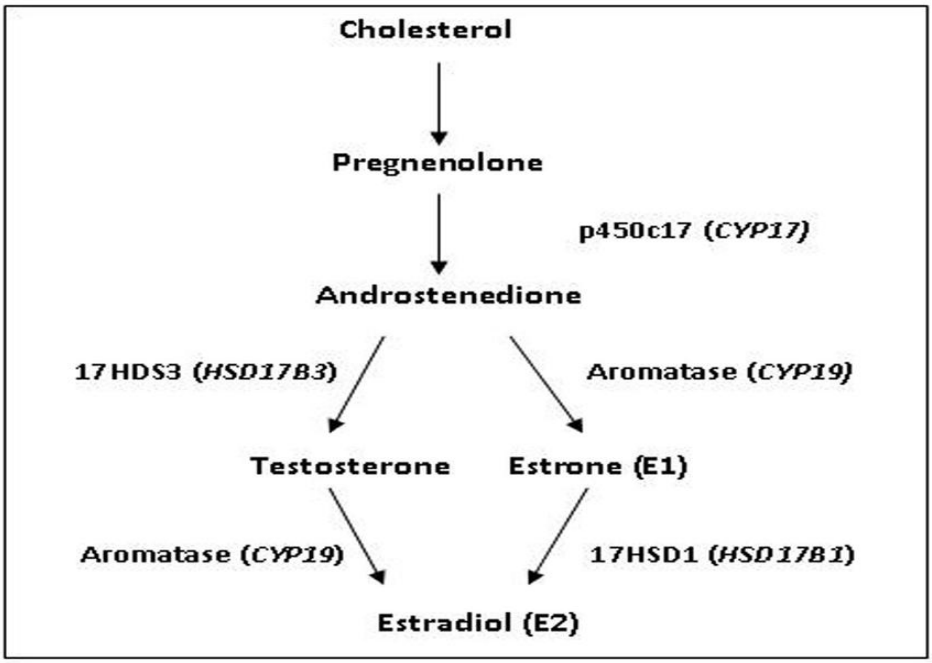


Figure (2.4) : Biosynthesis of estradiol (Upadhyay,2023)

2.5.2. Estrogen Metabolism:

Physiologically, estrogens can be eliminated from the body by urine, feces, and/or bile due to their metabolic conversion. Additionally, estrogen analogs are produced, which have been demonstrated to have antiproliferative properties (**Stanczyk, 2024)**. Estradiol and estrone can be metabolized by various mechanisms in target cells. CYP1A1, CYP1B1, and CYP1A2 are members of the cytochrome P450 superfamily of enzymes that catalyze the hydroxylation of estrone and estradiol at sites C2, C4. (**Denison, 2024)**.

2.5.3. Physiological Functions of Estrogens :

As sex steroid hormones, estrogens have a wide range of physiological effects. These include bone density, cognitive function, cholesterol mobilization, development of breast tissue and sexual organs, management of the menstrual cycle and reproduction, and inflammatory control (**Tokatli *et al* ., 2022**). Although estrogens have different functions in normal male and female physiology, they can have comparable functions in both sexes under specific physiological conditions (**Tiberi *et al* ., 2023**).

2.5.4. The Estrogen Receptors: History and Discovery :

By demonstrating that reproductive female cells could absorb estrogen from the bloodstream by attaching to proteins, Gorski identified the estrogen receptor in 1968—the first receptor ever found for any hormone. He subsequently showed that estrogen-bound receptors may go to the nucleus and activate the transcription of genes there. The first human estrogen receptor, now called ERα, was cloned more than 20 years later (**Gorski *et al*., 1968**).

2.5.5. Structural Properties of Estrogen Receptors :

ERα is 595 amino acids and 67 kDa in complete length. ERβ is 59 kDa and has 530 amino acids. The primary distinction between the two proteins is that ERβ's amino terminal domain is shorter than ERα (**Scobie *et al* ., 2002**) . The estrogen receptors ERα and ERβ share multiple structural regions and are made up of different functional domains, as they belong to the nuclear hormone receptors superfamily of transcription regulators the major functional (**Rollerova & Urbancikova , 2000)**.

2.5.6. Mechanism of Estrogen Receptor Signaling

Estrogen is a steroid hormone that can penetrate the plasma membrane and interact with intracellular ERα and ERβ to bind to DNA sequences and produce direct effects **(Hsu *et al* ., 2024)**. On the other hand, estrogen can interact with GPER1 and/or ERα and ERβ to initiate intracellular signaling cascades **(Tirado-Garibay *et al* ., 2023)** .Estrogen-mediated signaling events, in which estrogen-receptor complexes can bind to DNA directly or indirectly, are caused by variations in the cellular and molecular processes that regulate gene expression (**Yu, *et al* ., 2022)**.

2.5.7. Nuclear Estrogen Receptors:

Genomic signaling that is indirect as was previously mentioned, estradiol can also control the transcription of a number of genes without the estrogen receptors directly interacting to the DNA. These genes do not have EREs in their promoter regions. Recent results indicate that ERE-like sequences are absent from an estimated 35% of genes that estrogen targets **(AltweggA & Vadlamudi , 2021)**."Indirect genomic signaling" is the collective term for the ways in which estrogen influences gene expression **(B artkowiak-Wieczorek *et al* ., 2024)**.

2.5.8. Membrane Receptor:

Non-genomic indirect signaling not every estrogen response fits the traditional genetic model of steroid action, as was previously mentioned. Following the identification of the GPER1, the theory that estrogen might be operating through mechanisms other than direct target gene transcription and protein synthesis was developed in response to the observation of abnormally rapid estrogen-induced biological responses

(**Rishabh *et al* .,2024)**.Estrogen's non-genomic effects frequently entail signal-transduction pathway activation **(Gonçalves *et al* ., 2023)**.

2.6. Progesterone :

One important steroid hormone, progesterone, is primarily produced by the ovary's corpus luteum and then by the placenta during pregnancy. It is crucial for maintaining pregnancy, the menstrual cycle, neuroendocrine processes, and bone metabolism **(Das *et al* ., 2023)** . The ovary, adrenal glands, and central nervous system (CNS) synthesize pregnenolone from cholesterol (as a neurosteroid) **(Marwein *et al* ., 2020)** . controlled by luteinizing hormone (LH) in the ovary. During the luteal phase of the menstrual cycle, levels are at their peak, and they drastically decline with menopause **(Sinha *et al* ., 2022)** .

Osteoblasts, or bone-forming cells, have been shown to have progesterone receptors, indicating a direct anabolic action. It might increase bone production by encouraging osteoblast differentiation and proliferation. works in concert with estrogen to preserve the equilibrium of bone remodeling **(Imai *et al* ., 2013 ; Mohanty *et al* ., 2025)**.

2.7. Luteinizing hormone (LH) :

The anterior pituitary gland secretes the glycoprotein hormone known as luteinizing hormone (LH). In both males and females, it is essential for to control the reproductive system. Together with follicle-stimulating hormone (FSH), LH is a component of the hypothalamic-pituitary-gonadal (HPG) axis **(Al-Suhaimi *et al* ., 2022)**. However, recent studies have drawn attention to elevated gonadotropins, particularly LH, as possible contributors to postmenopausal bone deterioration.

Loss of estrogen's negative feedback on the hypothalamic-pituitary axis is a hallmark of menopause. As a result, LH and FSH levels rise noticeably **(Alasmi , 2022)**.

LH receptors (LHR) have been found in bone tissue by studies, including in osteoblasts (cells that build bone) and osteoclasts (cells that resorb bone). This implies that LH, rather than estrogen, may have a direct impact on bone metabolism **(Tedjawirja *et al* ., 2021)** .

2.8. Follicle-stimulating hormone (FSH):

The anterior pituitary gland secretes the glycoprotein hormone known as follicle-stimulating hormone (FSH). It is essential for controlling both male and female reproductive processes. FSH encourages ovarian follicle growth in females and spermatogenesis in males **(Oduwole *et al* ., 2021; Al-Suhaimi *et al* ., 2022)** . FSH may affect adiposity and bone metabolism systemically **(Ye *et al* ., 2023)** .

FSH is a heterodimer made up of α and β subunits; its biological specificity is provided by the β-subunit. The hypothalamus's gonadotropin-releasing hormone (GnRH) controls its secretion. Inhibin B and testosterone in men and inhibin B and estradiol in women are the main sources of negative feedback (**Das & Kumar, 2018)**.

FSH may affect bone resorption because osteoclasts have FSH receptors, and high FSH may promote bone loss even in the absence of estrogen shortage **(Spaziani *et al* .,2023)**.High FSH levels may be a contributing factor to decreased bone mass in postmenopausal osteoporosis **(Lu *et al* ., 2025)** .

2.9. Calcium :

Throughout the human body, with the bones and teeth store more than 99 percent of it (**Raskh, 2020)**. It is essential for preserving the strength and integrity of bones. Particularly in older and postmenopausal populations, calcium insufficiency is a significant risk factor for bone-related conditions, including osteoporosis **(Tang *et al* ., 2023)** .

Calcium helps maintain healthy bones through: Bone mineralization: Calcium gives bones their strength and rigidity, mostly in the form of hydroxyapatite crystals and is necessary for the activity of osteoblasts, which make new bone, and osteoclasts, which break down existing bone. Bones are constantly changing **(Wawrzyniak & Balawender, 2022)**.

Vitamin D, calcitonin, and parathyroid hormone (PTH) all play a key role in maintaining calcium homeostasis by controlling calcium mobilization from bone, absorption, and excretion **(Shaker & Deftos ,2023)**. Estrogen levels drastically drop following menopause or ovariectomy, which removes the ovaries surgically. This results in increased bone resorption, decreased calcium retention, and an increased risk of osteoporosis **(Hossain *et al* ., 2025)** .

2.10. Osteoporotic Pathway:

2.10.2 Receptor Activator of Nuclear Factor kappa-B (RANK)

The protein known as Receptor Activator of Nuclear Factor kappa-B (RANK) is essential for controlling bone metabolism. It is a member of the tumor necrosis factor (TNF) receptor superfamily of cell surface receptors. The primary surface of osteoclast precursor cells, which are engaged in bone resorption—the breakdown and removal of aged or damaged bone tissue—

is where RANK is expressed (**Aek *et al* ., 2023**). RANK promotes signaling pathways that result in osteoclast differentiation and activation when it interacts with its ligand, RANKL (Receptor Activator of Nuclear Factor kappa-B Ligand). Bone tissue is broken down by osteoclasts, which permits the skeleton to continuously remodel (**Holliday *et al* ., 2021)**.

2.10.1 Receptor Activator of Nuclear Factor kappa-B Ligand (RANKL)

Although RANKL is first generated as an integral membrane-bound protein, proteases have the ability to break it into a useful soluble form (**Elango *et al* ., 2021**). RANKL increases osteoclast development and function by binding to its receptor RANK on osteoclast progenitors (**Qiu, 2022)**. RANKL has a role in the fusing of osteoclast precursors into multinucleated cells, their development into mature osteoclasts, and their ongoing survival. As a cofactor for osteoclast differentiation, RANKL depends on macrophage-colony stimulating factor (M-CSF, often referred to as CSF-1) (**Niu *et al* ., 2022**).

Either proteolytic cleavage or alternative splicing can produce the membrane form of RANKL, a type II homotrimeric trans membrane protein that is produced as a membrane-bound and secreted protein (**Takegahara *et al* ., 2022**). ADAM (a disintegrin and metalloprotease domain) and matrix metalloproteases are necessary for the proteolytic cleavage of RANKL (**Cuffaro *et al* ., 2024**). The majority of the stimuli known to promote osteoclast development and activity also boost RANKL expression in osteoblast/stromal cells (**Taskozhina *et al* ., 2024**). it is expressed at low levels in a number of different organs, such as the spleen

and bone marrow, and at high levels in the lymph nodes, thymus, and lung **(Kramar *et al* ., 2024)**.

Cells of the osteoblast lineage, including matrix-embedded osteocytes, generate RANKL. Proteases break down membrane-bound RANKL to produce soluble RANKL **(Boyce & Xing ,2007)**. The majority of osteoblasts release OPG, which binds to RANKL to inhibit its activity and control osteoclastic bone resorption (**Ono *et al* ., 2020)**.

2.10.3 Osteoprotegerin (OPG)

In addition to osteoblasts, OPG is expressed in the heart, kidney, liver, spleen, and bone marrow, among other organs (**Freeman *et al* ., 2024**). The majority of the variables that cause osteoblasts to express RANKL also control its expression. The ratio of RANKL to OPG shifts in favor of osteoclastogenesis, despite conflicting results, because upregulation of RANKL is generally linked to downregulation of OPG, or at least reduced induction of OPG **(Boyce & Xing ,2008**). As seen in **Figure (2.5)**, numerous studies have substantiated the claim that the RANKL/OPG ratio is a significant driver of bone mass (**Ekeuku *et al* .,2023)**.

One protein that is essential for controlling bone density is osteoprotegerin (OPG). It plays a role in controlling bone remodeling, which is the breakdown and regrowth of bone tissue. Osteoclasts are cells that break down bone tissue, or resorb bone, and OPG helps regulate their activity (**Wang *et al* ., 2023)**. For a protein known as RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand), OPG serves as a "decoy receptor" . The development and activation of osteoclasts depend on RANKL. OPG

inhibits bone resorption by binding to RANKL and preventing it from connecting with its receptor on osteoclasts (**Di Cicco *et al* ., 2024**).

This regulation is crucial for maintaining the balance between bone formation and bone breakdown, helping to ensure the integrity and strength of the skeletal system. Imbalances in this system can lead to conditions such as osteoporosis or excessive bone loss (**Wawrzyniak & Balawender, 2022**).

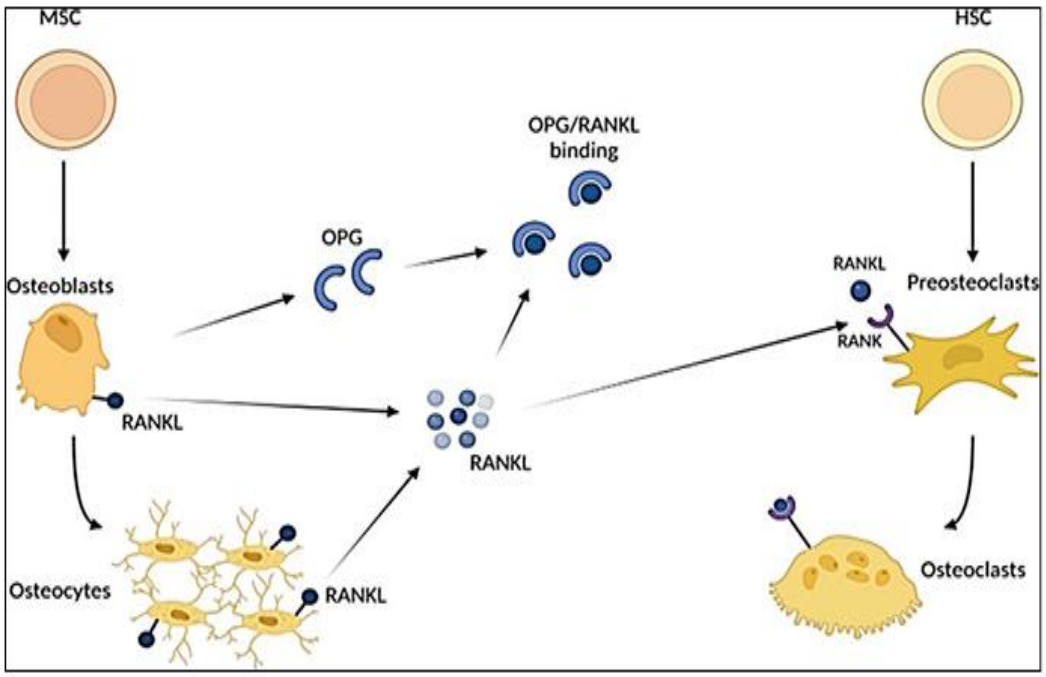


Figure (2.5) : RANKL/RANK/OPG pathway (Kim *et al* .,2022).

2.10.4 Cathepsin K :

The breakdown of type I collagen in osteoclast-mediated bone resorption is caused by cathepsin K. The cysteine protease cathepsin K aids in osteoclast-mediated bone resorption by breaking down the proteins that comprise the bone matrix **(Deaton *et al*., 2005)**. Most cells include the

endopeptidase cathepsins, which aid in tissue self-digestion and cell autolysis (**XIE *et al*., 2023**). Cathepsin B, C, F, H, K, L, O, S, V, W, and Z are the 11 members of the human cathepsin family. They are identified by their structures, catalytic processes, and the proteins they cleave (**Gureeva *et al* ., 2022**).

The primary enzyme thought to be in charge of breaking down the organic bone matrix is Cathepsin K (Cat K), a cysteine protease belonging to the papain family **(Dai *et al*., 2020**). It has the unusual capacity to break down type I collagen helical sections in acidic environments and is highly and specifically expressed in osteoclasts (**Celik *et al* ., 2024**). As seen in **Figure (2.6)**.

When cathepsin K changes from its inactive to its active state, it can be activated. The existence of additional enzymes and variations in acidity could be potential activators **(Dai *et al*., 2020)**. Osteoporosis develops as a result of damaged bone structure and decreased bone density brought on by Cathepsin K proteolytic action on the bone matrix. This makes fractures and bone degradation more likely **(Ahmad *et al*., 2025)** .

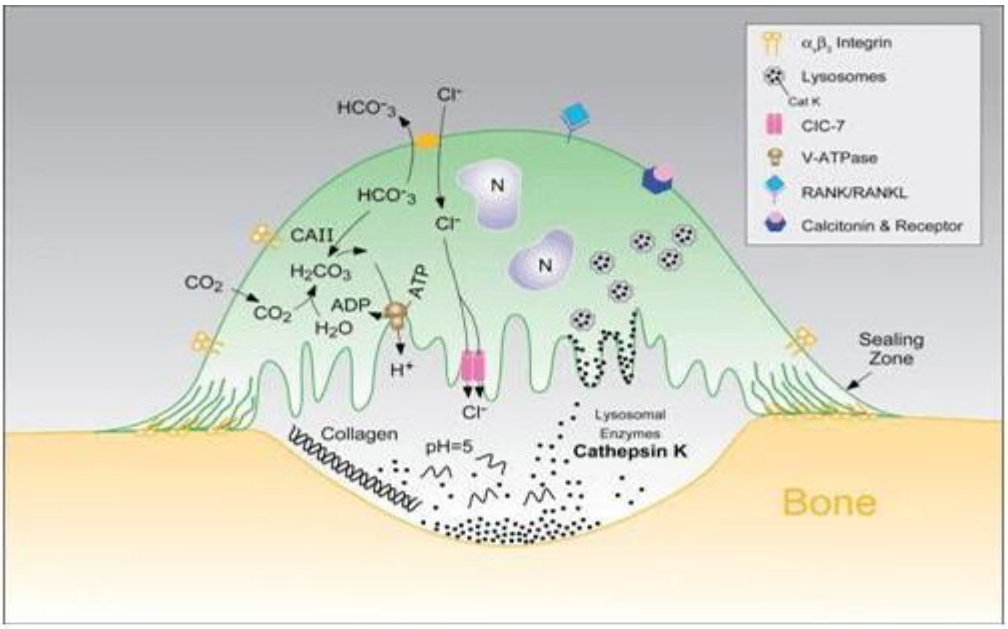


Figure (2.6): Cathepsin K - A new molecular target for osteoporosis (Rodan & Duong , 2008)

2.11. Ovariectomy:

The rate of surgical ovarian removal in the Iraq is estimated at 46 – 50 21 35% of all women, in addition to hysterectomy operations (**Gaughan *et al* .,2025 ; Abd Ali et al ., 2023)** 90% of tumors are benign tumors where there was an increasing trend in Ovariectomy before the age of 45 years, and the percentage between 45-65 years is between 78% (**Zhu *et al* ., 2023)**

The complications observed after the Ovariectomy procedure are an increase in cardiovascular disease, the negative effect of lipid-protein and blood clotting parameters Clotting and increased signs of osteoporosis, increased incidence of hip fractures, memory loss, and surgical removal of the ovaries for various reasons, including ovarian cancer, breast cancer, ovarian cysts or remnant ovarian syndrome (**Rawlinson, 2009 ; Johansen *et al* ., 2020)** . About 20% of women who undergo surgical menopause develop symptoms of hypertension and

atherosclerosis. It has been observed that women who undergo bilateral oophorectomy before the age of 45 years are associated with higher mortality rates due to these Cardiovascular diseases (**Madika *et al*., 2021**).

The process of removing the tumor leads to a decrease in the concentration of the hormone estrogen Hypoestrogen, loss of the menstrual cycle, and increases in the levels of thyroid hormones Pituitary gonadotropins in the blood as a result of loss of the feedback mechanism (**Rawlinson, 2009)**. "Therefore, ovariectomy is similar to menopause in women, which is the period during which menstrual cycles and uterine changes stop, typically between the ages of 45 and 55. During this time, the brain does not respond effectively to the gonadotropin hormones secreted by the pituitary gland, and the secretion of estrogen and progesterone decreases significantly.and the incidence bone diseases increases as a result of a decrease in the level of estrogen hormone in humans, especially since estrogen hormone is known for its antioxidant properties. Therefore, the process of removing the ovaries leads to increased oxidative stress. **(Yang & Toriola ,2024)**.

Chapter Three

Materials and Methods

3. Materials and Methods

3.1. Materials

3.1.1. Instruments and Equipment:

All the devices utilized in this study are summarized in **Table 3.1**.

Table 3.1. Apparatus and equipment with their manufacturers.

No.	Apparatus & Equipment	Company	Manufactures
1.	Anatomicalset(Scissors, Forceps, Scalpel)	Chemo lab	China
2.	Balance	Denver	Germany
3.	Beakers (100, 250, 500, 1000)	Chemo lab	India
4.	Centrifuge	Hettich	Germany
5.	Colony flask	Chemo lab	India
6.	Cotton	India	Entrepreneur
7.	Digital balance	Denver	Germany
8.	Digital camera	Canon	China
9.	ELIZA printer	Epson	Japan
10.	ELIZA reader	Biotek	USA
11.	Eppendorf's tubes	Chemo lab	India
12.	Filter paper	Chemo lab	India
13.	Gel tube	Chemo lab	India
14.	Incubator	Lab tech	Korea
15.	Insulin syringe	Eldawlia	Egypt
16.	Light Microscope	Olympus	Japan
17.	Micropipettes(different volumes)	Dragonmed	China
18.	Microscope with camera	Olympus	Japan
19.	Microtome	Leica RM	USA
20.	Pipette tips (10 – 1000) µl volume	Chemo lab	China
21.	Refrigerator	Denka	Japan
22.	Sensitive balance	Sartorius	Germany
23.	Slide & cover slip	Chemo lab	China
24.	Spectrophotometer	EMCLAB	Germany
25.	Surgical gloves	Chemo lab	China

26	Syringe (1 ml, 5 ml)	Chemo lab	China
27	Test tubes	Chemo lab	China
28	Vortex	Sturat	United
29	Water bath	Labtech	Korea

3. 1.2. Chemicals and Kits

All the chemicals and the standard kits used in this study are shown in **Table 3.2**.

Table 3.2: Chemicals and Kits with their suppliers.

No.	Chemicals & Kits	Company	Suppliers
1.	Absolute ethanol	Haymankimia	Uk
2.	Calcium kit	ELK bioticnology	Spain
3	Cathepsin K	ELK bioticnology	China
4.	Eosin Stain	Himedia Lab	India
5.	Estrogen kit	ELK bioticnology	Korea
6.	Formalin 37 %	Chemanol	SA
7.	FSH hormone kit	ELK bioticnology	Korea
8.	Hematoxylin Stain	Himedia Lab	India
9.	LH hormone kit	ELK bioticnology	Spain
10.	Osteoprotegerin	ELK bioticnology	China
11.	progesterone kit	ELK bioticnology	Korea
12.	RANk (Receptor Activator Of Nuclear Factor Kappa B)	ELK bioticnology	China
13.	RANKL(SolubleReceptor Activator of Nuclear factor-kB Ligand)	ELK bioticnology	China

Table 3-3 Materials used in molecular studies .

