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Mechanisms of Prolactin Function and Related Disorders: A Comprehensive Review

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Abstract: ***Prolactin (PRL) is an essential peptide hormone primarily involved in lactation. This paper explores the molecular characteristics of prolactin, genetic mutations in prolactin receptors, and their implications in hyperprolactinemia and related disorders. Key challenges in prolactin measurement, such as the high-dose hook effect and macroprolactinemia, are discussed. Additionally, current dopamine agonist treatments and novel antibody-based therapies are reviewed. The need for improved diagnostic accuracy and future therapeutic advancements in prolactin-related disorders is emphasized.***

Keywords: prolactin, prolactin receptor, growth hormone, pituitary gland, immunoassay

1. Introduction

Prolactin is a hormone secreted by the lactotroph cells in the anterior pituitary gland. It acts primarily on the mammary glands, where it plays a crucial role in lactation by stimulating milk production (Figure 1) [1]. Additionally, prolactin is a crucial hormone in reproductive health, metabolism, and immune system regulation [2], [3]. Misregulation of prolactin levels can lead to various disorders such as hyperprolactinemia and multiple fibroadenomas of the breast (MFAB). Hyperprolactinemia, marked by elevated levels of prolactin, can cause infertility and galactorrhea. It is often associated with pituitary tumors such as prolactinomas [4], [5]. MFAB is characterized by the presence of multiple benign tumors in breast tissue, which are usually painless [6]. Understanding the mechanisms underlying prolactin regulation is essential for diagnosing and managing related endocrine disorders.

2. Prolactin Molecular Characteristics

Prolactin is classified under the prolactin/growth hormone/placental lactogen family, which belongs to group I of the helix bundle protein hormones, due to its genetic, structural, binding, and functional characteristics [7], [8]. In human prolactin, its single chain of amino acids forms three intramolecular disulfide bonds between six cysteine residues: Cys4-Cys11, Cys58-Cys174, and Cys191-Cys199 [9], [10].

Prolactin exists in three distinct forms in the bloodstream: monomeric prolactin, dimeric prolactin, and macroprolactin (Table 1) [4], [11], [12]. Dimeric prolactin consists of two monomeric prolactin molecules, while macroprolactin is a complex formed with IgG autoantibody. Both dimeric prolactin and macroprolactin are stabilized through non-covalent interactions [2].

Monomeric prolactin, with a molecular weight of 23 kDa, is the most prevalent form in serum, constituting approximately 80-95% of the total prolactin. Dimeric prolactin, which has a molecular weight ranging from 48 to 56 kDa, accounts for about 5-10% of the serum prolactin. Similarly, macroprolactin, with a molecular weight exceeding 150 kDa represents around 5-10% of circulating prolactin [4], [11], [12]. Macroprolactin is a form of prolactin that is bound to an immunoglobulin molecule, most commonly IgG [12], [14], [15]. This antigen-antibody complex formation increases the molecular weight of the complex, which is cleared from the body at a slower rate. Due to its large size, macroprolactin remains within the blood vessels and cannot cross the capillary endothelium to reach target tissues [16], [17]. Therefore, even though macroprolactin is naturally present in the serum, it is considered to be biologically inactive or less functional compared to the monomeric form of prolactin [18].

