

Vaginal administration of progesterone in twin gestation: influence on bone turnover and oxidative stress

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ABSTRACT

Twin pregnancies are associated with significantly higher obstetric and maternal risks, including preterm birth, altered maternal metabolic demands, placental dysfunction, and increased oxidative stress. Progesterone plays a crucial role in the maintenance of pregnancy and has been widely investigated for the prevention of preterm birth, particularly through vaginal administration. Although the benefits and clinical outcomes of progesterone therapy in singleton pregnancies are relatively well established, its broader effects in twin pregnancies remain less clearly understood. Emerging evidence suggests that progesterone may influence maternal physiological processes beyond pregnancy maintenance, including bone turnover, calcium metabolism, inflammatory pathways, and oxidative stress, all of which undergo substantial changes during gestation and may be further amplified in multiple pregnancies. This review summarizes current evidence regarding the use of vaginal progesterone in twin pregnancies, with particular emphasis on its potential impact on bone metabolism and oxidative stress markers. A better understanding of these interactions may provide insight into the wider maternal implications of progesterone therapy and help guide future research and clinical management strategies in high-risk pregnancies.

Keywords: Twin pregnancy, Preterm birth, Bone remodelling, Oxidative stress, Progesterone, Vaginal Progesterone, Estrogen

Objectives:

- To review and analyse how vaginal progesterone affects maternal bone turnover and oxidative stress in twin pregnancies.
- To describe the changes in bone metabolism and oxidative stress during a normal pregnancy.
- To examine how these processes change in twin pregnancies.
- To evaluate how progesterone, particularly through vaginal administration, influences bone turnover.
- To assess the possible antioxidant and anti-inflammatory effects of progesterone in twin pregnancies.
- To identify gaps in current knowledge and suggest directions for future research.

INTRODUCTION:

Over the past twenty years, the global incidence of twin pregnancy has increased. Since 1980, The rate of multiple births has increased significantly; with an 88% increase in twin and 573% increase in triplet and higher order multiple births, including quadruplets and quintuplets. During this same period, the percentage of

births among women aged 40 years and older has increased more than threefold for singletons, sixfold for twins, and nearly twelvefold for triplet and higher-order multiple gestation.[25]

The prevalence of twin pregnancies has risen, largely due to increased maternal age at conception, wider use of assisted reproductive technology (ART), and changes in population diversity.[28]

Compared to singleton pregnancies, twin pregnancies are associated with increased risk of obstetric and perinatal complications such as preeclampsia, post-partum hemorrhage and pre term birth, gestational diabetes, miscarriage, which are associated with increased risk of maternal and perinatal mortality.[35] Additionally, the risk of preterm birth is usually higher in twin pregnancies which results in increased morbidity and mortality in twins. [33,34]

Infants born prematurely, particularly those born before 34 weeks, are at greater risk of having difficulties at birth and health problems during infancy including greater risks of dying during their first year. They are also more likely to experience long-term morbidity such as cerebral palsy, epilepsy, blindness or hearing loss. Preterm birth can therefore have lifelong impact for individuals and families.[26]

During pregnancy Progesterone plays a crucial role in maintaining uterine quiescence, and is thought to act by suppressing smooth muscle activity in the uterus which is essential for the continuation of gestation.

The most effective approach to managing preterm birth (PTB) is the prediction and prevention of its risks. Currently progesterone supplementation therapy is the most representative method of prevention of pre term birth in women with history of pre term birth and/or short cervical length (CL). [29,30] Although the exact role and mechanism of progesterone have not yet fully understood. Progesterone is known to exert estrogen antagonism by inhibiting estrogen receptors in uterine myometrial cells, blocking or down-regulating oxytocin receptors, and inhibiting prostaglandin synthesis and inflammation.

Beyond its obstetric role, progesterone is also involved in bone remodelling, oxidative stress metabolism. While its obstetric benefits have been extensively evaluated, its impact on maternal bone turnover and oxidative stress, particularly in twin gestations, remains poorly defined.[11] Understanding these effects is crucial, as pregnancy-related bone loss and oxidative stress may have long-term maternal health implications.

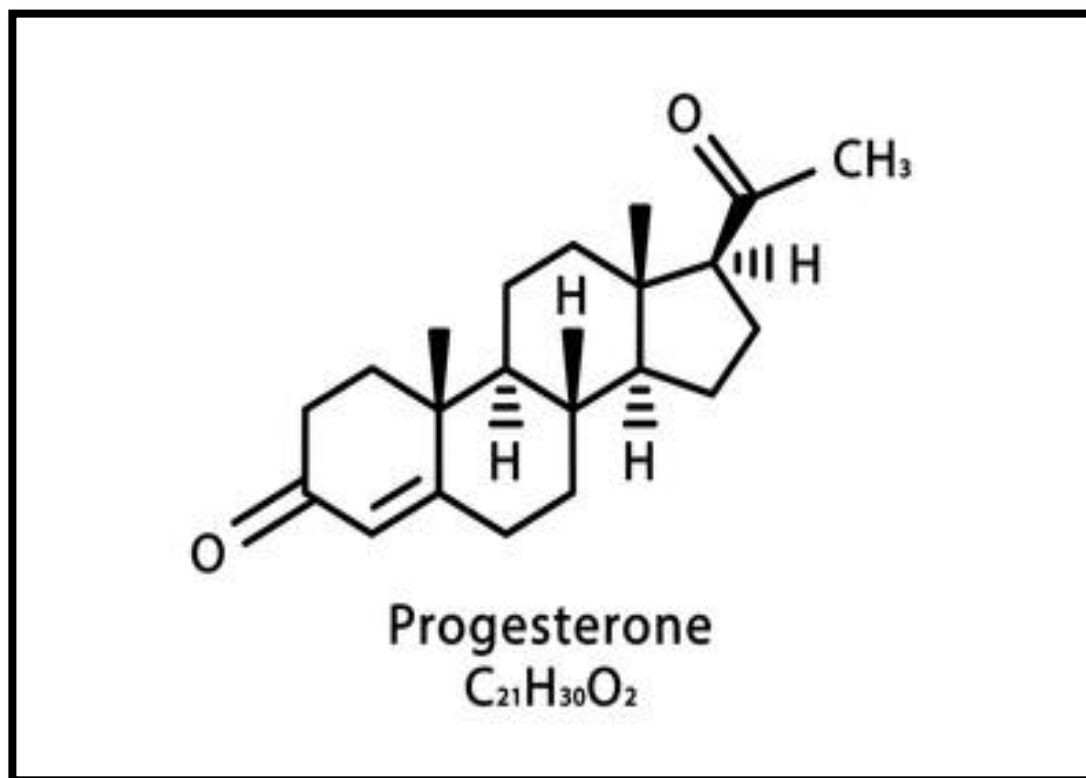


Fig 1: Biochemical structure of Progesterone

Progesterone is characterized by a four-ring cyclopentanoperhydrophenanthrene structure.

PROGESTERONE AND PREGNANCY PHYSIOLOGY:

Progesterone is an endogenous steroid hormone with 21 carbon atoms originating from cholesterol and regulates normal female reproductive functions. Progesterone is mainly produced by the adrenal cortex and the gonads, including the ovaries and testes. Progesterone is also secreted by the ovarian corpus luteum during the first ten weeks of pregnancy, followed by the placenta in the later phase of pregnancy.[36] The conversion of progesterone generation from the corpus luteum to the placenta generally occurs after week ten.

Biosynthesis of Progesterone:

The molecule progesterone is a derivative of cholesterol and has numerous functions in the human body, especially within the reproductive system. Its production happens through a series of enzyme reactions known as steroidogenesis, with cholesterol being the starting molecule.

The process starts when cholesterol is transported into the mitochondria. Inside the inner mitochondrial membrane, cholesterol is converted to pregnenolone by the enzyme cholesterol side-chain cleavage enzyme (P450_{scc}, also called CYP11A1). Pregnenolone is then changed into progesterone in the smooth endoplasmic reticulum.