No.	Chemicals & Kits	Company	Suppliers
1.	BioneerKorea	Trizol Reagent 100 ml	AccuZolTM Total RNA extraction kit
2.	BiobasicCanada	DNase I enzyme	DNase I enzyme set kit
		10X buffer	
		Free nuclease water	
3.	BioneerKorea	-RocketScript Reverse Transcriptase (200µ)	AccuPower ® RocketScript TM RT PreMix
		-5x Reaction Buffer (1x)	
		-DTT (0.25 Mm)	
		-dNTP (250 µM each)	
		-RNase Inhibitor (1µ)	
4.	BioneerKore	2x Greenstar Master mix	AccuPower® GreenStarTM qPCR PreMix
		-8 Well strips x 12 each	
		-DEPC-D.W. 1.8 ml x 4 Tubes	

3.1.3 - Primers

Table 3-4. The primers that were used in this study are:

Primer	Sequence		Product size
Estrogen Receptor α rabbit (ESR1)	**F**	**5-CATCCTCCTCCTCCTTGTGG-3**	**88 bq**
	R	**5-CAAAGGGTTCCTCGGAGACTG-3**	
Estrogen Receptor Beta rabbit (ESR2)	**F**	**5-CTCGATGTTTCCTTGGATGGTCCT-3**	**95 bq**
	R	**5-ATTGCCTCCGGCTACCACTAC-3**	

F=forward \ R=reverse

3.2. Examination methods :

3.2.1. Experimental Protocol:

Twenty rabbits four-month-old female New Zealand White rabbits (weighing 3–3.25 kg) were randomly divided into two groups (10 each): a control group and an ovariectomized group. The rabbits were housed in clean, comfortable wooden cages and acclimatized to the environment for two weeks before the start of the experiment. They were provided with free food and water ad libitum. The light/dark cycle was 14 h, the relative humidity was 50 ± 5%, and the temperature was maintained at 25 ± 2 °C. With adequate ventilation, the rabbits were divided into two groups and received broiler feed in the second phase, containing 3200 kcal/kg of energy, 19% crude protein, 4% crude fat, and no more than 1.9% fiber.

1. The first group control group consists of Ten healthy female rabbits. 2 Ten healthy female rabbits in the second group bilateral ovariectomy (OVX undergo surgery to remove both of their ovaries, and they are treated with antibiotics for five days after the procedure. As seen in **Figure (3.1).**

3.2.2. Experimental Design

Twenty female local rabbits were randomly divided into two groups: a control group and bilateral ovariectomy (OVX)group.

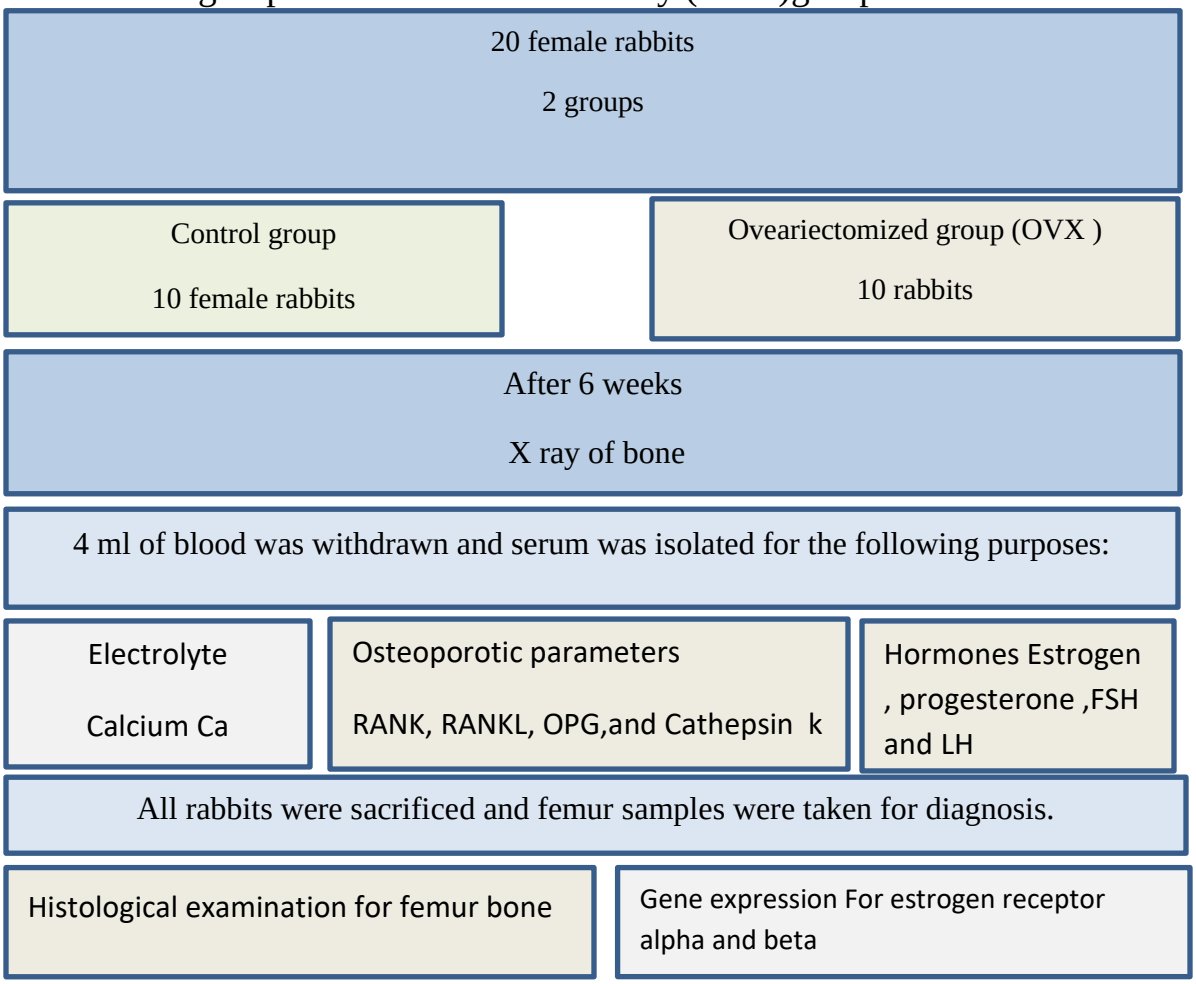


Figure (3.1) : Study design

3.3. Ethical approve

Under the reference number UOK.VET. PH.2024.095, the study was conducted at the Karbala University, College of Veterinary Medicines' anatomical facility in Iraq.

3.4. Surgical Ovariectomy

The operation was performed under sterile surgical conditions; the animals were anesthetized using a mixture of ketamine (40 mg/kg) and xylazine (10 mg/kg). For 30 minutes. The animal was placed in the dorsal recumbent position. The abdomen of the animal was prepared for surgery. After anesthesia, shaving and the skin is first cleaned with a special surgical antiseptic. A 4 cm surgical incision was made in the skin extending from the navel towards the back. The incision was then extended to include the tunica albuginea and peritoneum after pushing the cecum aside to reveal the uterus. After following the uterus upwards from the left side, the left ovary can be reached.

Two ligatures were placed around the ovarian arteries above the ovary in the ovarian mesentery and two other ligatures below the ovary in the true ligament using a 4/0 Catgut suture. Then it was cut between the two ligatures and then the ovarian suspensory ligament was cut to release the ovary.

The remaining stump of the blood vessels was then examined to ensure that there was no bleeding. Then the sutures were tied together and the operation was repeated on the right ovary. Then the abdominal wall was closed with continuous sutures using a 4/0 Catgut suture and the skin was closed with interrupted sutures using silk sutures.4/0. The suture was

removed after 10 days (**Kabakchiev *et al* .,2021**). **Figure (3-2)**

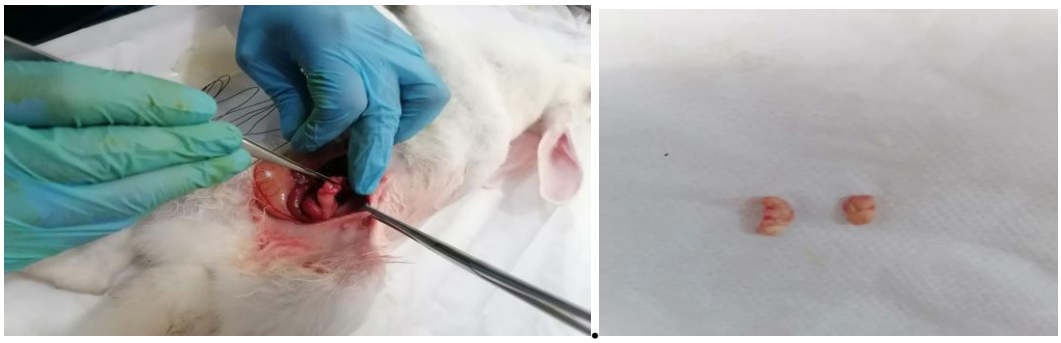


Figure (3.2): Ovariectomy procedure and excised bilateral ovaries during surgical intervention.

3.5. Examination of bone mineral density by X-ray:

Rabbits were anaesthetized using a mixture of I/M ketamine (40 mg/kg) xylazine (10 mg/kg) **(Saha *et al*.,2005)**. The anesthetized Rabbit was positioned in dorsally recumbence on the scan. All scans were performed using DXA(Hologic QDR-1000 System, Hologic Inc., Waltham, USA) . The high- resolution scan was performed to evaluate the bone mineral density (BMD) at the femur of rabbits.

3.6. Collection of Blood Samples.

Blood samples were drawn after starving the animals overnight, after six weeks of the experiment, the animals anesthetized by ketamine & xylazine in order to control and calm the animal before the blood draw. Four ml blood was drawn by heart puncture directly and the animal was lying on its back, and sterile medical syringes of 5 ml were used, then the blood was placed In special gel tube not containing an anticoagulant, the serum was separated by a centrifuge at a speed of 3000 r / min for 5 minutes, the separated serum put in a Eppendorf's tubes and kept in freeze at -20 ° C until the completion of the measurements.

3.7. Detection of serum Hormones and electrolytes that effect on bone:

3.7.1. Detection of serum estrogen:

Serum estrogen was detected by using a special ELISA kit according to the method of (**Faupel-Badger *et al* ., 2010)** as shown in Appendix I .

3.7.2. Detection of serum progesterone:

Serum progesterone was detected by using a special ELISA kit according to the method of (**Simersky *et al* ., 2007**) as shown in Appendix II .

3.7.3. Detection of serum luteinizing hormone(LH):

Serum luteinizing hormone(LH) was detected by using a special ELISA kit according to the method of **(Mäkelä *et al* ., 2020)** as shown in Appendix III.

3.7.4.Detection of serum Follicle-stimulating hormone (FSH):

Serum Follicle-stimulating hormone (FSH) was detected by using a special ELISA kit according to the method of (**Ongaro *et al* ., 2021)** as shown in Appendix IV .

3.7.5. Detection of serum Calcium Ca:

Serum Calcium Ca was detected by using a special ELISA kit according to the method of **(Li *et al* ., 2015)** as shown in Appendix V .

3.8. Detection of serum Biomarkers of Bone:

3.8.1. Detection of serum RANK:

Serum RANK was detected by using a special ELISA kit according to the method of **(Crisafulli *et al* .,2005)** as shown in Appendix VI.

3.8.2. Detection of serum s RANKL:

Serum RANKL was detected by using a special ELISA kit according to the method of **(Crisafulli *et al* ., 2005)** as shown in Appendix VII.

3.8.3 .Detection of serum osteoprotegerin OPG:

Serum osteoprotegerin was detected by using a special ELISA kit according to the method of **(Szalay *et al* ., 2003)** as shown in Appendix VIII.

3.8.4. Detection of serum Cathepsin K:

Serum Cathepsin K was detected by using a special ELISA kit according to the method of **(Sun *et al* ., 2013)** as shown in Appendix I**X**.

3.9 Molecular study

3.9.1. Quantitative Real-Tim PCR qRT-PCR

A quantitative real-time polymerase chain reaction (qRT-PCR) assay was performed to measure the quantitative levels of mRNA and RNA messenger RNA, which indicate the extent of gene expression.

Total RNA extraction Total RNA extraction was performed from bone tissue using the TRIzol® reagent kit. Before the Korean company, Ioneer, and the work was carried out according to the company's instructions as follows:

1. Grinded the rabbit's thigh bone and homogenize it with liquid nitrogen, then transfer the bone marrow to the tubes of Ibn Druf and add 1 ml of RTRIzol. kit reagent, then shake the tubes for 30 seconds.

2. Added 200 µl of chloroform to each tube and shake for 30 seconds as well.

3. Prepared the mixture on ice for 5 minutes. After that, Heat the centrifuge at 12000 rpm at 4°C for 15 minutes.

4. Transfered the upper transparent layer to a new test tube and add 500 µl of D9 and D2L to it 5 ml Mix the mixture by stirring in a tube 4

5. 1ml ethanol and prepare it at 4 °C for 10 minutes. Place the mixture in a centrifuge for 10 minutes to get rid of the supernatant and the remaining sediment in the tube.

6. mix it well by centrifuge. vortex

7. The tubes were placed in a centrifuge at a temperature of 4°C for 5 minutes at a speed of 12000 rpm

8. The supernatant were removed and take the RNA pellet and leave it to air dry.

9. Added 50 µl of DEPC (Dethyl Pyrocarbonate) to the RNA precipitate and mix by continuous shaking with a vortex device until it separates.

10. Stored the RNA extract at -20°C.

3.9.2. DNase I Treatment with Enzyme Treatment

Treatment of the RNA extract by using the DNase I enzyme to get rid of the remaining DNA in the extraction process, depending on the method of making the enzyme kit as in the following table:

Table 3-5 : The enzyme kit :

Mix	Volume
Total RNA	10 µl
DNase I enzyme	1 µl
10x buffer	2 µl
Free RNase water	7 µl
Total	20 µl

After that, the mixture was prepared in an incubator at a temperature of 37 °C for half an hour and then 1 microliter of 25 m M EDTA was added and incubated for 10 minutes at a temperature of 65 °C to inhibit the activity of the DNase enzyme.

3.9.3. Assessment of Total RNA concentration and purity

The total RNA concentration and purity were measured by a Nanodrop spectrophotometer (USA, Thermo) to determine the concentration of RNA (µL/ng). First, the purity of RNA and nucleic acid were measured by reading the absorbance at 1.8% at (260/280 nm). To confirm the presence of nucleic acid, using electrophoresis as follows: -

After running the Nanodrop software, select the RNA concentration and measure the RNA concentration using a special blotting paper. Place 1

microliter of N(O2ddH) on the surface of the substrate and perform zeroing. Then, clean the substrate to measure the samples. Press the OK button to start the RNA concentration measurement process using 1 Microlitres of RNA were extracted and then purified by cleaning the substrate of the measuring device again. Then, the sample of RNA was purified and the absorbance was read using a Nanodrop Spectrum at 260 nm (since the nucleic acid and the sample were purified from the blood). The absorbance ratio was (1.8).

Then, the sample was purified using the method Electrophoresis gel Agarose gel electrophoresis used for determining the integrity of RNA by analyzing the enzymes. This method is performed as follows: Prepare 1% agarose gel by dissolving it in TBE x1 buffer and boil it using a Magnetic Hot stirrer for 15 minutes until the crystals dissolve in the agarose solution. After that, the solution is cleaned in 3 m microliters of ethyl bromide diluted and placed on the surface of the glass.

After that, the solution is placed on a clean surface and the edges are covered with a thin layer of water. After that, the solution is placed on a clean surface and left to stand for 30 minutes. Then, it is transferred to the electrophoresis apparatus containing TBE buffer solution x1. RNA samples were prepared for the loading process by mixing 25 µl of nucleic acid RNA sample with 5 µl of loading buffer. The sample was transferred to a separate gelatinous gelatinization chamber. The sample was then incubated under a 100 V diode for 1 h. The nucleic acid RNA sample was separated by a wavelength of 260 nm.

3.9.4. cDNA Synthesis Method

The cDNA synthesis method was used to synthesize complementary DNA from RNA samples extracted using the Rockcript Accupower Kit Premix RT, which was prepared by the Korean company, Bionair. This process was carried out according to the method of operation of the kit, as shown in the following table.

Table (3.6): The cDNA synthesis :

cDNA master mix	Volume
Total RNA	10 Ml
Oligo(dT) 15 primer 10 pmole	2 Ml
DEPC water	8 Ml
Total	20 Ml

After that, the Mix Master RT components mentioned in the table were added to the tubes after making the cDNA containing the reverse transcription enzyme. Then, all the tubes were placed in a centrifuge such as the Exispin (Vortex Centerifuge) at a speed of 3000 rpm for three minutes. Then, the tubes were transferred to the Thermocycler (Korea.Mygene) and applied. Thermal imaging for the cDNA manufacturing process according to the method of the kit as in the following table:

Table 3.7 : the cDNA manufacturing process :

Step	Cycle	Temperature	Time
cDNA synthesis	1	50°C	1 hour
Heat Inactivation	1	95°C	5 minutes

After that, the sample was transferred to a storage unit of 20-degrees for use in Time-Real examination. (PCR)

3.9.5. Quantitative Real Time PCR (qPCR) Assay

A qPCR assay was performed on cDNA samples to determine the expression level of the gene (ESR1 , ESR2). The standard conservative gene (AccuPower® 2X GreenStarTM qPCR Master mix kit, Bioneer. Korea) was used to conduct this assay, which contained the green squirrel pigment and the interaction between The amplified genes were detected using Real-Time PCR (RT-PCR) and the examination was performed according to the method of (**Prasanthi *et al*, 2011**).

Table 3.8 : qPCR master mix :

qPCR master mix	Volume
2X Green star master mix	25µL
cDNA template (10ng/µL)	10µL
Forward primer (10pmol)	1µL
Reverse primer (10pmol)	1µL
DEPC water	13 µL
Total	50µL

After that, add these components mentioned in the table to the tubes of the Greenstar x2Accupower qPCR Mix Master containing the green sera reaction mixture for Time-Real Quantitative PCR testing. Then, place all the tubes in a centrifuge Vortex (Exispin) at a speed of 3000 rPM for three minutes. Then, transfer the plate to a Real (USA) PCR Time (-) device. The thermal conditions were applied to all genes according to the method of several operations, as in the following table :

Table 3.9 : The thermal conditions were applied to all genes :

Step	Condition	Cycle
Pre-Denaturation	95 °C 5 min	1
Denaturation	95 °C 20 sec	45
Annealing/Extension	60 °C 30 sec	
Detection (Scan)		
Melting curve	60-95°C	1

3.9.6. Real-Time PCR Data Analysis: Data Analysis Method.

The data resulting from the interaction of the polymerase chain reaction in real time is analyzed quantitatively by using the Livak Method, which was developed by **(Livak Schmittgen, 2001)**, which depends on extracting the relative quantity (Quantitative Relative) and the absolute quantity (Quantitive Absolute) through the process of correcting and equivalence of the target genes on the sample of the control until they are The results are meaningful to the biological sample, and the relative expression level is determined by the following equations:

Table 3.10 : expression level :

	Test	Calibrator (cal)
Target gene	CT(target, test)	CT(target, cal)
Reference gene	CT(ref, test)	CT(ref, cal)

First, normalize the CT of the reference (ref) gene to that of the target gene, for calibrator sample:

ΔCT (calibrator) = CT (ref, calibrator) – CT (target, calibrator)

Ratio (reference/target) = $2^{\text{CT (reference) – CT (target)}}$

Second, normalize the CT of the reference (ref) gene to that of the target gene, for the test sample:

ΔCT (Test) = CT (ref, test) – CT (target, test)

Ratio (reference/target) = $2^{\text{CT (reference) – CT (target)}}$

So, the relative expression was divided by the expression value of a chosen calibrator for each expression ratio of test sample.

3.10. Histopathological exam:

The bone was decalcified by use special acidic formalin then the tissue proceeded according to method mentioned in **(Suvarna ET AL .,2018).** Slides were then stained with hematoxylin and eosin (H&E) and examined by use light microscope .

The bone sections were initially preserved after removal from the animal in a 10% formalin solution. After 4-5 days, they were removed from the formalin

and washed several times with 70% ethyl alcohol.. The bone and uterus tissue sections were then photographed using a high-resolution digital camera.

1- Dehydration and leaching

Water was removed from the tissue by passing the samples through a series of ascending concentrations of ethyl alcohol (70, 80, 90%, 95%, 100% for two hours at each concentration. The samples were then leached by placing them in xylene for two hours.

2- Infiltration

After the leaching process was completed, the samples were transferred to bottles containing a mixture of paraffin wax with a melting point of (57-60°C) The melted, filtered and xylene in a ratio of 1:1 for half an hour inside an electric oven at a temperature of 60°C in order to keep the wax melted and to ensure the complete impregnation of the models with wax. They were transferred to other bottles containing paraffin wax inside the oven also for one hour, then transferred again to other bottles containing paraffin wax for one hour as well.

3- Embedding

Moulds of wax were made to contain the sample models by pouring wax into special plastic molds in which the models were embedded and left at laboratory temperature to solidify, then separated from the mold and stored until cutting.

4- Sectioning

A hand-held microtome was used to cut the models with a thickness ranging between 5-6 micrometers, then the sections were carried on clean glass slides wiped with a microscope after being placed in a water bath at a temperature of 45-50 m for one to two minutes to ensure the sections are brushed, then left on a hot plate to dry at a temperature of 37 m.

5. Staining:

The wax is removed and the slides are rehydrated.Stain with hematoxylin and eosin (H&E):Hematoxylin: stains the nuclei blue/violet.Eosin: stains the cytoplasm and tissue pink.

6. Final Mounting:

Cover the slide with a cover glass using a clear mounting medium to protect it.

3.11. Statistical analysis:

Statistical analyses were performed using GraphPad Prism (version 8.0). Graphs and charts were created using the ggplot2 R package and GraphPad Prism (version 8.0). Statistical analysis was then performed using the t-test.

To compare data, P values were calculated using a t-test with the median value used as the cut-off value. Data were presented as mean values ± standard error (SEM), and a statistically significant P value was set at <0.05. **(Ma *et al* .,2022)**

Chapter Four

Results and Discussion

4-1 : Effect of ovariectomy on femoral bone X-Ray:

In the current study the control group shows (0.69cm) a normal bone density at 6 weeks of the experiment while the ovariectomy group shows (0.42cm) a significant decrease in the bone density of osteoporosis femoral and pelvic bones especially at the middle and epiphysis of bones, as shown in the **figure (4-1)**

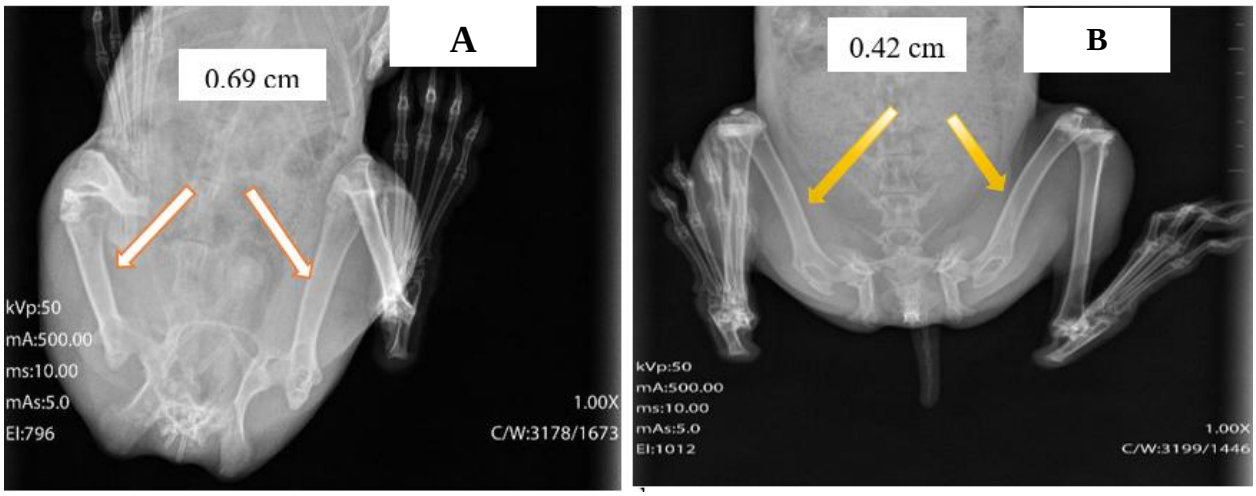


Figure (4-1): Radiographic image of the bones with white arrow of control group showing normal bone density (A). While Radiographic image of the bones with ovariectomy group (B) show a reduced bone density as compare with control group (after six weeks).

The images presented in **Figure (4-1)** X-rays provide important information about the effect of low estrogen levels on osteoporosis. Compared to the control group which had normal bones with adequate density, the bones in the OVX group were less dense. The bone appears more translucent or less white on images, and the cortex is thinned. The outer layer of the femur is very thin, and the bone marrow is dilated. The inner layer appears wider, and there is a loss of structural details in the trabecular bone.

X-rays are used to identify the possibility of osteoporosis. Osteoporosis results in a loss of bone mass and an increased risk of fractures and falls. Millions of people worldwide are affected by this serious health condition

(Balla *et al*., 2019). To maintain bone density, estrogen slows the rate of bone resorption, or breakdown. It also affects osteoclasts, the cells that break down bone (**Abd-Al-Ameer *et al*., 2024)**.

4.2: Hormonal and Biomarker effects by ovariectomized:

4.2.1. Estrogen level :

In the current study there was a significant ($p \leq 0.05$) decrease in the level of estrogen in the serum the OVX group compared to the control group. Shows the estrogen level in the control group 34.37±0.28 ng/mL , while the estrogen level in the OVX group 13.44±0. 49 ng/mL A bilateral ovariectomy is a factor affecting the estrogen level , as shown in **Figure (4.2)**.

