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premature ovarian insufficiency; reproductive function; ovarian reserve; precision medicine; personalized fertility management; ovarian biomarkers; reproductive endocrinology; fertility restoration.

Authors
[1]Sulaymanova Madinabonu Shukhrat qizi, Doctoral Student of the Department of Obstetrics and Gynecology No. 2, Samarkand State Medical University
E-mail: msrigina1@gmail.com

[2]Muzaffarova Mukhiba Hamzaevna - Doctor D ivf reproductive and genetic institute, Tashkent, Uzbekistan, https://orcid.org/0009-0004-0658-6089?lang=ru, Email: muxibam@gmail.com

[3]Nodira Khakimovna Ruzieva, MD, Professor, Department of Obstetrics, Gynecology, and Pediatric Gynecology, Tashkent State Medical University. Tashkent, Uzbekistan.
https://orcid.org/0000-0003-2131-2663

[4]Mohira Bakhtiyorovna Tursunova, PhD candidate, Department of Obstetrics, Gynecology, and Pediatric Gynecology, Tashkent State Medical University. Obstetrician-gynecologist and reproductologist. Tashkent, Uzbekistan,
E-mail: mokhira.tursunova.96@bk.ru

[5]Bakhadirova Shakhnoza Fayzullaevna, Genecologist reproductologist of Clinic Doctor Shahnoza- fayz, Shurtepa Block9, Houses 9 and 9 A, Mirzo - Ulugbec District, Tashkent 100124, Republic of Uzbekistan
Orcid009-001-2805-2123
E-mail: shahnozafayzivf@gmail.com

[6]Janna Pakhomova, https://orcid.org/0000-0002-8610-493X. Chief Physician Tashkent Inter-district Perinatal Center,
E-mail: pahomovaje@mail.ru

Address for correspondence: Sulaymanova Madinabonu Shukhrat qizi, Doctoral Student of the Department of Obstetrics and Gynecology No. 2, Samarkand State Medical University
E-mail: msrigina1@gmail.com

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Improvement of Methods for Restoring Reproductive Function in Women with Premature Ovarian Insufficiency Syndrome: An Integrated Precision Medicine Approach

Abstract
Premature ovarian insufficiency (POI) is a complex reproductive endocrine disorder characterized by the loss of normal ovarian function before the age of 40 years, resulting in infertility, menstrual dysfunction, hypoestrogenism, and long-term metabolic and psychosocial consequences. Despite significant advances in reproductive medicine, current therapeutic strategies remain limited in their ability to restore ovarian function and achieve successful pregnancy, highlighting the need for more individualized and multidisciplinary treatment approaches. This study aimed to develop and evaluate an integrated precision-based strategy for optimizing reproductive function in women with POI through comprehensive assessment of ovarian reserve, endocrine status, inflammatory and oxidative stress biomarkers, reproductive imaging, and personalized fertility management. A prospective comparative clinical design was employed, integrating hormonal profiling, transvaginal ultrasonography, ovarian reserve assessment, laboratory biomarker analysis, and advanced statistical modeling to identify the principal determinants of reproductive recovery. A novel conceptual model, the Integrated Precision Reproductive Restoration Framework (IPRRF), was developed to combine clinical, biochemical, imaging, and individualized therapeutic parameters into a unified decision-support system. The findings demonstrated that successful restoration of reproductive function depends on the dynamic interaction among ovarian reserve, endocrine regulation, inflammatory status, oxidative balance, and personalized clinical intervention rather than on any single therapeutic modality. The integrated model improved risk stratification, facilitated individualized treatment planning, and enhanced prediction of reproductive outcomes compared with conventional management approaches. The proposed framework represents an original contribution by introducing a comprehensive precision medicine paradigm that integrates biological, clinical, and technological determinants of ovarian recovery into a single analytical structure. This approach has the potential to improve fertility counseling, optimize treatment selection, support earlier clinical intervention, and advance personalized reproductive healthcare for women with premature ovarian insufficiency. The study provides a theoretical and clinical foundation for future multicenter validation studies and the development of evidence-based precision fertility management strategies.

INTRODUCTION (Part 1)

Premature ovarian insufficiency (POI) represents one of the most challenging disorders in contemporary reproductive medicine because it combines endocrine dysfunction, impaired fertility, accelerated ovarian aging, and long-term systemic health consequences in women younger than 40 years. Unlike natural menopause, which occurs as part of physiological aging, POI is characterized by intermittent or complete loss of ovarian activity before the expected reproductive lifespan has ended. The disorder is clinically defined by menstrual irregularity or amenorrhea accompanied by persistently elevated follicle-stimulating hormone (FSH) concentrations and reduced ovarian estrogen production. Importantly, ovarian function in POI is often intermittent rather than completely absent, allowing spontaneous ovulation and pregnancy to occur in a minority of women. Consequently, the concept of "insufficiency" rather than "failure" has become the preferred terminology because it more accurately reflects the biological variability of ovarian activity and reproductive potential. Recent international consensus guidelines jointly developed by the European Society of Human Reproduction and Embryology (ESHRE), the American Society for Reproductive Medicine (ASRM), the International Menopause Society (IMS), and CRE WHiRL further reinforce the adoption of standardized terminology and emphasize individualized clinical management throughout the patient's reproductive lifespan.

POI affects women during the most socially, psychologically, and reproductively active period of life. Beyond infertility, the disorder is associated with hypoestrogenism-related complications, including accelerated bone loss, increased cardiovascular risk, metabolic disturbances, sexual dysfunction, cognitive changes, and reduced quality of life. Psychological consequences—including anxiety, depression, emotional distress, and concerns regarding femininity and reproductive identity—are frequently reported and substantially influence long-term wellbeing. Accordingly, modern reproductive medicine increasingly recognizes POI as a multisystem disorder requiring comprehensive multidisciplinary management rather than isolated hormonal replacement or infertility treatment alone. The recently published Nature Reviews Disease Primers review describes POI as a heterogeneous condition involving endocrine, genetic, immunological, metabolic, and environmental mechanisms that interact throughout the course of ovarian aging and reproductive decline.

Recent epidemiological evidence suggests that POI is more common than previously believed. Earlier estimates indicated that approximately 1% of women develop POI before 40 years of age, whereas contemporary international evidence demonstrates that the global prevalence may approach 3–4% when broader diagnostic criteria and diverse populations are considered. The updated international evidence-based guideline reports an estimated prevalence of approximately 3.5%, emphasizing that many women continue to experience delayed diagnosis because menstrual disturbances are frequently attributed to stress, polycystic ovary syndrome, weight changes, or other endocrine disorders before ovarian insufficiency is recognized. Diagnostic delay contributes to loss of fertility opportunities, postponement of hormone replacement therapy, and progression of long-term complications affecting skeletal, cardiovascular, neurological, and reproductive health.

Infertility remains one of the most devastating consequences of POI. Declining ovarian reserve progressively reduces the quantity and quality of available follicles, ultimately compromising natural conception and limiting the success of assisted reproductive technologies. Although spontaneous pregnancy remains possible in approximately 5–15% of women because intermittent follicular activity may persist, prediction of reproductive potential remains highly uncertain. Conventional ovarian reserve markers provide useful information but do not fully explain the remarkable heterogeneity observed among women with apparently similar endocrine profiles. Consequently, clinicians face substantial challenges when counseling patients regarding fertility preservation, timing of conception, in vitro fertilization (IVF), oocyte donation, and emerging regenerative therapies. The uncertainty surrounding reproductive prognosis further highlights the need for more sophisticated prediction models integrating endocrine, molecular, imaging, genetic, and clinical variables rather than relying solely on isolated hormonal measurements.

Understanding the biological basis of POI requires appreciation of the normal process of ovarian aging. Human females are born with a finite number of primordial follicles that progressively decline throughout life through physiological follicular recruitment and atresia. Ovarian aging is therefore determined by both the quantity and functional competence of remaining follicles. Accelerated depletion of the primordial follicle pool or disruption of follicular maturation may precipitate ovarian insufficiency decades before the expected age of natural menopause. Importantly, ovarian aging represents a multidimensional biological process involving endocrine regulation, mitochondrial metabolism, oxidative stress, DNA repair capacity, immune homeostasis, vascular function, and genetic susceptibility. Rather than representing isolated pathological events, these mechanisms collectively influence follicular survival, oocyte competence, and endocrine function throughout reproductive life. The 2024 international POI guideline recognizes the heterogeneity of these mechanisms and recommends individualized diagnostic evaluation based on each patient's clinical presentation and suspected etiology.

Assessment of ovarian reserve has consequently become central to modern reproductive endocrinology. Anti-Müllerian hormone (AMH), antral follicle count (AFC), serum FSH, luteinizing hormone (LH), estradiol, inhibin B, ovarian volume, and ultrasonographic characteristics collectively provide valuable information regarding residual ovarian function. Nevertheless, each marker possesses important biological and clinical limitations. AMH reflects the population of growing follicles rather than the total primordial follicle pool, while FSH demonstrates considerable cycle-to-cycle variability. AFC depends on operator expertise and imaging quality, whereas ovarian volume alone has relatively limited predictive performance. Accordingly, no single biomarker

can accurately estimate reproductive prognosis in women with POI. Current evidence therefore favors integrated assessment combining hormonal, imaging, genetic, and clinical information to improve diagnostic precision and individualized treatment planning.

The endocrine mechanisms underlying POI are equally complex. Progressive follicular depletion results in diminished estradiol production, reduced inhibin secretion, and subsequent loss of negative feedback at the hypothalamic-pituitary level. Elevated circulating FSH concentrations stimulate residual follicles; however, in many patients the remaining follicles exhibit impaired responsiveness because of intrinsic ovarian dysfunction rather than inadequate gonadotropin stimulation. LH secretion may also become dysregulated, while altered androgen production, impaired granulosa-cell function, and disrupted local ovarian signaling further compromise follicular maturation. Increasing evidence indicates that endocrine alterations should not be interpreted solely as consequences of follicular depletion but also as active contributors to continued ovarian deterioration through disrupted intraovarian communication and abnormal follicular recruitment. This endocrine complexity explains why hormone replacement therapy effectively alleviates estrogen-deficiency symptoms but rarely restores physiological ovarian reserve or sustained fertility.

Folliculogenesis itself represents one of the most tightly regulated biological processes within human reproduction. Recruitment of primordial follicles, granulosa-cell proliferation, oocyte maturation, angiogenesis, and ovulation depend upon coordinated interactions among endocrine hormones, intraovarian growth factors, mitochondrial metabolism, immune regulation, and local cellular signaling pathways. Disturbances affecting any component of this regulatory network may accelerate follicular apoptosis, impair oocyte competence, or reduce reproductive lifespan. Recent advances in ovarian biology increasingly support the concept that successful restoration of reproductive function cannot rely upon hormonal correction alone but requires comprehensive understanding of the molecular, immunological, metabolic, and regenerative mechanisms governing follicular development. This emerging perspective provides the scientific foundation for precision reproductive medicine and forms the conceptual basis for the integrated framework proposed in the present study.

1.1 Introduction (Part 2)

Increasing evidence indicates that oxidative stress is a fundamental biological mechanism underlying the development and progression of premature ovarian insufficiency (POI). Physiologically, reactive oxygen species (ROS) participate in follicular growth, ovulation, luteal function, and intracellular signaling. However, excessive ROS production or insufficient antioxidant defense disrupts cellular homeostasis, leading to oxidative damage to lipids, proteins, mitochondrial DNA, and nuclear DNA within ovarian tissue. Granulosa cells, which provide metabolic and endocrine support for developing oocytes, are particularly susceptible to oxidative injury because of their high metabolic activity. Persistent oxidative stress accelerates granulosa-cell apoptosis, impairs steroidogenesis, reduces mitochondrial ATP production, and promotes premature follicular atresia, ultimately contributing to depletion of the ovarian reserve. Recent experimental and clinical investigations have further demonstrated that oxidative stress is closely associated with chronic inflammation, mitochondrial dysfunction, and reproductive aging, suggesting that these processes represent interconnected rather than independent pathological pathways (Bentov & Casper, 2023; European Society of Human Reproduction and Embryology [ESHRE], 2024).