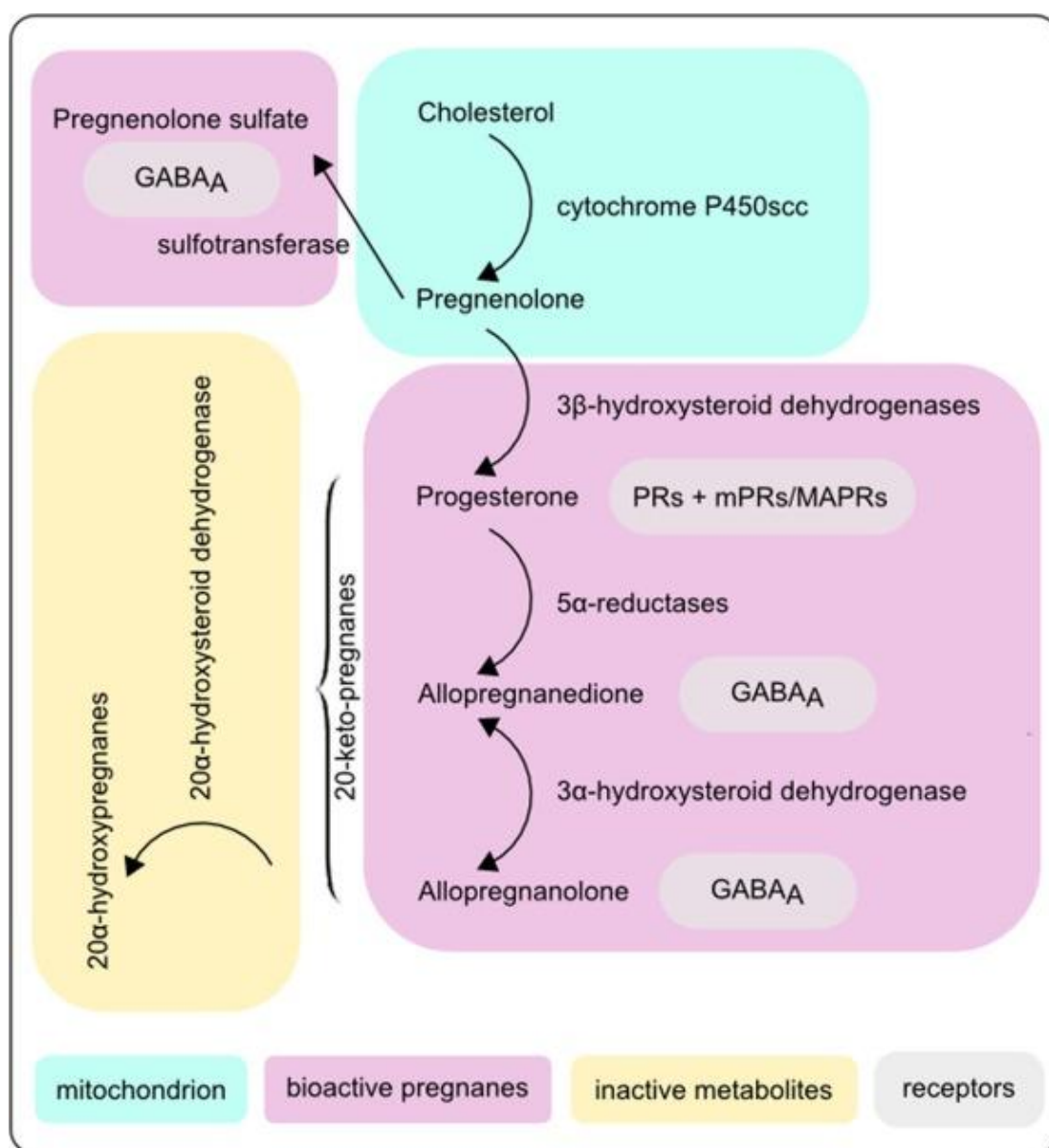


Fig. 2. A schematic representation of the progesterone synthesis, metabolism route, as well as the interaction with progesterone receptors.

Pregnenolone is synthesized from cholesterol by cytochrome P450_{scc}, and then converted to progesterone by the 3β-hydroxysteroid dehydrogenase. Progesterone is metabolized to allopregnanedione by the 5α-reductases,

further converted to allopregnanolone by the 3α -hydroxysteroid dehydrogenase. Progesterone exert its action by binding to intracellular receptors (PRs) to regulate gene transcription, by interacting with membrane receptors (mPRs) and membrane-associated kinases (MAPRs) in extranuclear compartments. Allopregnanolone and other progesterone metabolites act as positive modulators on the GABA_A receptors. The enzyme 20α -hydroxysteroid dehydrogenase catalyzes the conversion of the GABA-active progesterone metabolites into inactive metabolites. Additionally, pregnenolone is converted into pregnenolone sulfate by sulfotransferase. The metabolic pathways involving the conversion of progesterone to androgens, estrogens and corticosteroids are not mentioned in the present review.

Biological Role of Progesterone in Pregnancy:

Progesterone is primarily known as the pregnancy hormone in females, and most of its function relates to maintaining pregnancy specifically by preparing the endometrium, decreasing myometrial contractions for implantation, promoting gestation, and inhibition of lactation during pregnancy.

In addition to maintenance of the endometrium during pregnancy and vascularization of the endometrium during ovulation, progesterone also plays a role in bone formation. The process of resorption and building of new bone requires hormones such as estradiol and progesterone, as well as bone-forming cells, such as osteoblasts. Progesterone increases the process of bone formation by stimulating P-4-receptor mediated osteoblastic growth during the initiation of bone modelling.

Progesterone plays a vital role in the maintenance of the uterus during pregnancy. One of the primary responsibilities of progesterone throughout pregnancy is to maintain a decreased level of vascular tone in the myometrium. Progesterone also influences the production of inflammatory mediators, such as human T-cells within the uterine cavity. Thus, a loss of progesterone leads to an increase in myometrial contractility coupled with a decrease in fighting off immunologic threats, ultimately leading to a higher risk of miscarriage and early delivery of the fetus.

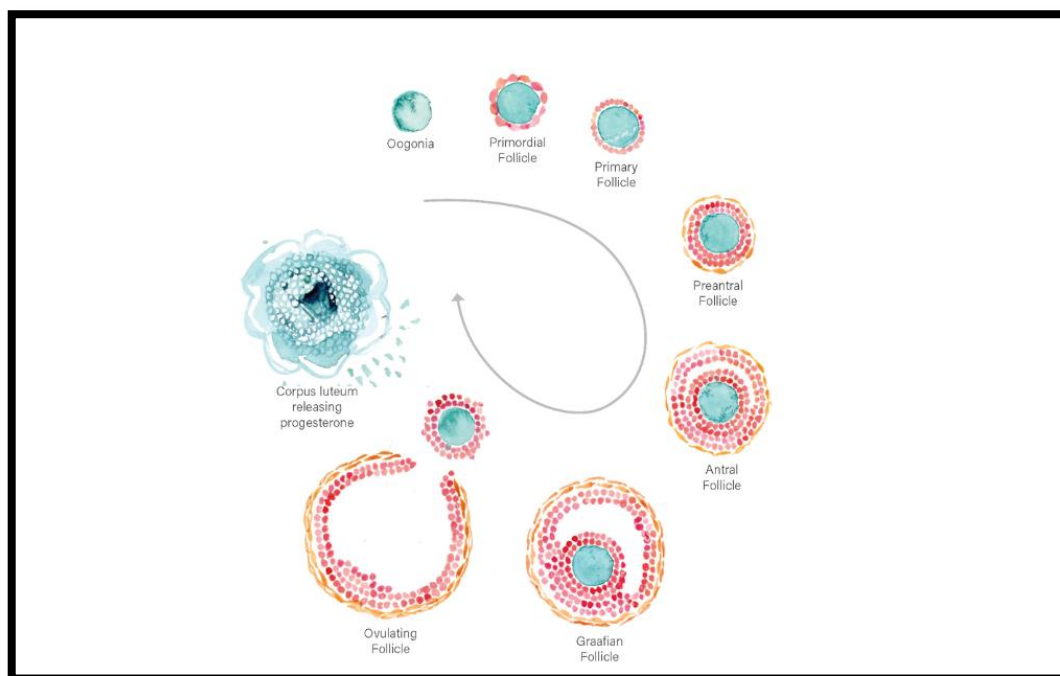


Fig 3: The journey of an ovarian follicle—from early formation to ovulation and progesterone production.