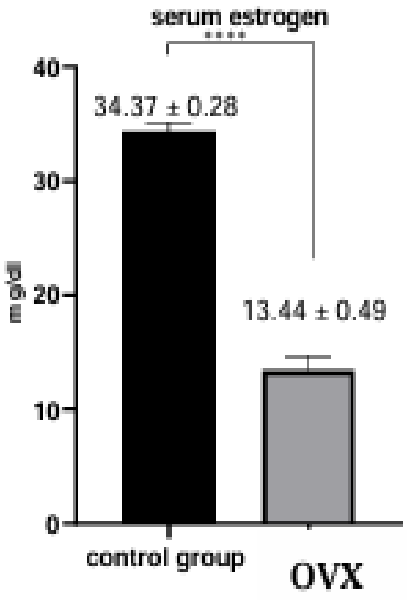


Figure (4.2): Estrogen serum levels in the control group and the OVX group. Values expressed as mean±, standard deviation, control group, and ovariectomized

The researchers reached a conclusion similar to what we have reached (**Feng *et al* .,2023; Abdi , 2024 ; Angulo Castro , 2024)**.The hypothesis that hypoestrogenic hormones act by stimulating the pituitary gland which is

considered a negative feedback mechanism. (**Yoon and Kim, 2024**). Estrogen deprivation by bilateral OVX alone has been previously described as a pure model of postmenopausal osteoporosis in rabbits (**Pennypacker *et al* ., 2011 ;Ye *et al* .,2014**).

Estrogen has a significant effect on calcium homeostasis in bones. Research has shown that estrogen deficiency in postmenopausal women is one of the causes of osteoporosis (**Cheng *et al*., 2022**). Estrogen is also an important regulator of bone metabolism in both females and males (**Noirrit-Esclassan *et al*., 2021**). Estrogen has an important role in the development and growth of bones and later in the maintenance of the bone mass. It is believed that the major action of estrogen on the skeleton in vivo is through the inhibition of bone resorption (**Hsu *et al* ., 2024**). Estrogen deficiency effects in bone remodeling, which is characterized by increased osteoclastic activity (bone resorption), This causes rapid loss of bone mass and micro-architecture, resulting in osteoporosis (**Ali *et al* ., 2022**). "Therefore, ovariectomy is similar to menopause in women, which is the period during which menstrual cycles and uterine changes stop, typically between the ages of 45 and 55. During this time, the brain does not respond effectively to the gonadotropin hormones secreted by the pituitary gland, and the secretion of estrogen and progesterone decreases significantly (**Bray *et al* ., 1999)**.

The level of estrogen in the blood serum of rabbits after removed ovaries has reached low levels and has not completely disappeared from the blood serum due to the presence of other sources that produce it in a limited manner, such as the adrenal cortex, as well as other main sites of estrogen production, such as fatty tissue, skin, muscles, and others. They have the ability to convert C19 steroid to C18 steroid, but they lose the ability to build and manufacture the

primary or primitive C19 steroid , but rather depend on circulating C19 steroid to manufacture estrogen (**Kratena , 2019)**.

4.2.2. Progesterone level:

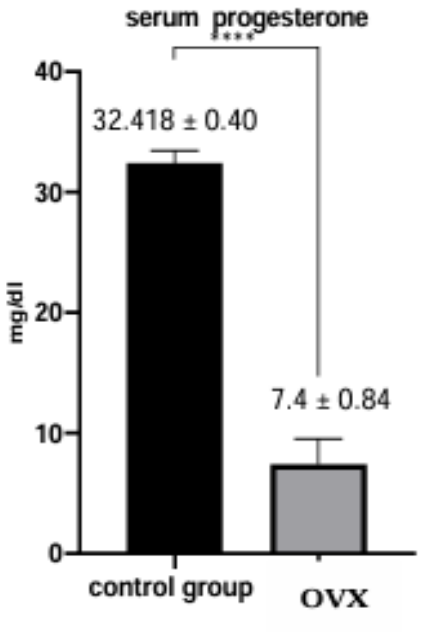


Figure (4.3) : progesterone serum levels in the control group and the OVX group. Values expressed as mean±, standard deviation, control group, and ovariectomized

In the current study, there was a significant decrease ($p \leq 0.05$) in serum progesterone levels in the OVX group compared to the control group. The progesterone level in the control group was 32.418±0.40 ng/mL, while the progesterone level in the OVX group was 7.4±0.84 ng/mL. A bilateral ovariectomy is a factor affecting the progesterone level , as shown in is a factor affecting progesterone levels, as shown in **Figure (4.3)**.

This is consistent with our results (**Tunheim, 2022 ; Hart ,2023)** Since the ovaries are the primary source of progesterone in premenopausal women, A bilateral ovariectomy, results in a marked drop in progesterone levels (**Sochocka *et al* ., 2023)** . The ovaries produce progesterone and estrogen, which are

essential for controlling the menstrual cycle, sustaining pregnancy, and sustaining a number of physiological processes outside of reproduction. Following the surgical excision of both ovaries, the body's levels of progesterone (and estrogen) suddenly decline (**Cable & Grider ,2020)**. Low progesterone levels, such as those caused by menopause or ovulatory disorders, reduce this bone-forming activity and raise the risk of osteoporosis and fragility fractures (**Palacios *et al* .,2024**) . Thus, progestins have complex effects on bone: they can promote bone formation in experimental settings but may not alone prevent osteoporosis in humans, particularly postmenopausal women. Combining progestins with estrogens or other therapies often yields better bone health outcomes (**Caplan, 2024)** .

Since Fuller Albright, estrogen insufficiency at menopause has been thought to be the main cause of osteoporosis (**Forbes, 1991)**. Most scientists view estradiol as women's sole bone-active gonadal steroid. In reality, estradiol and progesterone work together in every tissue in women's normal physiology (**Geller *et al* ., 2025**).

Estradiol's role in human bone health is unmistakable. However, progesterone is usually a present, but an unrecognized partner in bone. With amenorrhea and surgical or natural menopause, not only are estradiol levels low or dropping, progesterone levels are also low. While, in these conditions, estrogen and progesterone deficiency are nearly indistinguishable, progesterone deficiency precedes low estradiol levels in perimenopause (**Levin & Hammes , 2011)**. while estrogen and progestin regulate bone turnover through distinct mechanisms. Estrogen primarily inhibits osteoclast-mediated resorption via the RANKL/RANK (receptor activator of nuclear factor-kB) and OPG (osteoprotegerin) axis (**Chew *et al*., 2018)**, and is directly controlled by

progesterone receptors in osteoclasts (**Sandor *et al*., 2023)**. Both osteoblast and osteoclasts contain these receptors (**Mills *et al*., 2021**). Progesterone has also been reported to directly inhibit bone resorption through matrix metalloproteinases and glucocorticoid receptors, in addition to directly affecting osteoclasts (**Wang *et al* ., 2023** ; **Sharifi, 2023** ; **Banoriya *et al.*, 2025**).

4.2.3. LH level:

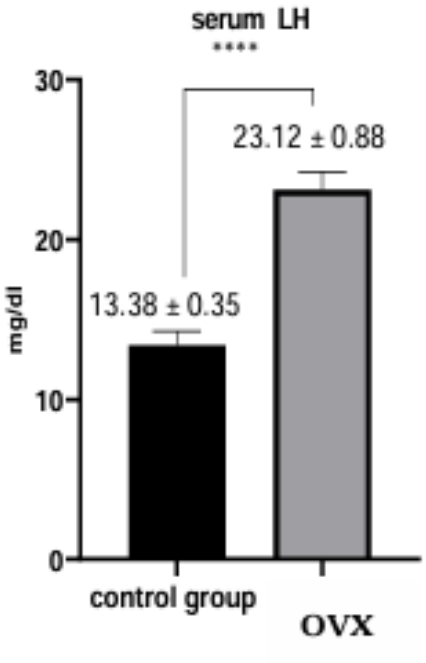


Figure (4.4): LH serum levels in the control group and the OVX group. Values expressed as mean±, standard deviation, control group , and ovariectomized .

In the current study, there was a significant increase ($p \leq 0.05$) in serum LH levels in the OVX group compared to the control group. The LH level in the control group was 13.38±0.35 ng/mL , while the LH level in the OVX group was 23.12 ±0.88 ng/mL. A bilateral ovariectomy is a factor affecting LH levels, as shown in **Figure (4.4)**.

This is consistent with our results (**Calik-Ksepka *et al* ., 2022**; **Niwczyk *et al* ., 2023 ; Wang *et al* ., 2023)**. After bilateral ovariectomy the levels of luteinizing hormone (LH) in the blood typically rise significantly. This is because the ovaries normally produce estrogen and other hormones that exert negative feedback on the hypothalamic - pituitary- gonadal axis regulating LH secretion **(Jayasena *et al* ., 2024)** . Menopause-related bone loss results primarily from estrogen deficiency, which causes increased bone resorption. Elevated LH and FSH reflect this hormonal state but the direct mechanistic role of LH on bone cells is less established compared to FSH (**Yang *et al* ., 2024**).

When ovaries are removed and there is a significant lack of estrogen and progesterone, the hypothalamus senses this hormone shortfall, the negative feedback is eliminated, and the hypothalamus secretes more gonadotropin-releasing hormone, The pituitary gland secretes more of the hormone LH and FSH as a result of this stimulation **(Suhaimi *et al* ., 2022; Jayasena *et al* ., 2024**). According to certain research, both bone-forming (osteoblasts) and bone-destroying (osteoclasts) cells carry LH receptors, Increased bone resorption can result from LH's direct stimulation of osteoclast activity or its stimulation of cytokines like RANKL (**Yuan *et al* ., 2015)** . The increase in the level of LH, FSH hormones in the group whose ovaries were removed LH may have a supporting role in osteoporosis by promoting bone resorption, although this role is less clear than FSH (**Behary& Comninos,2022**).

4.2.4. FSH level:

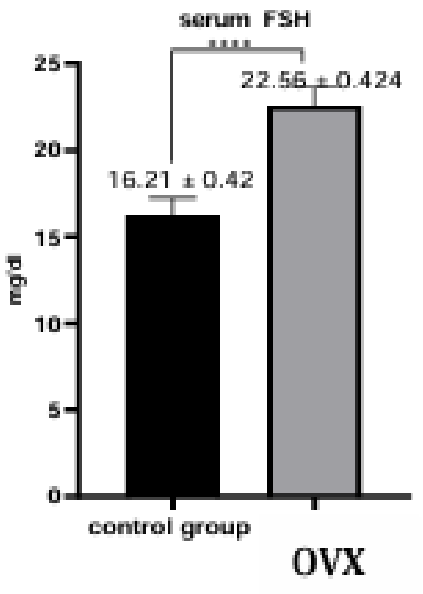


Figure (4.5): FSH serum levels in the control group and the OVX group. Values expressed as mean±, standard deviation, control group, and ovariectomized

In the current study, there was a significant increase ($p \leq 0.05$) in serum FSH levels in the OVX group compared to the control group. The FSH level in the control group was 16.21±0.42 ng/mL while the FSH level in the OVX group was 22.56 ±0.424 ng/mL . A bilateral ovariectomy is a factor affecting FSH levels, as shown in **Figure (4.5)**.

The researchers reached a conclusion similar to what we have reached **(Liu *et al* ., 2017 ; Juel Mortensen *et al*., 2019)**. After bilateral ovariectomy, follicle-stimulating hormone (FSH) levels rise significantly due to decreased ovarian function and low estrogen production **(Li *et al* ., 2021)** . To regulate the release of LH and follicle-stimulating hormone (FSH), the ovaries generate progesterone and estrogen. The hypothalamic-pituitary-gonadal axis controls the secretion of FSH

(Babaniyi *et al* ., 2024). The hypothalamus detects a considerable drop in estrogen and progesterone after ovaries are removed, removes the negative feedback, and increases the amount of gonadotropin-releasing hormone secreted. Because of this stimulation, the pituitary gland releases more LH and FSH **(Zaidi *et al* ., 2018 ; Alasmi, 2022 ; Huang *et al* ., 2024)**. FSH causes osteoclast precursors to produce more TNFα and express more RANK. Additionally, it improved the processes that lead to the differentiation of osteoclasts **(Umur *et al* ., 2024)**. One of the most provocative hypotheses in recent years is that follicle-stimulating hormone (FSH) has important extragonadal actions, particularly on bone **(Bartkowiak-Wieczorek *et al* ., 2024)**.

This is supported by substantial evidence in rodents, although some findings remain conflicting. In addition, observational human data are consistent with an important role for rising FSH levels in the peri- and postmenopausal period potentially driving the bone loss previously attributed to estrogen deficiency **(Zaidi *et al* ., 2018)**. Studies indicate that FSH has a direct role in osteoporosis through its receptors found in osteoclasts, where it enhances their activity and increases bone resorption (**Zhou, 2021)**.

This has led to enthusiasm for using FSH neutralization (e.g., FSH antibodies) to simultaneously treat the age-related comorbidities of osteoporosis and obesity **(Zaidi *et al* ., 2018)**.

4.2.5. Calcium level:

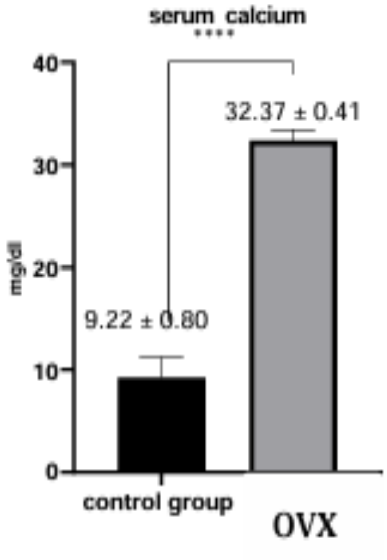


Figure (4.6): Calcium serum levels in the control group and the OVX group. Values expressed as mean±, standard deviation, control group, and ovariectomized .

In the current study, there was a significant increase ($p \leq 0.05$) in serum calcium levels in the OVX group compared to the control group.The calcium level in the control group was 9.22 ±0.80 ng/mL , while the calcium level in the OVX group was 32.37 ±0.41 ng/mL . A bilateral ovariectomy is a factor affecting calcium levels, as shown in **Figure (4.6)**.

This is consistent with our results (**Yang *et al* ., 2025; Suliburska *et al* ., 2023 ; Zhang *et al* ., 2022)** . High calcium levels after a bilateral ovariectomy mainly result from hormonal changes, particularly the reduction of estrogen, which plays a key role in calcium metabolism and bone health **(Elghareeb *et al* ., 2022)** . Removal of ovaries causes a sudden drop in estrogen levels, leading to decreased calcium absorption into bones and increased calcium loss. Estrogen normally promotes calcium retention and entry into the bone matrix. Its loss results in excessive bone calcium loss, raising the risk of osteoporosis and

hypocalcemia **(Bhattarai *et al* .,2020)**. The results are attributed the increase in calcium to the effect of ovariectomy on the intestine, which causes a decrease in its sensitivity to vitamin D, as well as a decrease in calcium reabsorption in the renal tubules, which leads to an increase in calcium secretion in urine, thus decreasing its level **(Seijo *et al* ., 2022)** .

All of these factors lead to the activation of bone loss and thus the incidence of osteoporosis. The results are not consistent with all of them **(Xie *et al* ., 2005 ; Aspray *et al* ., 2005)**. The deficiency of Ca is the main risk factor for osteoporosis. Changes in serum Ca levels involve adaptations in bone remodeling, as in the case of enhanced bone resorption caused by low serum Ca levels. Based on research on the effect of increased Ca intake on bone health in peri- and postmenopausal as well as elderly women, it was found that supplementation with Ca for more than five years reduces bone loss in postmenopausal women, especially those whose usual Ca intake is low (< 400 mg / day) (**Cannata-Andía *et al* ., 2021)**.

Calcium is an essential component for bone formation, with teeth and bone containing more than 99 percent of it (**Mao *et al*., 2021)**. When estrogen levels decline after menopause, the cells that make bone (osteoblasts) are unable to efficiently produce bone mass (**Lu and Tian, 2023**). Therefore, it is not surprising that the loss of ovarian estrogen after menopause is associated with decreased bone mineral density (BMD) and an increased risk of osteoporosis (**Camon *et al*., 2024)** .

The findings of this investigation are aligned with those of (**Mustafa *et al*., 2018**). who observed a notable reduction in the weight of femur ash and a drop in calcium and phosphorus in the bone ash of ovx rabbits. Furthermore, the results of this study are consistent with those of (**Puzio *et al*., 2021)**. who showed

a decrease in bone ash weight, ash percentage, calcium, phosphorus, and magnesium in the ovx rabbits.

After removing the ovaries, estrogen levels decrease, leading to an increase in osteoclasts, a decrease in bone density (osteoporosis), and an imbalance of calcium, as calcium is drawn from the bones into the blood to compensate for the deficiency (**Abdi, 2024)**.

4.2.6. RANK level:

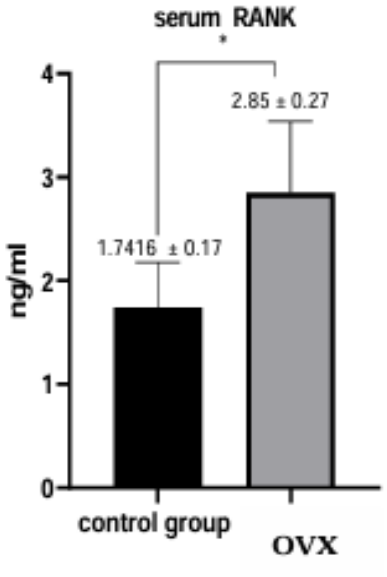


Figure (4.7): RANK serum levels in the control group and the OVX group. Values expressed as mean±, standard deviation, control group, and ovariectomized

In the current study, there was a significant increase ($p \leq 0.05$) in serum RANK levels in the OVX group compared to the control group.The RANK level in the control group was 1.7416± 0.17 ng/mL , while the RANK level in the OVX group was 2.85 ± 0.27 ng/mL A bilateral ovariectomy is a factor affecting RANK levels, as shown in **Figure (4.7)**.

The researchers reached a conclusion similar to what we have reached (**Wang *et al* ., 2022 ; Jin *et al* .,2023 ; Ribeiro *et al* ., 2025**) . RANK is expressed as a tumor necrosis factor, and we observed an increase in the expression of RANK and RANKL, the association between which leads to the activation of osteoclasts. These cells break down bone, causing bone loss. RANKL is mostly expressed on the surface of osteoclasts, which are involved in bone resorption. Ovariectomy leads to a decrease in estrogen, a major factor in osteoporosis and osteoclast activation (**Jin *et al*., 2023**). Osteoclasts, giant multinucleated cells, are differentiated from hematopoietic stem cells by the cooperative action of receptor activator of nuclear factor (NF)-κB ligand (RANKL) and macrophage-colony stimulating factor (M-CSF) (**Sabe *et al* ., 2024)**. RANKL, a critical cytokine of the tumor necrosis factor (TNF) family, can directly bind to its receptor RANK and subsequently activate downstream signaling pathways, including NF-κB and mitogen-activated protein kinase (MAPK), which induce osteoclast differentiation via activation of nuclear factor of activated T cell c1 (NFATc1) and c-Fos (**AlQranei *et al* ., 2021**).

During osteoclastogenesis, these transcription factors regulate the expression of osteoclast-related marker genes and proteins, including tartrate-resistant acid phosphatase (TRAP), c-Src, matrix metallopeptidase-9 (MMP-9), and cathepsin K (**Ren *et al* ., 2024**). Estrogen deficiency leads to a high bone turnover, due to the low production of estrogen by ovaries. Multiple lines of research have also revealed that estrogen deficiency activates bone resorption by triggering various factors, such as proinflammatory cytokines (e.g., interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α)) and the RANKL/RANK/OPG system (**Hsu *et al* ., 2024)**.The RANKL/RANK/OPG system, regarded for its important role in osteoclastogenesis and osteolysis, controls osteoclast differentiation and survival, thereby affecting bone metabolism resorption (**Boyce and Xing, 2008;**

Zhang *et al*., 2022). Within the microenvironment niche, the cytokine receptor activator of nuclear factor (NF)-κB-ligand (RANKL) binds to the receptor activator of NF-kB (RANK) on the surface of osteoclasts and recruits the TNF receptor-associated factor-6 (TRAF6), which triggers an intracellular signaling cascade to induce differentiation and activation of osteoclasts (**Engelmann *et al* ., 2023)**.

4.2.7.s RANKL level:

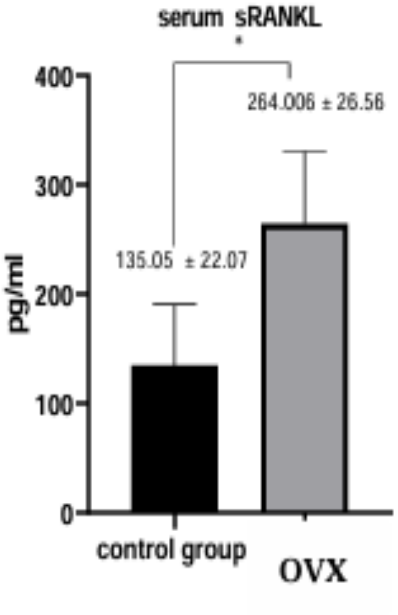


Figure (4.8): s RANKL serum levels in the control group and the OVX group. Values expressed as mean±, standard deviation, control group, and ovariectomized

In the current study, there was a significant increase ($p \leq 0.05$) in serum s RANKL levels in the OVX group compared to the control group. The s RANKL level in the control group was 135.05± 22.07 ng/mL , while the s RANKL level in the OVX group was 264.006±26.56 ng/mL . A bilateral ovariectomy is a factor affecting s RANKL levels, as shown in **Figure (4.8)**.

The researchers reached a conclusion similar to what we have reached (**Ma *et al* ., 2018 ; He *et al* ., 2022 ; Dong *et al* ., 2025**).

Estrogen normally suppresses RANKL expression in bone-forming cells. After ovariectomy, the resulting estrogen deficiency leads to a pronounced increase in RANKL production, particularly by bone lining cells and osteoblasts **(Mohanty *et al* ., 2025)**. This upregulation of RANKL enhances the formation and activity of osteoclasts, cells responsible for bone resorption, thereby increasing bone turnover and leading to bone loss **(Kitaura *et al* ., 2020)** .

It is commonly acknowledged that the RANK/RANKL/OPG system is a crucial signal transduction route for preserving the balance of bone metabolism and OC differentiation **(Sobacchi *et al* .,2025)**.

Estrogen inhibits osteoclastogenesis, which involves numerous signaling pathways, including RANK ligand (RANKL), receptor activator of nuclear factor-KB (RANK), and osteoprotegerin (OPG). RANKL promotes the activation and differentiation of osteoclasts. And act as substrates for the ECM degrading protein TRAP (**Chawla, 2024**). A late upregulation of the gene RANKL, which encodes the protein RANKL important for bone resorption by osteoclasts (**El-Masri *et al* ., 2024**).

During estrogen deficiency, osteoblasts alter paracrine regulation of osteoclast differentiation (TRAP, CTSK, NFATc1) and resorption **(Jiang, *et al* ., 2023)**. estrogen deficiency results in an increase in PGE2 production in bone marrow stromal cells, which then induces the expression of RANKL leading to accelerated osteoclastogenesis through interaction with RANK **(Freeman *et al* ., 2024)**. Meanwhile, osteoprotegerin (OPG) neutralizes RANKL, and correspondingly the ratio of RANKL to OPG determines the direction of bone formation or resorption **(Li *et al*., 2022**).

4.2.8. OPG level:

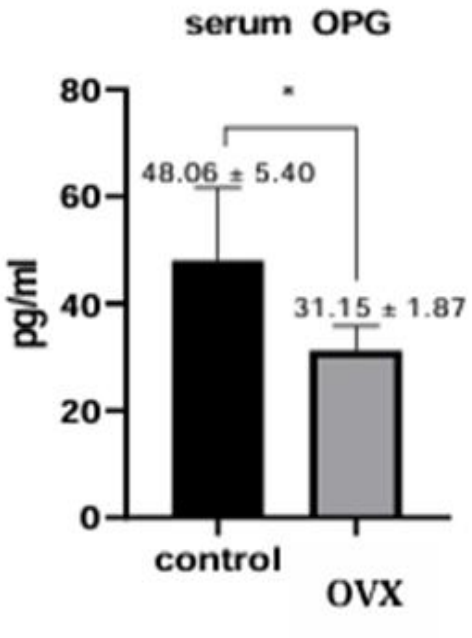


Figure (4.9): OPG serum levels in the control group and the OVX group. Values expressed as mean±, standard deviation, control group , and ovariectomized

In the current study, there was a significant decrease ($p \leq 0.05$) in serum OPG levels in the OVX group compared to the control group. The OPG level in the control group was 48.06±5.40 ng/mL while the OPG level in the OVX group was 31.15±1.87 ng/mL A bilateral ovariectomy is a factor affecting OPG levels, as shown in **Figure (4.9).** This is consistent with our results (**Xu *et al* ., 2018 ; Ozturk *et al* ., 2023 ; Liao *et al* ., 2025)**

Estrogen loss after ovarian removal downregulates OPG expression in osteoblasts, which disrupts the balance of bone remodeling. This results in increased RANKL activity, enhanced osteoclast formation, and accelerated bone loss **(Mohanty *et al* ., 2025)** . Estrogen typically binds to its receptors (ERα and ERβ) in osteoblasts, which directly stimulate OPG gene transcription. The OPG gene contains estrogen response elements (EREs) in its promoter, making

OPG expression highly sensitive to estrogen levels. Removal of ovaries eliminates the primary source of estrogen, leading to reduced stimulation of these receptors and lower transcription of the OPG gene **(Hsu *et al* ., 2024)** .