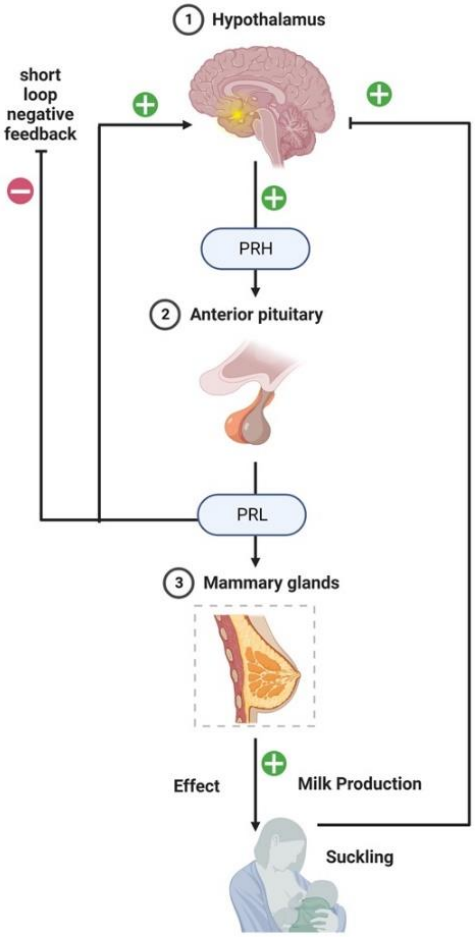

Figure 1: The hypothalamus-anterior pituitary gland-mammary gland axis showing regulation of prolactin secretion and milk production.

The prolactin-releasing hormone (PRH) from the hypothalamus signals the anterior pituitary for prolactin (PRL) secretion, which further binds to prolactin receptor

(PRLR) in mammary epithelial cells (MECs) to stimulate milk protein synthesis. Reprints with permission from Jena et al. 2023 [13].

Table 1: Forms of circulating prolactin.

Type of Prolactin	*Molecular Weight (kDa)*	*Prevalence in Serum (%)*
Monomeric	23	80-95
Dimeric (Big)	48-56	5-10
Macro (Big-big)	>150	5-10

Macroprolactinemia occurs when the concentration of macroprolactin exceeds 60% of the total serum prolactin [19]. It is generally benign in patients with normal levels of active prolactin, show few or no symptoms of elevated prolactin, and have normal pituitary imaging [20]. The pathogenesis of macroprolactinemia is not well understood. However, it is proposed that post-translational modifications (PTMs) might increase prolactin's immunogenicity. This can contribute to the production of anti-prolactin autoantibodies, resulting in the accumulation of macroprolactin in the bloodstream [21]. While macroprolactinemia is usually harmless, an imbalance in the proportion of macroprolactin to monomeric prolactin can create diagnostic difficulties [22]. Macroprolactin can be detected by standard immunoassays, producing falsely high total prolactin levels [12]. When high total prolactin results from increased macroprolactin levels rather than elevated monomeric prolactin, it can cause misdiagnosis in clinical settings. This occurs because standard immunoassays might inaccurately measure elevated total prolactin levels without differentiating between biologically active monomeric prolactin and inactive macroprolactin [18]. This can lead to misdiagnosis and incorrect treatment, leading to wasted healthcare resources and increased concern for both patients and healthcare providers [23].

The prolactin receptor (PRLR) is a dimer that consists of three main domains: an extracellular domain (EC, residues 1-210), a transmembrane domain (TM, residues 211-234), and an intracellular domain (IC, residues 235-598) [24]. The EC binds to the ligand, initiating PRLR activation [25], [26], [27], [28], [29]. The TM anchors the receptor to the cell membrane, while the IC is involved in signal transduction such as the JAK2-STAT5 pathway [28].

The prolactin receptor, a member of the class I cytokine receptor superfamily [30], [31], gets activated when binding to the prolactin [32], [33]. One of the main downstream signaling pathways for prolactin signaling is the JAK2-STAT5 pathway [34]. Upon prolactin binding to PRLR, signaling is initiated through dimerization and activation of a tyrosine kinase, Janus Kinase 2 (JAK2) [35]. JAK2 activation triggers the phosphorylation of tyrosine residues, recruiting signal transducer and activator of transcription 5 (STAT5) proteins [36]. Phosphorylated STAT5 then dimerizes and get translocated to the nucleus and regulates gene transcription [36]. This pathway is critical for the regulation of various physiological processes such as lactation, reproduction, metabolism, growth, electrolyte transport, and behavior, as well as pathological processes like immunity and carcinogenesis [30], [37], [38].

3. Human Body and Prolactin

Prolactin is a hormone primarily responsible for stimulating lactation in postpartum females [1], [39]. It also plays a crucial role in breast development during pregnancy [39]. In pregnancy, prolactin levels rise significantly, influenced by placental hormones like human placental lactogen, to prepare the mammary glands for lactation [40], [41]. After childbirth, prolactin level surges in response to infant suckling, maintaining milk production, but eventually decreases when lactation frequency declines [40], [42]. In contrast, males generally have lower and more stable prolactin levels, with no significant cyclical changes [43]. Prolactin in males plays a role in regulating the immune system and reproductive function [44].