The relationship between oxidative stress and ovarian aging has important therapeutic implications. Traditional endocrine treatment primarily addresses estrogen deficiency without directly targeting the intracellular mechanisms responsible for progressive follicular degeneration. Consequently, growing attention has been directed toward antioxidant therapy, mitochondrial protection, and regenerative interventions capable of preserving residual ovarian function. Although antioxidants such as coenzyme Q10, melatonin, resveratrol, vitamin D, and N-acetylcysteine have demonstrated promising biological effects in experimental models, current clinical evidence remains inconsistent because of differences in study design, patient selection, treatment duration, and outcome measures. These inconsistencies highlight the necessity for individualized therapeutic strategies based on comprehensive biological assessment rather than empirical supplementation alone (ESHRE, 2024).

Autoimmune mechanisms constitute another major etiological pathway in POI. Approximately 10–30% of women with POI exhibit evidence of autoimmune disease or circulating autoantibodies directed against ovarian tissue or associated endocrine organs. Autoimmune thyroid disease, Addison's disease, type 1 diabetes mellitus, systemic lupus erythematosus, rheumatoid arthritis, and autoimmune polyendocrine syndromes occur more frequently among women diagnosed with POI than in the general female population. Immune-mediated destruction of ovarian follicles involves complex interactions among T lymphocytes, B cells, macrophages, inflammatory cytokines, complement activation, and disruption of immune tolerance. These processes accelerate follicular apoptosis and impair ovarian endocrine function long before complete follicular depletion becomes clinically evident. International guidelines therefore recommend evaluation for autoimmune disorders, particularly adrenal and thyroid disease, during the diagnostic assessment of women with POI, emphasizing that early identification of immune abnormalities may improve long-term clinical management (ESHRE, 2024).

Genetic susceptibility has emerged as another essential determinant of ovarian insufficiency. While Turner syndrome and FMR1 premutation remain the most widely recognized genetic causes, advances in genomic sequencing have identified numerous genes involved in folliculogenesis, DNA repair, meiosis, mitochondrial metabolism, apoptosis, and hormonal signaling. Variants affecting NOBOX, BMP15, GDF9, FOXL2, FIGLA, MCM8, MCM9, STAG3, and several DNA repair genes

have been associated with impaired ovarian reserve and accelerated follicular depletion. Nevertheless, the genetic architecture of POI remains highly heterogeneous, with many women demonstrating polygenic susceptibility rather than single-gene disorders. Consequently, current research increasingly favors comprehensive genomic profiling combined with endocrine and clinical assessment to improve etiological diagnosis and individualized reproductive counseling (ESHRE, 2024).

Mitochondrial dysfunction provides an additional mechanistic explanation linking genetic, metabolic, and oxidative pathways. Oocytes contain the highest mitochondrial density of any human cell because fertilization, meiotic maturation, spindle formation, and early embryonic development require exceptionally high energy production. Age-related mitochondrial DNA mutations, impaired oxidative phosphorylation, reduced ATP synthesis, and excessive ROS production collectively compromise oocyte competence and embryonic developmental potential. Experimental studies further indicate that mitochondrial dysfunction contributes to impaired granulosa-cell communication, altered follicular metabolism, and premature activation of apoptotic pathways. Accordingly, preservation of mitochondrial integrity has become an important therapeutic target in reproductive medicine, particularly in women experiencing diminished ovarian reserve or POI.

The irreversible decline in ovarian reserve associated with POI has substantially increased interest in fertility preservation. Contemporary reproductive medicine emphasizes early counseling immediately after diagnosis because residual ovarian activity may persist for variable periods. Fertility preservation strategies include embryo cryopreservation, mature oocyte cryopreservation, ovarian tissue cryopreservation, and, in selected circumstances, in vitro maturation of immature oocytes. Ovarian tissue cryopreservation has attracted particular attention because it offers reproductive potential for women requiring gonadotoxic therapy or those at high genetic risk for ovarian failure. However, in women already diagnosed with established POI, the opportunity for successful fertility preservation is frequently limited because ovarian reserve has already undergone significant depletion before diagnosis. This emphasizes the importance of earlier recognition of menstrual irregularities and prompt referral to reproductive specialists.

Regenerative medicine has emerged as one of the most rapidly developing areas of reproductive science. Mesenchymal stem cell (MSC) therapy has demonstrated encouraging experimental results through several biological mechanisms, including immunomodulation, angiogenesis, anti-inflammatory activity, mitochondrial protection, inhibition of granulosa-cell apoptosis, and stimulation of residual follicular growth. Bone marrow-derived, adipose-derived, umbilical cord-derived, menstrual blood-derived, and amniotic-derived mesenchymal stem cells have each demonstrated the potential to improve ovarian morphology and endocrine function in experimental models. Early clinical investigations have reported partial restoration of ovarian activity, increased estrogen production, reductions in FSH concentrations, and occasional spontaneous pregnancies following stem cell therapy. Nevertheless, evidence remains preliminary, with substantial heterogeneity regarding cell source, transplantation technique, dosage, patient selection, and long-term safety. Consequently, stem cell therapy should currently be regarded as an investigational regenerative strategy rather than an established standard treatment for POI.

Platelet-rich plasma (PRP) therapy represents another innovative regenerative approach receiving increasing scientific attention. PRP contains concentrated platelets capable of releasing numerous bioactive growth factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF-1), epidermal growth factor (EGF), and fibroblast growth factor (FGF). These molecules participate in angiogenesis, tissue repair, cellular proliferation, extracellular matrix remodeling, and anti-inflammatory regulation. In reproductive medicine, intraovarian PRP administration has been proposed as a method for stimulating dormant follicles, improving ovarian vascularization, and enhancing granulosa-cell function. Preliminary observational studies have reported improvements in AMH concentrations, antral follicle count, menstrual cyclicity, and occasional successful pregnancies following PRP administration. However, systematic reviews consistently conclude that current evidence remains insufficient to recommend routine clinical application because most available studies involve small sample sizes, limited follow-up, absence of randomized controlled trials, and substantial methodological heterogeneity. Well-designed multicenter clinical trials are therefore required before PRP can be incorporated into evidence-based clinical guidelines.

Despite remarkable technological progress, assisted reproductive technologies (ART) remain the principal fertility treatment for women with POI. IVF using autologous oocytes generally demonstrates limited success because of severe ovarian depletion, whereas oocyte donation continues to achieve the highest pregnancy and live-birth rates. Nevertheless, many women strongly prefer preservation of their own genetic motherhood whenever possible. Consequently, clinicians increasingly combine individualized ovarian stimulation protocols, endocrine optimization, regenerative interventions, fertility preservation strategies, and advanced laboratory technologies to maximize the probability of obtaining viable oocytes before recommending donor oocytes. The continuing evolution of reproductive endocrinology therefore reflects a transition from standardized treatment protocols toward precision-based fertility management tailored to each patient's biological characteristics and reproductive goals. Collectively, these developments indicate that successful restoration of reproductive function in women with POI depends upon comprehensive integration of oxidative stress biology, immune regulation, genomic medicine, mitochondrial physiology, regenerative therapeutics, fertility preservation, and advanced reproductive technologies. Rather than functioning as isolated interventions, these components represent complementary elements of an individualized therapeutic

strategy. This multidimensional perspective provides the scientific rationale for the Integrated Precision Reproductive Restoration Framework (IPRRF) proposed in the present study, which seeks to unify biological mechanisms with personalized clinical decision-making in order to improve reproductive outcomes among women affected by premature ovarian insufficiency.

2. Literature Review (Part 1)

2.1. Epidemiology of Premature Ovarian Insufficiency

Premature ovarian insufficiency (POI) has become an increasingly important public health issue because of its profound reproductive, endocrine, metabolic, cardiovascular, and psychological consequences. Traditionally regarded as a relatively rare reproductive disorder affecting approximately 1% of women younger than 40 years, contemporary epidemiological evidence indicates that the prevalence of POI is considerably higher than previously estimated. The most recent international evidence-based guideline jointly developed by the European Society of Human Reproduction and Embryology (ESHRE), the American Society for Reproductive Medicine (ASRM), the International Menopause Society (IMS), and the Centre for Research Excellence in Women's Health in Reproductive Life (CRE WHiRL) estimates that approximately 3.5% of women worldwide may experience POI during their reproductive years (ESHRE et al., 2024).

The apparent increase in prevalence reflects several factors. First, improvements in diagnostic criteria and endocrine testing have enabled earlier recognition of ovarian dysfunction. Second, delayed childbearing has increased clinical attention to ovarian reserve assessment among women presenting with infertility. Third, advances in cancer treatment have improved survival among young women exposed to gonadotoxic chemotherapy and radiotherapy, resulting in a larger population at risk for secondary POI. Finally, expanding genetic and autoimmune investigations have improved identification of previously unrecognized etiological subgroups (Touraine et al., 2024).

Although POI occurs globally, considerable regional variation has been reported. Population-based studies suggest that socioeconomic conditions, environmental exposures, nutritional status, ethnicity, healthcare accessibility, and genetic background all influence disease frequency. Women living in low- and middle-income countries may experience delayed diagnosis because menstrual irregularities are often attributed to stress, malnutrition, or reproductive aging without comprehensive endocrine evaluation. Conversely, women in high-income countries may receive earlier hormonal assessment but continue to face uncertainty regarding reproductive prognosis because current biomarkers incompletely predict residual ovarian function (ESHRE et al., 2024).

One of the major challenges identified throughout recent epidemiological research concerns diagnostic delay. Several studies report that women frequently consult multiple healthcare professionals before receiving a definitive diagnosis. Delayed recognition contributes not only to psychological distress but also to reduced opportunities for fertility preservation, delayed initiation of hormone replacement therapy, and progression of long-term complications including osteoporosis and cardiovascular disease. Consequently, recent international recommendations advocate greater awareness among primary healthcare providers, gynecologists, endocrinologists, and reproductive specialists to facilitate earlier diagnosis and individualized counseling (Touraine et al., 2024).

Despite improved epidemiological understanding, important uncertainties remain. Most prevalence studies rely upon retrospective clinical databases rather than prospective population-based cohorts. Diagnostic criteria also differ among studies, particularly regarding hormonal thresholds and duration of menstrual disturbance. These methodological differences limit direct comparison between countries and complicate estimation of the true global burden of disease. Future multinational cohort studies using standardized diagnostic criteria are therefore required.

2.2. Ovarian Aging

Ovarian aging represents the central biological process underlying the development of POI. Unlike most organs, the ovary contains a finite pool of primordial follicles established before birth. Throughout reproductive life, continuous follicular recruitment and atresia progressively reduce this reserve until menopause occurs. In women with POI, this physiological process is markedly accelerated or prematurely disrupted.

Historically, ovarian aging was viewed primarily as quantitative depletion of follicles. Contemporary research, however, demonstrates that ovarian aging is considerably more complex, involving qualitative deterioration of oocytes, granulosa-cell dysfunction, impaired mitochondrial activity, altered endocrine regulation, chronic inflammation, oxidative stress, vascular remodeling, and epigenetic modifications (Touraine et al., 2024).

Recent molecular studies suggest that accelerated ovarian aging results from interactions among several biological pathways rather than a single pathogenic mechanism. DNA damage accumulation, impaired DNA repair capacity, telomere shortening, mitochondrial dysfunction, oxidative injury, and chronic inflammatory signaling collectively reduce follicular survival. Simultaneously, altered communication between granulosa cells and oocytes impairs follicular maturation, leading to reduced developmental competence even before complete follicular depletion occurs.

Nevertheless, disagreement remains regarding the relative importance of individual mechanisms. Some investigators argue that mitochondrial dysfunction represents the primary initiating event because impaired ATP production compromises meiotic spindle formation and chromosomal stability. Others propose that oxidative stress precedes mitochondrial injury by directly damaging mitochondrial DNA and intracellular proteins. Additional studies emphasize chronic low-grade inflammation ("inflammaging") as the principal mediator linking immune dysregulation with accelerated follicular apoptosis.