Overview of functions of progesterone in different tissues and systems [3]

Target system/tissue	Function
Endometrium	● The transition of endometrium from proliferative to secretory phase (Taraborrelli S 2015)
	● Promotes ovulation (Taraborrelli S 2015)
Pregnancy	● Essential for implantation and maintenance of early pregnancy
	● Progesterone suppresses myometrial contractility during pregnancy (Conneely OM et al. 2002)
Mammary gland	● Promotes lobular-alveolar development in preparation for milk secretion (Taraborrelli S 2015)
Brain	● Progesterone controls neurobehavioral expression associated with sexual responsiveness (González-Orozco JC, Camacho-Arroyo I 2019)
	● It is related to neuroprotection, neuromodulation, myelination, neurogenesis, neuronal plasticity, and mood (Genazzani AR et al. 2000)
	● Progesterone can act as a neuroprotective agent to treat traumatic brain injury (Wei J, Xiao GM 2013)
Bone	● Prevent bone loss (Balasch J 2003)
Cardio vascular system	● Exert protective effect on the cardiovascular system, induces vasodilation, and decreases blood pressure (Pang Y et al. 2015)
Metabolism	● Progesterone induces hyperinsulinemia and stimulates deposition of body fat
	● It also influences ketone body production (Kalkhoff RK 1982)
Immune system	● Progesterone inhibits inflammatory innate immune response
	● It also alters the distribution and activity of T cells (Hall OJ, Klein SL 2017)

Routes of Progesterone Administration:

Progesterone can be administrated orally, through an injection, or vaginally.[31] Many doctors prefer vaginal administration because it avoids first-pass metabolism in the liver. This method reaches high local levels in the uterus and cervix while causing few side effects throughout the body. It is often used in assisted reproduction and for women at risk of preterm birth.

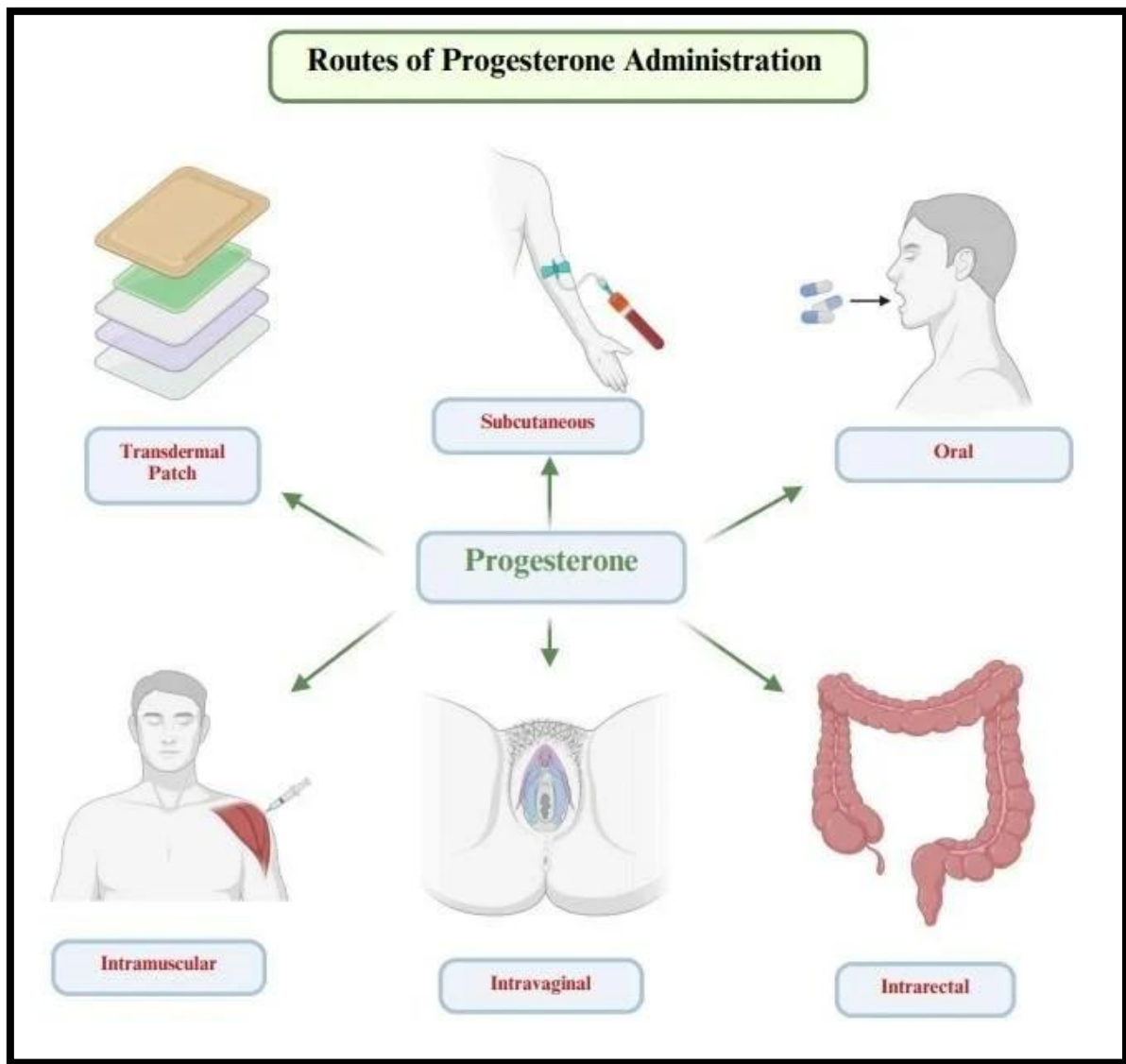


Fig 4: Routes of progesterone administration

PROGESTERONE IN TWIN GESTATION:

Multiple pregnancies make up 1 to 6% of all births, with twin births accounting for over 98% of these cases. The survival rate of premature infants is closely related to their gestational age at birth [17]. The chances of survival rise from below 50% before reaching 24 weeks to over 95% by the 33rd week of gestation. In addition, there is a negative relationship between the risk of severe disability in surviving infants and their gestational age at birth [30]. Several strategies have been proposed to prevent preterm births in twin pregnancies, including lifestyle changes, medical treatments, and surgical options like cervical cerclage. However, none of these methods have been successful [17]. Preterm birth is the most important factor determining neonatal morbidity and mortality among twins.

Different studies have shown that administration of vaginal progesterone was proposed for the prevention of preterm birth in women with sonographic short cervix in the mid-trimester based on its biologic effects on the cervix, myometrium, and chorio-amniotic membranes. It has been observed that treatment with vaginal progesterone in women with a cervical length $\leq 15\text{mm}$ was associated with a significant 44% reduction in the

rate of spontaneous preterm birth before 34 weeks of gestation [32]. For all of these reasons, vaginal progesterone administration is widely recommended for pregnancies at high risk of pre-term delivery.