Estrogen also exerts a post-transcriptional effect on OPG by modulating microRNAs, specifically miR-145. In the absence of estrogen, miR-145 expression increases, which then binds to the 3′-UTR region of OPG mRNA and suppresses its translation **(Puppo *et al* ., 2021)** . Estrogen downregulates miR-145, thereby allowing more OPG protein to be produced; this protective effect is lost after ovariectomy (**Jiménez-Ortega *et al* ., 2024)** .

Osteoblasts and bone marrow stromal cells are the primary sites for OPG and RANKL production. Estrogen withdrawal not only suppresses OPG gene expression but shifts the balance toward higher RANKL levels, aggravating osteoclastogenesis and bone resorption (**Yu & Wang , 2022)**. This change is sustained by a modified cellular signaling environment, which also affects OPG expression through interactions with immune cells and the release of pro-inflammatory cytokines including TNF and IL-6 **(Sadek *et al* .,2023)** .

On the other hand, IL-6 prompts the development of osteoclasts from hemopoietic precursors **(Nava-Valdivia *et al*., 2021)**. Additionally, bone resorption could be determined by the balance between these cytokines, RANKL, OPG, and bone mineral density **(Umur *et al* ., 2024**) .

Enabling bone formation. Osteocytes also regulate osteoclastogenesis and bone resorption through the upregulation or downregulation of receptor activator of NFκB ligand (RANKL) and osteoprotegerin (OPG-RANKL is a ligand for receptor activator of NFκB (RANK) present in the cell membrane of osteoclast precursors. The binding between RANKL and RANK triggers signaling

pathways, which promote proliferation and differentiation of mature osteoclasts (**Tanaka, 2019 ; Al-Bari & Al Mamun ,2020 ; Cheng *et al*., 2022)**.

Which are responsible for bone resorption. OPG is a soluble glycoprotein that acts as a decoy receptor for RANKL, by binding to RANKL and prevents RANK-RANKL association, thus antagonizing the signaling that promotes osteoclastogenesis **(Radhi *et al* ., 2025)**.

4.2.9. Cathepsin K level:

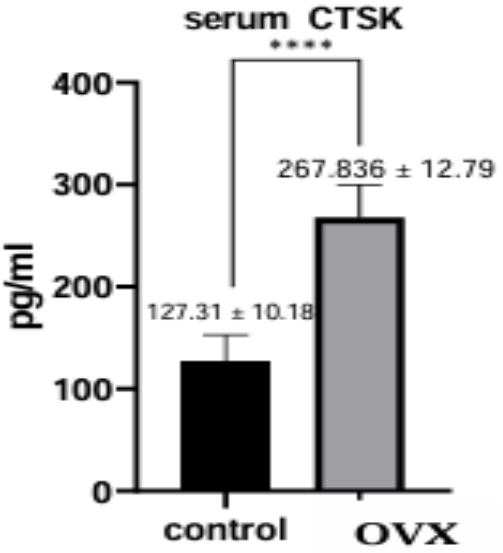


Figure (4.10): cathepsin K serum levels in the control group and the OVX group. Values expressed as mean±, standard deviation, control group , and ovariectomized

In the current study, there was a significant increase ($p \leq 0.05$) in serum cathepsin K levels in the OVX group compared to the control group. The cathepsin K level in the control group was 127.31±10. 18,ng/mL while the cathepsin K level in the OVX group was 267.836±12.79 ng/mL. A bilateral ovariectomy is a factor affecting cathepsin K levels, as shown in **Figure (4.10)**.

The researchers reached a conclusion similar to what we have reached (**Costa *et al* ., 2011; Mijanović *et al* ., 2022; Demeuse *et al* ., 2023)**. Following ovarian excision, elevated cathepsin K is mostly caused by estrogen deprivation **(Pennypacker *et al* ., 2011)** .Osteoclasts are the cells that break down bone, and estrogen is crucial in preventing their activit (**Langton & Njeh, 2016)**. Estrogen levels sharply decline following ovarian excision, which causes an increase in osteoclast activity and creation, elevated release of osteolytic enzymes, including cathepsin K, a protease enzyme required for the degradation of bone's type I collagen **(Canuas Landero, 2023)**.

Bone resorption is osteoclast-driven but osteoblasts control their formation and activity via the receptor activator of NF-κB ligand (RANKL) system (**Győri & Mócsai , 2020)** .At resorbing state, osteoclasts attach to bone surfaces and form different plasma membrane domains such as ruffled border containing vacuolar H+-ATPase that enables production of an acidic subcellular space **Knight, 2025)** . After demineralization, proteinases, in particular cathepsin K (**Lin *et al* ., 2023**), initiate the degradation of the organic collagen type I-rich osteoid matrix in subosteoclastic spaces **(Mandelin *et al* ., 2006)** .

Type I collagen is broken down via osteoclast-driven bone resorption, which is mediated by cathepsin K (**Shariati *et al* ., 2025)**. It is now believed that the primary enzyme responsible for degrading the organic bone matrix is cathepsin K, a cysteine protease belonging to the papain family. It is highly and specifically expressed in osteoclasts and possesses the unique ability to degrade type I collagen helical sections in acidic conditions. In contrast to the other cathepsins, osteoclasts were shown to express Cat K in high amounts **(Bautista-Carbajal *et al* ., 2023)**.

4.3 . Rustls of genes expression

Table (4.1): Downregulation of ERα in Ovariectomy Group Ct Values for ERα

Rabbit ID	Ct (ERα)	Ct (Housekeeping)	ΔCt (ERα)
Ovariectomy 1	28.5	22.0	28.5−22.0=6.5
Ovariectomy 2	29.0	21.8	29.0−21.8=7.2
Ovariectomy 3	27.8	22.3	27.8−22.3=5.5
Ovariectomy 4	28.2	22.1	28.2−22.1=6.1
Average ΔCt			(6.5+7.2+5.5+6.1)/4 =6.3

Rabbit ID	Ct (ERα)	Ct (Housekeeping)	ΔCt (ERα)
Control 1	25.0	22.0	25.0−22.0=3.0
Control 2	25.5	22.2	25.5−22.2=3.3
Control 3	25.3	22.1	25.3−22.1=3.2
Control 4	24.8	22.0	24.8−22.0=2.8
Average ΔCt			(3.0+3.3+3.2+2.8)/4 =3.1

ΔΔCt and Relative Expression (ERα)

- **ΔΔCt: ΔCtOvariectomy−ΔCtControl=6.3−3.1=3.2**
- **Relative Expression: 2−3.2=0.11 (Downregulated in ovariectomy group)**
- **Livak formula 2- ΔΔCt**

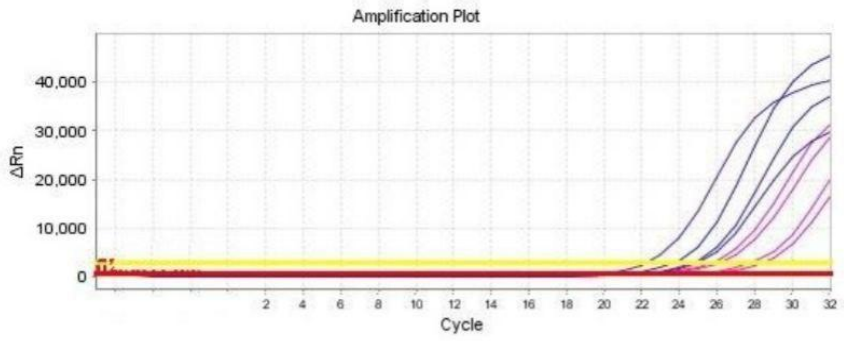


Figure (4.11): amplification curve of the tested samples represented the ERα gene. This indicate a successful RNA extraction and cDNA synthesis .

Table (4.2) .Upregulation of ERβ in Ovariectomy Group Ct Values for ERβ

Rabbit ID	Ct (ERβ)	Ct (Housekeeping)	ΔCt (ERβ)
Ovariectomy 1	23.5	22	23.5−22.0=1.5
Ovariectomy 2	24	22.2	24.0−22.2=1.8
Ovariectomy 3	23.8	22.3	23.8−22.3=1.5
Ovariectomy 4	23.7	22.1	23.7−22.1=1.6
Average ΔCt			(1.5+1.8+1.5+1.6)/4 =1.6

Rabbit ID	Ct (ERβ)	Ct (Housekeeping)	ΔCt (ERβ)
Control 1	25.5	22	25.5−22.0=3.5
Control 2	25.8	22.1	25.8−22.1=3.7
Control 3	26	22.2	26.0−22.2=3.8
Control 4	25.9	22	25.9−22.0=3.9
Average ΔCt			(3.5+3.7+3.8+3.9)/4 =3.725

ΔΔCt and Relative Expression (ERβ)

- **ΔΔCt: ΔCtOvariectomy−ΔCtControl=1.6−3.725=-2.125**
- **Relative Expression: 2−(-2.125)=4.36**
- **(Upregulated in ovariectomy group)**
- **Livak formula 2- ΔΔCt**

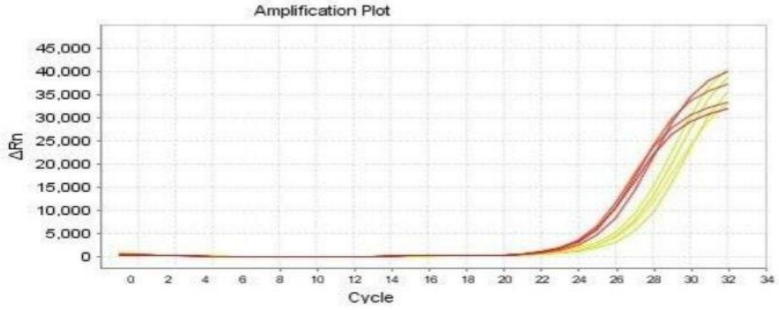


Figure (4.12): amplification curve of the tested samples represented the ERβ gene. This indicate a successful RNA extraction and cDNA synthesis

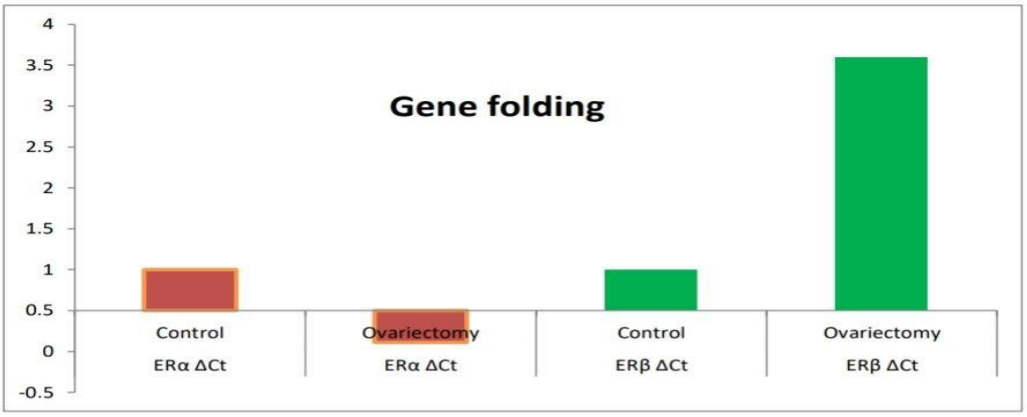


Figure (4.13): fold change comparison between the group expressing ERα and ERβ gene.

The current study demonstrated a significant alteration in estrogen receptor alpha gene expression after 6 weeks in the control group (mean ΔCt 3.1) compared to the ovariectomized group (mean ΔCt 6.3). Downregulation: In ovariectomized animal models, estrogen receptor alpha gene expression in bone cells may be decreased when estrogen is deficient This is consistent with our results (**Meng *et al* ., 2022; Elghareeb *et al* ., 2022 ; Qi *et al* ., 2023)** . A bilateral ovariectomy lowers the amount of estrogen in the blood, which in turn lowers the expression of ERα in bone cells. Increased osteoclast activity and

bone microstructure degradation are linked to this drop in ERα, which results in a loss of bone mass (**Manolagas *et al* ., 2013 ; Cheng *et al* ., 2022**) . ER-α is more important than ER-β for the regulation of bone metabolism (**Streicher *et al*., 2017)**. Osteoblasts and osteocytes express estrogen receptor α and β (ERα and ERβ), which play a role in regulating both cell survival and mechanosensation (**Shi & Morgan , 2024**; **Geoghegan *et al* ., 2019)**. After menopause, estrogen levels decline, and the promotion of cellular antioxidant function by estrogen is diminished. Excessive ROS produced by OS activates the NF-κB signaling pathway, up-regulates RANKL, and inhibits OPG, resulting in an elevated RANKL/OPG ratio (**Boyce *et al* ., 2005)**. This imbalance allows for increased RANKL-RANK binding, accelerated OC differentiation and activation, and enhanced bone resorption (**Almeida *et al* ., 2010)**.

In osteoblasts, estrogen has been shown to play a protective role by inhibiting osteoblast apoptosis and increasing its lifespan. Estrogen exerts these effects due to activation of the Src/Shc/ERK signaling pathway and downregulating JNK, which alter the activity of transcription factors such as CREB and c-Jun/cFos (**Naqvi *et al* ., 2020**). Supplementation of estrogen in osteoblasts has also been shown to increase OPG expression (**Wang *et al* ., 2023)**. and augment Cox-2 (via β1 integrins and ERs) response to fluid shear stress (**Shi & Morgan ,2024**). and decrease RANKL and SOST expression (**Jiao *et al* ., 2023**). By contributing to an imbalance in osteoclast and osteoblast activity, this decline makes bone loss worse **(Forte *et al*., 2023; Cheng *et al*., 2022).** Nonetheless, these molecular and cellular expressions have different patterns. For example, the distribution of ERα in osteocytes is more prevalent in cortical bone than that in trabecular bone **(Davis *et al* ., 2001; Lara-Castillo, 2021)**.

According to the current study, the average ΔCt for beta estrogen receptor gene expression after six weeks was 3.725 in the control groups and 1.6 in the animals who had their ovaries removed (upregulated in the ovariectomy group). Following osteoporosis, changes in hormonal signals led to an increase in beta estrogen receptors in bone cells.

The researchers reached a conclusion similar to what we have reached (**Farman, 2018 ; Balla *et al*. 2019; Shi & Morgan, 2024)**. Following ovarian excision, bone exhibits increased expression of the estrogen receptor beta (ERβ) in response to estrogen deprivation. However, since estrogen receptor alpha (ERα) plays a key role in bone protection, this increase is insufficient to stop bone loss (**Khalid & Krum, 2016 ;Mohanty *et al* ., 2025)** . while ERβ in osteocytes shows higher expression in trabecular bone (**Hsu *et al* ., 2024**). Both osteoclasts and cells of the osteoblast lineage express ER-β. The latter seem to be rather prevalent in this receptor, which may allow estrogen to act directly on osteoclasts (**Braidman *et al* ., 2001; Wang *et al* ., 2024)**.

Different ERα and ERβ polymorphisms have been proposed in a number of studies as being linked to osteoporotic fractures and BMD, although these have not yet been mechanistically verified **(Kian *et al* ., 2004 ; Dong *et al* ., 2025)** .The sensitivity of bone cells to mechanical loading and the ensuing bone cell mechanotransduction—that is, the activation of basic mechanosensitive bone remodeling pathways—have also been demonstrated to be influenced by estrogen and ERs **(Steppe *et al* ., 2021)**. While earlier reviews have examined the impact of estrogen and ERs on bone biology and health more generally, a prior analysis focused on the consequences of estrogen shortage in osteocyte mechanobiology **(Price *et al* ., 2011; Naqvi *et al* ., 2020)**.

4.4: Histological results:

The control group of the present study showed a normal bone structure with a compact osteophyte and prominent osteolytic canals surrounding normal marrow cavities as shown in **Figure (4.14, 4.15)**. Animals treated with a bilateral ovariectomy in **Figure (4.16)** also revealed significant histological changes manifested by marked thinning of the osteophytes, characterized by a thin wall with longitudinal views with irregular eroded borders, and severe resorption and perforation of the compact bone with irregular atrophic osteocytes and marked cavity formations with numerous typical multinucleated osteocytes emanating from the surface of the osteophytes and typical large multinucleated osteocytes in a osteophyte with irregular lamellae margins .

Also in **Figure (4.17)**, the histological section of the longitudinal femur of rabbits with bilateral ovaries removed shows lacunae with osteocytes and without osteocytes osteophyte plate. Irregular borders with osteoclast infiltration and dilated Haversian canal The researchers reached a conclusion similar to what we have reached (**Chen *et al* ., 2024; Hsu *et al* ., 2024; Elahmer *et al* ., 2024)**.

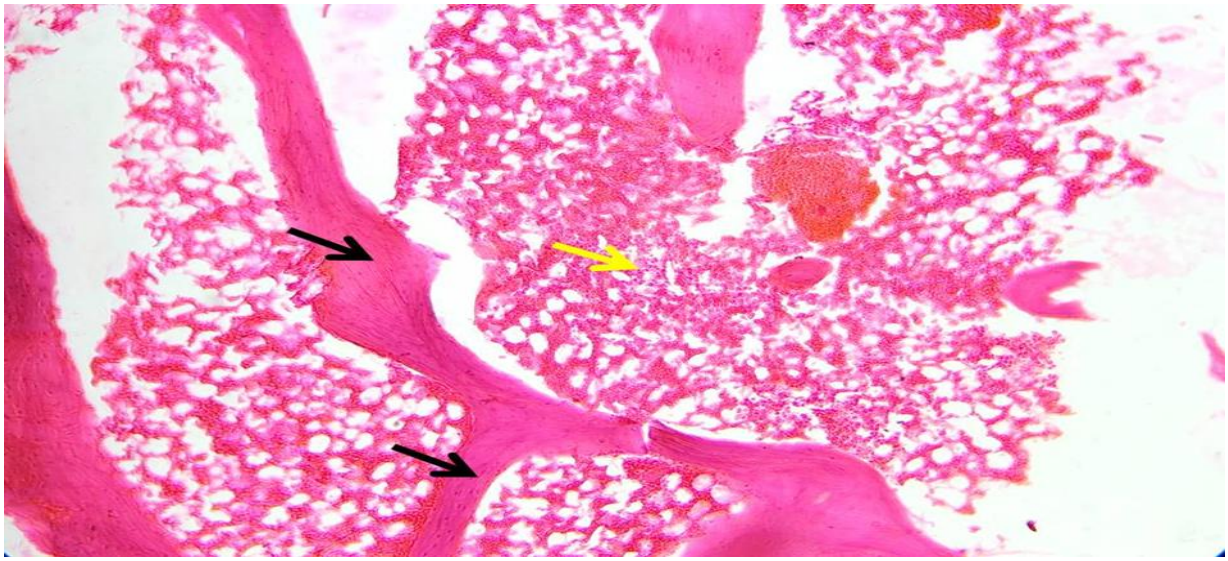


Figure (4.14): Photography of spongy bone (control group) showing the normal state of histological features, thin trabecular (black arrows) and large spaces of bone marrow (yellow arrow). H&E stain, 400x.

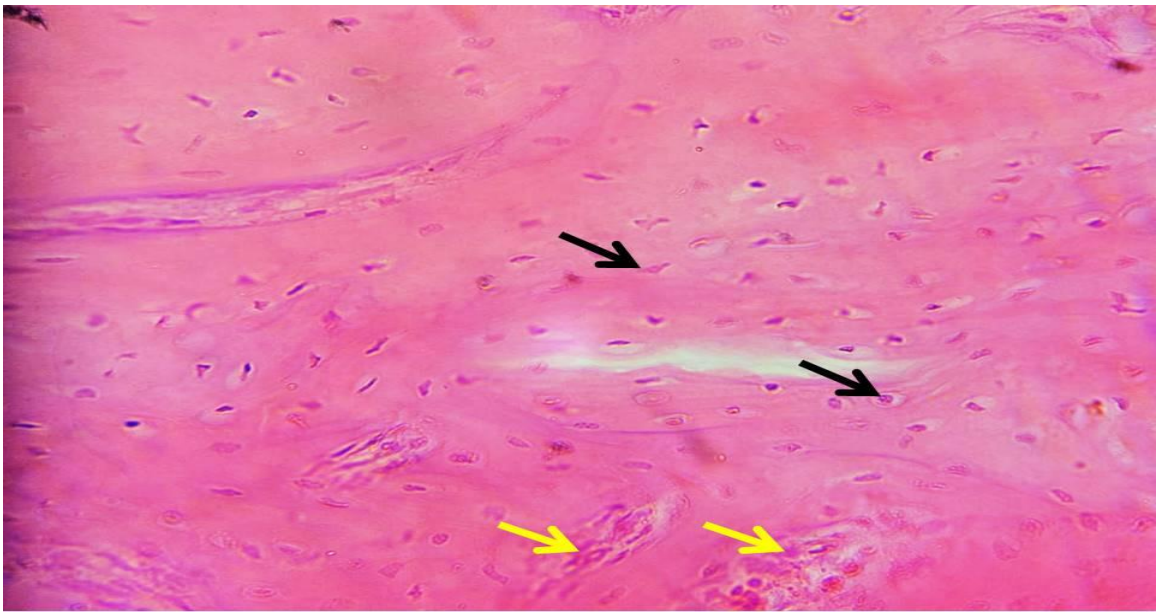


Figure (4.15) : Photography of compact bone (control group) shows activity of osteocytes (black arrows) and increased of osteoblast with normal state of osteoid tissue. H&E stain. 400X.

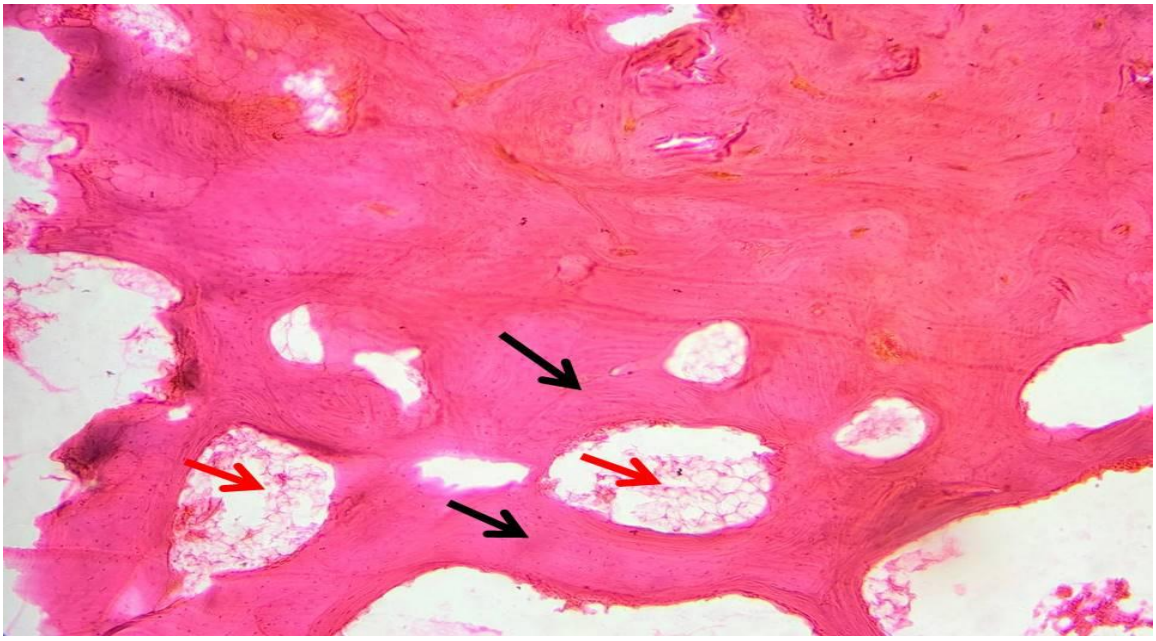


Figure (4.16): Photography of (osteoporosis group) shows thickness of spongy bone becomes less dense, with a decrease in trabecular number (black arrows). Decrease of bone marrow spaces (red arrows). H&E stain,400x.