These apparently conflicting hypotheses should not be interpreted as mutually exclusive. Increasing evidence

supports a systems biology perspective in which oxidative stress, mitochondrial dysfunction, immune activation, endocrine dysregulation, and genetic susceptibility reinforce one another through positive feedback mechanisms. Such complexity explains why therapeutic strategies targeting only one biological pathway often produce inconsistent clinical outcomes.

The concept of biological ovarian age has therefore emerged as an important advance. Chronological age alone inadequately reflects reproductive potential because women of identical age frequently exhibit markedly different ovarian reserves and responses to fertility treatment. Consequently, individualized evaluation based on endocrine biomarkers, imaging, molecular characteristics, and clinical history is increasingly recommended for reproductive decision-making (ESHRE et al., 2024).

2.3. Ovarian Reserve Biomarkers

Assessment of ovarian reserve has become one of the most extensively investigated topics in reproductive endocrinology. Reliable biomarkers are essential for diagnosis, fertility counseling, prediction of ovarian response, and evaluation of therapeutic interventions. Nevertheless, no single marker accurately reflects the complex biological status of ovarian reserve.

Anti-Müllerian hormone (AMH) has emerged as the most widely accepted biochemical marker because it is secreted by granulosa cells of small growing follicles and demonstrates relatively limited variation throughout the menstrual cycle. Numerous clinical studies consistently demonstrate lower AMH concentrations among women with POI compared with healthy reproductive-age controls. Consequently, AMH has become an integral component of international diagnostic recommendations (ESHRE et al., 2024).

However, AMH possesses important limitations. It reflects the population of growing follicles rather than the dormant primordial follicle pool. Extremely low AMH concentrations do not necessarily indicate complete absence of residual ovarian activity, and spontaneous ovulation has been documented even among women with undetectable serum AMH. Conversely, women with moderately preserved AMH may still experience poor reproductive outcomes because oocyte quality cannot be inferred solely from follicle number.

Antral follicle count (AFC), determined by transvaginal ultrasonography, represents another important marker of ovarian reserve. AFC provides direct visualization of small antral follicles and correlates with ovarian responsiveness during controlled ovarian stimulation. Nevertheless, AFC depends heavily upon operator expertise, ultrasound equipment, and interobserver reproducibility. Moreover, AFC primarily estimates follicular quantity rather than developmental competence.

Follicle-stimulating hormone (FSH) remains one of the classical diagnostic markers for POI. Elevated serum FSH reflects reduced ovarian estrogen and inhibin production leading to diminished hypothalamic-pituitary negative feedback. Although persistently elevated FSH constitutes a diagnostic criterion for POI, its predictive value for future fertility remains limited because concentrations fluctuate considerably and may temporarily normalize during intermittent ovarian activity.

Other endocrine biomarkers, including luteinizing hormone (LH), estradiol, inhibin B, ovarian volume, and ovarian stromal blood flow, have also been investigated. Individually, each provides only partial information regarding ovarian physiology. Consequently, several investigators advocate multimarker assessment combining endocrine, ultrasonographic, molecular, and clinical variables to improve individualized prediction of reproductive outcomes.

Recent developments in reproductive biomarker research have also focused on inflammatory cytokines, oxidative stress indicators, extracellular vesicles, metabolomics, proteomics, and circulating microRNAs. While these emerging biomarkers demonstrate promising biological associations with ovarian aging, their clinical utility remains uncertain because standardized laboratory methods and prospective validation studies are lacking. Therefore, contemporary evidence favors integrated biomarker panels rather than dependence upon isolated laboratory measurements.

2.4. Hormonal Mechanisms

The endocrine regulation of ovarian function depends upon highly coordinated interactions among the hypothalamus, pituitary gland, ovaries, and peripheral endocrine tissues. Disruption of this hypothalamic-pituitary-ovarian (HPO) axis constitutes one of the principal mechanisms underlying POI.

Under physiological conditions, pulsatile gonadotropin-releasing hormone (GnRH) stimulates secretion of FSH and luteinizing hormone (LH). FSH promotes granulosa-cell proliferation and follicular growth, whereas LH regulates theca-cell steroidogenesis and ovulation. Developing follicles subsequently produce estradiol and inhibin B, which exert negative feedback upon pituitary gonadotropin secretion.

In POI, progressive follicular depletion reduces estradiol and inhibin synthesis, leading to persistent elevation of FSH concentrations. However, endocrine dysregulation extends beyond simple loss of negative feedback. Contemporary ovarian biology demonstrates that local intraovarian signaling—including activins, inhibins, bone morphogenetic proteins (BMPs), growth differentiation factor-9 (GDF-9), insulin-like growth factors (IGFs), anti-Müllerian hormone, and transforming growth factor-beta (TGF-β)—plays equally important roles in regulating follicular recruitment and maturation.

Disturbance of these local signaling pathways contributes to impaired granulosa-cell differentiation, abnormal steroidogenesis, altered follicular metabolism, and accelerated apoptosis. Consequently, restoration of endocrine balance through hormone replacement therapy effectively alleviates hypoestrogenic symptoms but rarely restores physiological folliculogenesis or sustained fertility.

Recent investigations further demonstrate close interactions between endocrine regulation and immune signaling. Pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and interferon-γ influence granulosa-cell survival, steroid hormone synthesis, and follicular angiogenesis. Similarly, oxidative stress modifies

steroidogenic enzyme activity, further disrupting endocrine homeostasis. These findings support the concept that endocrine dysfunction represents both a consequence and a contributor to progressive ovarian deterioration.

Although hormone replacement therapy remains the cornerstone of long-term management, considerable debate continues regarding optimal therapeutic strategies for fertility restoration. Conventional estrogen replacement effectively protects bone and cardiovascular health but has limited influence on residual follicular activity. Conversely, experimental regenerative approaches—including stem cell therapy, platelet-rich plasma, mitochondrial-targeted interventions, and precision medicine strategies—seek to modify the underlying biological mechanisms responsible for ovarian insufficiency rather than simply correcting hormonal deficiency.

Collectively, recent literature demonstrates that endocrine mechanisms cannot be understood independently of oxidative stress, immune regulation, mitochondrial function, and molecular signaling. This multidimensional understanding provides the scientific basis for individualized reproductive medicine and supports development of integrated precision models capable of improving prediction and restoration of reproductive function in women with POI.

2. Literature Review (Part 2)

2.5. Autoimmune Premature Ovarian Insufficiency

Autoimmunity is recognized as an important but incompletely understood cause of premature ovarian insufficiency. In autoimmune POI, ovarian function may be impaired through immune-mediated damage to steroid-producing cells, developing follicles, granulosa cells, or ovarian stromal tissue. The condition frequently occurs alongside autoimmune disorders involving the thyroid, adrenal glands, connective tissues, gastrointestinal tract, and other endocrine organs. However, reported estimates of autoimmune involvement vary considerably because studies use different definitions, antibody panels, diagnostic thresholds, and patient populations.

Recent population-level evidence has strengthened the association between POI and autoimmune disease. A Finnish registry study involving 3,972 women with spontaneous POI and 15,708 matched controls found that women with POI were substantially more likely to have severe autoimmune disorders both before and after their POI diagnosis. The strongest associations were observed with autoimmune polyglandular syndromes, Addison's disease, systemic lupus erythematosus, vasculitis, rheumatoid arthritis, inflammatory bowel disease, and hyperthyroidism. The risk of receiving a new diagnosis of severe autoimmune disease was particularly elevated during the first three years after POI diagnosis (Savukoski et al., 2024).

These results support the hypothesis that immune dysregulation may precede or accompany ovarian insufficiency. Nevertheless, association does not establish that all autoimmune diseases directly cause POI. Shared genetic susceptibility, systemic inflammation, treatment exposure, surveillance bias, and common endocrine pathways may partly explain the observed coexistence. A recent bidirectional Mendelian-randomization study provided more specific evidence by identifying genetically predicted Addison's disease and systemic lupus erythematosus as potential causal risk factors for POI. However, evidence for other autoimmune diseases was weaker, suggesting that the relationship is not uniform across all immune-mediated disorders (Chen et al., 2024).

One unresolved question concerns the identification of true ovarian autoimmunity. Circulating ovarian antibodies have been described, but many available assays lack sufficient specificity, reproducibility, or clinical validation. Positive antibodies may reflect generalized autoimmune activation rather than direct follicular destruction. Histological confirmation through ovarian biopsy is invasive, affected tissue may be distributed unevenly, and biopsy is not recommended for routine diagnosis. Consequently, autoimmune POI is usually inferred from clinical context, associated disorders, and selected endocrine investigations rather than confirmed through a single diagnostic marker.

The 2024 international evidence-based guideline recommends evaluation for relevant autoimmune conditions, particularly adrenal and thyroid disease, while cautioning against indiscriminate use of poorly validated ovarian antibody tests. The guideline also emphasizes that immune-modifying treatments have not yet been established as routine fertility-restoration therapies for POI (Panay et al., 2024).

This creates a major clinical contradiction. Autoimmune mechanisms appear highly relevant in a subgroup of patients, yet standard care remains primarily supportive through hormone therapy, fertility counseling, and management of associated autoimmune disease. Immunosuppression might theoretically preserve remaining follicles during active immune-mediated injury, but uncontrolled treatment could expose patients to serious adverse effects without reliable evidence of ovarian benefit. Future studies therefore need validated immune phenotyping, prospective identification of early-stage autoimmune POI, and carefully controlled trials targeting biologically selected subgroups rather than all women with POI.

2.6. Genetic Factors

Genetic factors constitute one of the most rapidly developing areas of POI research. Established causes include X-chromosome abnormalities, Turner syndrome, fragile X messenger ribonucleoprotein 1 (FMR1) premutation, and pathogenic variants affecting ovarian development, meiosis, DNA repair, folliculogenesis, mitochondrial function, and gonadotropin signaling. However, the genetic architecture of POI is highly heterogeneous, and the majority of apparently idiopathic cases remain without a clearly defined molecular diagnosis.

A large whole-exome sequencing study of 1,030 women with POI identified pathogenic or likely pathogenic variants in recognized POI genes in approximately 18.7% of cases. When newly associated genes were included, the proportion increased to 23.5%. The implicated genes were involved in gonadal development, meiotic recombination,

DNA repair, follicular activation, oocyte maturation, and ovulation (Ke et al., 2023). These findings demonstrate that genomic analysis can substantially increase etiological yield beyond conventional karyotyping and FMR1 testing.

Nevertheless, the clinical interpretation of genetic variants remains challenging. A population-based analysis using exome data from more than 100,000 women found limited evidence that most previously reported heterozygous variants exert highly penetrant autosomal-dominant effects. Nearly all protein-truncating variants assessed were also found in reproductively healthy women. The authors concluded that many POI cases may be oligogenic or polygenic rather than caused by a single dominant mutation (Shekari et al., 2023).

These studies appear contradictory but may actually reflect different aspects of genetic susceptibility. Sequencing of clinically selected patients enriches the detection of rare pathogenic variants, whereas large population cohorts reveal incomplete penetrance and variable expressivity. A variant may contribute to ovarian aging without inevitably producing POI, particularly when its effect depends on other genes, environmental exposures, autoimmune activity, or gonadotoxic treatment.

Ethnic and geographic diversity presents another limitation. Genetic evidence is disproportionately derived from European and East Asian cohorts. A systematic review of nonsyndromic POI in Middle Eastern and North African populations highlighted substantial regional heterogeneity and a shortage of large, well-characterized genomic cohorts (Allouch et al., 2024). This limits the transferability of variant classifications across populations and may contribute to uncertain or conflicting interpretations.

From a clinical perspective, identifying a genetic cause may influence counseling, family screening, fertility preservation, assessment of associated syndromic conditions, and reproductive decision-making. Women with familial risk may benefit from earlier ovarian-reserve evaluation and timely fertility-preservation counseling. However, genetic testing also raises ethical concerns related to incidental findings, uncertain variants, psychological burden, discrimination, and the reproductive implications for relatives.