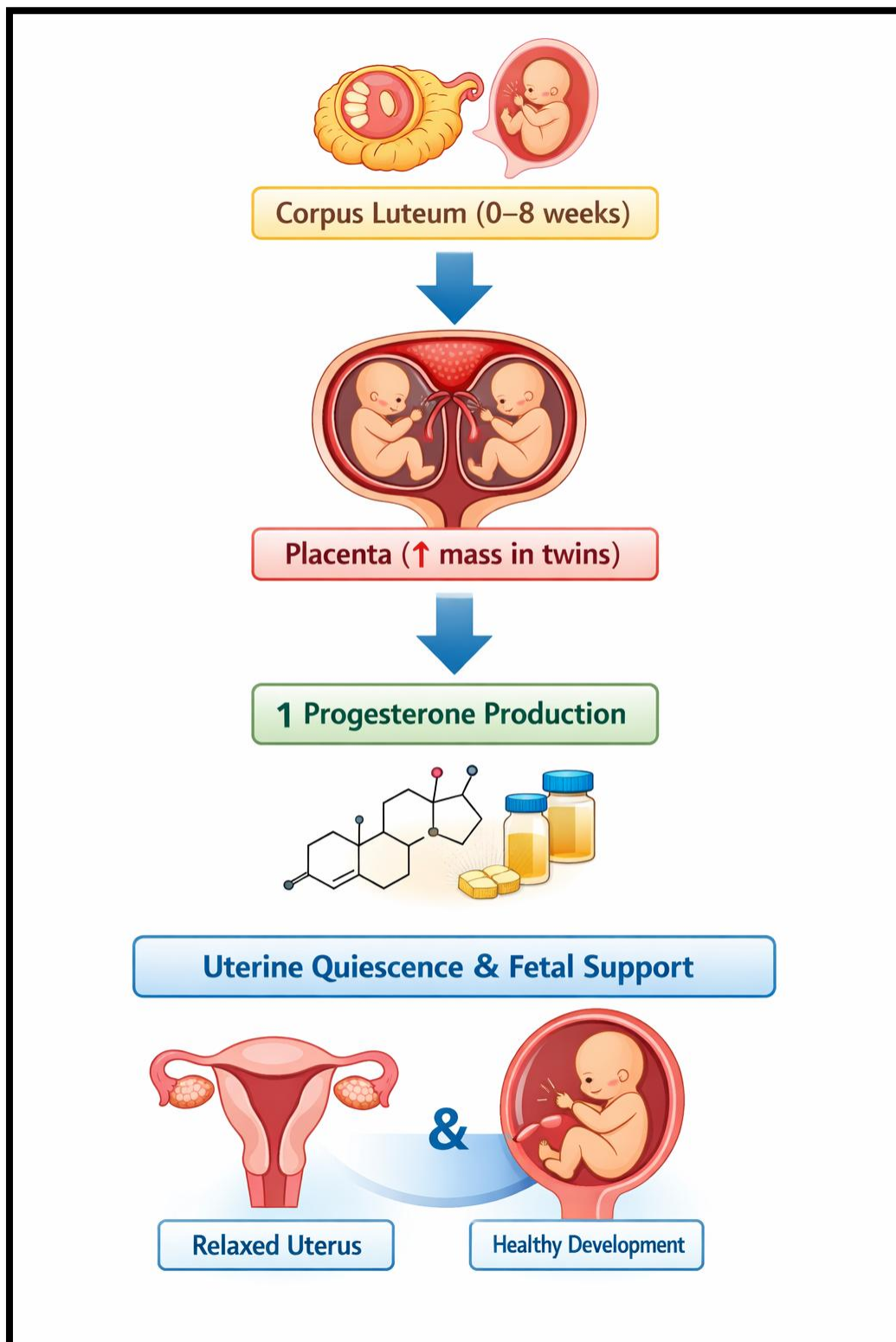


Fig 5: Mechanistic overview of progesterone dynamics in twin gestation.

During early pregnancy, the corpus luteum mainly produces progesterone. This changes to placental production later on. In twin pregnancies, the placental mass is larger, which increases progesterone synthesis. Higher progesterone levels help keep the uterus quiet and support proper fetal growth and development.

MATERNAL BONE TURNOVER IN TWIN PREGNANCY:

During normal human gestation, maternal bone turnover—as reflected by various biochemical serum molecules—increases, and a state of a reversible bone mineral density and bone mass reduction develops [1]. One of these biomarkers can be considered vitamin D (or calciferol). Furthermore, numerous studies suggest that maternal Vit-D status is related with adverse pregnancy outcomes, such as preeclampsia, gestational diabetes mellitus (GDM), low birth weight, prematurity, impaired offspring neurodevelopment, neonatal body composition, as well as newborn long-term metabolic health [2]

Physiology of Bone Turnover:

Bone turnover is markedly increased during pregnancy to support calcium demands of the growing fetus. Although the maximal fetal demand for calcium rises during the third trimester. Physiological responses to the demand for calcium include increased fractional absorption of calcium, which is doubled by the second trimester and most likely a consequence of the elevated 1,25-dihydroxyvitamin D concentration.[38] Pregnancy related changes may result in transient decreases in maternal bone mass density, with an increased risk of fracture and osteoporosis. These effects are more pronounced during twin pregnancy due to maximal fetal demand for calcium. It is well-known that the levels of estrogen and corticotropin in twin pregnancies are higher than in single pregnancies, and that the effect of hormonal changes on bone metabolism may be more pronounced during twin gestation ovarian. Pregnant women have a higher risk of anemia because of excessive menstruation, continuous low iron intake, and increased iron demand. In twins, this risk is even higher. Pregnancy anemia can easily cause oxidative stress, and oxidative stress may have adverse effects on both the mother and fetus.[39]

Bone turnover markers:

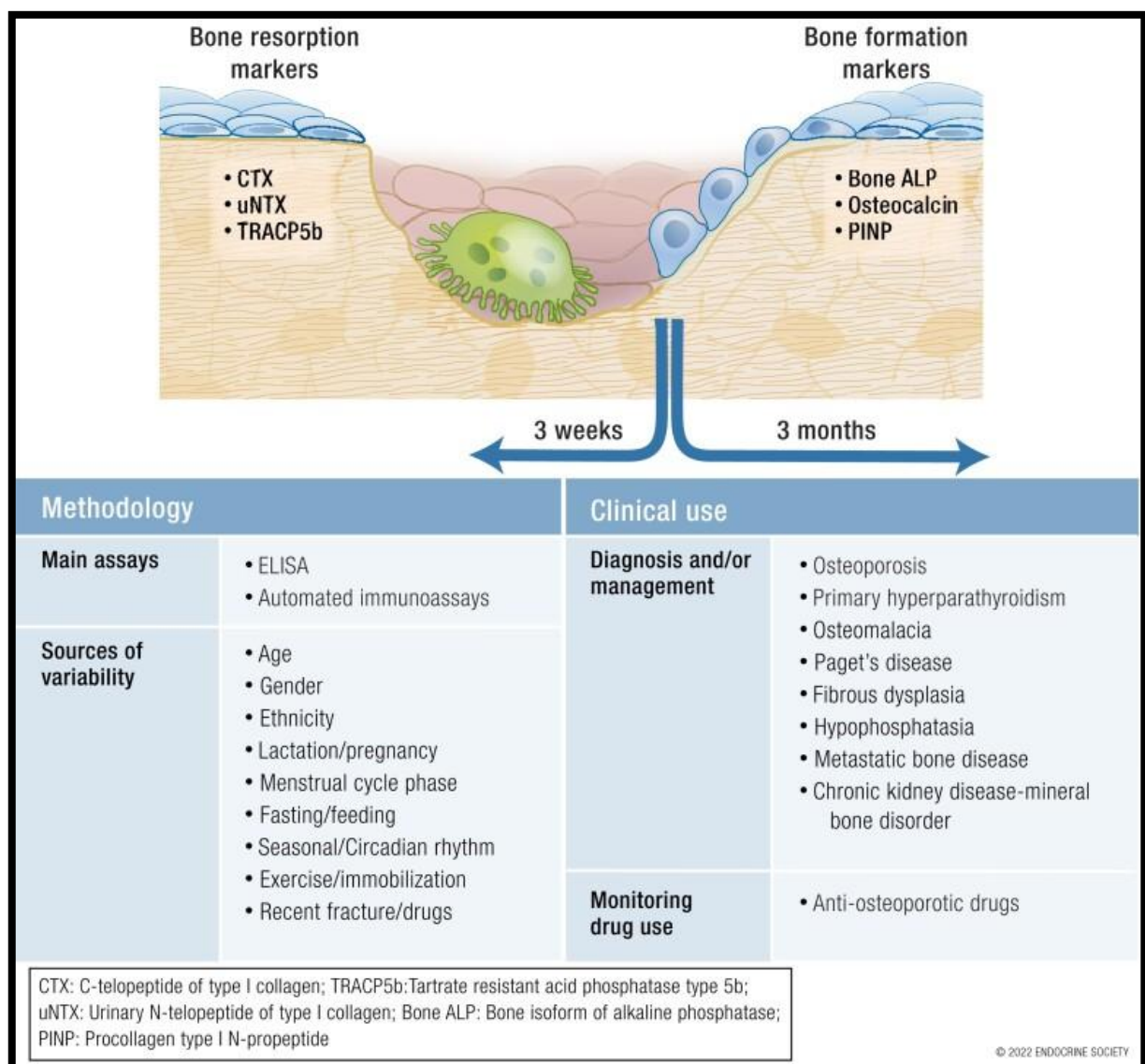
Bone turnover markers (BTMs) are released during the bone remodelling cycle and are measurable in blood or urine. BTMs are either products of osteoblasts secreted during bone formation (bone formation markers), or products of osteoclasts or bone degradation products released during bone resorption (bone resorption markers).[42]

Biochemical markers of bone turnover are noninvasive tools used to assess the dynamics of the skeletal system and are widely applied in the diagnosis and treatment control of osteoporosis. Several studies have measured changes in these markers during pregnancy and the postpartum period, as well as in relation to pregnancy complications. Serum C-terminal telopeptide of type I collagen (β -CTX) serves as a bone resorption marker, reflecting the activity and magnitude of bone loss, while biochemical markers of bone formation indicate the ability of bone-forming cells to synthesize bone proteins, especially type I collagen.[41] Osteocalcin (OC) is the most common marker of bone formation used in these settings.[40]

Osteoporotic fractures can be severe complications in pregnant women, potentially affecting both maternal and neonatal health. Among various osteoporosis-risk factors, parity and lactation are particularly noteworthy. The protective effects of pregnancy on the maternal skeleton may derive from the lack of regular menstrual cycles, while lactation induces high bone turnover, causing temporary bone loss. Twin pregnancies, characterized by increased calcium demand for fetal skeletal development, may theoretically lead to greater maternal bone loss compared with single pregnancies.