Prolactin differs between genders in its regulation and function. In women, prolactin levels rise significantly during pregnancy and lactation, playing an important role in reproductive health [40], [41], [42]. Men do not experience these increases, as prolactin levels remain lower and more stable [43]. This difference highlights the hormone's critical role in female reproductive physiology during pregnancy and lactation.

4. Prolactin-Related Disorders

Hyperprolactinemia refers to the prolactin level in the blood that exceeds the reference interval, regardless of its magnitude [45], [46]. This condition can result in significant symptoms, such as infertility in both males and females [47]. Hyperprolactinemia can arise from various factors, including physiological, pathological, and pharmacological background, leading to a complex etiology [48], [49], [50]. Prolactinoma is the predominant cause of chronic hyperprolactinemia along with pregnancy, primary hypothyroidism, and medications that elevate serum prolactin levels [47], [51], [52], [53].

Prolactinomas are a type of pituitary tumor, primarily composed of benign adenomas, which originate from lactotroph cells in the pituitary gland [54], [55]. These tumors are characterized by their ability to produce and secrete prolactin, resulting in hyperprolactinemia [56], [57]. Prolactinomas exhibit a range of sizes, categorized as microadenomas (smaller than 10 mm) and macroadenomas (10 mm or larger) [58], [59], [60]. Microadenomas are more common in premenopausal women, while macroadenomas are more common in men and postmenopausal women [58], [61], [62]. Serum prolactin level is often used as a diagnostic criterion, with levels exceeding 250 ng/mL typically indicative of a macroprolactinoma [58].

Mutations in the prolactin receptor gene (*PRLR)* can alter the structure of the prolactin receptor, affecting its functions. There are natural variations of the *PRLR*, including His212Arg, Arg171Stop, Pro269Leu, and Ile170Leu (Table 2 and Figure 2). The His212Arg, Arg171Stop, and Pro269Leu variants result in a loss-of-function of the prolactin receptor. Specifically, His212Arg and Arg171Stop are the loss-of-function mutations in the extracellular domain, while Pro269Leu affects the cytoplasmic domain. All three mutations are associated with elevated prolactin levels,

contributing to hyperprolactinemia. In contrast, the Ile170Leu mutation, which is also in the extracellular domain, causes a gain-of-function of the receptor and contributes to the development of MFAB.

Table 2: Summary of PRLR Gene Mutations.

Mutation	*Amino Acid Change*	*Domain*	*Type of Mutation*	*Associated Disorder*	*Ref.*
His212Arg	Histidine to arginine	Extracellular	Loss-of-function missense mutation	Hyperprolactinemia	[63]
Arg171Stop	Arginine to stop codon	Extracellular	Loss-of-function nonsense mutation	Hyperprolactinemia	[64]
Pro269Leu	Proline to leucine	Cytoplasmic	Loss-of-function missense mutation	Hyperprolactinemia	[64]
Ile170Leu	Isoleucine to leucine	Extracellular	Gain-of-function missense mutation	Multiple fibroadenomas of the breast (MFAB)	[65]