The central unresolved problem is how to translate genomic information into effective fertility restoration. Genetic diagnosis can explain why ovarian reserve was lost, but it does not necessarily identify a reversible pathway. Future research should integrate whole-genome sequencing, transcriptomics, epigenetics, functional assays, and longitudinal reproductive phenotyping. Such an approach may help distinguish pathogenic mutations from low-penetrance susceptibility variants and support genuinely personalized reproductive management.

2.7. Oxidative Stress

Oxidative stress is increasingly regarded as a common mechanistic pathway linking ovarian aging, chemotherapy exposure, environmental toxicants, inflammation, mitochondrial dysfunction, and follicular apoptosis. Physiological concentrations of reactive oxygen species contribute to follicular growth, steroidogenesis, ovulation, and intracellular signaling. Pathology develops when reactive oxygen species exceed antioxidant defenses, leading to damage of mitochondrial DNA, nuclear DNA, lipids, proteins, membranes, and cellular signaling systems.

Granulosa cells and oocytes are especially vulnerable because follicular development requires high metabolic activity and efficient mitochondrial energy production. Excessive oxidative stress can activate apoptotic pathways, impair steroidogenic enzymes, disrupt meiotic spindle formation, reduce ATP generation, and accelerate follicular atresia. A recent review concluded that oxidative injury may contribute to POI through effects on genetic material, transcription factors, signaling pathways, mitochondrial integrity, granulosa-cell survival, and the ovarian microenvironment (Zhang et al., 2023).

Clinical evidence also suggests an imbalance between oxidative and antioxidative capacity in women with POI. Kakinuma and Kakinuma (2023) reported that mitochondrial dysfunction and reactive oxygen species are plausible contributors to follicular depletion and reduced follicular quality. However, the authors also emphasized that oxidative markers are influenced by numerous systemic and environmental factors, limiting their diagnostic specificity.

The principal controversy concerns whether oxidative stress is a primary cause of POI or a downstream consequence of ovarian dysfunction. Follicular depletion, hypoestrogenism, chronic inflammation, and metabolic change may themselves increase oxidative stress. Cross-sectional studies therefore cannot establish temporal direction. Oxidative stress is likely both a driver and an amplifier of ovarian damage, forming a self-reinforcing cycle with mitochondrial dysfunction and inflammation.

Antioxidant therapies have shown promising effects in animal models, including improved follicle counts, reduced apoptosis, and partial normalization of hormonal profiles. Agents investigated include melatonin, coenzyme Q10, resveratrol, N-acetylcysteine, vitamins, and plant-derived compounds. However, clinical evidence remains insufficient to recommend these interventions as established fertility-restoration therapies. Studies often use small samples, inconsistent formulations, different etiologies of POI, surrogate hormonal outcomes, and short follow-up periods.

Another unresolved issue is patient selection. Antioxidant treatment may be more biologically plausible in women with documented oxidative imbalance, mitochondrial dysfunction, or chemotherapy-related injury than in women whose POI is caused by complete gonadal dysgenesis or advanced follicular depletion. Precision approaches should therefore use validated oxidative biomarkers and mechanistic phenotyping rather than offering uniform supplementation.

2.8. Stem Cell Therapy

Stem cell therapy has emerged as a prominent experimental strategy for restoring ovarian endocrine and reproductive function. Most research focuses on mesenchymal stem cells derived from bone marrow, adipose tissue, umbilical cord, menstrual blood, placenta, or amniotic tissue. Proposed mechanisms include

paracrine signaling, angiogenesis, immune modulation, reduction of fibrosis, inhibition of granulosa-cell apoptosis, mitochondrial protection, and regulation of oxidative stress.

The prevailing interpretation is that mesenchymal stem cells do not restore fertility primarily by differentiating into new oocytes. Instead, they appear to modify the ovarian microenvironment through growth factors, extracellular vesicles, cytokines, and anti-inflammatory mediators. This distinction is important because claims of direct germ-cell replacement remain controversial and have not been convincingly established in humans.

A systematic review and meta-analysis of animal and clinical studies found that stem cell therapy improved several measures of ovarian structure and function. However, the evidence was dominated by animal experiments, while human studies were generally small, uncontrolled, and methodologically heterogeneous (Hu et al., 2024a). Recent reviews describe potential benefits through angiogenesis, fibrosis reduction, anti-apoptotic signaling, immune regulation, and paracrine repair, but continue to classify the approach as investigational (Hu et al., 2024b).

Experimental research provides increasingly detailed mechanistic evidence. Human umbilical-cord mesenchymal stem cells have been reported to improve ovarian function in animal POI models through pathways involving oxidative stress and endoplasmic-reticulum stress signaling. Nevertheless, findings from induced mouse models cannot be directly generalized to women with spontaneous, genetic, or long-standing POI (Wu et al., 2024).

Autoimmune POI represents a particularly relevant target because mesenchymal stem cells may combine tissue repair with immunomodulation. Preclinical studies have reported reduced inflammatory injury and improved ovarian parameters following bone marrow-derived mesenchymal stem-cell treatment. Yet the clinical translation of these findings remains uncertain because the timing, reversibility, and residual follicular reserve in autoimmune POI vary substantially (Ma et al., 2024).

Several unresolved issues prevent routine clinical application. These include the optimal cell source, dose, route of administration, treatment timing, cell survival, manufacturing standards, long-term safety, tumorigenic risk, immune reactions, and reproductive consequences for offspring. Improvement in AMH, FSH, estradiol, or menstrual regularity does not necessarily demonstrate restoration of competent oocytes or increased live-birth rates.

The greatest methodological weakness is the frequent reliance on surrogate outcomes. A credible fertility-restoration therapy must ultimately be evaluated through oocyte retrieval, embryo development, clinical pregnancy, live birth, maternal safety, and offspring health. Until sufficiently powered randomized trials demonstrate such benefits, stem cell therapy should remain within ethically approved clinical research rather than routine commercial fertility care.

Research Novelty and Original Contribution (Part 1)

The present study introduces an original conceptual and clinical framework entitled the Integrated Precision Reproductive Restoration Framework (IPRRF) for the comprehensive assessment and restoration of reproductive function in women with Premature Ovarian Insufficiency (POI). Unlike conventional approaches that primarily focus on endocrine replacement therapy or assisted reproductive technologies (ART), the proposed framework integrates biological, endocrine, molecular, imaging, computational, and personalized clinical variables into a unified precision medicine model. This multidimensional approach reflects the current understanding that ovarian insufficiency is a heterogeneous syndrome resulting from complex interactions among ovarian reserve depletion, endocrine dysregulation, immune activation, oxidative stress, mitochondrial dysfunction, genetic susceptibility, and environmental influences rather than a single pathological process.

The principal scientific innovation of the IPRRF lies in replacing isolated biomarker interpretation with a systems-based reproductive evaluation. Traditional clinical management frequently relies on individual parameters such as anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), or antral follicle count (AFC) to estimate ovarian reserve. Although these biomarkers provide valuable diagnostic information, each reflects only one aspect of ovarian physiology and demonstrates important biological limitations. AMH primarily represents the pool of growing follicles rather than the primordial follicle reserve; FSH exhibits substantial inter-cycle variability; AFC depends on operator expertise and ultrasound quality; while estradiol and inhibin B fluctuate according to residual follicular activity. Consequently, clinical decisions based on isolated biomarkers may inadequately represent the true reproductive potential of women with POI (Panay et al., 2024; Touraine et al., 2024).

The IPRRF addresses this limitation by integrating multiple endocrine indicators, including AMH, AFC, FSH, luteinizing hormone (LH), estradiol, and inhibin B, into a dynamic reproductive profile rather than interpreting each variable independently. This multidimensional endocrine assessment is further combined with oxidative stress biomarkers, inflammatory cytokines, mitochondrial functional indicators, reproductive imaging findings, and genomic susceptibility profiles to establish a comprehensive representation of ovarian biology. Instead of describing ovarian reserve as a static measurement, the proposed model conceptualizes reproductive capacity as a continuously evolving biological system influenced by interacting endocrine, metabolic, immunological, and molecular mechanisms.

A second major innovation is the incorporation of host biological heterogeneity into reproductive decision-making. Previous clinical models generally assume relatively homogeneous disease progression among women diagnosed with POI. However, contemporary genomic and reproductive biology studies demonstrate that POI represents a biologically heterogeneous condition arising from diverse etiological pathways, including chromosomal abnormalities, pathogenic gene variants, autoimmune disorders, environmental exposures, mitochondrial dysfunction, accelerated ovarian aging, and oxidative injury. The proposed

framework therefore introduces genetic susceptibility and mitochondrial functional status as integral components of reproductive assessment rather than optional research variables. By recognizing individual biological variability, the IPRRF supports precision reproductive medicine and facilitates patient-specific therapeutic planning instead of standardized treatment algorithms.

The framework further incorporates inflammatory and oxidative pathways as central determinants of ovarian function. Increasing evidence indicates that chronic oxidative stress, excessive reactive oxygen species production, mitochondrial dysfunction, and inflammatory cytokine activation contribute to granulosa-cell apoptosis, impaired steroidogenesis, accelerated follicular atresia, and reduced oocyte competence. Rather than considering these mechanisms secondary consequences of ovarian aging, the IPRRF identifies them as active biological processes capable of modifying reproductive prognosis and therapeutic response. This systems-oriented interpretation provides opportunities for integrating antioxidant interventions, immune evaluation, and regenerative strategies into individualized fertility management.

An additional novel component of the framework is the integration of artificial intelligence-assisted prediction with conventional reproductive assessment. Current fertility counseling frequently depends upon clinician experience and isolated laboratory findings, resulting in substantial variability in prediction of ovarian response and pregnancy outcomes. Within the proposed model, machine learning algorithms may integrate endocrine biomarkers, imaging parameters, mitochondrial indicators, inflammatory profiles, oxidative stress measurements, and genetic information to generate individualized reproductive risk estimates. Rather than replacing clinical judgment, AI functions as an advanced decision-support system capable of identifying complex multidimensional relationships that may not be evident through conventional statistical interpretation. This integration represents an important transition from descriptive reproductive endocrinology toward predictive and personalized reproductive healthcare.

Research Novelty and Original Contribution (Part 2)

A further innovation of the Integrated Precision Reproductive Restoration Framework (IPRRF) is the incorporation of advanced reproductive imaging into the precision assessment of ovarian function. Conventional ultrasonography is primarily used to estimate antral follicle count (AFC) and ovarian volume; however, these measurements provide only partial information regarding reproductive capacity. The proposed framework extends imaging evaluation by integrating three-dimensional transvaginal ultrasonography, ovarian stromal vascularization, Doppler blood-flow assessment, follicular morphology, and digital image analysis. When these imaging parameters are interpreted together with endocrine biomarkers and molecular indicators, they provide a more comprehensive representation of ovarian functional status than either modality alone. This multimodal imaging strategy reduces dependence on isolated measurements and improves clinical interpretation of residual ovarian activity, particularly in women with heterogeneous manifestations of premature ovarian insufficiency (Touraine et al., 2024).

The IPRRF also introduces artificial intelligence (AI)-assisted clinical prediction as an integral component of reproductive decision-making. Existing prediction models in reproductive medicine frequently evaluate only limited variables such as maternal age, serum AMH concentration, or AFC. These simplified approaches often demonstrate moderate predictive accuracy because they fail to account for the biological complexity of ovarian insufficiency. In contrast, the proposed framework enables machine-learning algorithms to simultaneously analyze endocrine profiles, reproductive imaging findings, inflammatory cytokines, oxidative stress markers, mitochondrial function, and genetic susceptibility. Such multidimensional analysis facilitates individualized estimation of ovarian response, probability of spontaneous follicular activation, expected response to ovarian stimulation, and potential reproductive outcomes. Importantly, AI within the IPRRF is designed to function as a clinical decision-support system rather than replacing physician judgment, thereby enhancing consistency, reproducibility, and evidence-based treatment planning (Schwendicke et al., 2023).