Table- Bone turnover markers

Bone Formation Markers	Bone Resorption Markers
Osteocalcin	C-terminal telopeptide of Type I collagen crosslinks
Bone Specific Alkaline Phosphatase	N-terminal telopeptide of Type I collagen crosslinks
Carboxy terminal propeptide of Type I collagen	Pyridinoline
Amino terminal propeptide of Type I collagen	Deoxypyridinoline
	Tartrate Resistant Acid Phosphatase

**Fig 6: Bone turnover markers in bone resorption and formation.**

Bone resorption markers, including C-terminal telopeptide of type I collagen (CTX), urinary N-telopeptide of type I collagen (uNTX), and tartrate-resistant acid phosphatase 5b (TRACP5b), reflect osteoclast activity and typically change within approximately 3 weeks. Bone formation markers, including bone-specific alkaline phosphatase (Bone ALP), osteocalcin, and procollagen type I N-propeptide (PINP), reflect osteoblast activity and usually respond over approximately 3 months. These biomarkers are measured using ELISA or automated

immunoassays and are influenced by factors such as age, sex, ethnicity, pregnancy/lactation, menstrual cycle phase, nutritional status, circadian rhythm, physical activity, fractures, and medications. Clinically, bone turnover markers are used in the diagnosis and management of metabolic bone diseases and in monitoring responses to anti-osteoporotic therapies.

EFFECTS OF VAGINAL PROGESTERONE ON BONE METABOLISM:

The regulation of bone metabolism by progesterone involves several key mechanisms that help with bone remodelling and maintaining bone density. Progesterone mainly works by stimulating osteoblast activity, which promotes bone formation. It may also inhibit osteoclast-mediated bone resorption. This hormonal action occurs through progesterone receptors on bone cells, influencing gene expression that controls bone growth and mineralization processes.[27]

Additionally, progesterone works with estrogen pathways to improve bone density. This makes it an important part of hormonal balance for skeletal health. In clinical settings, progesterone is a key component of hormone replacement therapy (HRT) aimed at reducing bone loss associated with menopause. Its positive effects on bone have made progesterone a promising option for preventing and treating osteoporosis, particularly in postmenopausal women and individuals facing hormonal shortages.

Understanding progesterone's complex role in bone metabolism is crucial for improving treatment methods that maintain or restore bone health. While this context does not provide detailed molecular pathways specific to progesterone alone, there is a general agreement on its importance in stimulating bone formation and working with estrogen to preserve bone mass and quality. More research into progesterone-related molecular mechanisms could improve clinical applications and strategies for reducing fracture risk in osteoporosis management.

RANK/RANKL/OPG Signaling Pathway in Bone Remodeling

The RANK/RANKL/OPG pathway is a central regulatory system controlling bone remodeling, particularly the balance between bone formation and bone resorption. It plays a crucial role in maintaining skeletal integrity and is highly relevant in physiological states such as pregnancy, including twin gestation.

The RANKL/RANK/OPG system is known for its roles in osteoclasts maturation, bone modeling, and bone remodeling. Receptor activator of NF- κ B (RANK), receptor activator of NF- κ B ligand (RANKL), and osteoprotegerin (OPG) are the main components of this signaling system. Interestingly, taking part in bone hemostasis is not the only effect of the RANKL/RANK/OPG pathway [4]. RANKL (also known as OPGL, ODF, and TRANCE), as a homotrimeric protein, is produced by osteoblasts and some other cells like activated T cells.

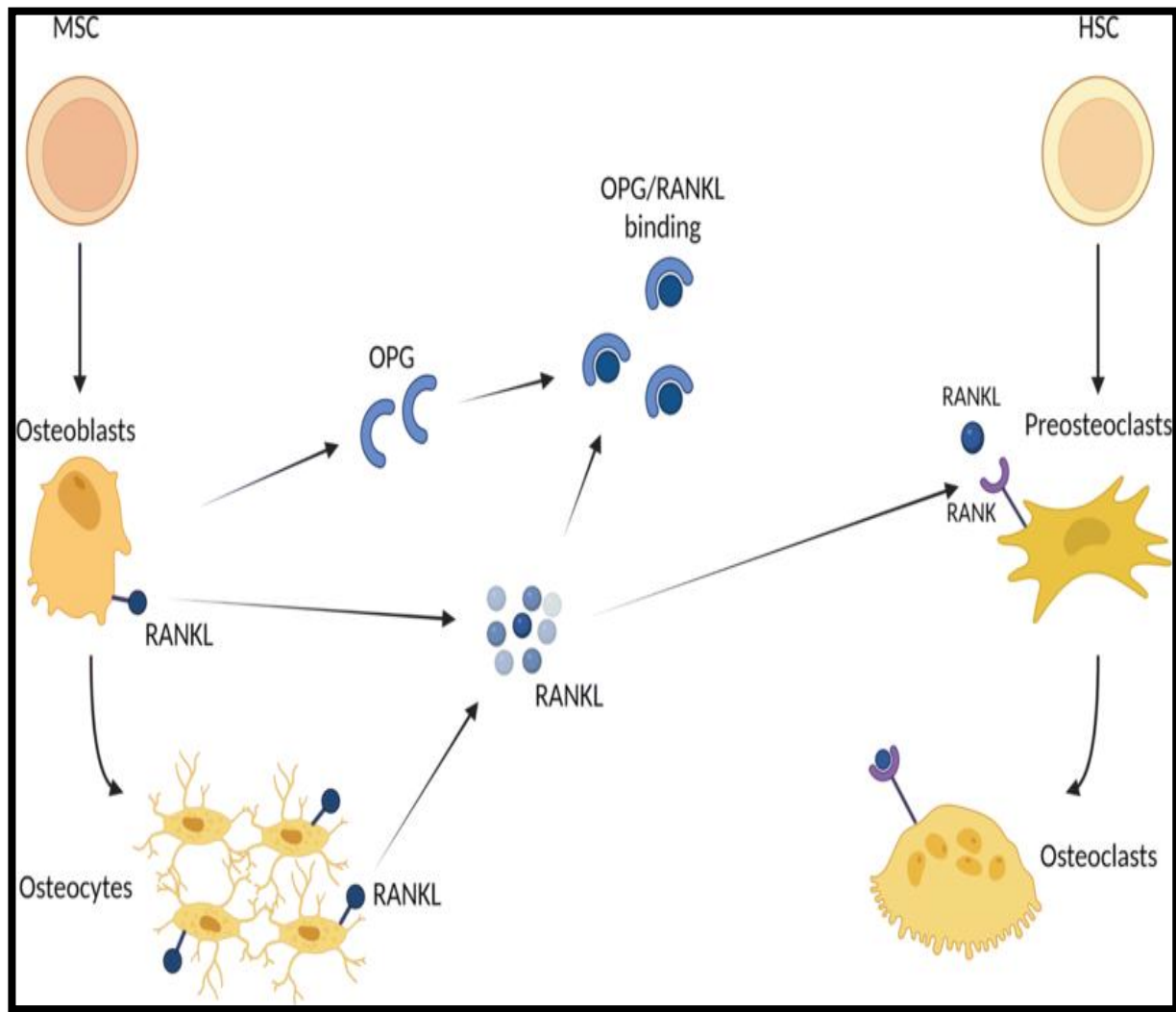


Fig 7: OPG/RANKL/RANK signaling pathway in bone remodeling.

Mesenchymal stem cells (MSCs) differentiate into osteoblasts, which produce receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG). Osteocytes also contribute to RANKL production. RANKL binds to its receptor RANK on preosteoclasts derived from hematopoietic stem cells (HSCs), promoting their differentiation into mature osteoclasts and stimulating bone resorption. OPG acts as a decoy receptor by binding RANKL, thereby preventing RANK activation and inhibiting osteoclastogenesis.

OXIDATIVE STRESS IN TWIN PREGNANCY:

Over the past few years, more and more attention has been devoted to issues of environmental impact, lifestyle, and comorbidities on the body's oxidative balance and its possible impact on abnormalities related to fetal development and pregnancy outcome. The term oxidative stress refers to the imbalance between the production of reactive oxygen species (ROS) and the ability of antioxidant mechanisms to neutralize them. It may be the result of an increase in ROS generation and/or a weakening of antioxidant defense [5,15].