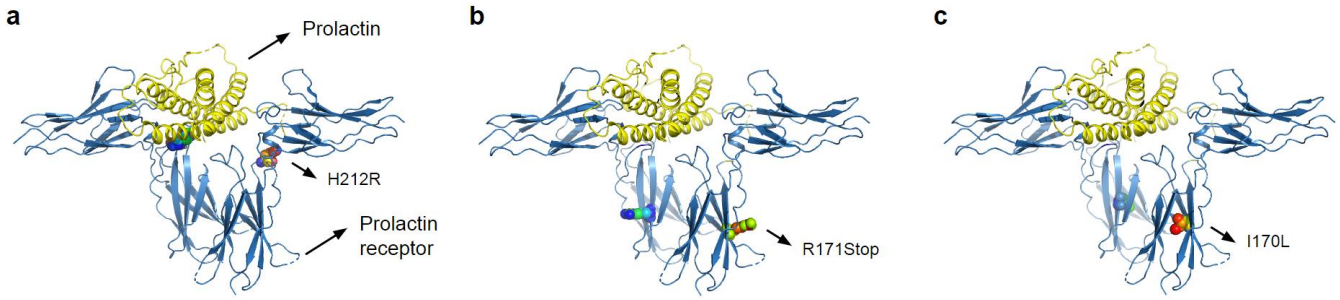

Figure 2: Structural alterations in the prolactin receptor due to specific mutations in the prolactin receptor gene (*PRLR*). The prolactin molecule (yellow) binds to the receptor (blue), with only the extracellular region of the PRLR (PDB: 3NPZ) [25]. (a) The His212Arg (H212R) mutation in the extracellular domain results in a loss-of-function by disrupting the high-affinity ligand-binding interface, leading to impaired downstream signaling and hyperprolactinemia. (b) The Arg171Stop (R171Stop) mutation introduces a premature stop codon in the extracellular domain, producing a truncated, non-functional receptor and causing hyperprolactinemia. (c) The Ile170Leu (I170L) mutation, also in the extracellular domain, results in a gain-of-function by producing a constitutively active receptor, which is associated with multiple fibroadenomas of the breast (MFAB). Illustrated in PyMOL v2.3.4. [66]

The His212Arg (H212R) mutation in the prolactin receptor gene is a missense mutation involving an A-to-G transition, which results in the substitution of histidine with arginine at codon 212. This loss-of-function mutation disrupts the high-affinity ligand-binding interface of the receptor, leading to a loss of downstream signaling via JAK2 and STAT5. Clinically, this mutation has been identified in a three-generation family with autosomal dominant hyperprolactinemia [63]. Normally, functional prolactin receptors on tuberoinfundibular dopaminergic neurons mediate a short-loop negative feedback mechanism. In this mechanism, prolactin binds to its receptors on these neurons, prompting the production and release of dopamine. Dopamine then acts back on pituitary lactotrophs to inhibit further prolactin secretion [39], [67]. The His212Arg mutation impairs prolactin receptor binding to prolactin, disrupting feedback regulation and causing elevated blood prolactin levels [64].

The Arg171Stop (R171Stop) mutation in the prolactin receptor (PRLR) gene is a nonsense mutation resulting from a C-to-T substitution in exon 6, causing the replacement of arginine with a stop codon at codon 171. This loss-of-function mutation produces a shorter, non-functional receptor that prevents signaling when prolactin binds. The absence of functional prolactin receptors due to the Arg171Stop mutation disrupts the feedback loop, leading to unregulated prolactin secretion from the pituitary gland and subsequently causing hyperprolactinemia [64].

The Pro269Leu (P269L) mutation in the prolactin receptor gene is a missense mutation characterized by a C-to-T substitution in exon 9, resulting in the substitution of proline with leucine at codon 269. This loss-of-function mutation in the prolactin receptor gene affects the protein's ability to function normally. It alters a key docking site for JAK2, a crucial component in prolactin signaling pathways. Consequently, the disrupted signaling cascade fails to effectively regulate prolactin secretion through negative feedback mechanisms [64].

The Ile170Leu (I170L) mutation in the prolactin receptor gene is a gain-of-function missense mutation involving an A-to-C substitution in exon 6, resulting in isoleucine being substituted by leucine at codon 170. This genetic change produces a constitutively active receptor capable of signaling without prolactin binding, leading to continuous activation. This mutation was identified in some patients with MFAB specifically [65]. While the Ile170Leu mutation causes constitutive activity in vitro, its clinical significance is debated due to its presence in 2.39% of the European American population, suggesting it might be a common polymorphism [63]. Nonetheless, it is associated with breast tissue abnormalities, MFAB [65].

Point mutations such as His212Arg, Arg171Stop, Pro269Leu, and Ile170Leu alter the PRLR gene, which contribute to various genetic disorders by disrupting receptor function. These mutations can cause either loss- or gain-of-function, affecting prolactin binding and subsequent signal transduction pathways [63], [64], [65]. In addition to these point mutations, alternative splicing can also impact the PRLR gene. This

process gives rise to different PRLR isoforms, each with distinct structural and functional properties, further contributing to the complexity of PRLR-related disorders [68], [69].