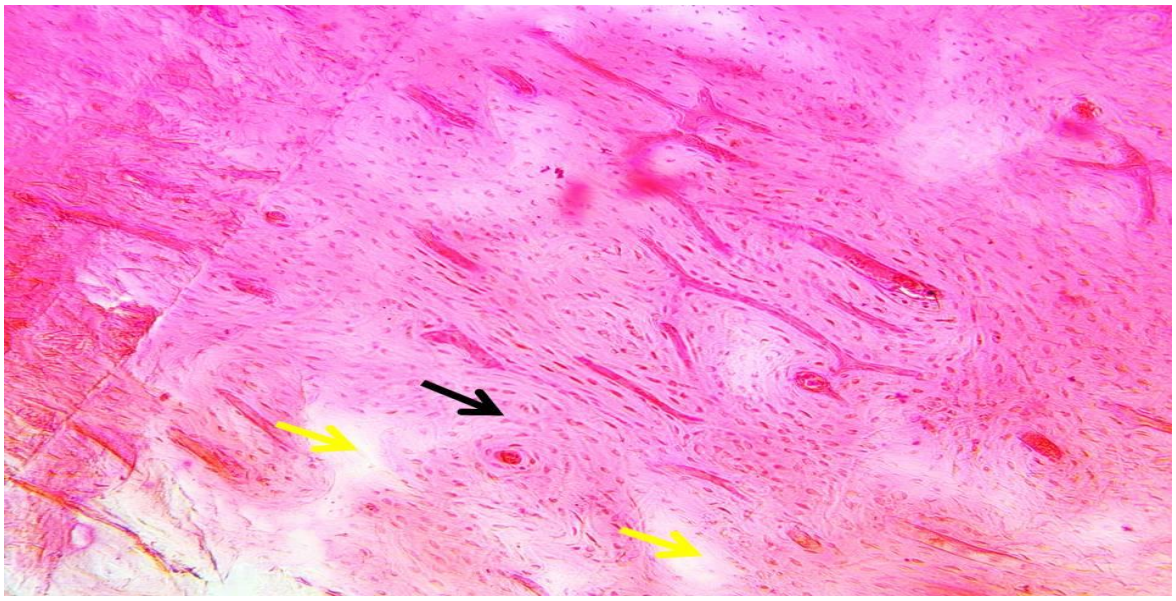


Figure (4.17): Photography of compact bone (osteoporosis group) shows un-mineralized osteoid tissue, with increased thickness in histological sections (yellow arrows) and decreased of osteoblast (black arrow). H&E stain. 400X.

Chapter five

Conclusions

&

Recommendations

5. Conclusions & Recommendations

5.1 Conclusions:

The current study concluded that bilateral ovariectomy OVX in rabbits for six weeks leads to the following:

1. X-ray examination showed lower bone density and greater bone translucency in the OVX group compared to the control group

2. Serum hormone tests showed a decrease in estrogen and progesterone and an increase in follicle-stimulating hormone FSH and Luteinizing hormone LH the hormones in the group OVX compared to the control group.

3. Increase in serum electrolytes calcium (Ca) in the group OVX compared to the control group

4.Bone biomarkers are affected, with both increasing RANK, RANKL, Cathepsin K and decreasing in serum OPG in the OVX group compared to the control group.

5. Gene expression of estrogen receptor alpha and beta in the OVX group showed downregulation of estrogen receptor alpha while upregulation of estrogen receptor beta. Compared with the control group.

6. Histological examination of the OVX group showed tissue erosion and an increase in osteoclasts, compared to the control group.

5.2 Recommendations:

1.A study on the possibility of treating postmenopausal osteoporosis with estrogen, balancing LH and FSH, and the biological effects of this on various body organs.

2. More studies on another animal model in the experiment to study the physiological changes that occur in the body.

3. Studying the physiological changes in other body organs after menopause.

4.Studying gene expression RUNX2 and its relationship with estrogen receptors alpha and beta.

5. Studying the effect of postmenopausal estrogen deficiency on other estrogen-secreting cells in the body.

Chapter Six

Reference

Abd Ali, F. K., Ismael, S. M., & Abduaali, D. K. (2024). Complications of Hysterectomy in Iraqi Women in the Department of Obstetrics and Gynecology. Journal Of Medicine And Surgery, 3(4), 25-31.

Abd-Al-Ameer DR , Albazi W. (2024). Monitoring of bone matrix acidificatio by TRAP and ERK biomarkers in the chronic hypercholesterolemia male rats. *Open Veterinary Journal*, 14(8), 1836.

Abdallah, H. M., Farag, M. A., Algandaby, M. M., Nasrullah, M. Z., Abdel Naim, A. B., Eid, B. G., ... & Malebari, A. M. (2020). Osteoprotective activity and metabolite fingerprint via UPLC/MS and GC/MS of Lepidium sativum in ovariectomized Rats. *Nutrients*, 12(7), 2075.

Abdi, S. (2024). Animal models for the study of osteoporosis. *Animal Models in Experimental Medicine*, 221-245.

Agoro, R., & White, K. E. (2023). Regulation of FGF23 production and phosphate metabolism by bone–kidney interactions. *Nature Reviews Nephrology*, 19(3), 185-193.

Ahmad, S. S., Ahmed, F., Ahmad, S., & Khan, M. A. (2025). Proteases and Osteoporosis: A Comprehensive Review of Their Role in Bone Health. *Current Drug Targets*, *26*(7), 489-505.

Aibar-Almazán, A., Voltes-Martínez, A., Castellote-Caballero, Y., Afanador-Restrepo, D. F., Carcelén-Fraile, M. D. C., & López-Ruiz, E. (2022). Current status of the diagnosis and management of osteoporosis. International *journal of molecular sciences*, 23(16), 9465.

Alasmi, S. (2022). Functional role (s) of gonadotropins in liver and bone metabolism.

Al-Bari, A. A., & Al Mamun, A. (2020). Current advances in regulation of bone homeostasis. *Faseb Bioadvances*, 2(11), 668-679.

Alcorta-Sevillano, N., Macías, I., Infante, A., & Rodríguez, C. I. (2020). Deciphering the relevance of bone ECM signaling. *Cells*, *9*(12), 2630.

Ali, D., Tencerova, M., Figeac, F., Kassem, M., & Jafari, A. (2022). The pathophysiology of osteoporosis in obesity and type 2 diabetes in aging women and men: The mechanisms and roles of increased bone marrow adiposity. *Frontiers in Endocrinology*, *13*, 981487.

Almeida M., Han L., Ambrogini E., Bartell S.M., Manolagas S.C.(2010).Oxidative stress stimulates apoptosis and activates NF-kappaB in osteoblastic cells via a PKCbeta/p66shc signaling cascade: Counter regulation by estrogens or androgens. Mol. *Endocrinol*.;24:2030–2037. doi: 10.1210/me.2010-0189.

Almeida, M. (2017). Estrogens and androgens in skeletal physiology and pathophysiology. *Physiol*. Rev. 97, 135–187.

AlQranei, M. S., Senbanjo, L. T., Aljohani, H., Hamza, T., & Chellaiah, M. A. (2021). Lipopolysaccharide-TLR-4 Axis regulates Osteoclastogenesis independent of RANKL/RANK signaling. *BMC immunology*, 22, 1-16.

Al-Suhaimi, E. A., Khan, F. A., & Homeida, A. M. (2022). Regulation of male and female reproductive functions. In Emerging concepts in endocrine structure and functions (pp. 287-347). Singapore: *Springer Nature Singapore.*

Altwegg, K. A., & Vadlamudi, R. K. (2021). Role of estrogen receptor coregulators in endocrine resistant breast cancer. *Exploration of targeted anti-tumor therapy*, *2*(4), 385.

Angulo Castro, A. G. (2024). The Influence of Ovariohysterectomy and Bisphosphonate Treatment on the Fatigue Behaviour of Whole-Bone and Bone Tissue.

Anwar, A., Sapra, L., Gupta, N., Ojha, R. P., Verma, B., & Srivastava, R. K. (2023). Fine-tuning osteoclastogenesis: An insight into the cellular and molecular regulation of osteoclastogenesis. *Journal of cellular physiology*, *238*(7), 1431-1464.

Aslam Khan, M. U., Aslam, M. A., Bin Abdullah, M. F., & Stojanović, G. M. (2024). Current perspectives of protein in bone tissue Engineering: bone structure, Ideal scaffolds, fabrication techniques, applications, Scopes, and future advances. *ACS Applied Bio Materials*, 7(8), 5082-5106.

Aspray T.J., L. Yan and A. Prentice(2005): Parathyroid hormone and rates of bone formation are raised in perimenopausal rural Gambian women. *Bone*, 36(4): 710-720.

Ayers, C., Kansagara, D., Lazur, B., Fu, R., Kwon, A., & Harrod, C. (2023). Effectiveness and safety of treatments to prevent fractures in people with low bone mass or primary osteoporosis: a living systematic review and network meta-analysis for the American College of Physicians. *Annals of internal medicine*, 176(2), 182-195.

Babaniyi, G. G., Ajao, B. H., Akor, U. J., & Babaniyi, E. (2024). Reproductive endocrinology drug development: Hormones, metabolism, and fertility in female reproductive health. In *Perspectives of Quorum Quenching in New Drug Development* (pp. 187-206). CRC Press.

Balla, B., Sárvári, M., Kósa, J. P., Kocsis-Deák, B., Tobiás, B., Árvai, K., ... & Lakatos, P. (2019). Long-term selective estrogen receptor-beta agonist treatment modulates gene expression in bone and bone marrow of ovariectomized rats. *The Journal of steroid biochemistry and molecular biology*, 188, 185-194.

Banoriya, G. K., Singh, V. K., Maurya, R., & Kharwar, R. K. (2025). Neuro-Immuno-Endocrine Regulation of Bone Homeostasis. *Discovery medicine*, *37*(194), 464-485.

Bartkowiak-Wieczorek, J., Jaros, A., Gajdzińska, A., Wojtyła-Buciora, P., Szymański, I., Szymaniak, J., ... & Bienert, A. (2024). The Dual Faces of Oestrogen: The Impact of Exogenous Oestrogen on the Physiological and Pathophysiological Functions of Tissues and Organs. *International Journal of Molecular Sciences*, 25(15), 8167.

Baseem, T., Albazi, W., Mousa, R. F., & Mahmood, H. B. (2024). Inhibition of the RANK RANK-L OPG/Cathepsin-K pathway on osteoclast activity by a treadmill in the osteoporosis induced by D-gal in the male rats. *Iraqi Journal of Veterinary Sciences*, 38, 17-26.

Bautista-Carbajal, A., Villanueva-Arriaga, R. E., Páez-Arenas, A., Massó-Rojas, F., Frechero Molina, N., & García-López, S. (2023). Nitrogen-Containing Bisphosphonates Downregulate Cathepsin K and Upregulate Annexin V in Osteoclasts Cultured In Vitro. *International Journal of Dentistry*, 2023(1), 2960941.

Behary, P., & Comninos, A. N. (2022). Bone perspectives in functional hypothalamic amenorrhoea: an update and future avenues. *Frontiers in Endocrinology*, 13, 923791.

Bhattarai, H. K., Shrestha, S., Rokka, K., & Shakya, R. (2020). Vitamin D, calcium, parathyroid hormone, and sex steroids in bone health and effects of aging. *Journal of osteoporosis*, *2020*(1), 9324505.

Bordukalo-Nikšić, T., Kufner, V., & Vukičević, S. (2022). The role of BMPs in the regulation of osteoclasts resorption and bone remodeling: from experimental models to clinical applications. *Frontiers in Immunology*, 13, 869422.

Boyce B.F., Yamashita T., Yao Z., Zhang Q., Li F., Xing L. Roles for NF-kappaB and c-Fos in osteoclasts. *J. Bone Miner. Metab*. 2005;23((Suppl. S1)):11–15. doi: 10.1007/BF03026317.

Boyce, B. F., & Xing, L. (2007). Biology of RANK, RANKL, and osteoprotegerin. *Arthritis research & therapy*, 9, 1-7.

Boyce, B. F., & Xing, L. (2008). Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Archives of biochemistry and biophysics*, 473(2), 139-146.

Braidman, I. P., Hainey, L., Batra, G., Selby, P. L., Saunders, P. T., & Hoyland, J. A. (2001). Localization of estrogen receptor β protein expression in adult human bone. *Journal of Bone and Mineral Research*, 16(2), 214-220.

Bray ,JJ.;Cragg, PA. ;Macknight,AC.;& Mills, RG. (1999). Effects of alcohol postmenopausal.

Briot K, Roux C (2015) Glucocorticoid-induced osteoporosis. *RMD Open* 1:e000014.

Brodetska, L., Natrus, L., Lisakovska, O., Kaniura, O., Iakovenko, L., Skrypnyk, I., & Flis, P. (2020). The regulatory role of the RANKL/RANK/OPG signaling pathway in the mechanisms of tooth eruption in patients with impacted teeth. *BMC oral health*, *20*(1), 261.

Cable, J. K., & Grider, M. H. (2020). Physiology, progesterone.

Calik-Ksepka, A., Stradczuk, M., Czarnecka, K., Grymowicz, M., & Smolarczyk, R. (2022). Lactational amenorrhea: neuroendocrine pathways controlling fertility and bone turnover. *International Journal of Molecular Sciences*, 23(3), 1633.

Camon, C., Garratt, M., & Correa, S. M. (2024). Exploring the effects of estrogen deficiency and aging on organismal homeostasis during menopause. *Nature aging*, *4*(12), 1731-1744.

Candelaria NR, Liu K, Lin CY. (2013). Estrogen receptor alpha: molecular mechanisms and emerging insights. *J Cell Biochem*.;114(10):2203–8.

Cannata-Andía, J. B., Carrillo-López, N., Messina, O. D., Hamdy, N. A. T., Panizo, S., & Ferrari, S. L. (2021). on behalf of the International Osteoporosis Foundation (IOF) Working Group on Bone and Cardiovascular Diseases. Pathophysiology of Vascular Calcification and Bone Loss: Linked Disorders of Ageing? *Nutrients 2021*, 13, 3835.

Canuas Landero, V. G. (2023). *Defining how oestrogen influences the anti-tumour effects of adjuvant bisphosphonates using in vivo models of breast cancer* (Doctoral dissertation, University of Sheffield).

Caplan, R. M. (2024). Menopause and Osteoporosis. In *Long Life Strategy: A Guide for Living a Longer, Healthier, and More Fulfilling Life* (pp. 91-107). Cham: Springer Nature Switzerland.

Capulli M., Paone R., Rucci N. Osteoblast and Osteocyte: Games without Frontiers. Arch. *Biochem. Biophys***.** 2014;561:3–12.

Celik, B., Leal, A. F., & Tomatsu, S. (2024). Potential targeting mechanisms for bone-directed therapies. *International Journal of Molecular Sciences*, *25*(15), 8339.

Chang, B., & Liu, X. (2022). Osteon: structure, turnover, and regeneration. *Tissue Engineering Part B: Reviews*, 28(2), 261-278.

Chawla, P. (2024). MRTF-dependent Paracrine Regulation of Osteoclast Differentiation and Bone Colonization of Breast Cancer Cells (Master's thesis, University of Pittsburgh).

Chen, J., Hao, Z., Li, H., Wang, J., Chen, T., Wang, Y., ... & Li, J. (2024). Osteoporotic osseointegration: therapeutic hallmarks and engineering strategies. *Theranostics*, *14*(10), 3859.

Chen, Y. J., Jia, L. H., Han, T. H., Zhao, Z. H., Yang, J., Xiao, J. P., ... & Yang, K. (2024). Osteoporosis treatment: current drugs and future developments. Frontiers in Pharmacology, 15, 1456796.

Cheng, C. H., Chen, L. R., & Chen, K. H. (2022). Osteoporosis due to hormone imbalance: an overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover. *International journal of molecular sciences*, 23(3), 1376.

Chew, C.K.; Clarke, B.L.(2018). Causes of low peak bone mass in women. *Maturitas*, 111, 61–68.

Costa, A. G., Cusano, N. E., Silva, B. C., Cremers, S., & Bilezikian, J. P. (2011). Cathepsin K: its skeletal actions and role as a therapeutic target in osteoporosis. *Nature Reviews Rheumatology*, 7(8), 447-456.

Crisafulli, A., Micari, A., Altavilla, D., Saporito, F., Sardella, A., Passaniti, M., & Squadrito, F. (2005). Serum levels of osteoprotegerin and RANKL in patients with ST elevation acute myocardial infarction. *Clinical Science*, 109(4), 389-395.

Cuffaro, D., Scilabra, S. D., Spanò, D. P., Calligaris, M., Nuti, E., & Rossello, A. (2024). A disintegrin and metalloproteinases (ADAMs) and tumor necrosis factor-alpha-converting enzyme (TACE). In *Metalloenzymes* (pp. 207-237). Academic Press.

Dai, R., Wu, Z., Chu, H. Y., Lu, J., Lyu, A., Liu, J., & Zhang, G. (2020). Cathepsin K: the action in and beyond bone. *Frontiers in cell and developmental biology*, 8, 433.

Dang, Y., Zhang, Y., Luo, G., Li, D., Ma, Y., Xiao, Y., ... & Wang, X. (2024). The decisive early phase of biomaterial-induced bone regeneration. *Applied Materials Today*, 38, 102236.

Das, N., & Kumar, T. R. (2018). Molecular regulation of follicle-stimulating hormone synthesis, secretion and action. *Journal of molecular endocrinology*, 60(3), R131-R155.

Das, P. K., Mukherjee, J., & Banerjee, D. (2023). Female reproductive physiology. In Textbook of veterinary physiology (pp. 513-568). Singapore: *Springer Nature Singapore.*

Davis, C.A.; Lavoie, T.B.(2001). Gonzalez, A. Estrogen Receptors Alpha and Beta Are Differentially Expressed in Developing Human Bone. *J. Clin. Endocrinol. Metab*, 86, 2309–2314.

Deaton, D. N., & Tavares, F. X. (2005). Design of cathepsin K inhibitors for osteoporosis. *Current Topics in Medicinal Chemistry*. 5[16]: 1639-1675.

Demeuse, J., Massonnet, P., Schoumacher, M., Grifnée, E., Huyghebaert, L., Dubrowski, T & Cavalier, E. (2023). Innovative workflow for the identification of cathepsin K cleavage sites in type I collagen. *Journal of Chromatography B*, 1228, 123864.

Denison, M. (2024). Exploiting Light to Probe Biological Processes (*Doctoral dissertation, Wayne State University*).

Desai, S., & Jayasuriya, C. T. (2020). Implementation of endogenous and exogenous mesenchymal progenitor cells for skeletal tissue regeneration and repair. *Bioengineering*, *7*(3), 86.

Di Cicco, G., Marzano, E., Mastrostefano, A., Pitocco, D., Castilho, R. S., Zambelli, R., ... & Perisano, C. (2024). The Pathogenetic Role of RANK/RANKL/OPG Signaling in Osteoarthritis and Related Targeted Therapies. *Biomedicines*, 12(10), 2292.

Dong, X., Liu, H., Yuan, D., Gulati, K., & Liu, Y. (2025). Re-engineering bone: pathogenesis, diagnosis and emerging therapies for osteoporosis. *Journal of Materials Chemistry B*.

Dong, Y., Wu, X., Hao, Y., Liu, W., Hu, X., Zhou, J., ... & Wang, B. (2025). Epiregulin ameliorates ovariectomy-induced bone loss through orchestrating the differentiation of osteoblasts and osteoclasts. *Journal of Bone and Mineral Research*, zjaf017.

Donsante, S., Palmisano, B., Serafini, M., Robey, P. G., Corsi, A., & Riminucci, M. (2021). From stem cells to bone-forming cells. *International journal of molecular sciences*, *22*(8), 3989.

Du, H., Jia, A., Gu, M., Li, S., Zhang, P., & Bai, J. (2021). Thyrotoxicosis and osteomalacia: from symptom to pathogenesis. *American Journal of Translational Research*, *13*(2), 803.

Ekeuku, S. O., Chin, K. Y., & Mohd Ramli, E. S. (2023). Effects of Piper sarmentosum on bone Health and fracture healing: a scoping review. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders)*, *23*(7), 908-916.

Elahmer, N. R., Wong, S. K., Mohamed, N., Alias, E., Chin, K. Y., & Muhammad, N. (2024). Mechanistic insights and therapeutic strategies in osteoporosis: a comprehensive review. *Biomedicines*, 12(8), 1635.

Elango, J., Bao, B., & Wu, W. (2021). The hidden secrets of soluble RANKL in bone biology. *Cytokine*, *144*, 155559.

Elbossaty W. F. Mineralization of bones in osteoporosis and osteomalacia. *Annals of Clinical and Laboratory Research*. 2017;5(4):3–6. doi: 10.21767/2386-5180.1000201.

Elghareeb, M. M., Elshopakey, G. E., Elkhooly, T. A., Salama, B., Samy, A., Bazer, F. W., ... & Rezk, S. (2022). Estradiol and zinc-doped nano hydroxyapatite as therapeutic agents in the prevention of osteoporosis; oxidative stress status, inflammation, bone turnover, bone mineral density, and histological alterations in ovariectomized rats. *Frontiers in Physiology*, *13*, 989487.

El-Haroun, H., Soliman, M., & El-Gawad, A. (2020). Comparative Study on the Possible Protective Effect of Lepidium Sativum versus Teriparatide in Induced Osteoporosis in Adult Male Guinea Pigs. *Egyptian Journal of Histology*, 43(3), 931-947.

El-Masri, B. M., Andreasen, C. M., Laursen, K. S., Kofod, V. B., Dahl, X. G., Nielsen, M. H., ... & Andersen, T. L. (2024). Mapping RANKL-and OPG-expressing cells in bone tissue: the bone surface cells as activators of osteoclastogenesis and promoters of the denosumab rebound effect. *Bone research*, 12(1), 62.

Elson, A., Anuj, A., Barnea-Zohar, M., & Reuven, N. (2022). The origins and formation of bone-resorbing osteoclasts. *Bone*, 164, 116538.

Emmanuelle, N. E., Marie-Cécile, V., Florence, T., Jean-François, A., Françoise, L., Coralie, F., & Alexia, V. (2021). Critical Role of Estrogens on Bone Homeostasis in Both Male and Female: From Physiology to Medical Implications. *International journal of molecular sciences*, 22(4), 1568.

Engelmann, J., Ragipoglu, D., Ben-Batalla, I., & Loges, S. (2023). The Role of TAM Receptors in Bone. *International Journal of Molecular Sciences*, 25(1), 233.

Fang, H., Deng, Z., Liu, J., Chen, S., Deng, Z., & Li, W. (2022). The mechanism of bone remodeling after bone aging. *Clinical Interventions in Aging*, 405-415.

Farman, H. (2018). The Role of Estrogen Receptor α Signaling in the Regulation of Bone Mass.

Faupel-Badger, J. M., Fuhrman, B. J., Xu, X., Falk, R. T., Keefer, L. K., Veenstra, T. D., ... & Ziegler, R. G. (2010). Comparison of liquid chromatography-tandem mass spectrometry, RIA, and ELISA methods for measurement of urinary estrogens. *Cancer epidemiology, biomarkers & prevention*, 19(1), 292-300.

Feng, N., Zhang, Y., & Li, Y. Site-Specific Response of Bone Tissue to Ovariectomy in a Rabbit Model. *Clin Surg*. 2023; 8, 3614.

Feng, S. T., Yang, Y., Li, X., Zuo, W. Y., & Sun, H. B. (2024). Risk factors of new symptomatic fractures after vertebroplasty: a retrospective cohort study of 268 patients with painful osteoporotic vertebral compression fracture. *World Neurosurgery*, 187, e890-e897.

Fink, D. A., Kilday, D., Cao, Z., Larson, K., Smith, A., Lipkin, C., ... & Rosenthal, N. (2023). Trends in maternal mortality and severe maternal morbidity during delivery-related hospitalizations in the United States, 2008 to 2021. *JAMA Network Open*, *6*(6), e2317641-e2317641.

Fitzgerald, C., Burley, D. C., Wright, D. K., McLeod, D. K., & Parmenter, A. P. B. (2024). Improving mental health, pain and quality of life in persons living with osteoporosis and depression or anxiety: a systematic review. *Journal of Clinical Exercise Physiology*, *13*(s2), 516-516.

Forbes, A. P. (1991). Fuller Albright: His Concept of Postmenopausal Osteoporosis and What Came of It. *Clinical Orthopaedics and Related Research (1976-2007)*, *269*, 128-141.

Foroutan, B. (2024). Osteoporosis Etiology, Epidemiology, Diagnosis, Diet, and Treatment: A Narrative Review. *OBM Geriatrics*, *8*(2), 1-60.

Forte, Y. S., Renovato-Martins, M., & Barja-Fidalgo, C. (2023). Cellular and molecular mechanisms associating obesity to bone loss. *Cells*, 12(4), 521.

Freeman, C., A. S, M. D., & A. S, P. (2024). Unraveling the intricacies of OPG/RANKL/RANK biology and its implications in neurological disorders—a comprehensive literature review. *Molecular Neurobiology*, 1-15.

Galanis, A., Dimopoulou, S., Karampinas, P., Vavourakis, M., Papagrigorakis, E., Sakellariou, E., & Vlamis, J. (2023). The correlation between transient osteoporosis of the hip and pregnancy: A review. *Medicine*, *102*(41), e35475.