Oxidative stress represents an overproduction of reactive oxygen species, like hydroxyl radicals, peroxide radicals, and superoxide anions, which is not appropriately balanced by both enzymatic and nonenzymatic antioxidant defense [6]. It negatively impacts fertility and plays a significant role in the pathogenesis of malignancies, neurological and cardiovascular degenerative diseases, and in general, in the aging processes [13,9].

Pregnancy is also associated with increased susceptibility to OS generated by the systemic inflammatory response, which plays a significant role during pregnancy, normal childbirth, and the initiation of preterm birth [11]. Common disorders during pregnancy, such as endothelial cell dysfunction, are likely caused by ROS, which attack cell membrane phospholipids and react with polyunsaturated fatty acids, creating LPO and causing cell damage [10].

Twin pregnancies involve a larger placental mass and higher oxygen consumption, which may increase oxidative stress. Studies have shown that twin pregnancies have higher levels of oxidative stress markers. These markers are associated with complications like preeclampsia and preterm birth.

Table:

Major Endogenous Oxidants (6)

Oxidant	Formula	Reaction Equation
Superoxide anion	$O_2^{\cdot -}$	$NADPH + 2O_2 \leftrightarrow NADP^+ + 2O_2^{\cdot -} + H^+$ $2O_2^{\cdot -} + H^+ \rightarrow O_2 + H_2O_2$
Hydrogen peroxide	H_2O_2	$Hypoxanthine + H_2O + O_2 \rightleftharpoons xanthine + H_2O_2$ $Xanthine + H_2O + O_2 \rightleftharpoons uric\ acid + H_2O_2$
Hydroxyl radical	$\bullet OH$	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$
Hypochlorous acid	$HOCl$	$H_2O_2 + Cl^- \rightarrow HOCl + H_2O$
Peroxyl radicals	$ROO\bullet$	$R\bullet + O_2 \rightarrow ROO\bullet$
Hydroperoxyl radical	$HOO\bullet$	$O_2^{\cdot -} + H_2O \rightleftharpoons HOO\bullet + OH^-$

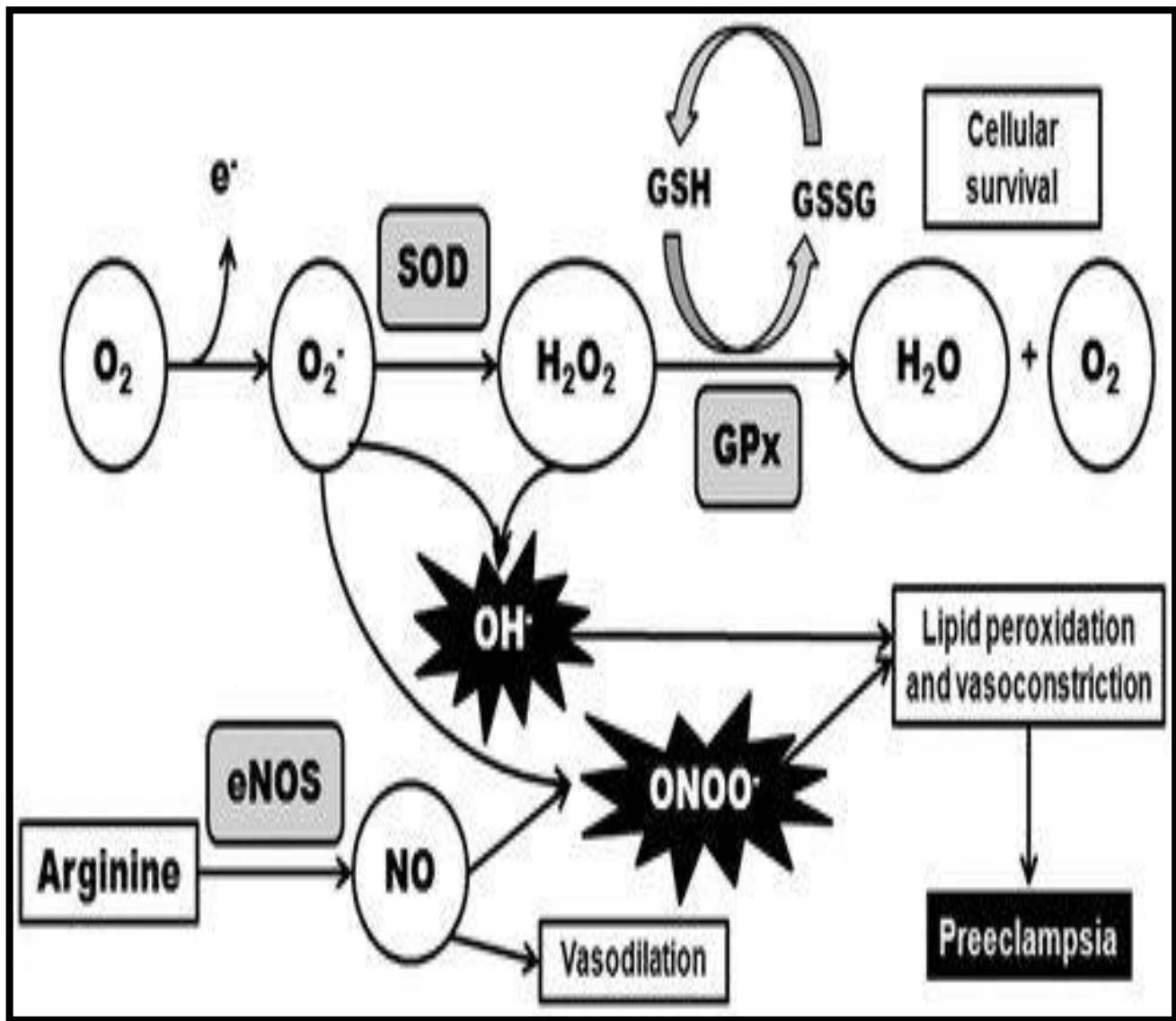


Fig 8: Schematic representation of oxidative stress pathways implicated in preeclampsia, showing the generation of reactive oxygen species (ROS), nitric oxide (NO) imbalance, formation of peroxynitrite (ONOO⁻), lipid peroxidation, vasoconstriction, and impaired cellular survival mechanisms.

ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS OF PROGESTERONE ON OXIDATIVE STRESS:

Progesterone plays an important role in maintaining pregnancy, and in twin pregnancies it may also have antioxidant and anti-inflammatory effects that help protect both the mother and fetuses from complications related to oxidative stress and inflammation. Twin pregnancies are associated with increased oxidative stress because the placenta is larger and metabolic demands are higher. Excess reactive oxygen species (ROS) may contribute to complications such as preterm birth, fetal growth restriction, and preeclampsia. [14,15]

Studies suggest progesterone can help reduce oxidative stress by decreasing lipid peroxidation and free radical production, improving mitochondrial function, enhancing antioxidant defense enzymes such as superoxide dismutase and catalase.

Progesterone also functions as an immunomodulatory hormone during pregnancy. Its anti-inflammatory effects help maintain maternal-fetal tolerance suppression of pro-inflammatory cytokines (such as TNF- α , IL-1 β , IL-6). The immunomodulatory mechanisms include - promotion of anti-inflammatory cytokines, reduction in activation of inflammatory immune cells, enhancement of regulatory T-cell activity to support fetal tolerance,

prevention of excessive uterine inflammatory signalling linked to preterm labor. These immunomodulatory actions are considered important in maintaining pregnancy and preventing premature uterine contractions.[12]

A recent study in twin gestations found that women receiving vaginal progesterone did not show the same increase in oxidative stress markers seen in untreated women, suggesting a protective antioxidant effect and decrease excessive immune activation. Twin pregnancies themselves often show altered antioxidant balance early in gestation, especially in pregnancies later complicated by fetal growth restriction or prematurity.[10]