Alternative splicing is a critical mechanism by which a single gene can result in multiple protein isoforms through the selective inclusion or exclusion of exons during mRNA processing [70], [71]. This process enhances the functional diversity of proteins and is crucial for cellular differentiation and organism development [68], [69]. However, alternative splicing can also link to diseases like Duchenne muscular dystrophy (DMD) [72] and spinal muscle atrophy (SMA) [73]. The PRLR gene, located on chromosome 5 and comprising at least 10 exons, can undergo alternative splicing that generates various PRLR isoforms with distinct structural and functional properties (Table 3 and Figure 3) [74]. The human prolactin receptor exists in several forms, including a long form (LF), an intermediate form (IF), and multiple short forms (SFs), each with distinct domain structures and functional properties [28], [75]. These isoforms differ in the length and composition of their extracellular and intracellular domains. The isoforms that do not contain a transmembrane domain are soluble prolactin receptors [74].

Table 3: Summary of human prolactin receptor (PRLR) splice variants and their functional characteristics.

Splice Variant	*Amino Acid Length*	*Exon Structure*	*Domain*	*Functional Characteristics*	*Ref.*
LF	622	Full length, exons 1-10	Full extracellular, transmembrane, intracellular	Full receptor function	[76]
IF	349	Major deletion of exon 10 and frameshift	Shortened intracellular	Induces minimal proliferation at high PRL	[77]
ΔS1	521	Lacks exons 4-5	Abbreviated extracellular	Reduced hormone affinity but effective signal transduction	[78]
S1a	376	Splicing from exon 10 to 11	Shortened intracellular	Fails to transmit prolactin signaling	[75], [79]
S1b	288	Splicing from exon 9 to 11	Shortened intracellular	Dominant-negative effect on differentiation signal transduced by LF	[75], [79]
Δ4-S1b	217	SF1b with deletion of exon 4	Lack of signal peptide and partial extracellular domain	Loss of PRL binding ability	[79]
S1c	309	Lacks exon 10	Shortened intracellular	Identified in spermatozoa	[80]
Δ7/11	268	Splicing from exon 7 to 11	No transmembrane domain	Soluble	[79]
Δ4-Δ7/11	197	Δ7/11 with deletion of exon 4	No transmembrane domain, lack of signal peptide and partial extracellular domain	Soluble, loss of PRL binding ability	[79]