Gan, X., Dai, G., Li, Y., Xu, L., & Liu, G. (2024). Intricate roles of estrogen and estrogen receptors in digestive system cancers: a systematic review. *Cancer Biology & Medicine*, 21(10), 898-915.

Gaughan, N. A., Rademacher, N., Rogers, C., Purdy, A., D MATTINGLY, J., Patten, C. R., ... & Cortina, C. S. (2025). Current insights into breast and ovarian cancer risk: a contemporary review. *Minerva surgery*, *80*(4), 334.

Geller, A., Salganicoff, A., Burke, S. P., & National Academies of Sciences, Engineering, and Medicine. (2025). The Biological Basis for Women's Health Through the Lens of Chromosomes and Hormones. In *A New Vision for Women's Health Research: Transformative Change at the National Institutes of Health*. National Academies Press (US).

Geoghegan, I. P., Hoey, D. A., & McNamara, L. M. (2019). Estrogen deficiency impairs integrin αvβ3-mediated mechanosensation by osteocytes and alters osteoclastogenic paracrine signalling. *Scientific reports*, 9(1), 4654.

Georgin, J., Franco, D. S. P., Manzar, M. S., Meili, L., & El Messaoudi, N. (2024). A critical and comprehensive review of the current status of 17β-estradiol hormone remediation through adsorption technology. *Environmental Science and Pollution Research*, 31(17), 24679-24712.

Gilsanz V, Roe TF, Gibbens DT, Schulz EE, Carlson ME, Gonzalez O, Boechat MI: Effect of sex steroids on peak bone density of growing rabbits. Am J *Physiol Endocrinol Metab*. 1988, 255: E416-421.

Gonçalves, F. J., Abrantes-Soares, F., Pouso, M. R., Lorigo, M., & Cairrao, E. (2023). Non-genomic effect of estradiol on the neurovascular unit and possible involvement in the cerebral vascular accident. *Molecular Neurobiology*, 60(4), 1964-1985.

Gorski J, et al. (1968). Hormone receptors: studies on the interaction of estrogen with the uterus. *Recent Prog Horm Res*.;24:45–80. doi: 10.1016/b978-1-4831-9827-9.50008-3.

Green S, et al. (1986). Cloning of the human oestrogen receptor cDNA. *J Steroid Biochem*.;24(1):77–83. doi: 10.1016/0022-4731(86)90035-x.

Grygorieva, N. V., Kovalenko, V. M., Korzh, M. O., Tatarchuk, T. F., Dedukh, N. V., Strafun, S. S. & Chaykivska, E. F. (2023). Guideline for diagnostic, prevention and treatment of postmenopausal osteoporosis. *Pain, joints, spine*, *13*(3), 128-154.

Gureeva, T. A., Timoshenko, O. S., Kugaevskaya, E. V., & Solovyova, N. I. (2022). Cysteine Cathepsins: Structure, Physiological Functions, and the Role in Carcinogenesis. *Biochemistry (Moscow), Supplement Series B: Biomedical Chemistry*, *16*(2), 91-103.

Győri, D. S. (2024). Research on Bone Cells in Health and Disease. *International Journal of Molecular Sciences*, *25*(16), 8758.

Győri, D. S., & Mócsai, A. (2020). Osteoclast signal transduction during bone metastasis formation. *Frontiers in cell and developmental biology*, *8*, 507.

Hart, D. A. (2023). Regulation of bone by mechanical loading, sex hormones, and nerves: Integration of such regulatory complexity and implications for bone loss during space flight and post-menopausal osteoporosis. *Biomolecules*, 13(7), 1136

He, Q., Fu, K., Yao, H., Wei, S., Xiang, L., Liu, S., ... & Gao, Y. (2022). Traditional Chinese decoction Si Zhi Wan attenuates ovariectomy (OVX)-induced bone loss by inhibiting osteoclastogenesis and promoting apoptosis of mature osteoclasts. *Frontiers in Pharmacology*, 13, 983884.

Henry, J. P., & Bordoni, B. (2020). Histology, osteoblasts.

Holliday, L. S., Patel, S. S., & Rody Jr, W. J. (2021). RANKL and RANK in extracellular vesicles: Surprising new players in bone remodeling. *Extracellular vesicles and circulating nucleic acids*, 2, 18.

Hossain, M., Sultana, T., Moon, J. E., Moon, G. S., & Jeong, J. H. (2025). Anti-osteoporotic potential of a probiotic mixture containing Limosilactobacillus reuteri and Weissella cibaria in ovariectomized rats. *Scientific Reports*, 15(1), 18586.

Hsu, S. H., Chen, L. R., & Chen, K. H. (2024). Primary Osteoporosis Induced by Androgen and Estrogen Deficiency: The Molecular and Cellular Perspective on Pathophysiological Mechanisms and Treatments. *International Journal of Molecular Sciences*, 25(22), 12139.

Huang, J., Zhang, Z., He, P., & Zhou, J. (2024). Possible mechanisms underlying the regulation of postmenopausal osteoporosis by follicle-stimulating hormone. *Heliyon*, 10(15).

Imai, Y., Youn, M. Y., Inoue, K., Takada, I., Kouzmenko, A., & Kato, S. (2013). Nuclear receptors in bone physiology and diseases. *Physiological reviews*, 93(2), 481-523.

Isojima, T., & Sims, N. A. (2021). Cortical bone development, maintenance and porosity: genetic alterations in humans and mice influencing chondrocytes, osteoclasts, osteoblasts and osteocytes. *Cellular and Molecular Life Sciences*, *78*(15), 5755-5773.

Ivory, A. R. (2021). Estrogens, the G-Protein Coupled Estrogen Receptor, and Human Endothelial Colony Forming Cell Function: *Implications for Post-Menopausal Cardiovascular Disease (Master's thesis, The Medical College of Wisconsin).*

Iwamoto, N., & Kawakami, A. (2022). The monocyte-to-osteoclast transition in rheumatoid arthritis: Recent findings. *Frontiers in Immunology*, 13, 998554.

Jayasena, C. N., Devine, K., Barber, K., Comninos, A. N., Conway, G. S., Crown, A., ... & Quinton, R. (2024). Society for endocrinology guideline for understanding, diagnosing and treating female hypogonadism. *Clinical Endocrinology*, *101*(5), 409-442.

Jiang, T., Xia, T., Qiao, F., Wang, N., Jiang, Y., & Xin, H. (2023). Role and regulation of transcription factors in osteoclastogenesis. *International Journal of Molecular Sciences*, 24(22), 16175.

Jiao, Z., Chai, H., Wang, S., Sun, C., Huang, Q., & Xu, W. (2023). SOST gene suppression stimulates osteocyte Wnt/β-catenin signaling to prevent bone resorption and attenuates particle-induced osteolysis. *Journal of Molecular Medicine*, 101(5), 607-620.

Jiménez-Ortega, R. F., Ortega-Meléndez, A. I., Patiño, N., Rivera-Paredez, B., Hidalgo-Bravo, A., & Velázquez-Cruz, R. (2024). The involvement of microRNAs in bone remodeling signaling pathways and their role in the development of osteoporosis. *Biology*, *13*(7), 505.

Jin, Y., Zhou, B. H., Zhao, J., Ommati, M. M., Wang, S., & Wang, H. W. (2023). Fluoride-induced osteoporosis via interfering with the RANKL/RANK/OPG pathway in ovariectomized rats: Oophorectomy shifted skeletal fluorosis from osteosclerosis to osteoporosis. *Environmental Pollution*, 336, 122407.

Jo'rayevna, S. M. (2023). PREVENTION AND TREATMENT OF OSTEOPOROSIS, ONE OF THE MOST COMMON DISEASES NOWADAYS. *Ethiopian International Journal of Multidisciplinary Research*, 10(09), 489-495.

Johansen, N., Tonstad, S., Liavaag, A. H., Selmer, R. M., Tanbo, T. G., & Michelsen, T. M. (2020). Risk of cardiovascular disease after preventive salpingo-oophorectomy. *International Journal of Gynecological Cancer*, 30(5), 575-582.

Juel Mortensen L, Lorenzen M, Jørgensen N, Andersson AM, Nielsen JE, Petersen LI, *et al* .(2019). Possible link between FSH and RANKL release from adipocytes in men with impaired gonadal function including Klinefelter syndrome. *Bone 123*: 103–114.

Kabakchiev, C., Singh, A., Dobson, S., & Beaufrère, H. (2021). Comparison of intra− and postoperative variables between laparoscopic and open ovariectomy in rabbits (Oryctolagus cuniculus). *American Journal of Veterinary Research*, *82*(3), 237-248.

Kater, C. E., Giorgi, R. B., & Costa-Barbosa, F. A. (2022). Classic and current concepts in adrenal steroidogenesis: a reappraisal. *Archives of endocrinology and metabolism*, *66*(1), 77-87.

Katsimbri P, (2017). The biology of normal bone remodelling. *Eur. J. Cancer Care (Engl.)*, 26(6) 10.1111/ecc.12740 PMID: 28786518.

Khalid, A. B., & Krum, S. A. (2016). Estrogen receptors alpha and beta in bone. *Bone*, 87, 130-135.

Khiyali, Z., Rashedi, V., Tavacol, Z., Dehghan, A., & Bijani, M. (2024). Smoking, alcohol consumption, drug abuse, and osteoporosis among older adults: a cross-sectional study on PERSIAN cohort study in Fasa. *BMC geriatrics*, *24*(1), 80.

Khoswanto, C. (2023). Role of matrix metalloproteinases in bone regeneration: Narrative review. *Journal of Oral Biology and Craniofacial Research*, *13*(5), 539-543.

Khotib, J., Marhaeny, H. D., Miatmoko, A., Budiatin, A. S., Ardianto, C., Rahmadi, M., ... & Tahir, M. (2023). Differentiation of osteoblasts: the links between essential transcription factors. *Journal of Biomolecular Structure and Dynamics*, *41*(19), 10257-10276.

Kian Tee, M., Rogatsky, I., Tzagarakis-Foster, C., Cvoro, A., An, J., Christy, R. J., ... & Leitman, D. C. (2004). Estradiol and selective estrogen receptor modulators differentially regulate target genes with estrogen receptors α and β. *Molecular biology of the cell*, 15(3), 1262-1272.

Kim, A. S., Girgis, C. M., & McDonald, M. M. (2022). Osteoclast recycling and the rebound phenomenon following denosumab discontinuation. *Current Osteoporosis Reports*, 20(6), 505-515.

Kitaura, H., Marahleh, A., Ohori, F., Noguchi, T., Shen, W. R., Qi, J., ... & Mizoguchi, I. (2020). Osteocyte-related cytokines regulate osteoclast formation and bone resorption. *International journal of molecular sciences*, *21*(14), 5169.

Knight, K. (2025). *Nanobody development for therapeutically targeting Vacuolar H+-ATPases* (Doctoral dissertation).

Koh, N. Y., Miszkiewicz, J. J., Fac, M. L., Wee, N. K., & Sims, N. A. (2024). Preclinical rodent models for human bone disease, including a focus on cortical bone. *Endocrine Reviews*, *45*(4), 493-520.

Kramar, S. B., Soroka, Y. V., Havryliuk-Skyba, H. O., Pyda, V. P., Nebesna, Z. M., & Lisnychuk, N. Y. (2024). Structural changes in the organs of the lymphoid system in terms of induced carcinogenesis. *Reports of Morphology*, 30(3), 5-14.

Kratena, N. (2019). Synthesis of steroid long-term metabolites with rearranged D-ring fragments (Doctoral dissertation, Technische Universität Wien).

Kuiper GG, et al. (1996) Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U S A* ;93(12):5925–30. doi: 10.1073/pnas.93.12.5925.

Kushchayeva, Y., Pestun, I., Kushchayev, S., Radzikhovska, N., & Lewiecki, E. M. (2022). Advancement in the treatment of osteoporosis and the effects on bone healing. *Journal of Clinical Medicine*, *11*(24), 7477.

Lalmanach, G., Saidi, A., Bigot, P., Chazeirat, T., Lecaille, F., & Wartenberg, M. (2020). Regulation of the proteolytic activity of cysteine cathepsins by oxidants. *International journal of molecular sciences*, *21*(6), 1944.

Langton, C. M., & Njeh, C. F. (Eds.). (2016). *The physical measurement of bone*. CRC Press.

Lara-Castillo, N. (2021). Estrogen signaling in bone. Applied Sciences, 11(10), 4439.

Levin, E. R., & Hammes, S. R. (2011). Estrogens and progestins. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, *12*, 1163-94.

Li, B., Wang, P., Jiao, J., Wei, H., Xu, W., & Zhou, P. (2022). Roles of the RANKL–RANK Axis in Immunity—Implications for Pathogenesis and Treatment of Bone Metastasis. *Frontiers in Immunology*, 13, 824117.

Li, H., Xiao, Z., Quarles, L. D., & Li, W. (2021). Osteoporosis: mechanism, molecular target and current status on drug development. *Current medicinal chemistry*, 28(8), 1489-1507.

Li, J., Wu, N., Li, Y., Ye, K., He, M., & Hu, R. (2015). Cross-sectional analysis of serum calcium levels for associations with left ventricular hypertrophy in normocalcemia individuals with type 2 diabetes. *Cardiovascular diabetology*, 14, 1-11.

Li, Q., Zheng, D., Lin, H., Zhong, F., Liu, J., Wu, Y., ... & Zhao, J. (2021). High circulating follicle-stimulating hormone level is a potential risk factor for renal dysfunction in post-menopausal women. *Frontiers in Endocrinology*, *12*, 627903.

Li, X., Jiang, Y., Liu, X., Fu, J., Du, J., Luo, Z., ... & Guo, L. (2023). Mesenchymal stem cell-derived apoptotic bodies alleviate alveolar bone destruction by regulating osteoclast differentiation and function. *International Journal of Oral Science*, *15*(1), 51.

Liao, N., Zeng, Z., Pang, X., Zhou, J., Liao, H., Qin, Z., ... & Shao, M. (2025). Cnicin Regulates Bone Turnover Homeostasis in Rats with Ovariectomy-Induced Osteoporosis via RANKL/RANK/OPG Pathway. *Revista Brasileira de Farmacognosia*, 1-14.

Lin, X., Tong, X., Chen, Y., Huang, Z., & Yu, H. (2023). The activity, distribution, and colocalization of cathepsin K and matrix metalloproteases in intact and eroded dentin. *Clinical Oral Investigations*, *28*(1), 1.

Lippuner, K., Golder, M., & Greiner, R. (2005). Epidemiology and direct medical costs of osteoporotic fractures in men and women in Switzerland. *Osteoporosis International*, 16(2), S8-S17.

Liu P, Ji Y, Yuen T, Rendina-Ruedy E, DeMambro VE, Dhawan S, *et al* .(2017). Blocking FSH induces thermogenic adipose tissue and reduces body fat. *Nature 546*: 107–112.

Livak, K.J., and Schmittgen, T.D. 2001.Analysis of relative geneexpression data using real-time quantitative PCR and the 2 –ΔΔCTmethod. *Methods*, 25: 402–408.

Lorente-Ramos, R., Azpeitia-Armán, J., Muñoz-Hernández, A., García-Gómez, J. M., Díez-Martínez, P., & Grande-Bárez, M. (2011). Dual-energy x-ray absorptiometry in the diagnosis of osteoporosis: a practical guide. *American Journal of Roentgenology*, 196(4), 897-904.

Lu, B., Han, Q., Zhao, S., Ding, S., Bao, G., & Liu, Y. (2025). Associations between hormones, metabolic markers, and bone mass in perimenopausal and postmenopausal women. *Journal of bone and mineral metabolism*, 1-10.

Lu, L., & Tian, L. (2023). Postmenopausal osteoporosis coexisting with sarcopenia: the role and mechanisms of estrogen. *Journal of Endocrinology*, 259(1).

Ma, X., Liu, J., Yang, L., Zhang, B., Dong, Y., & Zhao, Q. (2018). Cynomorium songaricum prevents bone resorption in ovariectomized rats through RANKL/RANK/TRAF6 mediated suppression of PI3K/AKT and NF-κB pathways. *Life Sciences*, 209, 140-148.

Ma, Z., Zhang, W., Dong, B., Xin, Z., Ji, Y., Su, R., ... & Xue, W. (2022). Docetaxel remodels prostate cancer immune microenvironment and enhances checkpoint inhibitor-based immunotherapy. *Theranostics*, *12*(11), 4965.

Madika, A. L., MacDonald, C. J., Gelot, A., Hitier, S., Mounier-Vehier, C., Beraud, G., ... & Bonnet, F. (2021). Hysterectomy, non-malignant gynecological diseases, and the risk of incident hypertension: The E3N prospective cohort. *Maturitas*, *150*, 22-29.

Mäkelä, R., Leinonen, A., & Suominen, T. (2020). Analysis of luteinizing hormone (LH): Validation of a commercial ELISA kit for LH analysis and quantification in doping control samples. *Drug Testing and Analysis*, 12(2), 239-246.

Mandelin, J., Hukkanen, M., Li, T. F., Korhonen, M., Liljeström, M., Sillat, T., ... & Konttinen, Y. T. (2006). Human osteoblasts produce cathepsin K. *Bone*, *38*(6), 769-777.

Manolagas, S. C., O'brien, C. A., & Almeida, M. (2013). The role of estrogen and androgen receptors in bone health and disease. *Nature Reviews Endocrinology*, 9(12), 699.

Mao, B., Xie, Y., Yang, H., Yu, C., Ma, P., You, Z., ... & Han, Q. (2021). Casein phosphopeptide-amorphous calcium phosphate modified glass ionomer cement attenuates demineralization and modulates biofilm composition in dental caries. *Dental Materials Journal*, *40*(1), 84-93.

Marini, F., Giusti, F., Palmini, G., & Brandi, M. L. (2023). Role of Wnt signaling and sclerostin in bone and as therapeutic targets in skeletal disorders. *Osteoporosis International*, 34(2), 213-238.

Marwein, S., Biswal, S., & Acharya, P. C. (2020). Hormones and steroids as neurotransmitters. In Frontiers in pharmacology of neurotransmitters (pp. 447-501). Singapore: *Springer Singapore*.

McNamara, L. M. (2021). Osteocytes and estrogen deficiency. *Current osteoporosis reports*, 1-12.

Meng, X., Lin, Z., Cao, S., Janowska, I., Sonomoto, K., Andreev, D., ... & Bozec, A. (2022). Estrogen-mediated downregulation of HIF-1α signaling in B lymphocytes influences postmenopausal bone loss. *Bone research*, *10*(1), 15.

Metzger, C. E., & Narayanan, S. A. (2019). The role of osteocytes in inflammatory bone loss. *Frontiers in endocrinology*, *10*, 285.

Mijanović, O., Jakovleva, A., Branković, A., Zdravkova, K., Pualic, M., Belozerskaya, T. A., ... & Zamyatnin Jr, A. A. (2022). Cathepsin K in pathological conditions and new therapeutic and diagnostic perspectives. *International journal of molecular sciences*, 23(22), 13762.

Mills EG, Yang L, Nielsen MF, Kassem M, Dhillo WS, Comninos AN.(2021). The Relationship Between Bone and Reproductive Hormones Beyond Estrogens and Androgens. *Endocrine Rev*. [epub]. pmid:33901271

Mohanty, S., Sahu, A., Mukherjee, T., Kispotta, S., Mal, P., Gupta, M., ... & Prabhakar, P. K. (2025). Molecular mechanisms and treatment strategies for estrogen deficiency-related and glucocorticoid-induced osteoporosis: a comprehensive review. *Inflammopharmacology*, 1-37.

Mohanty, S., Sahu, A., Mukherjee, T., Kispotta, S., Mal, P., Gupta, M., ... & Prabhakar, P. K. (2025). Molecular mechanisms and treatment strategies for estrogen deficiency-related and glucocorticoid-induced osteoporosis: a comprehensive review. *Inflammopharmacology*, 1-37.

Moon, D. O. (2024). Review of Cathepsin K Inhibitor Development and the Potential Role of Phytochemicals. *Molecules*, 30(1), 91.

Mustafa, R. A., Alfky, N. A., Hijazi, H. H., Header, E. A., and Azzeh, F. S. (2018). Biological effect of calcium and vitamin D dietary supplements against osteoporosis in ovariectomized rats. *J Nutr Int Med*, 20(1): 86-93.

Naqvi, S. M., Panadero Pérez, J. A., Kumar, V., Verbruggen, A. S., & McNamara, L. M. (2020). A novel 3D osteoblast and osteocyte model revealing changes in mineralization and pro-osteoclastogenic paracrine signaling during Estrogen deficiency. *Frontiers in Bioengineering and Biotechnology*, 8, 601.

Nava-Valdivia C., Ponce-Guarneros J., SALDAñA-Cruz A., Corona-Sanchez E., Ramirez-VILLAFAñA M., Perez-Guerrero E., et al. (2021). Assessment of serum sRANKL, sRANKL/OPG ratio, and other bone turnover markers with the estimated 10-year risk of major and hip osteoporotic fractures in rheumatoid arthritis: *A cross-sectional study. Biomed. Res*. Int. 2021, 5567666.

Nazia Fathima, S. M., Tamilselvi, R., & Parisa Beham, M. (2022). A Survey on Osteoporosis Detection Methods with a Focus on X-ray and DEXA Images. *IETE Journal of Research*, 68(6), 4640-4664.

Niu, Q., Gao, J., Wang, L., Liu, J., & Zhang, L. (2022). Regulation of differentiation and generation of osteoclasts in rheumatoid arthritis. *Frontiers in Immunology*, *13*, 1034050.

Niwczyk, O., Grymowicz, M., Szczęsnowicz, A., Hajbos, M., Kostrzak, A., Budzik, M., ... & Męczekalski, B. (2023). Bones and hormones: Interaction between hormones of the hypothalamus, pituitary, adipose tissue and bone. *International Journal of Molecular Sciences*, *24*(7), 6840.

Noirrit-Esclassan, E., Valera, M. C., Tremollieres, F., Arnal, J. F., Lenfant, F., Fontaine, C., & Vinel, A. (2021). Critical role of estrogens on bone homeostasis in both male and female: from physiology to medical implications. *International Journal of Molecular Sciences*, 22(4), 1568.

Oduwole, O. O., Huhtaniemi, I. T., & Misrahi, M. (2021). The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. *International journal of molecular sciences*, 22(23), 12735.

Omi, M., & Mishina, Y. (2022). Roles of osteoclasts in alveolar bone. remodeling. *genesis*, 60(8-9), e23490.

Ongaro, L., Alonso, C. A. I., Zhou, X., Brûlé, E., Li, Y., Schang, G., ... & Bernard, D. J. (2021). Development of a highly sensitive ELISA for measurement of FSH in serum, plasma, and whole blood in mice. *Endocrinology*, 162(4), bqab014.

Ono, T., & Nakashima, T. (2018). Recent advances in osteoclast biology. *Histochemistry and cell biology*, 149, 325-341.

Ono, T., Hayashi, M., Sasaki, F., & Nakashima, T. (2020). RANKL biology: bone metabolism, the immune system, and beyond. *Inflammation and regeneration*, *40*, 1-16.

Osterhoff, G.; Morgan, E.F.; Shefelbine, S.J.; Karim, L.; McNamara, L.M.; Augat, P.(2016). Bone mechanical properties and changes with osteoporosis. *Injury* , *47*, 11–20.

Ozturk, S., Cuneyit, I., Altuntas, F., Karagur, E. R., Donmez, A. C., Ocak, M., ... & Donmez, B. O. (2023). Resveratrol prevents ovariectomy-induced bone quality deterioration by improving the microarchitectural and biophysicochemical properties of bone. *Journal of Bone and Mineral Metabolism*, *41*(4), 443-456.

Palacios, S., González, S. P., Sánchez-Prieto, M., & Fasero, M. (2024). challenges and considerations in pharmacotherapy of osteoporosis due to menopause. *Expert Opinion on Pharmacotherapy*, *25*(10), 1359-1372.

Pathak, J. L., Bravenboer, N., & Klein-Nulend, J. (2020). The osteocyte as the new discovery of therapeutic options in rare bone diseases. *Frontiers in endocrinology*, *11*, 405.

Pennypacker BL, Duong LT, Cusick TE, et al.(2011). Cathepsin K inhibitors prevent bone loss in estrogen-deficient rabbits. *J Bone Miner Res* ;26:252–262. doi: 10.1002/jbmr.223

Piñar-Gutierrez, A., García-Fontana, C., García-Fontana, B., & Muñoz Torres, M. (2022). Obesity and bone health: a complex relationship. *International journal of molecular sciences*, 23(15), 8303.

Pouladvand, N., Azarnia, M., Zeinali, H., Fathi, R., & Tavana, S. (2024). An overview of different methods to establish a murine premature ovarian failure mode.

Prasanthi, J. R., Larson, T., Schommer, J., & Ghribi, O. (2011). Silencing GADD153/CHOP gene expression protects against Alzheimer's disease-like pathology induced by 27-hydroxycholesterol in rabbit hippocampus. *PloS one*, 6(10), e26420.