This specific P₄-dependent regulation may be due to the P₄-dependent production of at least two mediators of the immune response: PIBF (progesterone-induced blocking factor) and LIF (leukemia inhibitory factor). P₄ can influence T-cell activation through PIBF and LIF secretion, regulating it via membrane PR (mPR). It is known that T-cell activation is associated with a rapid increase in the intracellular free calcium concentration. Furthermore, the inhibition of T-cell activation by P₄ can be indirect. P₄ enhances the induction of IL-10-producing cells, particularly dendritic cells. Thus, IL-10 may inhibit Th1 differentiation [44]. Hormone replacement therapy (HRT) can also be an example of the anti-inflammatory action of P₄ since this hormone reverses the menopausal CD4/CD8 ratio, as well as limits the general peri- and postmenopausal inflammatory state.[45] The exact mechanism via which P₄ regulates T-cell activation is still unclear. Recent studies using the analysis of P₄-induced transcriptomic changes in an in vitro model of human CD4⁺ T cells demonstrated a profound direct effect of P₄ on T-cell activation and downregulation of the immune genes involved in autoimmune diseases such as multiple sclerosis and rheumatoid arthritis.[43]

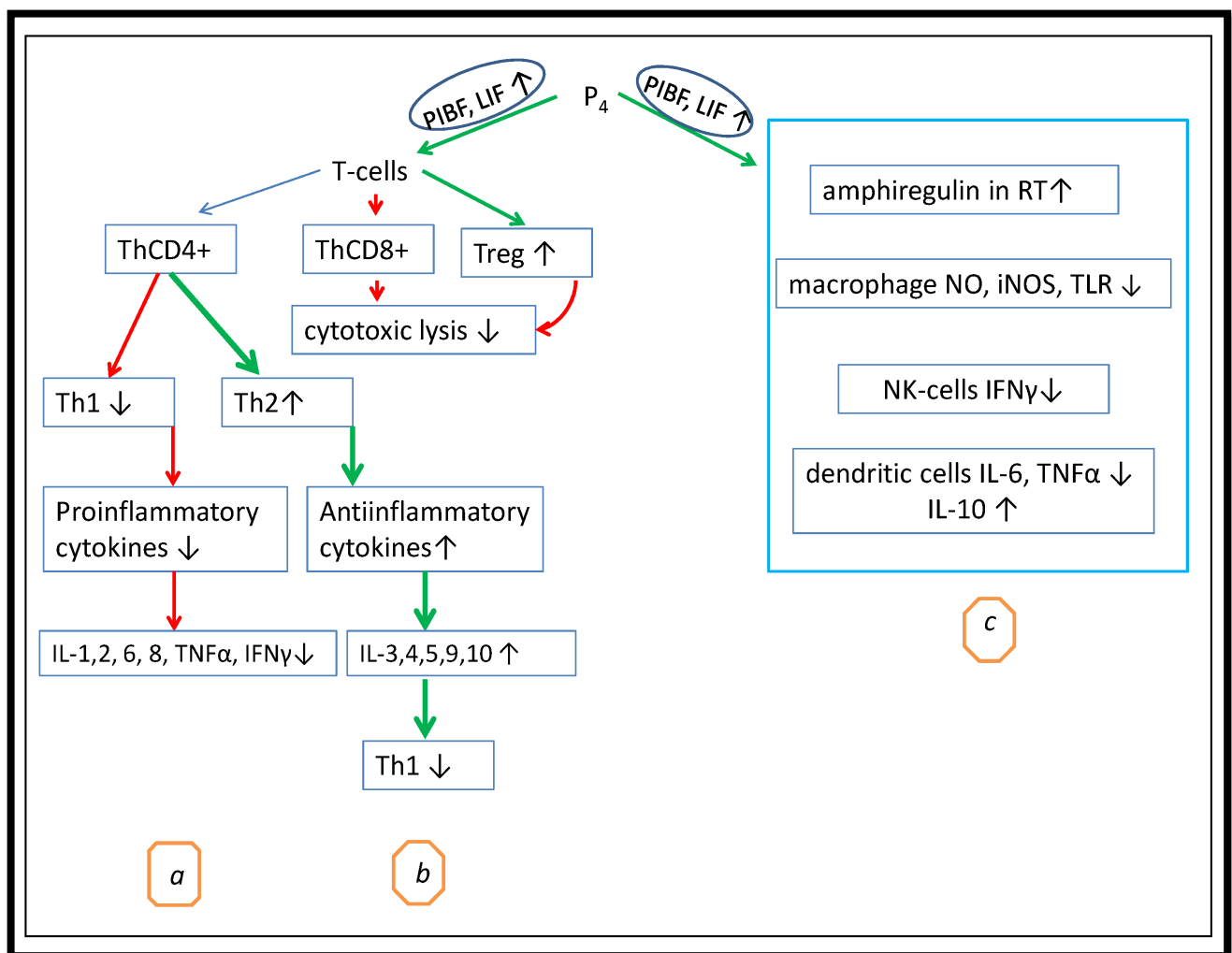


Fig 9: The proposed mechanisms of the P₄ immunomodulatory effects. (a) P₄ induces PIBF and LIF production, which inhibit the Th1 pathway and reduce the production of proinflammatory cytokines; (b) P₄ upregulates Th2 differentiation and anti-inflammatory cytokine synthesis, which downregulate Th1 differentiation; P₄ decreases CD8⁺ T cytotoxicity and upregulates Tregs, which also decrease CD8⁺ T cytotoxicity; (c) by stimulation of the secreting amphiregulin, P₄ activates proliferation and repair in the

respiratory tract (RT). P_4 decreases the production of proinflammatory cytokines $IFN-\gamma$, IL-6, and $TNF-\alpha$ by NK (natural killer) and dendritic cells, as well as decreases NO synthesis by macrophages. Red arrow— inhibition, green arrow—stimulation.

Progesterone Regulates Inflammation via FKBP51:

Progesterone can regulate inflammation partly through its interaction with FKBP51, a stress-response and immunoregulatory protein. The active cytosolic/nuclear progesterone receptor complex exists in dynamic interactions with chaperones HSP90, HSP70, and p23, as well as cochaperone immunophilins FKBP51 and FKBP52. FKBP51 and FKBP52 are members of the immunophilin protein family, named because they bind the immunosuppressive drugs tacrolimus FK506, sirolimus (rapamycin), and cyclosporin A (CsA), which play a role in immune regulation and basic cellular processes involving protein folding and trafficking.

FKBP51 interacts with mature progesterone receptor hetero complexes, as well as glucocorticoid and mineralocorticoid complexes with HSP90 and p23 protein. Via a short feedback loop, FKBP51 regulates hypothalamic–pituitary–adrenal axis activity. FKBP51 expression is increased by GC or P_4 and acts on GR or PR in an inhibitory manner; then, cortisol synthesis is decreased, which downregulates inflammation.[43] FKBP51 is also linked to the NF- κ B signaling pathway, which controls production of inflammatory cytokines such as $TNF-\alpha$ and IL-6.