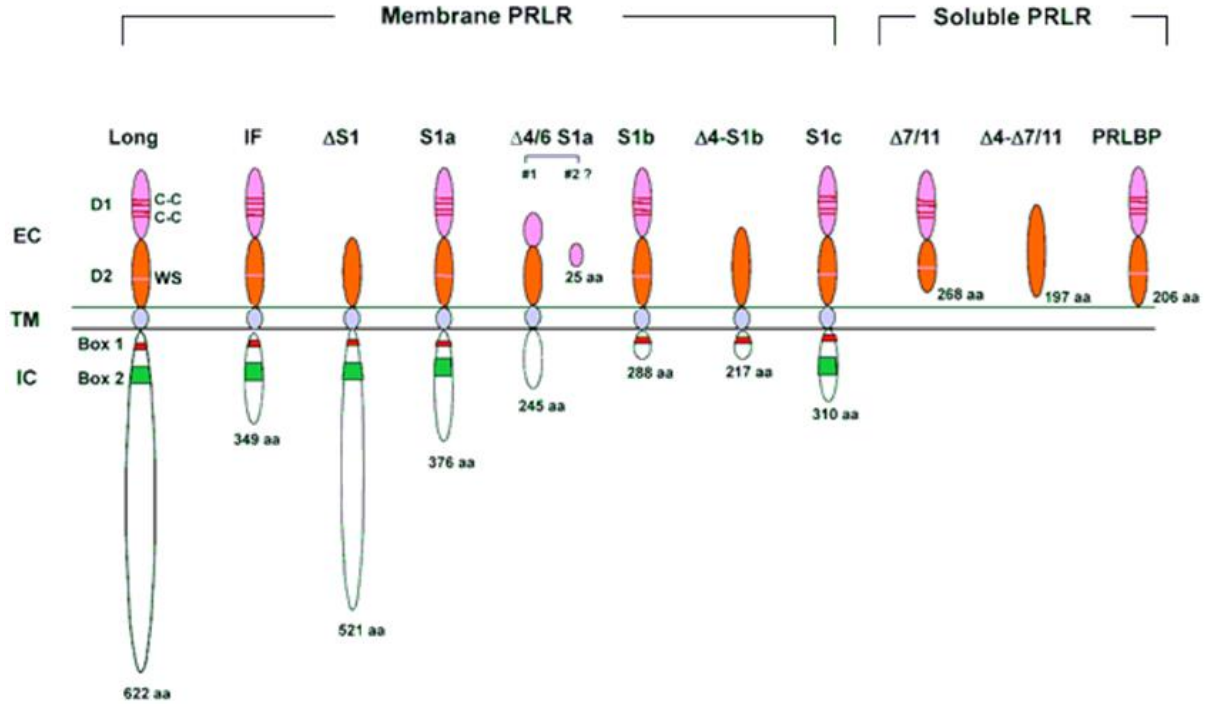

Figure 3: Structure of human prolactin receptor (PRLR) splicing variants. The various isoforms of the human PRLR are shown and divided into membrane PRLR and soluble PRLR. Membrane PRLRs contain the transmembrane domain (TM), which allows them to extend across the cell membrane. This category includes the long form (LF), intermediate form (IF), and some short forms (SFs). The LF contains a full extracellular domain (EC), TM, and intracellular domain (IC), while the IF has a major deletion in exon 10 and a shortened intracellular domain. Short forms, such as S1a, S1b, and S1c, differ in their exonal structures and domain compositions. ΔS1 refers to the long form lacking the D1 domain. Soluble PRLRs lack the TM and are found in the extracellular matrix. It includes variants like Δ7/11, Δ4-Δ7/11, and prolactin binding protein (PRLBP). Key components are

labeled: D1 and D2 denote the N-terminal subdomains; C stands for cysteine; WS represents the WSXWS motif; Δ indicates deleted exons. Reprints with permission from Tsai-Morris et al. [81]

Prolactin splice variants are crucial in understanding the diverse roles of the prolactin receptor in various physiological and pathological processes. In breast cancer, the balance between different PRLR isoforms is particularly significant. The SFs of the receptor, such as S1a and S1b, often act as dominant-negative inhibitors of the LF, modulating the receptor's overall activity. A decreased ratio of SFs to LF in breast cancer tissues compared to adjacent normal tissues suggests a loss of inhibitory control, potentially leading to increased tumor cell proliferation [82]. Understanding the expression patterns and functional implications of these splice variants can provide insights into the mechanisms driving breast cancer progression and might reveal novel targets for therapeutic intervention.

In endocrinology, accurate measurement of prolactin level is critical for diagnosing various disorders. Imbalances in prolactin levels can contribute to conditions such as hyperprolactinemia, which is often associated with pituitary tumors such as prolactinomas [4], [5]. Accurate measurement of prolactin in the blood is therefore essential for diagnosing these disorders and managing patient care effectively. However, there are challenges in measuring prolactin levels with different methodologies.

5. Methodology

In 1977, the Nobel Prize in Physiology or Medicine was given for the invention of the radioimmunoassay (RIA), an innovative technique for measuring peptide hormones. Afterward, RIA is applied in the detection of the non-immunogenic steroid hormones [83], [84], [85]. Notably, RIA can be used to measure prolactin levels [86]. This technique involves using antibodies labeled with radioactive isotopes, such as iodine-131, iodine-125, tritium, carbon-14, and sulfur-35, to detect and quantify specific antigens or hormones by measuring the radioactivity emitted from these tracers [87], [88]. Although the traditional advantage of RIA was its high sensitivity, modern enzyme immunoassay (EIA)-based methods now offer comparable sensitivity [89]. Besides, the RIA technique has several significant disadvantages. It involves using antibodies labeled with radioactive isotopes, such as iodine-131, which has a limited half-life of about 8 days [88]. Moreover, the radioiodination process is potentially hazardous [90]. Handling and disposing of radioactive materials require strict regulations and facilities, usually confined to only a few laboratories, making RIA less suitable for routine diagnostics [89], [90].