Presnell, J. K., Schreibman, M. P., & Humason, G. L. (1997). Humason's animal tissue techniques. *(No Title)*.

Price, J. S., Sugiyama, T., Galea, G. L., Meakin, L. B., Sunters, A., & Lanyon, L. E. (2011). Role of endocrine and paracrine factors in the adaptation of bone to mechanical loading. *Current osteoporosis reports*, 9, 76-82.

Puppo, M., Taipaleenmäki, H., Hesse, E., & Clézardin, P. (2021). Non-coding RNAs in bone remodelling and bone metastasis: Mechanisms of action and translational relevance. *British Journal of Pharmacology*, *178*(9), 1936-1954.

Puzio, I., Graboś, D., Bieńko, M., Radzki, R. P., Nowakiewicz, A., & Kosior-Korzecka, U. (2021). Camelina oil supplementation improves bone parameters in ovariectomized rats. *Animals*, *11*(5), 1343.

Qi, H., Shen, E., Shu, X., Liu, D., & Wu, C. A. (2023). ERK-estrogen receptor α signaling plays a role in the process of bone marrow mesenchymal stem cell-derived exosomes protecting against ovariectomy-induced bone loss. *Journal of Orthopaedic Surgery and Research*, *18*(1), 250.

Qiu, H. (2022). New insights into the mechanisms of RANKL bidirectional signalling.

Radhi, J. H., El-Hagrasy, A. M. A., Almosawi, S. H., Alhashel, A., & Butler, A. E. (2025). The Role of Osteoprotegerin in Breast Cancer: Genetic Variations, Tumorigenic Pathways, and Therapeutic Potential. *Cancers*, 17(3), 337.

Raskh, S. (2020). The importance and role of calcium on the growth and development of children and its complications. *International Journal for Research in Applied Sciences and Biotechnology (IJRASB)*, 7(6), 162-167.

Ratajczak, A. E., Rychter, A. M., Zawada, A., Dobrowolska, A., & Krela-Kaźmierczak, I. (2021). Do only calcium and vitamin D matter? Micronutrients in the diet of inflammatory bowel diseases patients and the risk of osteoporosis. *Nutrients*, *13*(2), 525.

Rawlinson SC, Boyde A, Davis GR, Howell PG, Hughes FJ, Kingsmill VJ.(2009). Ovariectomy vs. hypofunction: Their effects on rat mandibular bone. *J Dent Res*.;88(7):615-20.

Ren, M., Ahmed, A. F., Li, M., Li, M., Yan, Z., & Wang, J. (2024). A review: The mechanism of plant-derived polysaccharides on osteoblasts and osteoclasts. *Journal of Future Foods*, 4(3), 183-192.

Ribeiro, L. B., Machado, P. G., Reis-Canaan, J. C., Oliveira Júnior, I. M. D., Bertolini, N. O., Macari, S., ... & Pereira, L. J. (2025). Effects of aerobic and resistance training on bone, muscle hypertrophy and inflammation in OVX mice. *Climacteric*, 1-8.

Rishabh, Rohilla, M., Bansal, S., Bansal, N., Chauhan, S., Sharma, S., ... & Gupta, S. (2024). Estrogen signalling and Alzheimer's disease: Decoding molecular mechanisms for therapeutic breakthrough. *European Journal of Neuroscience*, *60*(1), 3466-3490.

Robling, A. G., & Bonewald, L. F. (2020). The osteocyte: new insights. *Annual review of physiology*, *82*(1), 485-506.

Rodan, S. B., & Duong, L. T. (2008). Cathepsin K-A new molecular target for osteoporosis. *IBMS BoneKEy*, 5, 16.

Rollerova, E., & Urbancikova, M. (2000). Intracellular estrogen receptors, their characterization and function. *Endocr Regul*, *34*(4), 203-218.

Sabe, H., Yahara, Y., & Ishii, M. (2024). Cell fusion dynamics: mechanisms of multinucleation in osteoclasts and macrophages. *Inflammation and Regeneration*, 44(1), 49.

Sachdeva, S., Saluja, H., Mahajani, M. J., Tripathi, V., Deo, A., & Mani, A. (2024). Periodontal medicine: The bidirectional relationship between osteoporosis and periodontal health. *Journal of Cellular Biotechnology*, *10*(2), 139-157.

Sadek, K. M., El Moshy, S., Radwan, I. A., Rady, D., Abbass, M. M., El-Rashidy, A. A., ... & Fawzy El-Sayed, K. M. (2023). Molecular basis beyond interrelated bone resorption/regeneration in periodontal diseases: a concise review. *International Journal of Molecular Sciences*, *24*(5), 4599.

Sadiq, N. M., & Tadi, P. (2023). Physiology, pituitary hormones. *In StatPearls. StatPearls Publishing.*

Saha, J. K., Xia, J., Grondin, J. M., Engle, S. K., & Jakubowski, J. A. (2005). Acute hyperglycemia induced by ketamine/xylazine anesthesia in rats: mechanisms and implications for preclinical models. *Experimental Biology and Medicine*, 230(10), 777-784.

Sale, J. E., Beaton, D. E., Sujic, R., & Bogoch, E. R. (2010). 'If it was osteoporosis, I would have really hurt myself.'Ambiguity about osteoporosis and osteoporosis care despite a screening programme to educate fragility fracture patients. *Journal of evaluation in clinical practice*, *16*(3), 590-596.

Sandor, L. F., Ragacs, R., & Gyori, D. S. (2023). Local effects of steroid hormones within the bone microenvironment. *International Journal of Molecular Sciences*, 24(24), 17482.

Saxena, Y., Routh, S., & Mukhopadhaya, A. (2021). Immunoporosis: role of innate immune cells in osteoporosis. *Frontiers in immunology*, *12*, 687037.

Schurman, C. A., Verbruggen, S. W., & Alliston, T. (2021). Disrupted osteocyte connectivity and pericellular fluid flow in bone with aging and defective TGF-β signaling. *Proceedings of the National Academy of Sciences*, *118*(25), e2023999118.

Scobie, G. A., Macpherson, S., Millar, M. R., Groome, N. P., Romana, P. G., & Saunders, P. T. (2002). Human oestrogen receptors: differential expression of ERalpha and beta and the identification of ERbeta variants. *Steroids*, *67*(12), 985-992.

Seijo, M., Bonanno, M. N., Bryk, G., Zeni Coronel, M. E., Pita Martin de Portela, M. L., & Zeni, S. N. (2022). Does vitamin D insufficiency influence prebiotic effect on calcium absorption and bone retention?. *Calcified Tissue International*, *111*(3), 300-312.

Shaker, J. L., & Deftos, L. (2023). Calcium and phosphate homeostasis.

Shapiro, I. M., & Landis, W. J. (2024). Form and Function of Tissues which Undergo Mineralization. In Mechanisms of Mineralization of Vertebrate Skeletal and Dental Tissues (pp. 71-130). Cham: *Springer International Publishing*.

Shariati, K., Bedar, M., Huang, K. X., Moghadam, S., Mirzaie, S., LaGuardia, J. S & Lee, J. C. (2025). Biomaterial Cues for Regulation of Osteoclast Differentiation and Function in Bone Regeneration. *Advanced Therapeutics*, 8(1), 2400296.

Sharifi, A. (2023). Targeted Peri-implant Crevicular Fluid Biomarkers in Osteoporotic Patients Receiving a Dental Implant (Doctoral dissertation, Queen Mary University of London).

Shi, V., & Morgan, E. F. (2024). Estrogen and estrogen receptors mediate the mechanobiology of bone disease and repair. *Bone*, 117220.

Simersky, R., Swaczynova, J., Morris, D. A., Franek, M., & Strnad, M. (2007). Development of an ELISA-based kit for the on-farm determination of progesterone in milk. *VETERINARNI MEDICINA-PRAHA*-, 52(1), 19.

Sinha, S., Sharan, A., Sinha, S., & SINHA, S. (2022). Anti-Mullerian hormone as a marker of ovarian reserve and function. *Cureus*, 14(9).

Sobacchi, C., Menale, C., Crisafulli, L., & Ficara, F. (2025). Role of RANKL signaling in bone homeostasis. *Physiology*, 40(1), 46-66.

Sobh, M. M., Abdalbary, M., Elnagar, S., Nagy, E., Elshabrawy, N., Abdelsalam, M., ... & El-Husseini, A. (2022). Secondary osteoporosis and metabolic bone diseases. *Journal of Clinical Medicine*, 11(9), 2382.

Sochocka, M., Karska, J., Pszczołowska, M., Ochnik, M., Fułek, M., Fułek, K., ... & Leszek, J. (2023). Cognitive decline in early and premature menopause. *International journal of molecular sciences*, *24*(7), 6566.

Spaziani, M., Carlomagno, F., Tenuta, M., Sesti, F., Angelini, F., Bonaventura, I & Pozza, C. (2023). Extra-gonadal and non-canonical effects of FSH in males. *Pharmaceuticals*, 16(6), 813.

Šromová, V., Sobola, D., & Kaspar, P. (2023). A brief review of bone cell function and importance. *Cells*, 12(21), 2576.

Stanczyk, F. Z. (2024). Metabolism of endogenous and exogenous estrogens in women. *The Journal of Steroid Biochemistry and Molecular Biology*, 106539.

Steppe, L., Krüger, B. T., Tschaffon, M. E. A., Fischer, V., Tuckermann, J., Ignatius, A., & Haffner-Luntzer, M. (2021). Estrogen receptor α signaling in osteoblasts is required for mechanotransduction in bone fracture healing. *Frontiers in Bioengineering and Biotechnology*, 9, 782355.

Storbeck KH, Schiffer L, Baranowski ES, Chortis ES, Prete A, Barnard L, et al.(2019). Steroid metabolome analysis in disorders of adrenal steroid biosynthesis and metabolism. *Endocr Rev* ;40:1605-25.

Streicher C., Heyny A., Andrukhova O., Haigl B., Slavic S., Schuler C., et al. (2017). Estrogen regulates bone turnover by targeting RANKL expression in bone lining cells. *Sci. Rep*. 7, 1–14. doi:10.1038/s41598-017-06614-0

Suliburska, J., Wawrzyniak, N., Gramza-Michałowska, A., & Kurzawa, P. (2023). Calcium-deficit diet improves iron content in ovariectomized rats. *Biological Trace Element Research, 201*(10), 4806-4811.

Sun, S., Karsdal, M. A., Bay-Jensen, A. C., Sørensen, M. G., Zheng, Q., Dziegiel, M. H., ... & Henriksen, K. (2013). The development and characterization of an ELISA specifically detecting the active form of cathepsin K. *Clinical biochemistry*, 46(15), 1601-1606.

Sun, Y., Li, J., Xie, X., Gu, F., Sui, Z., Zhang, K., & Yu, T. (2021). Macrophage-osteoclast associations: origin, polarization, and subgroups. *Frontiers in immunology*, *12*, 778078.

Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). Bancroft's theory and practice of histological techniques E-Book. *Elsevier health sciences.*

Szalay, F., Hegedus, D., Lakatos, P. L., Tornai, I., Bajnok, E., Dunkel, K., & Lakatos, P. (2003). High serum osteoprotegerin and low RANKL in primary biliary cirrhosis. *Journal of hepatology*, 38(4), 395-400.

Takegahara, N., Kim, H., & Choi, Y. (2022). RANKL biology. *Bone*, 159, 116353.

Tanaka, S. (2019). Molecular understanding of pharmacological treatment of osteoporosis. *EFORT Open Rev*, 4(4): 158-164. DOI: 10.1302/2058-5241-4-180018

Tang, G., Feng, L., Pei, Y., Gu, Z., Chen, T., & Feng, Z. (2023). Low BMI, blood calcium and vitamin D, kyphosis time, and outdoor activity time are independent risk factors for osteoporosis in postmenopausal women. *Frontiers in Endocrinology*, 14, 1154927.

Taskozhina, G., Batyrova, G., Umarova, G., Issanguzhina, Z., & Kereyeva, N. (2024). The manganese–bone connection: investigating the role of manganese in bone health. *Journal of Clinical Medicine*, *13*(16), 4679.

Tedjawirja, V. N., Nieuwdorp, M., Yeung, K. K., Balm, R., & de Waard, V. (2021). A novel hypothesis: A role for follicle stimulating hormone in abdominal aortic aneurysm development in postmenopausal women. *Frontiers in Endocrinology*, 12, 726107**.**

Tiberi, J., Cesarini, V., Stefanelli, R., Canterini, S., Fiorenza, M. T., & La Rosa, P. (2023). Sex differences in antioxidant defence and the regulation of redox homeostasis in physiology and pathology. *Mechanisms of ageing and development*, 211, 111802.

Tirado-Garibay, A. C., Falcón-Ruiz, E. A., Ochoa-Zarzosa, A., & López-Meza, J. E. (2023). GPER: an estrogen receptor key in metastasis and tumoral microenvironments. *International journal of molecular sciences*, 24(19), 14993.

Tokatli, M. R., Sisti, L. G., Marziali, E., Nachira, L., Rossi, M. F., Amantea, C., & Malorni, W. (2022). Hormones and sex-specific medicine in human physiopathology. *Biomolecules*, 12(3), 413.

Tunheim, E. G. (2022). Role of hormones in bone formation and resorption: A literature review [How hormones; adiponectin, angiotensin, cortisol, erythropoietin, insulin, parathyroid hormone, oxytocin, sex hormones, affect bone remodeling].

Uda, Y. (2022). The Role of Osteocytes in Mechanical Unloading and Age-Induced Osteopenia (*Doctoral dissertation, Boston University*).

Uenaka, M., Yamashita, E., Kikuta, J., Morimoto, A., Ao, T., Mizuno, H., ... & Ishii, M. (2022). Osteoblast-derived vesicles induce a switch from bone-formation to bone-resorption in vivo. *Nature Communications*, 13(1), 1066.

Ullah, R., Husain, S., Mohammed, F., & Khurram, S. A. (2021). Alveolar Bone. *An Illustrated Guide to Oral Histology*, 99-121.

Umur, E., Bulut, S. B., Yiğit, P., Bayrak, E., Arkan, Y., Arslan, F., ... & Ayan, B. (2024). Exploring the role of hormones and cytokines in osteoporosis development. *Biomedicines*, *12*(8), 1830.

Upadhyay, R. K. (2023). High cholesterol disorders, myocardial infarction and its therapeutics. *World Journal of Cardiovascular Diseases*, 13(8), 433-469.

Usgu, G., & Usgu, S. (2023). The bone and joint structure. In Functional Exercise Anatomy and Physiology for Physiotherapists (pp. 53-75). Cham: *Springer International Publishing*.

van Driel, M., & van Leeuwen, J. P. (2023). Vitamin D and bone: a story of endocrine and auto/paracrine action in osteoblasts. *Nutrients*, 15(3), 480.

Wang, J., Zhang, Y., Cao, J., Wang, Y., Anwar, N., Zhang, Z., ... & Wang, X. (2023). The role of autophagy in bone metabolism and clinical significance. *Autophagy*, 19(9), 2409-2427.

Wang, L. T., Chen, L. R., & Chen, K. H. (2023). Hormone-related and drug-induced osteoporosis: a cellular and molecular overview. *International Journal of Molecular Sciences*, 24(6), 5814.

Wang, L., Huang, X., Qin, J., Qi, B., Sun, C., Guo, X., ... & Zhang, Y. (2024). The role of traditional Chinese medicines in the treatment of osteoporosis. *The American Journal of Chinese Medicine*, 52(04), 949-986.

Wang, S., Tang, C., Chen, J., Tang, H., Zhang, L., & Tang, G. (2022). Changes in bone marrow fatty acids early after ovariectomy-induced osteoporosis in rats and potential functions. *Metabolites*, 13(1), 36.

Wang, Z., Wu, J., Li, L., Wang, K., Wu, X., Chen, H., ... & Pan, Z. (2023). Eicosapentaenoic acid supplementation modulates the osteoblast/osteoclast balance in inflammatory environments and protects against estrogen deficiency-induced bone loss in mice. *Clinical Nutrition*, 42(9), 1715-1727.

Wawrzyniak, A., & Balawender, K. (2022). Structural and metabolic changes in bone. *Animals*, 12(15), 1946.

Wend K, Wend P, Krum SA. (2012). Tissue-Specific Effects of Loss of Estrogen during Menopause and Aging. *Front Endocrinol (Lausanne)*;3:19.

Williams C, et al. (2008). A genome-wide study of the repressive effects of estrogen receptor beta on estrogen receptor alpha signaling in breast cancer cells. *Oncogene*;27(7):1019–32. doi: 10.1038/sj.onc.1210712.

Wu, Z., Xiao, C., Wang, J., Zhou, M., You, F., & Li, X. (2024). 17β-estradiol in colorectal cancer: friend or foe?. *Cell Communication and Signaling*, 22(1), 367.

Xie F., C.F. Wu, Y. Zhang, et al. (2005):Increase in bone mass and bone strength by Sambucus williamsii HANCE in ovareictomized rats. *Biol. Pharm. Bull*. 28(10): 1879-1885 .

Xie, Z., Zhao, M., Yan, C., Kong, W., Lan, F., Narengaowa, ... & Ni, J. (2023). Cathepsin B in programmed cell death machinery: mechanisms of execution and regulatory pathways. *Cell Death & Disease*, 14(4), 255.

Xu, J., Yu, L., Liu, F., Wan, L., & Deng, Z. (2023). The effect of cytokines on osteoblasts and osteoclasts in bone remodeling in osteoporosis: a review. *Frontiers in immunology*, 14, 1222129.

Xu, Z. S., Dai, F., Chen, J., Lv, M., Cheng, J. W., Zhang, X. M., & Lin, B. W. (2018). Experimental research into the potential therapeutic effect of GYY4137 on Ovariectomy-induced osteoporosis. *Cellular & Molecular Biology Letters*, 23, 1-13.

Yang, J. L., Hodara, E., Sriprasert, I., Shoupe, D., & Stanczyk, F. Z. (2024). Estrogen deficiency in the menopause and the role of hormone therapy: integrating the findings of basic science research with clinical trials. *Menopause*, 10-1097.

Yang, J., Shi, J., Jiang, L., Zhang, S., Wei, F., Guo, Z., ... & Liu, X. (2023). Co-occurrence network in core microorganisms driving the transformation of phosphorous fractionations during phosphorus recovery product used as soil fertilizer. *Science of the Total Environment*, *871*, 162081.

Yang, L., & Toriola, A. T. (2024, September). Menopausal hormone therapy use among postmenopausal women. In JAMA Health Forum (Vol. 5, No. 9, pp. e243128-e243128). *American Medical Association*.

Yang, L., Zhou, H., Wang, Q., Wang, Z., Bai, L., Wei, J., ... & Liang, T. (2025). Prevention of osteoporosis in ovariectomized mice with yak caseinate calcium. *Journal of Dairy Science*.

Ye X, Zhang P, Xue S, Xu Y, Tan J, Liu G.(2014). Adipose-derived stem cells alleviate osteoporosis by enhancing osteogenesis and inhibiting adipogenesis in a rabbit model. *Cytotherapy* ;16:1643–1655. doi: 10.1016/j.jcyt.2014.07.009.

Ye, S., Shi, L., & Zhang, Z. (2023). Effect of insulin resistance on gonadotropin and bone mineral density in nondiabetic postmenopausal women. *Frontiers in endocrinology*, 14, 1235102.

Yoon, S., & Kim, Y. K. (2024). Endocrinological Treatment Targets for Depressive Disorder. Recent Advances and Challenges in the Treatment of Major *Depressive Disorder*, 3-25.

Yu, B., & Wang, C. Y. (2022). Osteoporosis and periodontal diseases–An update on their association and mechanistic links. *Periodontology 2000*, *89*(1), 99-113.

Yu, K., Huang, Z. Y., Xu, X. L., Li, J., Fu, X. W., & Deng, S. L. (2022). Estrogen receptor function: impact on the human endometrium. *Frontiers in endocrinology*, 13, 827724.

Yuan FengLai, Y. F., Xu RuiSheng, X. R., Jiang DongLin, J. D., He XingLong, H. X., Su Qiang, S. Q., Jin Chen, J. C., & Li Xia, L. X. (2015). Leonurine hydrochloride inhibits osteoclastogenesis and prevents osteoporosis associated with estrogen deficiency by inhibiting the NF-κB and PI3K/Akt signaling pathways.

Zaidi M , Lizneva D, Kim SM, et al.(2018). FSH, bone mass, body fat, and biological aging. *Endocrinology* ;159(10):3503-3514

Zhang, Y. W., Cao, M. M., Li, Y. J., Lu, P. P., Dai, G. C., Zhang, M., ... & Rui, Y. F. (2022). Fecal microbiota transplantation ameliorates bone loss in mice with ovariectomy-induced osteoporosis via modulating gut microbiota and metabolic function. *Journal of orthopaedic translation*, 37, 46-60.

Zhang, Y., Liang, J., Liu, P., Wang, Q., Liu, L., & Zhao, H. (2022). The RANK/RANKL/OPG system and tumor bone metastasis: Potential mechanisms and therapeutic strategies. *Frontiers in Endocrinology*, 13, 1063815.

Zhou, Z. S. (2021). Follicle-stimulating hormone (FSH) does not regulate bone via the FSH receptor in osteoclasts. *McGill University (Canada).*

Zhu, X., Xi, H., Li, Z., & Wang, X. (2023). Trends in surgical approaches and adnexal surgeries during hysterectomies for benign diseases between 2015–2021. *Frontiers in Surgery*, *10*, 1068776.

Appendix

Appendix I

Detection of serum estrogen

The hormone concentration was measured by following the steps attached to the estrogen test kit, which consists of the following materials:

1-(STR) Strips for Estradiol hormone, which are used with 10 wells covered with a thin plate marked with the Estradiol symbol for the purpose of distinguishing them.

2-(SPRs) Solid phase receptacles are ready to use and are exactly like the (Tip)used in the micropipette, except that they are marked on their wide end with the symbol Estradiol for the purpose of identifying them as well.

3-(C1) Estradiol control (S1) Estradiol calibrator(R1) Estradiol dilutant These materials are ready to use.

4-MIe card is a ready-made card that contains the main encrypted information for the calibration data used in the evaluation of the estrogen concentration test.

5-The principle of measuring estrogen concentration is based on enzyme immunoassay sandwich methods with a final fluorescent detection.

Working methods

The method of work includes the following steps:

1-Place the M/e card after the test in the designated place in the Minividas device so that it can automatically recognize the test. Without it, the device

cannot display the result and then print it.

2- One STR & SPR strip was used for each of the standard and control serum and placed in the designated place in the device.

3- 100 µL serum samples were drawn and placed in the respective holes on the (STR) Strip. This is done for the standard and control serum.

4-The device's specific steps in the device's MANUAL were followed, and the device began the calibration process automatically, which takes 45 minutes.

5- After calibration and printing the results, the STR and SPR strips were extracted from the device and placed in a special container, as these strips are used only once.

Appendix II

Detection of serum progesterone

The hormone concentration was measured by following the steps attached to the progesterone test kit, which consists of the following materials:

1-(STR) Strips for progesterone hormone, which are used with 10 wells covered with a thin plate marked with the progesterone symbol for the purpose of distinguishing them.

2- (SPRs) Solid phase receptacles are ready to use and are exactly like the (Tip) used in the micropipette, except that they are marked on their wide end with the symbol progesterone for the purpose of identifying them as well.

3-(C1) progesterone control(S1) progesterone calibrator(R1) progesterone dilutant These materials are ready to use.

4-MIe card is a ready-made card that contains the main encrypted information for the calibration data used in the evaluation of the progesterone concentration test.

5-The principle of measuring progesterone concentration is based on enzyme immunoassay sandwich methods with a final fluorescent detection.

Working methods

The method of work includes the following steps:

1-Place the M/e card after the test in the designated place in the Minividas device so that it can automatically recognize the test. Without it, the device cannot display the result and then print it.

2- One STR & SPR strip was used for each of the standard and control serum and placed in the designated place in the device.

3- 100 µL serum samples were drawn and placed in the respective holes on the (STR) Strip. This is done for the standard and control serum.

4-The device's specific steps in the device's MANUAL were followed, and the device began the calibration process automatically, which takes 45 minutes.

5- After calibration and printing the results, the STR and SPR strips were extracted from the device and placed in a special container, as these strips are used only once.

Appendix III

Detection of serum LH

The hormone concentration was measured by following the steps attached to the LH test kit, which consists of the following materials:

1-(STR) Strips for LH hormone, which are used with 10 wells covered with a thin plate

marked with the LH symbol for the purpose of distinguishing them.

2-(SPRs) Solid phase receptacles are ready to use and are exactly like the(Tip)used in the micropipette, except that they are marked on their wide end with the symbol LH for the purpose of identifying them as well.

3-(C1) LH control (S1) LH calibrator (R1) LH dilutant These materials are ready to use.

4-MIe card is a ready-made card that contains the main encrypted information for the calibration data used in the evaluation of the LH concentration test.

5-The principle of measuring LH concentration is based on enzyme immunoassay sandwich methods with a final fluorescent detection.