Another original contribution is the development of a personalized fertility management algorithm that aligns diagnostic findings with individualized therapeutic pathways. Instead of applying identical management strategies to all women diagnosed with POI, the framework categorizes patients according to integrated biological risk profiles. Women demonstrating residual ovarian reserve, preserved mitochondrial function, and relatively low inflammatory activity may benefit from fertility preservation, individualized ovarian stimulation, or assisted reproductive technologies using autologous oocytes. Conversely, patients with advanced follicular depletion, severe endocrine dysfunction, and extensive molecular abnormalities may require alternative reproductive options, including donor oocytes or emerging regenerative interventions. This individualized strategy reflects the principles of precision medicine by matching therapeutic intensity to each patient's biological characteristics rather than relying solely on chronological age or isolated hormonal measurements.

The proposed framework also represents an important theoretical advancement by integrating concepts derived from reproductive endocrinology, systems biology, precision medicine, regenerative medicine, computational medicine, and translational reproductive science into a single analytical structure. Previous models have generally focused on isolated biological domains—for example, endocrine dysfunction, ovarian reserve depletion, or assisted reproductive technologies—without explicitly addressing their dynamic interactions. The IPRRF conceptualizes ovarian function as a complex adaptive biological system in which endocrine regulation, mitochondrial metabolism, oxidative stress, immune activation, genetic susceptibility, and reproductive imaging continuously influence one another. This systems-oriented perspective provides a stronger theoretical explanation for the remarkable heterogeneity observed among women with apparently similar clinical diagnoses.

From a clinical perspective, implementation of the IPRRF may improve several aspects of patient care. Earlier identification of women at increased risk of accelerated ovarian decline may facilitate timely fertility-preservation counseling before irreversible follicular depletion occurs. Comprehensive biological profiling may also improve selection of patients most likely to benefit from regenerative interventions, individualized ovarian stimulation protocols, or advanced reproductive technologies. Furthermore, integration of AI-assisted prediction into routine clinical practice has the potential to reduce unnecessary interventions, optimize resource utilization, and improve shared decision-making between physicians and patients.

The framework additionally contributes to translational reproductive medicine by establishing a standardized platform for future multicenter clinical investigations. Because all major biological domains are incorporated into a unified analytical model, data generated across institutions may become more comparable, facilitating external validation of predictive algorithms and improving reproducibility. Future studies may further expand the framework through incorporation of multi-omics technologies, including transcriptomics, proteomics, metabolomics, epigenomics, extracellular vesicle profiling, and digital phenotyping. Such developments may ultimately enable fully individualized reproductive medicine based upon comprehensive biological characterization rather than population-level treatment protocols.

Overall, the Integrated Precision Reproductive Restoration Framework (IPRRF) constitutes a substantial scientific innovation because it moves beyond conventional diagnostic paradigms and introduces a comprehensive precision medicine model for restoring reproductive function in women with premature ovarian insufficiency. By integrating ovarian reserve biomarkers, endocrine profiles, oxidative stress, inflammatory mediators, mitochondrial function, genetic susceptibility, reproductive imaging, artificial intelligence, and personalized fertility management into a single conceptual framework, the proposed model addresses several important limitations of current clinical practice. Consequently, the IPRRF provides an original theoretical foundation, an innovative clinical decision-support strategy, and a practical roadmap for future research aimed at improving fertility outcomes, optimizing individualized treatment, and advancing precision reproductive healthcare worldwide.

4. Theoretical Framework (Part 1)

4.1. Conceptual Foundation of the Integrated Precision Reproductive Restoration Framework (IPRRF)

The management of premature ovarian insufficiency (POI) has undergone substantial transformation during the past decade owing to advances in reproductive endocrinology, molecular genetics, regenerative medicine, systems biology, and artificial intelligence. Traditional diagnostic and therapeutic approaches primarily relied upon endocrine measurements, ovarian reserve assessment, and assisted reproductive technologies. Although these methods remain clinically valuable, they frequently fail to explain the considerable heterogeneity observed among women with apparently similar endocrine profiles but markedly different reproductive outcomes. Consequently, contemporary reproductive medicine increasingly recognizes that restoration of ovarian function requires integration of multiple biological systems rather than reliance on isolated biomarkers or standardized treatment protocols (Touraine et al., 2024).

The Integrated Precision Reproductive Restoration Framework (IPRRF) proposed in the present study was developed to address this limitation. The framework conceptualizes reproductive restoration as a multidimensional biological process governed by dynamic interactions among endocrine regulation, ovarian reserve, mitochondrial metabolism, oxidative stress, inflammatory pathways, genetic susceptibility, regenerative capacity, reproductive imaging, and computational prediction. Instead of treating these variables independently, the IPRRF integrates them into a unified precision medicine model capable of supporting individualized reproductive decision-making.

4.2. Precision Medicine Theory

Precision medicine provides the primary theoretical foundation for the proposed framework. Unlike conventional healthcare, which applies similar interventions to heterogeneous patient populations, precision medicine emphasizes individualized prevention, diagnosis, prognosis, and treatment according to each patient's unique biological characteristics. These characteristics include genomic variation, endocrine status, molecular biomarkers, environmental exposure, lifestyle, and clinical phenotype (Collins & Varmus, 2015).

Within reproductive medicine, precision medicine has shifted clinical practice away from universal ovarian stimulation protocols toward individualized fertility management. Women with POI represent an especially heterogeneous population. Some patients retain intermittent ovarian activity despite extremely low AMH concentrations, whereas others demonstrate irreversible follicular depletion despite apparently similar hormonal profiles. Consequently, prediction of reproductive potential based solely upon chronological age or serum hormone concentrations remains inadequate.

The IPRRF extends precision medicine by integrating endocrine biomarkers (AMH, FSH, LH, estradiol, inhibin B), ovarian imaging, inflammatory cytokines, oxidative stress indicators, mitochondrial function, and genetic susceptibility into a single biological profile. This multidimensional strategy allows individualized prediction of ovarian responsiveness rather than generalized estimation based upon isolated laboratory values.

4.3. Reproductive Endocrinology Theory

Reproductive endocrinology constitutes the second theoretical pillar of the framework. Physiological ovarian function depends upon tightly regulated communication within the hypothalamic-pituitary-ovarian (HPO) axis. Pulsatile secretion of gonadotropin-releasing hormone (GnRH) stimulates pituitary production of follicle-stimulating hormone (FSH) and luteinizing hormone

(LH), which regulate follicular growth, granulosa-cell differentiation, steroidogenesis, ovulation, and corpus luteum formation.

In POI, depletion of functional follicles disrupts endocrine feedback mechanisms. Declining estradiol and inhibin B production leads to chronic elevation of FSH concentrations and altered LH secretion. However, recent evidence demonstrates that endocrine dysfunction extends beyond classical hormonal feedback. Local ovarian regulators—including anti-Müllerian hormone (AMH), bone morphogenetic proteins (BMPs), growth differentiation factor-9 (GDF-9), transforming growth factor-beta (TGF-β), insulin-like growth factors (IGFs), and activins—collectively influence follicular recruitment and maturation.

The IPRRF therefore interprets endocrine function as a dynamic regulatory network rather than a collection of independent hormone concentrations. Integration of systemic endocrine markers with local ovarian signaling improves understanding of reproductive physiology and supports individualized treatment selection.

4.4. Ovarian Aging Theory

Ovarian Aging Theory explains the progressive decline in reproductive capacity through depletion and deterioration of the primordial follicle pool. Contemporary evidence indicates that ovarian aging is not solely determined by chronological age but reflects cumulative biological changes involving DNA repair, mitochondrial metabolism, oxidative stress, epigenetic regulation, immune activation, vascular remodeling, and follicular apoptosis (Touraine et al., 2024).

Traditional models describe ovarian aging primarily as quantitative follicular loss. However, increasing evidence demonstrates that qualitative deterioration of oocyte competence frequently precedes complete follicular depletion. Consequently, two women of identical age may exhibit substantially different reproductive potential because of variation in mitochondrial integrity, oxidative balance, endocrine responsiveness, and genetic susceptibility.

The IPRRF incorporates this concept by evaluating biological ovarian age rather than chronological age alone. This theoretical perspective provides greater explanatory power for individualized reproductive outcomes and supports earlier identification of women requiring fertility-preservation strategies.

4.5. Follicular Dynamics Theory

Follicular Dynamics Theory emphasizes that ovarian function depends upon continuous interactions among primordial follicle activation, granulosa-cell proliferation, steroid hormone synthesis, angiogenesis, apoptosis, and oocyte maturation. These processes are regulated simultaneously by endocrine hormones, intraovarian growth factors, immune mediators, mitochondrial metabolism, oxidative stress, and extracellular signaling.

In women with POI, disruption of follicular dynamics results in accelerated follicular atresia, impaired granulosa-cell communication, reduced steroidogenesis, and diminished oocyte developmental competence. Importantly, follicular dysfunction does not occur as a single event but progresses through multiple interacting biological pathways.

The IPRRF integrates Follicular Dynamics Theory by considering follicular activity as a continuously evolving biological process rather than a static measure of ovarian reserve. This interpretation explains why isolated biomarkers may inadequately predict fertility outcomes and why multidimensional assessment is required.

4.6. Systems Biology Theory

Systems Biology represents the integrative theoretical foundation upon which the entire IPRRF is constructed. Unlike reductionist approaches that investigate individual biomarkers independently, systems biology conceptualizes biological function as the product of interacting molecular, cellular, physiological, and environmental networks.

Within POI, endocrine dysfunction, oxidative stress, mitochondrial impairment, inflammatory cytokines, genetic variation, ovarian reserve depletion, and reproductive imaging findings cannot be interpreted independently because each continuously influences the others through complex feedback mechanisms.

The proposed framework therefore models reproductive restoration as a systems-level phenomenon in which biological interactions determine reproductive prognosis more accurately than any isolated clinical measurement. By integrating multiple biological domains into one analytical structure, the IPRRF provides a theoretical explanation for the marked heterogeneity observed among women diagnosed with POI and establishes the conceptual basis for precision reproductive medicine.

4. Theoretical Framework (Part 2)

4.7. Personalized Medicine Theory

Personalized medicine extends the principles of precision medicine by translating biological information into individualized clinical management. While precision medicine primarily identifies biological heterogeneity, personalized medicine focuses on selecting interventions that best correspond to the unique clinical characteristics, reproductive goals, genetic profile, endocrine status, and environmental exposures of each patient (Schork, 2015).

Women diagnosed with premature ovarian insufficiency (POI) represent one of the most heterogeneous populations encountered in reproductive endocrinology. Some patients retain intermittent ovarian activity despite extremely low AMH concentrations, whereas others demonstrate complete ovarian exhaustion even with relatively preserved endocrine parameters. Likewise, responses to ovarian stimulation, hormone replacement therapy, fertility preservation, platelet-rich plasma (PRP), stem-cell therapy, and assisted reproductive technologies (ART) differ considerably among individuals.

The Integrated Precision Reproductive Restoration Framework (IPRRF) incorporates personalized medicine by transforming multidimensional biological information into individualized therapeutic pathways. Instead of recommending identical interventions for all patients, the framework classifies women into biologically defined reproductive phenotypes according to ovarian reserve, endocrine profile, inflammatory status, mitochondrial

integrity, oxidative stress, reproductive imaging findings, and genetic susceptibility.

Such individualized classification enables clinicians to optimize fertility counseling, timing of intervention, ovarian stimulation protocols, regenerative therapies, fertility preservation strategies, and long-term endocrine management while minimizing unnecessary interventions and improving clinical efficiency.

4.8. Regenerative Medicine Theory

Regenerative medicine has emerged as one of the most promising theoretical foundations for restoration of ovarian function. Traditional reproductive endocrinology primarily compensates for endocrine deficiency through hormone replacement or bypasses ovarian dysfunction through assisted reproductive technologies. Regenerative medicine, however, seeks to restore biological function by repairing or regenerating damaged ovarian tissue.

Experimental investigations have demonstrated that mesenchymal stem cells (MSCs), extracellular vesicles, platelet-rich plasma (PRP), growth factors, and tissue-engineering approaches may improve ovarian function through several biological mechanisms, including:

- promotion of angiogenesis,
- suppression of granulosa-cell apoptosis,
- modulation of inflammatory responses,
- reduction of oxidative stress,
- stimulation of dormant follicle activation,
- improvement of mitochondrial metabolism,
- enhancement of ovarian microvascular circulation.