Nowadays, endocrine issues are evaluated using a variety of laboratory techniques. Mass spectrometry has been utilized for measuring prolactin levels, allowing differentiation between various prolactin isoforms due to its high specificity and sensitivity [91]. However, mass spectrometry is relatively expensive and not typically used for routine testing. As a result, immunoassays remain the most widely used method for assessing hormonal disorders [92].

One widely used method for measuring serum prolactin levels in modern clinical laboratories is the automated, high-throughput, 2-site "sandwich" enzyme-linked immunosorbent assays (ELISA) [93]. The analytical ranges for prolactin measurement usually fall between 10 mU/l to 5,000 mU/l, covering a broad range of clinical situations [45]. In the non-competitive sandwich ELISA method, capture antibodies are attached to a solid phase in the microtiter plate. Next, the serum sample containing prolactin is added, where the capture antibodies on the microtiter plate bind prolactin from serum samples. Following this, a detection antibody is introduced. It binds to the captured prolactin, forming a "sandwich" structure. After washing away any unbound detection antibodies, an enzyme-labeled secondary antibody is added, which binds to the detection antibody. The signal is then measured by adding a substrate that reacts with the enzyme, generating a colored signal proportional to the prolactin concentration [94]. Quantification is achieved by comparing the signal to a standard curve generated from known prolactin concentrations (Figure 4).

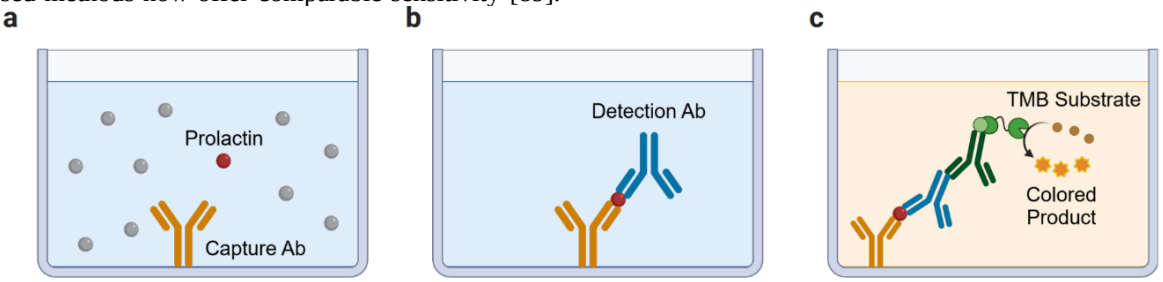

Figure 4: Steps of the prolactin sandwich ELISA assay. (a) Capture antibody is immobilized on a solid phase within the microtiter plate. The serum sample containing prolactin is added. (b) The capture antibody binds to prolactin in serum samples. Detection antibody is then introduced, forming a "sandwich" with the captured prolactin during incubation. (c) An enzyme-labeled secondary antibody binds to the detection antibody, producing a colored signal proportional to the prolactin concentration in the sample. Figure created with BioRender.

6. Challenges in Measuring Prolactin Levels

Despite advancements in diagnostic techniques, hyperprolactinemia presents significant diagnostic challenges, particularly due to potential pitfalls such as the high-dose hook effect and macroprolactinemia when measured by non-competitive sandwich ELISA.

The high-dose hook effect occurs when increasing the concentration of an analyte initially raises the test signal.