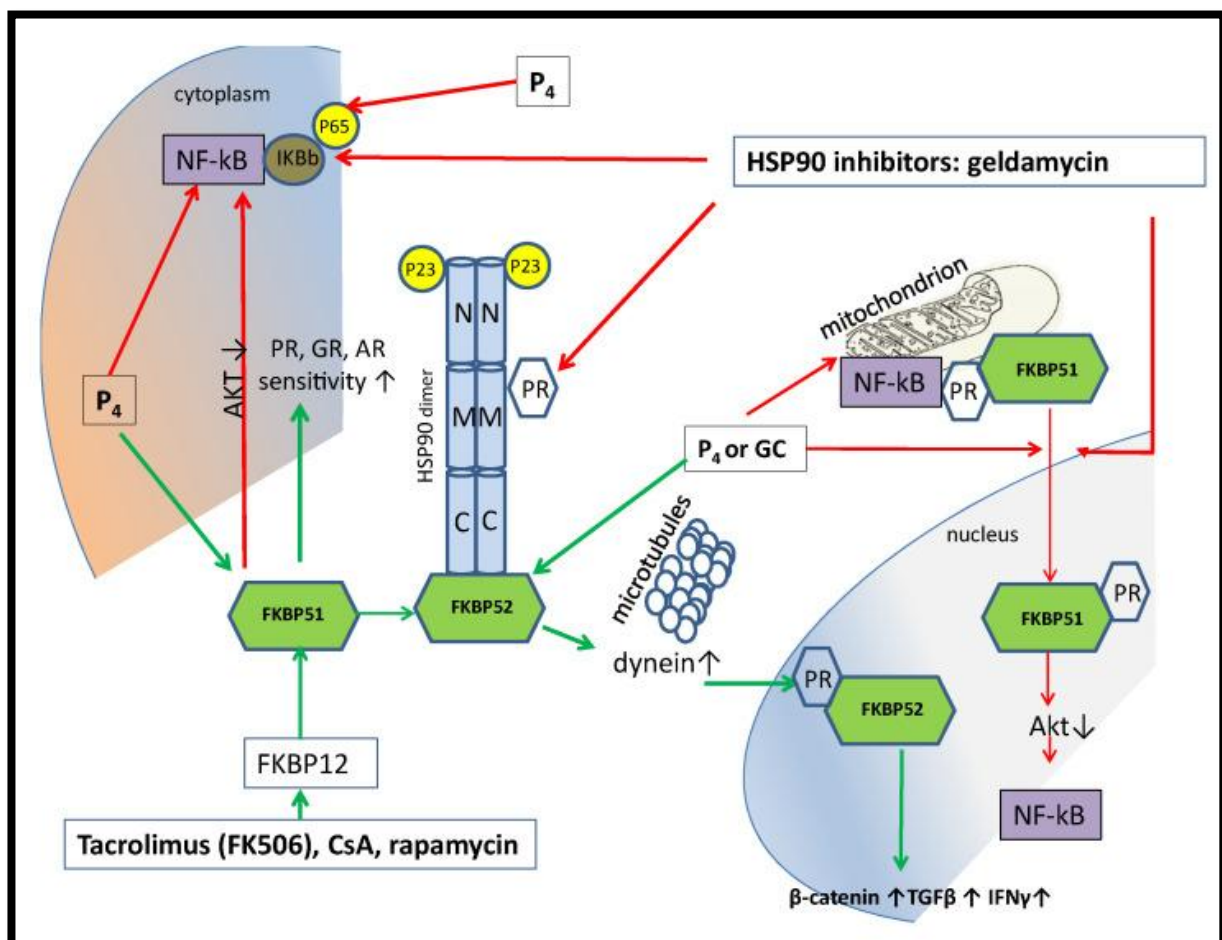


Fig 10: The probable mechanism of the P_4 -mediated PR and GR sensitization by FKBP-targeted drugs. Tacrolimus (FK506), CsA, and rapamycin bind FKBP12 and then inhibit FKBP51, which inhibits supplantation of FKBP52; in this way, FKBP52 does not induce dynein and disturbs FKBP52-mediated signaling (β -catenin, $TGF\beta$, and $IFN-\gamma$). Simultaneously, FKBP51 inhibits NF- κ B through an Akt-inhibitory mechanism. P_4 induces FKBP51, thus activating silenced PR, GR, and AR signaling. HSP90 inhibitors reduce the activation of FKBP52-mediated signaling. HSP90 inhibitors also decrease FKBP51 shuttling from the mitochondria to the nucleus; thus, NF- κ B is not activated in the nucleus. Red arrow— inhibition, green arrow—stimulation.

VAGINAL PROGESTERONE, BONE TURNOVER, OXIDATIVE STRESS IN TWIN PREGNANCY: CURRENT EVIDENCE

Clinical studies directly assessing the effects of vaginal progesterone on bone turnover and oxidative stress in twin pregnancies are limited. Most available data come from studies on singleton pregnancies or non-pregnant people.[16] Evidence shows that progesterone supplementation does not negatively impact bone metabolism and may help achieve a better oxidative balance [17]. However, the absence of large, well-designed studies in twin pregnancies prevents us from drawing firm conclusions.

Observational studies looking at biochemical markers like osteocalcin, bone-specific alkaline phosphatase, malondialdehyde, and total antioxidant capacity could offer important insights. The relationship between progesterone therapy, nutritional status, and basic maternal health is likely significant as well.

It has been confirmed by many studies that markers of bone turnover change significantly during pregnancy. The main difference is the direction of change of these markers, and different markers show different changing trends at different stages of pregnancy. Meanwhile, pregnancy and anemia can cause oxidative stress. Therefore, the study explored whether vaginal progesterone would be beneficial in improving bone metabolism and antioxidant levels of women with twin pregnancies.

Multiple high-quality randomized controlled trials and meta-analyses demonstrate that vaginal progesterone does not significantly reduce the overall risk of preterm birth in unselected twin pregnancies. For example, a randomized controlled trial comparing 200 mg and 400 mg daily doses of vaginal natural progesterone from 20 to 34 weeks in unselected dichorionic diamniotic twin pregnancies showed no difference in preterm birth rates or neonatal outcomes versus placebo, and higher doses conferred no additional benefit [18]. Similarly, a sub-analysis focusing on high-risk twin pregnancies defined by short cervical length or prior preterm delivery history also found no significant benefit of vaginal progesterone in prolonging gestation or improving neonatal complications [19].

However, vaginal progesterone has shown more promising results in the subset of twin pregnancies with a short cervix (typically ≤ 25 mm) identified in mid-trimester ultrasound screening. Individual patient data meta-analyses and updated meta-analyses of randomized trials revealed that vaginal progesterone in twin pregnancies with a short cervix significantly reduces preterm birth before 33 weeks, neonatal mortality, and several neonatal morbidities including respiratory distress syndrome, mechanical ventilation requirement, and very low birth weight (<1500 g) [20].

A notable retrospective cohort study showed that combining vaginal progesterone with cervical pessary in twin pregnancies with very short cervix (<20 mm) was associated with longer pregnancy duration, lower preterm birth <32 weeks, and reduced severe neonatal morbidity compared with progesterone alone, suggesting potential synergistic benefits warranting further research [21].

CLINICAL IMPLICATIONS:

As mentioned earlier, the effects of progesterone use in twin pregnancies on bone turnover and oxidative status have not been studied. Given how important these factors are for the health of mothers, babies, and newborns, it is vital to investigate these effects further. [11]

The predominant clinical goal of vaginal progesterone use in twin pregnancies has been to reduce preterm birth risk, a major cause of neonatal morbidity and mortality in multiple gestations. Although some randomized trials show no significant prolongation of pregnancy with vaginal progesterone in unselected twin pregnancies [22,18] higher benefit appears evident in specific subgroups.

In women with twin gestations and a mid-trimester sonographic short cervix (≤ 25 mm), vaginal progesterone notably reduces preterm birth before 33 weeks and improves neonatal outcomes including mortality, respiratory distress syndrome, and severe neonatal morbidity [16].

Vaginal progesterone also shows secondary benefits such as decreased rates of very low birthweight and reduced need for mechanical ventilation in twins [23].

Additionally, for twin pregnancies after arrested preterm labor, high-dose vaginal micronized progesterone (400 mg daily) prolongs pregnancy, delays delivery, decreases neonatal intensive care length of stay, and increases birth weight [24].

Vaginal progesterone therapy in twin gestation shows clear benefits in prevention of preterm birth and neonatal morbidity primarily when targeted to women with short cervix or after preterm labor.[29] Its bone-trophic effects suggest positive implications for maternal bone health, while its potential to modulate oxidative stress pathways could address critical pathophysiological processes in pregnancy complications. Future research should emphasize personalized approaches, optimal dosing, mechanistic biomarker integration, and long-term outcome studies to maximize clinical benefits and safely incorporate vaginal progesterone into comprehensive twin pregnancy management.

CONCLUSION AND FUTURE PERSPECTIVES:

Twin gestation comes with specific physiological changes that increase the mother's metabolic needs, affect bone metabolism, and raise oxidative stress compared to singleton pregnancies. Vaginal progesterone has become an important treatment in obstetric care because it helps maintain pregnancy and lower the chances of preterm birth. Although research has thoroughly examined its effectiveness and safety concerning obstetric outcomes, we still don't fully understand how it affects maternal bone turnover and oxidative stress in twin pregnancies.

Current evidence indicates that progesterone may have either helpful or neutral impacts on bone metabolism through its effects on osteoblastic activity and bone remodelling. Moreover, progesterone seems to have antioxidant and anti-inflammatory effects that might help reduce the higher oxidative stress seen in twin pregnancies. However, much of what we know comes from studies on singleton pregnancies or experimental models, limiting our ability to make clear conclusions specifically about twin pregnancies.

Future studies should focus on large-scale prospective studies and randomized controlled trials to evaluate how vaginal progesterone affects biochemical markers of bone turnover and oxidative stress in women with twin pregnancies. Long-term follow-up studies are also necessary to examine postpartum bone recovery and its potential effects on maternal bone health. Additionally, exploring the relationship between progesterone therapy, nutrition, calcium and vitamin D supplements, and antioxidant defenses may lead to a better understanding of how mothers adapt during twin pregnancy. This research will help formulate evidence-based clinical guidelines and improve outcomes for both mothers and babies in this higher-risk group.

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