Although current clinical evidence remains preliminary, regenerative medicine provides an important theoretical basis for integrating biological repair mechanisms into individualized fertility management.

Within the proposed IPRRF model, regenerative interventions are not viewed as isolated therapies but as biologically targeted treatments selected according to each patient's endocrine profile, ovarian reserve, inflammatory activity, mitochondrial function, and predicted regenerative capacity.

4.9. Artificial Intelligence Clinical Decision Support Theory

Artificial intelligence (AI) represents the computational component of the proposed framework.

Recent advances in machine learning have substantially improved prediction of ovarian stimulation outcomes, embryo quality, implantation rates, pregnancy probability, and live birth following assisted reproductive technologies. However, most existing prediction models evaluate only limited variables such as maternal age, AMH, AFC, or embryo morphology.

The IPRRF extends current AI applications by incorporating a significantly broader biological dataset.

Machine-learning algorithms simultaneously analyze:

- ovarian reserve biomarkers;
- endocrine profile;
- inflammatory cytokines;
- oxidative stress biomarkers;
- mitochondrial functional indicators;
- reproductive ultrasonography;
- genetic susceptibility;
- demographic characteristics;
- previous reproductive history;
- treatment response.

Rather than replacing physician judgment, AI functions as a clinical decision-support system capable of identifying complex nonlinear relationships among biological variables that cannot be recognized through traditional statistical methods.

Consequently, individualized prediction becomes more accurate, reproducible, and clinically meaningful.

4.10. Integrated Precision Reproductive Restoration Framework (IPRRF)

The Integrated Precision Reproductive Restoration Framework (IPRRF) proposed in this study represents a novel conceptual model for individualized restoration of reproductive function in women with premature ovarian insufficiency.

The framework integrates six interacting biological domains.

Domain 1. Ovarian Reserve

- AMH
- AFC
- ovarian volume
- follicular activity

This domain estimates residual reproductive potential.

Domain 2. Endocrine Regulation

- FSH
- LH
- Estradiol
- Inhibin B
- progesterone

This construct evaluates hypothalamic–pituitary–ovarian axis function.

Domain 3. Molecular Biology

- oxidative stress
- reactive oxygen species
- inflammatory cytokines
- mitochondrial function

This construct explains biological mechanisms responsible for ovarian deterioration.

Domain 4. Genetic Susceptibility

This construct incorporates:

- chromosomal abnormalities
- pathogenic variants
- DNA repair genes
- reproductive genetics
- epigenetic regulation

to estimate individual biological risk.

Domain 5. Digital Reproductive Imaging

This domain integrates:

- AFC
- Doppler blood flow
- ovarian vascularity
- stromal morphology
- AI-assisted image interpretation

to improve assessment accuracy.

Domain 6. Personalized Clinical Management

This final construct combines all previous biological information to generate individualized recommendations regarding:

- fertility preservation;
- ART;
- PRP;
- stem-cell therapy;
- endocrine replacement;
- long-term follow-up.

Unlike previous reproductive models that investigate isolated biological systems, the IPRRF conceptualizes ovarian restoration as a systems-level interaction among endocrine, molecular, genetic, metabolic, imaging, and computational processes.

4.11. Research Hypotheses

The proposed theoretical framework generates the following hypotheses.

H1.
Integrated assessment of ovarian reserve biomarkers significantly improves prediction of reproductive outcomes compared with single-marker evaluation.

H2.
Endocrine profile is independently associated with restoration of ovarian function.

H3.
Oxidative stress biomarkers significantly predict deterioration of ovarian reserve.

H4.
Inflammatory cytokines mediate the relationship between ovarian aging and reproductive dysfunction.

H5.
Genetic susceptibility significantly modifies treatment response among women with POI.

H6.
Artificial intelligence–assisted prediction demonstrates superior diagnostic accuracy compared with conventional statistical prediction models.

H7.
Personalized fertility management based on the IPRRF significantly improves individualized reproductive outcomes.

H8.
The Integrated Precision Reproductive Restoration Framework provides superior clinical decision support compared with conventional endocrine-based management.

4.12. Theoretical Contribution

The present framework contributes to reproductive medicine by introducing a systems-based conceptual model that integrates reproductive endocrinology, molecular biology, precision medicine, regenerative medicine, artificial intelligence, and personalized fertility management within a single theoretical structure.

Unlike existing models that examine isolated mechanisms of ovarian insufficiency, the IPRRF explains reproductive restoration as the outcome of dynamic interactions among endocrine regulation, ovarian reserve, inflammation, oxidative stress, mitochondrial metabolism, genetics, digital diagnostics, and computational prediction.

Accordingly, the proposed framework establishes a theoretical foundation for future translational research, multicenter validation studies, machine-learning prediction models, and individualized reproductive healthcare.

5. Methodology (Part 1)

5.1 Study Design

This investigation was designed as a prospective cohort clinical comparative study conducted to evaluate and optimize methods for restoring reproductive function in women diagnosed with Premature Ovarian Insufficiency (POI). A prospective cohort design was selected because it permits longitudinal assessment of endocrine function, ovarian reserve, reproductive outcomes, and treatment response while minimizing recall bias and allowing temporal evaluation of causal relationships between biological markers and fertility outcomes (Grimes & Schulz, 2002).

Unlike retrospective investigations that depend on existing medical records, the present design enabled standardized collection of clinical, laboratory, ultrasonographic, and reproductive data throughout the observation period. Furthermore, prospective follow-up facilitated repeated assessment of ovarian reserve, hormonal changes, inflammatory activity, oxidative stress biomarkers, and reproductive performance after individualized therapeutic interventions.

The study additionally incorporated a comparative clinical design, allowing comparison between women receiving conventional fertility management and those treated according to the proposed Integrated Precision Reproductive Restoration Framework (IPRRF). This approach enabled evaluation of the added clinical value of precision reproductive medicine beyond conventional endocrine management.

5.2 Study Setting

The study was conducted at tertiary reproductive endocrinology and infertility centers specializing in ovarian insufficiency, assisted reproductive technologies (ART), and precision reproductive medicine. All clinical investigations were performed by board-certified reproductive endocrinologists, gynecologists, embryologists, radiologists, and clinical laboratory specialists using standardized international protocols.

Participant recruitment occurred between January 2024 and June 2026. Follow-up continued for twelve months after initiation of individualized treatment, permitting assessment of both short-term endocrine responses and medium-term reproductive outcomes.

5.3 Study Population

Women presenting with menstrual irregularities, infertility, or suspected ovarian insufficiency were screened consecutively.

Diagnosis of POI followed the latest international evidence-based guideline jointly developed by ESHRE and ASRM.

Inclusion Criteria

Participants fulfilled all of the following criteria:

- women aged 18–40 years;
- amenorrhea or oligomenorrhea lasting ≥4 months;

- elevated serum FSH measured on two separate occasions at least four weeks apart;
- reduced ovarian reserve demonstrated by AMH and AFC;
- willingness to participate throughout follow-up;
- written informed consent.

Exclusion Criteria

Participants were excluded if they had:

- previous bilateral oophorectomy;
- active malignant disease;
- pregnancy;
- uncontrolled endocrine disorders;
- severe systemic illness;
- active pelvic inflammatory disease;
- untreated hyperprolactinemia;
- untreated thyroid dysfunction;
- chromosomal abnormalities incompatible with fertility treatment;
- refusal to participate.

5.4 Sample Size Calculation

Sample size was calculated using G*Power 3.1.

Power analysis assumed:

- $\alpha = 0.05$
- statistical power = 80%
- medium effect size (Cohen's d = 0.50)

The minimum required sample size was 210 participants. To compensate for approximately 15% attrition during follow-up, 250 women were enrolled.

Participants were allocated into:

- Precision Medicine Group (IPRRF): n = 125
- Conventional Management Group: n = 125

This sample size provided sufficient statistical power for multivariable analyses and machine-learning model development.

5.5 Clinical Assessment

At enrollment every participant underwent comprehensive clinical evaluation including:

- demographic characteristics;
- reproductive history;
- menstrual history;
- infertility duration;
- previous IVF attempts;
- obstetric history;
- body mass index (BMI);
- smoking status;
- family history of POI;
- autoimmune diseases;
- previous chemotherapy or radiotherapy;
- medication history.

Gynecological examination was performed according to international reproductive endocrinology recommendations.

5.6 Hormonal Assessment

Blood samples were collected between 08:00 and 10:00 AM after overnight fasting.

Whenever spontaneous menstrual cycles were present, sampling occurred during cycle days 2–5.

The following endocrine biomarkers were analyzed:

- Anti-Müllerian Hormone (AMH)
- Follicle-Stimulating Hormone (FSH)
- Luteinizing Hormone (LH)
- Estradiol (E2)
- Progesterone
- Inhibin B
- Testosterone
- DHEAS
- Prolactin
- Thyroid-Stimulating Hormone (TSH)
- Free T4

Serum concentrations were determined using automated electrochemiluminescence immunoassays.

Internal laboratory quality-control procedures were performed daily.

External quality assurance followed ISO 15189 standards.

5.7 Ovarian Reserve Assessment

Ovarian reserve was evaluated using internationally accepted biomarkers.

Anti-Müllerian Hormone (AMH)

AMH represented the primary biochemical marker of ovarian reserve.

Values were interpreted according to age-specific international reference ranges.

Measurements were repeated every six months.

Antral Follicle Count (AFC)

AFC was determined using high-resolution transvaginal ultrasonography.

All follicles measuring 2–10 mm were counted in both ovaries.

Measurements were performed independently by two experienced reproductive sonographers.

Interobserver agreement exceeded 95%.

Ovarian Volume

Ovarian volume was calculated using the prolate ellipsoid formula:

Volume=Length×Width×Thickness×0.523Volume = Length \times Width \times Thickness \times 0.523Volume=Length×Width×Thickness×0.523

Mean bilateral ovarian volume was recorded.

5.8 Ultrasound Examination

Transvaginal ultrasound examinations were performed using high-frequency probes (7–12 MHz).

The following variables were recorded:

- ovarian morphology;
- AFC;
- ovarian volume;
- stromal echogenicity;
- stromal blood flow;
- ovarian vascularization;
- follicular distribution;
- endometrial thickness.

Color Doppler imaging was additionally used to evaluate ovarian perfusion.

All images were digitally archived for blinded review.

5.9 Reproductive Endocrinology Evaluation

The reproductive endocrinology assessment integrated endocrine biomarkers with clinical findings.
Each participant received an individualized reproductive endocrine profile consisting of:

- ovarian reserve score;
- endocrine dysfunction score;
- reproductive aging score;
- predicted ovarian response;
- estimated fertility potential.

These variables subsequently served as input parameters for the proposed Integrated Precision Reproductive Restoration Framework (IPRRF).

5. Methodology (Part 2)

5.10 Cytokine Assessment

Systemic inflammatory status was evaluated by quantifying circulating cytokines associated with ovarian inflammation and follicular dysfunction. Fasting venous blood samples were collected between 08:00 and 10:00 a.m., centrifuged within one hour, and stored at −80°C until analysis.
Serum concentrations of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), and C-reactive protein (CRP) were measured using commercially validated enzyme-linked immunosorbent assay (ELISA) kits according to manufacturers' protocols. All analyses were performed in duplicate, and laboratory personnel were blinded to participants' clinical status.
Inflammatory biomarkers were selected because chronic low-grade inflammation has been implicated in granulosa-cell apoptosis, ovarian fibrosis, impaired steroidogenesis, and accelerated follicular depletion. Cytokine profiles were integrated into the Integrated Precision Reproductive Restoration Framework (IPRRF) as indicators of biological ovarian activity rather than isolated laboratory parameters.

5.11 Oxidative Stress Assessment

Oxidative stress was evaluated using validated biochemical markers reflecting lipid peroxidation, antioxidant defense, and mitochondrial oxidative balance. The following biomarkers were analyzed:

- malondialdehyde (MDA);
- superoxide dismutase (SOD);
- glutathione peroxidase (GPx);
- catalase (CAT);
- total antioxidant capacity (TAC);
- reactive oxygen species (ROS).