Working methods

The method of work includes the following steps:

1-Place the M/e card after the test in the designated place in the Minividas device so that it can automatically recognize the test. Without it, the device cannot display the result and then print it.

2- One STR & SPR strip was used for each of the standard and control serum and placed in the designated place in the device.

3- 100 µL serum samples were drawn and placed in the respective holes on the (STR) Strip. This is done for the standard and control serum.

4-The device's specific steps in the device's MANUAL were followed, and the device began the calibration process automatically, which takes 45 minutes.

5- After calibration and printing the results, the STR and SPR strips were extracted

from the device and placed in a special container, as these strips are used only once.

Appendix IV

Detection of serum FSH

The hormone concentration was measured by following the steps attached to the FSH test kit, which consists of the following materials:

1-(STR) Strips for FSH hormone, which are used with 10 wells covered with a thin plate marked with the FSH symbol for the purpose of distinguishing them.

2- (SPRs) Solid phase receptacles are ready to use and are exactly like the (Tip) used in the micropipette, except that they are marked on their wide end with the symbol FSH for the purpose of identifying them as well.

3-(C1) FSH control(S1) FSH calibrator(R1) FSH dilutant These materials are ready to use.

4-MIe card is a ready-made card that contains the main encrypted information for the calibration data used in the evaluation of the FSH concentration test.

5-The principle of measuring FSH concentration is based on enzyme immunoassay sandwich methods with a final fluorescent detection.

Working methods

The method of work includes the following steps:

1-Place the M/e card after the test in the designated place in the Minividas device so that it can automatically recognize the test. Without it, the device cannot display the result and then print it.

2- One STR & SPR strip was used for each of the standard and control serum and placed in the designated place in the device.

3- 100 µL serum samples were drawn and placed in the respective holes on the (STR) Strip. This is done for the standard and control serum.

4-The device's specific steps in the device's MANUAL were followed, and the device began the calibration process automatically, which takes 45 minutes.

5- After calibration and printing the results, the STR and SPR strips were extracted from the device and placed in a special container, as these strips are used only once.

Appendix V

Detection of serum Calcium(Ca)

Total Calcium exists in 3 physiochemical states in plasma, of which approximately 50 % is free or ionised calcium, 40 % is bound to plasma proteins, and 10 % are bound with small anions.The level of serum calcium may be affected by intestinal malabsorption, by alterations in plasma proteins level, especially albumin, which should be measured concurrently with calcium. Hypercalcemia is found in hyperparathyroïdism, multiple myeloma, bone and parathyroïdal neoplasms and in states with bones demineralisation.Hypocalcemia is encountered in hypoparathyroïdism and in several cases of necrosis and acute pancreatitis.

PRINCIPLE

Moorehead and Briggs derived CPC (O-Cresol Phtalein Complexone) method allows to determinate total Calcium concentration in serum, plasma or urines.In alkaline solution CPC reacts with calcium to form a dark-red coloured complex which absorbance measured at 570 nm is proportional to the amount of calcium in the

specimen

PROCEDURE

Detailed Kenza 240TX procedure is available on request Wavelength: 570 nm Temperature: 37°C.Temperature should be held constant as the absorbance of the dye is temperature sensitive.

	Automated Analyzer	**Manual procedure**
Reagents	**120 µL R1** **120 µL R2**	**WR :1000 µL**
Standard, Controls, Specimen	**6 µL**	**25 µL**
Mix well. Incubate for 5 minutes at room temperature. **Read absorbance at 570 nm (550-590) against reagent blank.** **The coloration is stable for 1 hour away from light**		

CALCULATION

Calculate the result as follows:

Result = $\frac{\text{Abs(Assay)}}{\text{Abs(Standard}}$ x Standard concentration

Appendix VI

Detection of serum RANK

Reagent Preparation:

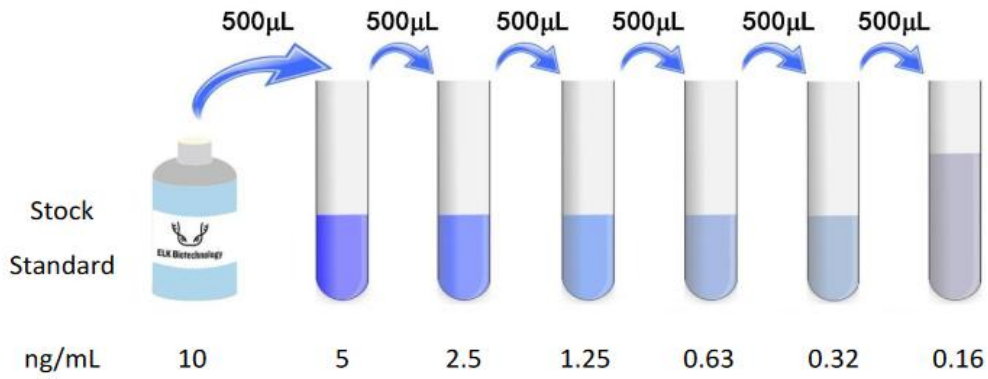
1. Bring all kit components and samples to room temperature (18-25°C) before use.Make sure all components are dissolved and mixed well before using the kit.

2. If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.

3. Dilute the 25× Wash Buffer into 1× Wash Buffer with double-distilled Water.

4. Standard Working Solution - Centrifuge the Standard at 1000 × g for 1 minute. Reconstitute the Standard with 1.0 mL of Standard Diluent Buffer, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the Standard in the stock solution is 10 ng/mL. Pleas prepare 7 tubes containing 0.5 mL Standard Diluent Buffer and use the Di luted Standard to produce a double dilution series according to the picture shown below. To mix each tube thoroughly before the next transfer, pipette the solution up and down several times. Set up 7 points of Diluted Standard such as 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.32 ng/mL, 0.16 ng/mL, and the last EP tubes with Standard Diluent is the Blank as 0 ng/mL. In order to guarantee the experimental results validity, please use the new Standard Solution for each experiment. When diluting the Standard from high concentration to low concentration,

replace the pipette tip for each dilution. Note: the last tube is regarded as a Blank and do not pipette solution into it from the former tube.



5. Biotinylated Antibody and 1× Streptavidin-HRP - Briefly spor centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute them to working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.

Samples Preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

3. Please predict the concentration before assaying. If values for these are not within the range of the Standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Procedure

1. Determine wells for Diluted Standard, Blank and Sample. Prepare 7

wells for Standard, 1 well for Blank. Add 100 μL each of Standard Working Solution (please refer to Reagent Preparation), or 100 μL of samples into the appropriate wells. Cover with the Plate Cover. Incubate for 80 minutes at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Pour out the liquid of each well. Aspirate the solution and wash with 200 μL of 1× Wash Solution to each well and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent

paper. Notes: (a) When adding Washing Solution, the pipette tip should not touch the wall of the wells to a void contamination. (b) Pay attention to pouring the washing liquid directly to ensure that the washing liquid does not contaminate other wells.

3. Add 100 μL of Biotinylated Antibody Working Solution to each well, cover the wells with the Plate http://www.elkbiotech.com elkbio@elkbiotech.com10 Cover and incubate for 50 minutes at 37°C.

4. Repeat the aspiration,wash process for total 3 times as conducted in step 2.

5. Add 100 μL of Streptavidin-HRP Working Solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.

6. Repeat the aspiration, wash process for total 5 times as conducted in

step 2.

7. Add 90 μL of TMB Substrate Solution to each well. Cover with a new Plate Cover. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes) in the dark. The liquid will turn blue by the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement.

8. Add 50 μL of Stop Reagent to each well. The liquid will turn yellow by the addition of Stop Reagent. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution.

9. Wipe off any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.

Calculation of Results

Average the duplicate readings for each Standard, Control, and Samples and subtract the average zero Standard optical density. Construct a Standard curve with the rabbit RANK concentration on the y-axis and absorbance on the x-axis, and draw a best fit curve through the points on the graph. If samples have been diluted, the concentration read from the Standard curve must be multiplied by the dilution.

Appendix VII

Detection of serum sRANKL

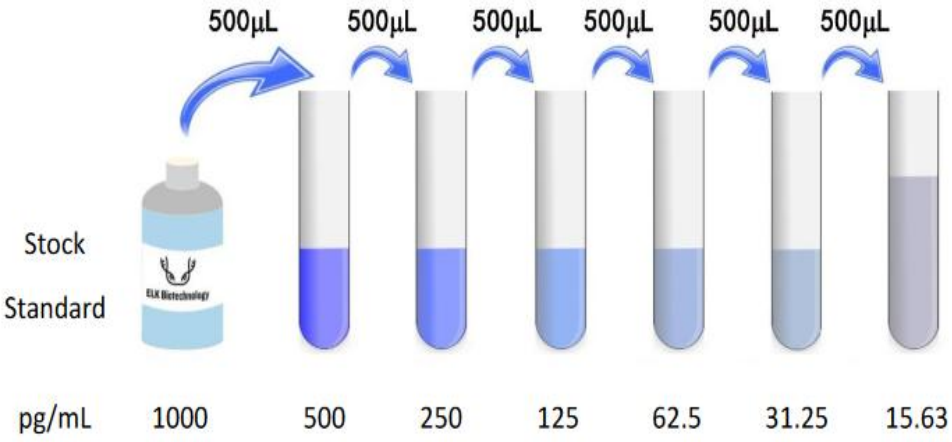
Reagent Preparation:

1. Bring all kit components and samples to room temperature (18-25°C) before use.Make sure all components are dissolved and mixed well before using the kit.

2. If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.

3. Dilute the 25× Wash Buffer into 1× Wash Buffer with double-distilled Water.

4. Standard Working Solution - Centrifuge the Standard at 1000 × g for 1 minute. Reconstitute the Standard with 1.0 mL of Standard Diluent Buffer, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the Standard in the stock solution is 10 ng/mL. Pleas prepare 7 tubes containing 0.5 mL Standard Diluent Buffer and use the Diluted Standard to produce a double dilution series according to the picture shown below. To mix each tube thoroughly before the next transfer, pipette the solution up and down several times. Set up 7 points of Diluted Standard such as 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.32 ng/mL, 0.16 ng/mL, and the last EP tubes with Standard Diluent is the Blank as 0 ng/mL. In order to guarantee the experimental results validity, please use the new Standard Solution for each experiment. When diluting the Standard from high concentration to low concentration, replace the pipette tip for each dilution. Note: the last tube is regarded as a Blank and do not pipette solution into it from the former tube.



5- Biotinylated Antibody and 1× Streptavidin-HRP - Briefly spor centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute them to working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.

6. TMB Substrate Solution - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Samples Preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

3. Please predict the concentration before assaying. If values for these are not within the range of the Standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Procedure

1. Determine wells for Diluted Standard, Blank and Sample. Prepare 7 wells for Standard, 1 well for Blank. Add 100 μL each of Standard Working Solution (please refer to Reagent Preparation), or 100 μL of samples into the appropriate wells. Cover with the Plate Cover. Incubate for 80 minutes at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Pour out the liquid of each well. Aspirate the solution and wash with 200 μL of 1× Wash Solution to each well and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent

paper. Notes: (a) When adding Washing Solution, the pipette tip should not touch the wall of the wells to a void contamination. (b) Pay attention to pouring the washing liquid directly to ensure that the washing liquid does not contaminate other wells.

3. Add 100 μL of Biotinylated Antibody Working Solution to each well, cover the wells with the Plate http://www.elkbiotech.com elkbio@elkbiotech.com10 Cover and incubate for 50 minutes at 37°C.

4. Repeat the aspiration,wash process for total 3 times as conducted in step 2.

5. Add 100 μL of Streptavidin-HRP Working Solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.

6. Repeat the aspiration, wash process for total 5 times as conducted in step 2.

7. Add 90 μL of TMB Substrate Solution to each well. Cover with a new Plate Cover. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes) in the dark. The liquid will

turn blue by the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement.

8. Add 50 μL of Stop Reagent to each well. The liquid will turn yellow by the addition of Stop Reagent. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution.

9. Wipe off any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.

Calculation of Results

Average the duplicate readings for each Standard, Control, and Samples and subtract the average zero Standard optical density. Construct a Standard curve with the rabbit RANKL concentration on the y-axis and absorbance on the x-axis, and draw a best fit curve through the points on the graph. If samples have been diluted, the concentration read from the Standard curve must be multiplied by the dilution.

Appendix VIII

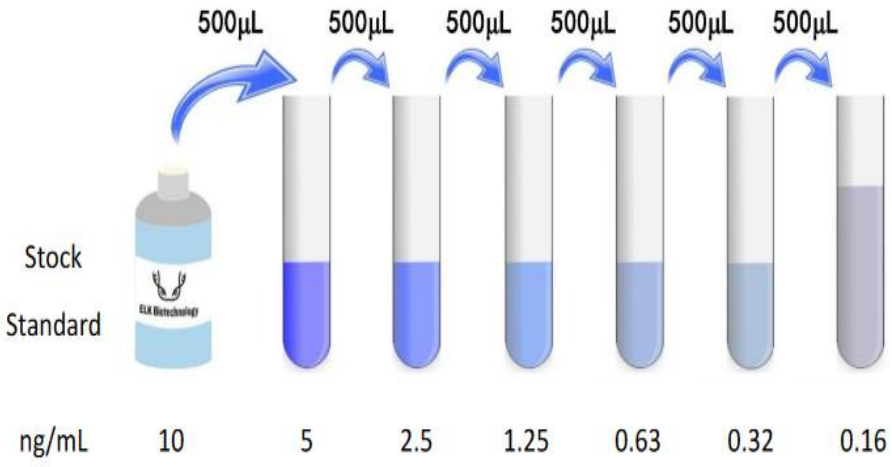
Detection of serum OPG

Reagent Preparation:

1. Bring all kit components and samples to room temperature (18-25°C) before use. Make sure all components are dissolved and mixed well before using the kit.
2. If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.

3. Dilute the 25× Wash Buffer into 1× Wash Buffer with double-distilled Water.

4. Standard Working Solution - Centrifuge the Standard at 1000 × g for 1 minute. Reconstitute the Standard with 1.0 mL of Standard Diluent Buffer, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the Standard in the stock solution is 10 ng/mL. Pleas prepare 7 tubes containing 0.5 mL Standard Diluent Buffer and use the Diluted Standard to produce a double dilution series according to the picture shown below. To mix each tube thoroughly before the next transfer, pipette the solution up and down several times. Set up 7 points of Diluted Standard such as 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.32 ng/mL, 0.16 ng/mL, and the last EP tubes with Standard Diluent is the Blank as 0 ng/mL. In order to guarantee the experimental results validity, please use the new Standard Solution for each experiment. When diluting the Standard from high concentration to low concentration, replace the pipette tip for each dilution. Note: the last tube is regarded as a Blank and do not pipette solution into it from the former tube.



5. Biotinylated Antibody and 1× Streptavidin-HRP - Briefly spor centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute them to working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.

Samples Preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

3. Please predict the concentration before assaying. If values for these are not within the range of the Standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Procedure

1. Determine wells for Diluted Standard, Blank and Sample. Prepare 7 wells for Standard, 1 well for Blank. Add 100 μL each of Standard Working Solution (please refer to Reagent Preparation), or 100 μL of samples into the appropriate wells. Cover with the Plate Cover. Incubate for 80 minutes at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Pour out the liquid of each well. Aspirate the solution and wash with 200 μL of 1× Wash Solution to each well and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent

paper. Notes: (a) When adding Washing Solution, the pipette tip should not touch the wall of the wells to a void contamination. (b) Pay attention to pouring the washing liquid directly to ensure that the washing liquid does not contaminate other wells.

3. Add 100 μL of Biotinylated Antibody Working Solution to each well, cover the wells with the Plate http://www.elkbiotech.com elkbio@elkbiotech.com10 Cover and incubate for 50 minutes at 37°C.

4. Repeat the aspiration,wash process for total 3 times as conducted in step 2.

5. Add 100 μL of Streptavidin-HRP Working Solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.

6. Repeat the aspiration, wash process for total 5 times as conducted in step 2.

7. Add 90 μL of TMB Substrate Solution to each well. Cover with a new Plate Cover. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes) in the dark. The liquid will turn blue by the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement.

8. Add 50 μL of Stop Reagent to each well. The liquid will turn yellow by the addition of Stop Reagent. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution.

9. Wipe off any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.

Calculation of Results

Average the duplicate readings for each Standard, Control, and Samples and subtract the average zero Standard optical density. Construct a Standard curve with the rabbit OPG concentration on the y-axis and absorbance on the x-axis, and draw a best fit curve through the points on the graph. If samples have been diluted, the concentration read from the Standard curve must be multiplied by the dilution.

Appendix IX

Detection of serum Cathepsin K

Reagent Preparation:

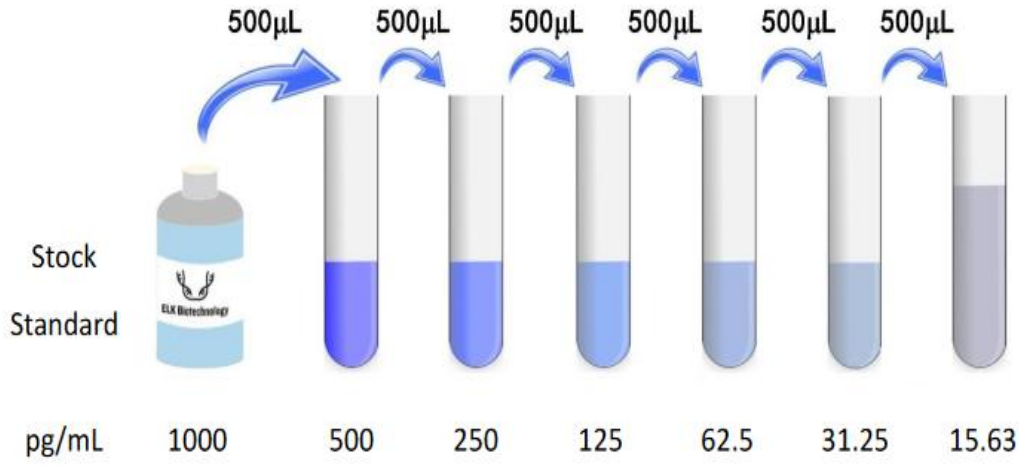
1. Bring all kit components and samples to room temperature (18-25°C) before use.Make sure all components are dissolved and mixed well before using the kit.

2. If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.

3. Dilute the 25× Wash Buffer into 1× Wash Buffer with double-distilled Water.

4. Standard Working Solution - Centrifuge the Standard at 1000 × g for 1 minute. Reconstitute the Standard with 1.0 mL of Standard Diluent Buffer, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the Standard in the stock solution is 10 ng/mL. Pleas prepare 7 tubes containing 0.5 mL Standard Diluent Buffer and use the Diluted Standard to produce a double dilution series according to the picture shown below. To mix each tube thoroughly before the next transfer, pipette the solution up and down several times. Set up 7 points of Diluted Standard such as 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.32 ng/mL, 0.16 ng/mL, and the last EP tubes with Standard Diluent is the Blank as 0 ng/mL. In order to guarantee the experimental results validity, please use the new Standard Solution for each experiment. When diluting the Standard from high concentration to low concentration,

replace the pipette tip for each dilution. Note: the last tube is regarded as a Blank and do not pipette solution into it from the former tube.



5- Biotinylated Antibody and 1× Streptavidin-HRP - Briefly spor centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute them to working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.

6. TMB Substrate Solution - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Samples Preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

3. Please predict the concentration before assaying. If values for these are

not within the range of the Standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Procedure

1. Determine wells for Diluted Standard, Blank and Sample. Prepare 7 wells for Standard, 1 well for Blank. Add 100 μL each of Standard Working Solution (please refer to Reagent Preparation), or 100 μL of samples into the appropriate wells. Cover with the Plate Cover. Incubate for 80 minutes at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Pour out the liquid of each well. Aspirate the solution and wash with 200 μL of 1× Wash Solution to each well and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent

paper. Notes: (a) When adding Washing Solution, the pipette tip should not touch the wall of the wells to a void contamination. (b) Pay attention to pouring the washing liquid directly to ensure that the washing liquid does not contaminate other wells.

3. Add 100 μL of Biotinylated Antibody Working Solution to each well, cover the wells with the Plate http://www.elkbiotech.com elkbio@elkbiotech.com10 Cover and incubate for 50 minutes at 37°C.

4. Repeat the aspiration,wash process for total 3 times as conducted in step

2.

5. Add 100 µL of Streptavidin-HRP Working Solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.

6. Repeat the aspiration, wash process for total 5 times as conducted in step 2.

7. Add 90 µL of TMB Substrate Solution to each well. Cover with a new Plate Cover. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes) in the dark. The liquid will turn blue by the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement.

8. Add 50 µL of Stop Reagent to each well. The liquid will turn yellow by the addition of Stop Reagent. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution.

9. Wipe off any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.

Calculation of Results

Average the duplicate readings for each Standard, Control, and Samples and subtract the average zero Standard optical density. Construct a Standard curve with the rabbit CTSK concentration on the y-axis and absorbance on the x-axis, and draw a best fit curve through the points on the graph. If samples have been diluted.

Appendix……

الخلاصة

الخلاصة:

تلعب هرمونات الإستروجين دورًا حاسمًا في عملية التمثيل الغذائي للعظام والحفاظ على كثافة المعادن في العظام. كان الهدف من الدراسة الحالية هو التحقيق في تأثير مستقبلات الإستروجين ألفا وبيتا مع مسار RANK وRANK-L وOPG في هشاشة العظام الناجمة عن استئصال المبيض لدى إناث الأرانب. تم تقسيم عشرين أرنبًا من إناث الأرانب البيضاء النيوزيلندية بعمر أربعة أشهر (وزنها 3-3.25 كجم) عشوائيًا إلى مجموعتين (10 لكل منهما): مجموعة ضابطة ومجموعة مستأصلة المبيض (.(OVXبعد ستة أسابيع من التجربة، خضعت الأرانب لتصوير شعاعي للفخذ. تم سحب عينات الدم للتحليل الكيميائي، بما في ذلك مستويات هرمونات المصل الإستروجين والبروجسترون والهرمون الملوتن ((LHوالهرمون المنبه للجريب ((FSHومستويات أملاح الكالسيوم ((Caفي المصل، والعلامات الحيوية للعظام RANK وRANKL و(OPG) و .Cathepsin kتم أخذ عينات من الفخذ أيضًا للفحص النسيجي واختبار التعبير الجيني لمستقبلات هرمون الاستروجين ألفا وبيتا. أظهرت النتائج انخفاضًا في مستويات هرمون الاستروجين والبروجسترون في مجموعة OVX مقارنةً بالمجموعة الضابطة وزيادة واضحة في مستويات الهرمون الملوتن ((LHوالهرمون المنبه للجريب ((FSH ومستوى الكالسيوم ((Caفي الأرانب المصابة بهشاشة العظام في OVX مقارنةً بالمجموعة الضابطة، بينما أظهر المصل زيادة واضحة في مستويات RANK وRANKL وCathepsin k في الأرانب المصابة بهشاشة العظام. ومع ذلك، أظهرت الدراسة أن OVX في هذه المجموعة انخفض بشكل ملحوظ مقارنةً بالمجموعة الضابطة. انخفضت كثافة العظام في مجموعة OVX بشكل ملحوظ، وفقًا للتصوير بالأشعة السينية مقارنةً بالمجموعة الضابطة. أظهر فحص التعبير الجيني لمستقبلات هرمون الاستروجين ألفا وبيتا في العظام انخفاضًا في التعبير الجيني لمستقبلات هرمون الاستروجين ألفا وزيادة في مستقبلات هرمون الاستروجين بيتا في مجموعة OVX مقارنةً بالمجموعة الضابطة. أظهر الفحص النسيجي لعظم الفخذ لدى الأرانب في مجموعة OVX إعادة امتصاص مع ترقق الصفائح العظمية ووجود العديد من الخلايا الناقضة للعظم مقارنة بالمجموعة الضابطة.

الخلاصة: وجدت الدراسة الحالية أن هشاشة العظام وتدهور الأنسجة والخلايا المكونة للعظام يتأثران بشكل مباشر بانخفاض هرمون الإستروجين. ويعني انخفاض تنظيم التعبير الجيني لمستقبلات الإستروجين ألفا فقدان أهم مسار يحافظ على التوازن بين تكوين العظام وهدمها، مما يؤدي إلى ضعف بنية العظام وزيادة خطر الإصابة بهشاشة العظام بعد استئصال المبيضين أو انقطاع الطمث. ويُعوّض هذا الفقد جزئيًا بمستقبلات الإستروجين بيتا، إلا أن تعويضها لا يكفي لتعويض الوظائف المحددة لمستقبلات الإستروجين ألفا.

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دراسة تأثير مستقبلات الاستروجين ألفا وبيتا مع مسارات RANK وRANK-L وOPG في هشاشة العظام الناجمة عن استئصال المبيض في الأرانب الإناث.

رسالة مقدمة إلى مجلس كلية الطب البيطري جامعة كربلاء

كجزء من متطلبات درجة الماجستير في الطب البيطري / الفسلجة

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هـ1447 **2025 م**