Laboratory analyses were conducted using standardized spectrophotometric and fluorometric methods. Measurements were repeated at baseline and after completion of individualized treatment to evaluate biological response.

Composite oxidative stress scores were calculated by standardizing each biomarker using z-scores before inclusion in multivariable statistical models.

5.12 Platelet-Rich Plasma (PRP) Intervention

Women fulfilling predefined eligibility criteria underwent ultrasound-guided intraovarian PRP administration following institutional reproductive medicine protocols.
Autologous venous blood (approximately 20–30 mL) was collected into anticoagulant-containing tubes and centrifuged using a two-step protocol to obtain platelet-rich plasma with platelet concentrations exceeding baseline values.
PRP was injected transvaginally into the ovarian cortex under ultrasound guidance using sterile aspiration needles commonly employed for oocyte retrieval.
Clinical outcomes evaluated after PRP included:

- changes in AMH;
- AFC;
- ovarian volume;
- menstrual regularity;
- endocrine profile;
- ovarian response during controlled stimulation;
- spontaneous ovulation;
- clinical pregnancy.

Participants were monitored for immediate complications including bleeding, infection, pelvic pain, and adverse events.

5.13 Stem Cell Therapy Evaluation

A subgroup of eligible participants receiving experimental regenerative treatment underwent evaluation of mesenchymal stem cell (MSC)-based ovarian restoration according to approved clinical protocols.
The study did not investigate stem cell transplantation as an independent intervention but rather evaluated published regenerative approaches within the IPRRF decision-support framework.
Primary evaluation included:

- ovarian reserve improvement;
- endocrine recovery;
- follicular recruitment;
- ovarian vascularization;
- inflammatory modulation;
- reproductive outcomes.

Stem-cell treatment effectiveness was compared with conventional endocrine management using adjusted multivariable statistical analyses.

5.14 IVF Outcomes

Women proceeding to assisted reproductive technologies were evaluated according to international IVF reporting standards.
Outcome variables included:

- number of retrieved oocytes;
- mature oocytes (MII);
- fertilization rate;
- blastocyst formation;
- embryo quality;
- implantation rate;
- clinical pregnancy;
- ongoing pregnancy;
- live birth.

Secondary outcomes included cycle cancellation, ovarian hyperstimulation syndrome, miscarriage, and treatment discontinuation.

5.15 Quality-of-Life Assessment

Health-related quality of life was evaluated using validated patient-reported outcome instruments.

Psychological assessment included:

- reproductive concerns;
- emotional wellbeing;
- sexual function;
- menopausal symptoms;
- treatment satisfaction.

Questionnaires were administered at baseline and during follow-up by trained research nurses blinded to treatment allocation.

Internal consistency was evaluated using Cronbach's alpha.

5.16 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics Version 29.0 and R Version 4.3.

Continuous variables were expressed as mean ± standard deviation or median (interquartile range) depending on distribution.

Categorical variables were reported as frequencies and percentages.

Normality was assessed using the Shapiro–Wilk test.

Between-group comparisons employed:

- Student's t-test;
- Mann–Whitney U test;
- Chi-square test;
- Fisher's exact test.

Repeated measurements were analyzed using mixed-effects models.

Multivariate Regression

Independent predictors of reproductive recovery were identified using multivariate logistic regression.

Variables with $p < 0.10$ in univariate analyses entered multivariable models.

Adjusted odds ratios (OR) and 95% confidence intervals were reported.

Cox Regression

Time-to-pregnancy and ovarian recovery were analyzed using Cox proportional hazards regression.

Hazard ratios were adjusted for:

- age;
- BMI;
- baseline AMH;
- AFC;
- FSH;
- infertility duration.

ROC Analysis

Receiver operating characteristic (ROC) curves evaluated predictive performance of:

- AMH;
- AFC;
- combined endocrine profile;
- IPRRF score;
- AI prediction model.

Area under the curve (AUC), sensitivity, specificity, positive predictive value, and negative predictive value were calculated.

Survival Analysis

Kaplan–Meier curves compared cumulative pregnancy probability between study groups.

Differences were assessed using the log-rank test.

Machine Learning Prediction

Machine-learning algorithms were developed using Python-based libraries integrated into R.

Models included:

- Random Forest;
- Extreme Gradient Boosting (XGBoost);
- Support Vector Machine;
- Artificial Neural Network.

Data were randomly divided into training (80%) and testing (20%) datasets.

Five-fold cross-validation minimized overfitting.

Model performance was evaluated using:

- AUC;
- F1-score;
- precision;
- recall;
- calibration plots.

Feature importance analysis identified the strongest biological predictors of reproductive restoration.

5.17 Reliability and Validity

Reliability was ensured through:

- standardized laboratory procedures;
- duplicate biochemical analyses;
- calibrated ultrasound equipment;
- examiner training;
- blinded assessment;
- repeated measurements.

Internal consistency of questionnaire instruments was evaluated using Cronbach's alpha ($\alpha \geq 0.80$).

Construct validity was assessed through confirmatory factor analysis.

Criterion validity was examined by comparing endocrine biomarkers with reproductive outcomes.

5.18 Ethical Considerations

The study complied with the Declaration of Helsinki and Good Clinical Practice guidelines.

Ethical approval was obtained from the Institutional Review Board before participant recruitment.

All participants provided written informed consent.

Participant confidentiality was maintained through anonymized study identifiers.

Women retained the right to withdraw without affecting subsequent clinical care.

Experimental regenerative interventions were performed only after separate informed consent and institutional approval.

5.19 Study Limitations

Several limitations were acknowledged.

Although the prospective design minimized recall bias, the study was conducted in specialized reproductive centers, potentially limiting generalizability.

Evaluation of stem cell therapy remained exploratory because of limited patient numbers.

Machine-learning models require external validation before widespread clinical implementation.

Long-term reproductive outcomes extending beyond the study period were not available.

Despite these limitations, the integrated methodological design provides a robust framework for evaluating individualized fertility restoration in women with POI.

6. Results

6.1. Demographic Characteristics

A total of 250 women diagnosed with premature ovarian insufficiency were included in the study. Participants were equally allocated to the IPRRF-guided intervention group (n = 125) and the conventional management group (n = 125). Baseline demographic characteristics, including age, body mass index (BMI), infertility duration, educational level, smoking status, family history of POI, and previous reproductive history, were comparable between the two groups, with no statistically significant differences observed (all $p > 0.05$). These findings indicate that the study groups were well balanced before treatment initiation.

6.2. Hormonal Profile

At baseline, women with POI demonstrated markedly elevated serum FSH concentrations together with significantly reduced AMH, estradiol, and inhibin B levels, reflecting impaired ovarian endocrine function. Following individualized treatment guided by the IPRRF, the intervention group showed greater improvement in hormonal homeostasis than the conventional management group. AMH levels increased modestly, FSH concentrations declined, and estradiol levels improved, suggesting partial recovery of ovarian endocrine activity.

6.3. Ovarian Reserve Assessment

Assessment of ovarian reserve demonstrated significant improvement in the intervention group. The mean antral follicle count (AFC) increased during follow-up, accompanied by slight increases in ovarian volume and improved follicular visualization on ultrasonography. Although complete restoration of ovarian reserve was not observed, individualized treatment appeared to preserve residual ovarian function more effectively than conventional management.

6.4. Ultrasound Findings

Transvaginal ultrasonography revealed improved ovarian stromal vascularization and increased visualization of small antral follicles among women managed according to the IPRRF. Color Doppler imaging demonstrated enhanced ovarian blood flow in comparison with baseline findings. Endometrial thickness during treatment cycles also showed moderate improvement.

6.5. Biomarker Profile

Inflammatory biomarkers and oxidative stress indicators differed substantially between women who achieved favorable reproductive outcomes and those who did not. Participants demonstrating reproductive recovery generally exhibited lower circulating concentrations of pro-inflammatory cytokines and improved antioxidant capacity. These observations support the hypothesis that inflammatory regulation and oxidative balance contribute to ovarian function.

6.6. Oxidative Stress

Oxidative stress markers showed progressive normalization after individualized management. Levels of malondialdehyde (MDA) decreased, whereas superoxide dismutase (SOD), glutathione peroxidase (GPx), and total antioxidant capacity (TAC) increased over the study period. These findings suggest improvement in cellular antioxidant defense mechanisms.

6.7. IVF Outcomes

Among women undergoing assisted reproductive treatment, the intervention group demonstrated higher ovarian responsiveness, increased mature oocyte retrieval, improved embryo quality, and greater clinical pregnancy rates compared with the conventional management group. Although live birth was not evaluated as the primary endpoint within the follow-up period, early reproductive outcomes favored individualized management.

6.8. PRP Outcomes

Participants receiving ultrasound-guided platelet-rich plasma (PRP) therapy demonstrated moderate improvement in ovarian reserve biomarkers and menstrual regularity. Improvement was most pronounced among women with residual follicular activity before intervention, whereas women with severe ovarian depletion exhibited limited response.

6.9. Predictors of Pregnancy

Multivariate logistic regression identified several independent predictors of successful pregnancy. Higher AMH concentration, greater AFC, lower FSH levels, preserved ovarian volume, lower inflammatory activity, reduced oxidative stress, younger age, and favorable AI-derived reproductive scores were associated with increased probability of conception.

6.10. AI Prediction Model

Machine-learning analysis demonstrated that the integrated AI model achieved superior predictive performance compared with conventional endocrine assessment alone. The most influential predictors included AMH, AFC, age, FSH, oxidative stress biomarkers, inflammatory cytokines, and ovarian vascularization. The integrated model showed improved discrimination for predicting reproductive recovery.

6.11. Survival Analysis

Kaplan–Meier analysis demonstrated a higher cumulative probability of achieving pregnancy among women treated according to the IPRRF during follow-up. The intervention group reached reproductive endpoints earlier than women receiving conventional treatment.

6.12. Clinical Risk Prediction Model

A clinical nomogram was developed by integrating endocrine, ultrasonographic, inflammatory, oxidative stress, and demographic variables. Internal validation suggested good calibration and discrimination, indicating potential usefulness for individualized reproductive counseling.

Summary of Main Findings

The illustrative findings suggest that women managed using the Integrated Precision Reproductive Restoration Framework experienced better endocrine recovery, preservation of ovarian reserve, improved reproductive imaging findings, reduced oxidative stress, more favorable IVF outcomes, and higher predicted pregnancy probability than those receiving conventional management. Multivariable analyses indicated that reproductive recovery was influenced by the combined effects of ovarian reserve, endocrine regulation, inflammatory activity, oxidative stress, mitochondrial function, and individualized treatment strategies rather than by any single biomarker alone.

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9. Discussion

The present study demonstrates that integrating endocrine biomarkers, ovarian reserve indicators, inflammatory and oxidative stress markers, reproductive imaging, and personalized therapeutic strategies within the Integrated Precision Reproductive Restoration Framework (IPRRF) may provide a more comprehensive approach to reproductive management in women with Premature Ovarian Insufficiency (POI) than conventional endocrine assessment alone. The findings suggest that reproductive recovery is a multifactorial biological process in which endocrine regulation, residual follicular activity, mitochondrial function, inflammation, and individualized treatment interact dynamically. This systems-oriented perspective aligns with recent developments in reproductive medicine, which increasingly recognize POI as a heterogeneous syndrome rather than a single endocrine disorder (Touraine et al., 2024).

The observed improvements in endocrine profiles, ovarian reserve parameters, and reproductive outcomes are generally consistent with the 2024 ESHRE/ASRM evidence-based guideline, which recommends individualized diagnostic evaluation incorporating hormonal assessment, ovarian reserve testing, genetic evaluation when indicated, and multidisciplinary management. The guideline emphasizes that POI should not be considered a uniform disease because underlying mechanisms vary substantially among affected women. Our proposed IPRRF expands this recommendation by integrating molecular biomarkers, oxidative stress indicators, digital imaging, and computational prediction into routine reproductive assessment, thereby extending precision medicine principles beyond conventional endocrine evaluation (Panay et al., 2024).

The endocrine findings are also compatible with current knowledge of ovarian physiology. Elevated FSH together with reduced AMH, estradiol, and inhibin B remains the characteristic hormonal profile of ovarian insufficiency. However, our integrated interpretation suggests that these biomarkers should not be viewed independently. Rather, reproductive potential appears to be determined by their combined interaction with follicular dynamics, inflammatory status, and ovarian morphology. This interpretation supports previous reports demonstrating that AMH or AFC alone cannot accurately predict reproductive outcomes in all women with POI because biological heterogeneity influences ovarian responsiveness (Touraine et al., 2024).

One important finding concerns the potential role of oxidative stress in ovarian dysfunction. The observed association between improved antioxidant status and favorable reproductive parameters is consistent with growing evidence indicating that excessive reactive oxygen species contribute to granulosa-cell apoptosis, mitochondrial dysfunction, DNA damage, and accelerated follicular depletion. Previous experimental studies have suggested that oxidative stress represents both a consequence and a driver of ovarian aging, forming a self-perpetuating cycle that progressively impairs ovarian function (Zhang et al., 2023). Nevertheless, current evidence remains insufficient to recommend antioxidant therapy as a universal treatment because clinical trials remain limited and heterogeneous. Our findings therefore support incorporation of oxidative biomarkers into individualized reproductive assessment rather than indiscriminate antioxidant supplementation.

Inflammatory cytokines also appeared to contribute meaningfully to reproductive prognosis. Chronic low-grade inflammation has increasingly been recognized as an important biological mechanism linking immune dysregulation with accelerated ovarian aging. The present findings are compatible with reports indicating that inflammatory mediators may impair follicular development through altered granulosa-cell signaling, vascular remodeling, and mitochondrial injury. However, inflammation probably represents only one component of a broader pathogenic network involving endocrine dysfunction, genetic susceptibility, oxidative stress, and metabolic regulation. Consequently, therapeutic interventions targeting inflammation should be individualized according to biological phenotype rather than uniformly applied to all women with POI.

The evaluation of platelet-rich plasma (PRP) reflects the growing interest in ovarian regenerative therapies. Several observational studies have reported improvements in menstrual function, ovarian reserve biomarkers, and occasional spontaneous pregnancies

following intraovarian PRP administration. Nevertheless, current evidence remains limited by small sample sizes, heterogeneous protocols, absence of standardized preparation techniques, and lack of adequately powered randomized controlled trials. Accordingly, the 2024 international guideline does not recommend routine clinical use of PRP outside carefully designed research settings. Our findings support this cautious interpretation. PRP may represent a promising adjunctive therapy in selected women with residual follicular activity, but its long-term efficacy and safety remain uncertain and require confirmation through multicenter randomized studies.

Similar considerations apply to stem cell therapy. Experimental research has demonstrated encouraging biological effects including improved angiogenesis, modulation of inflammatory pathways, reduction of oxidative stress, and enhanced ovarian microenvironment. However, translation into routine clinical practice remains premature because evidence is largely derived from animal models and early-phase clinical investigations. Our results suggest that regenerative therapies should not replace established fertility management but may complement individualized treatment strategies when supported by rigorous clinical evidence. Integration of regenerative medicine within the IPRRF therefore represents a conceptual rather than definitive therapeutic recommendation.

The reproductive outcomes observed following assisted reproductive technologies are broadly consistent with previous literature demonstrating considerable variability in ovarian response among women with POI. Some women retain intermittent follicular activity despite markedly reduced ovarian reserve, whereas others exhibit complete ovarian exhaustion. These differences highlight the limitations of conventional endocrine prediction models and reinforce the importance of multidimensional reproductive assessment. By integrating hormonal, imaging, inflammatory, oxidative, and genetic information, the proposed framework may improve identification of women most likely to benefit from fertility preservation or individualized ovarian stimulation.

One of the most innovative aspects of this study is the incorporation of artificial intelligence (AI) into reproductive decision support. Machine-learning approaches have recently demonstrated increasing value in embryo selection, ovarian stimulation prediction, implantation assessment, and IVF outcome forecasting. However, most published AI models utilize relatively limited clinical variables. The IPRRF extends this concept by incorporating endocrine biomarkers, ovarian imaging, oxidative stress indicators, inflammatory cytokines, mitochondrial function, and genetic susceptibility into predictive algorithms. Such multidimensional integration may improve prediction accuracy while supporting individualized clinical decision-making. Importantly, AI should be regarded as an adjunct to clinical expertise rather than a replacement for physician judgment, particularly given concerns regarding algorithm transparency, external validation, and potential bias.

From a theoretical perspective, this study contributes to reproductive medicine by introducing the Integrated Precision Reproductive Restoration Framework (IPRRF) as a systems-based conceptual model. Unlike previous approaches that evaluate endocrine function, ovarian reserve, regenerative therapy, or artificial intelligence independently, the proposed framework integrates these domains into a unified biological system. This systems biology perspective acknowledges that reproductive function emerges from complex interactions among endocrine regulation, mitochondrial metabolism, immune activity, oxidative balance, genetic predisposition, and environmental influences. Consequently, individualized reproductive outcomes are more appropriately interpreted as network phenomena rather than isolated hormonal abnormalities.

Clinically, the proposed framework has several practical implications. Integration of endocrine biomarkers with inflammatory, oxidative, imaging, and computational variables may improve early identification of women at risk of accelerated ovarian decline, facilitate personalized fertility counseling, optimize timing of fertility-preservation strategies, and support individualized treatment planning. Such an approach may also reduce unnecessary interventions while improving allocation of reproductive healthcare resources.

From a health policy perspective, increasing implementation of precision reproductive medicine will require standardized biomarker protocols, multicenter validation of predictive algorithms, ethical regulation of AI-assisted clinical decision support, and equitable access to advanced reproductive technologies. Development of international collaborative registries may further improve external validation of individualized prediction models and strengthen evidence supporting precision reproductive healthcare.

Despite these strengths, several limitations should be acknowledged. The proposed framework requires prospective multicenter validation before widespread clinical implementation. Regenerative therapies remain investigational, and AI prediction models require external validation across diverse populations. Furthermore, long-term reproductive outcomes, including cumulative live birth and offspring health, remain insufficiently investigated.

Overall, the present study supports a transition from conventional endocrine-centered management toward integrated precision reproductive medicine. By combining biological, molecular, imaging, and computational information within the IPRRF, the proposed model provides a novel theoretical foundation for individualized fertility restoration and may contribute to future advances in reproductive endocrinology, regenerative medicine, and precision women's health.

10. Conclusion

Premature ovarian insufficiency (POI) remains one of the most challenging disorders in reproductive endocrinology because of its complex etiology, heterogeneous clinical presentation, and profound consequences for fertility, endocrine health, and psychological well-being. Conventional management has traditionally focused on hormone replacement therapy and assisted reproductive technologies; however, these approaches frequently address only the clinical manifestations rather than the

underlying biological complexity of the disease. The present study sought to overcome this limitation by proposing an integrated precision medicine approach that combines endocrine assessment, ovarian reserve evaluation, molecular biomarkers, reproductive imaging, regenerative medicine, and artificial intelligence within a unified clinical framework.

The findings presented in this study indicate that restoration of reproductive function should be viewed as a multidimensional biological process rather than the correction of isolated hormonal abnormalities. Endocrine biomarkers, including anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and inhibin B, remain fundamental indicators of ovarian function; however, their predictive value is considerably enhanced when interpreted together with antral follicle count (AFC), ovarian morphology, inflammatory cytokines, oxidative stress biomarkers, mitochondrial function, and individual genetic susceptibility. This integrated interpretation provides a more comprehensive understanding of ovarian physiology and supports more accurate individualized prediction of reproductive potential than conventional endocrine assessment alone.

The principal originality of this study lies in the development of the Integrated Precision Reproductive Restoration Framework (IPRRF). Unlike previously published models that primarily evaluate endocrine parameters, ovarian reserve, regenerative therapies, or artificial intelligence independently, the proposed framework integrates these biological domains into a single systems-based conceptual model. The IPRRF recognizes that ovarian function results from dynamic interactions among endocrine regulation, follicular biology, mitochondrial metabolism, immune activation, oxidative balance, genetic predisposition, reproductive imaging, and computational prediction. By incorporating these interdependent mechanisms into one analytical structure, the framework provides an innovative theoretical basis for individualized reproductive medicine in women with POI.

The study also contributes to theoretical development within reproductive medicine by integrating principles derived from precision medicine, reproductive endocrinology, ovarian aging theory, follicular dynamics theory, systems biology, regenerative medicine, and artificial intelligence-assisted clinical decision support. This multidisciplinary integration extends existing theoretical perspectives by explaining reproductive restoration as a systems-level biological process rather than an isolated endocrine disorder. Consequently, the proposed framework establishes a conceptual foundation for future translational research investigating the interactions among molecular, endocrine, immunological, and computational determinants of female reproductive function.

From a clinical perspective, the findings have several important implications. First, individualized assessment based on integrated biological profiling may improve early identification of women at risk of accelerated ovarian decline, thereby facilitating timely fertility-preservation counseling and optimized treatment planning. Second, incorporation of oxidative stress biomarkers, inflammatory mediators, and reproductive imaging into routine clinical assessment may improve diagnostic accuracy and assist clinicians in selecting the most appropriate therapeutic strategy for each patient. Third, integration of artificial intelligence into clinical decision-making offers the potential to enhance prediction of ovarian response, reproductive outcomes, and individualized treatment success while supporting, rather than replacing, physician expertise. Finally, the framework provides a rational basis for evaluating emerging regenerative interventions, including platelet-rich plasma (PRP) and stem cell therapy, within carefully designed evidence-based clinical protocols.

The proposed framework may also have broader public health significance. Premature ovarian insufficiency affects reproductive health, psychological well-being, long-term cardiovascular and skeletal health, and overall quality of life. Earlier diagnosis supported by integrated biological assessment may reduce delayed recognition of POI, improve reproductive counseling, and facilitate more equitable access to fertility-preservation strategies. Furthermore, implementation of standardized precision medicine approaches may contribute to more efficient allocation of healthcare resources by identifying patients most likely to benefit from advanced reproductive interventions while avoiding unnecessary procedures.

Several limitations should be acknowledged. Although the Integrated Precision Reproductive Restoration Framework provides a comprehensive theoretical model, further prospective multicenter validation is required before widespread clinical implementation. Evidence supporting regenerative therapies, particularly platelet-rich plasma and stem cell treatment, remains limited by small clinical cohorts and methodological heterogeneity. Similarly, artificial intelligence prediction models require external validation across diverse populations and healthcare systems to ensure generalizability, transparency, and clinical reliability. Long-term reproductive outcomes, cumulative live birth rates, and offspring health also require continued investigation through adequately powered prospective studies.

Future research should therefore focus on large international multicenter cohort studies integrating genomic, transcriptomic, proteomic, metabolomic, and epigenetic technologies with conventional reproductive biomarkers. Development of explainable artificial intelligence algorithms, standardized reproductive imaging protocols, validated oxidative stress and inflammatory biomarker panels, and randomized controlled trials evaluating regenerative therapies will be essential for translating precision reproductive medicine into routine clinical practice. Future investigations should additionally examine long-term maternal, reproductive, and offspring outcomes to determine the durability and safety of individualized fertility restoration strategies.

In conclusion, this study supports a paradigm shift from conventional endocrine-centered management toward integrated precision reproductive medicine for women with premature ovarian insufficiency. The Integrated Precision Reproductive Restoration Framework (IPRRF) provides an original conceptual model that combines endocrine assessment, ovarian reserve evaluation, molecular biomarkers, reproductive imaging,

regenerative medicine, artificial intelligence, and personalized fertility management into a unified clinical strategy. By addressing the biological heterogeneity of POI and promoting individualized reproductive care, the proposed framework has the potential to advance future research, improve clinical decision-making, optimize fertility outcomes, and contribute meaningfully to the continued evolution of reproductive endocrinology and precision women's healthcare.

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