However, once the analyte concentration surpasses a certain limit, the test becomes saturated and the signal begins to decrease. This results in a hook-shaped curve on the graph, where the signal drops instead of continuing to rise with higher analyte levels [95]. The high-dose hook effect can happen when using immunoassays to measure a specific hormone (analyte). This is particularly noticeable when using the two-site "sandwich" assay [96]. In normal cases, where the prolactin level is either normal or increased within the tolerance of the assay (i.e., not exhibiting a high-dose hook effect), an antibody-hormone-antibody "sandwich" is formed. Prolactin binds to the capture antibody on one end and the detection antibody at the other end, generating a signal directly proportional to the prolactin concentration in the sample [97]. However, when the prolactin concentration is abnormally high or the antibodies get depleted, prolactin saturates both the capture and detection antibodies, thus inhibiting the formation of the sandwich structure. After washing away unbound detection antibodies, only a few "sandwiches" remain attached to the solid surface, resulting in a detected signal that indicates a low or only mildly increased prolactin concentration [95]. To overcome the high-dose hook effect, serial dilution of the sample can be employed. By diluting the serum sample before testing, the concentration of prolactin is reduced, which prevents the saturation of antibodies and facilitates the proper formation of the antibody-prolactin-antibody complexes. This approach enables the assay to return to a range where the signal is directly proportional to the analyte concentration, thereby improving the accuracy of the test results [98].

Macroprolactinemia is a condition characterized by elevated levels of macroprolactin in the bloodstream, which is a complex formed by prolactin bound to IgG autoantibodies [12], [13], [14], [19]. Although macroprolactin is often less biologically active than monomeric prolactin, it can still lead to misleadingly high total prolactin measurements in standard immunoassays, complicating the diagnosis of prolactin-related disorders [12]. One effective solution to this diagnostic challenge is to perform Polyethylene Glycol (PEG, Molecular Weight 200-6,000 Da [99]) precipitation, a method that selectively removes macroprolactin from serum samples. When PEG is added to the serum, it precipitates the larger macroprolactin complexes, allowing for the more accurate measurement of biologically active monomeric prolactin levels [100]. This procedure enhances the specificity of prolactin assays and improves diagnostic accuracy, particularly in cases where high total prolactin levels are detected.

We explored the diagnostic challenges in hyperprolactinemia related to the high-dose hook effect and macroprolactinemia and identified a few case studies. The first case involves a 45-year-old man with clinical symptoms and MRI findings indicative of a macroadenoma. Initial prolactin measurements were falsely low due to the high-dose hook effect, but serial dilution confirmed a diagnosis of macroprolactinoma [101]. The second case study explores macroprolactinemia in Thai hyperprolactinemic patients, finding a 20% prevalence. It emphasizes the importance of performing PEG precipitation in diagnostic protocols to prevent misdiagnosis and improper treatment, ensuring accurate assessment and appropriate management of hyperprolactinemia [102].

7. Current Therapies, Future Directions and Novel Therapies for Prolactin-Related Disorders

Treatment for hyperprolactinemia primarily aims to restore and maintain normal gonadal function and fertility, as well as to prevent osteoporosis [103], [104]. Prolactinomas, which are the most common cause of hyperprolactinemia, can be treated with medications, surgery, or, in rare cases, radiation therapy [55], [105]. The most common treatment for prolactinomas is medication. Dopamine agonists like bromocriptine (BRC) and cabergoline (CAB) are the preferred first-line treatments for patients with idiopathic hyperprolactinemia and prolactinomas [51], [106]. Surgery and radiation therapy are considered for refractory and medication-intolerant patients [106], [107].

Dopamine agonists are divided into two types: (1) ergot derivatives, including bromocriptine (BRC), cabergoline (CAB), and pergolide (PER), and (2) non-ergot derivatives such as quinagolide (QG) [108]. These medications bind to dopamine receptors and work by mimicking the action of dopamine, a neurotransmitter that inhibits prolactin production [109]. Through various molecular pathways mediated by dopamine receptor D2, dopamine agonists cause lactotrophs to undergo apoptosis, autophagic cell death, and paraptosis, which reduces prolactin secretion and shrinks tumors [110]. However, these medications can cause severe side effects, including nausea, headaches, dizziness, fatigue, and hypotension [111]. A summary of dopamine agonist therapies for prolactinoma is provided in Table 4.

Table 4: Comparison of dopamine agonist therapies in prolactinoma.

Drug	*Class*	*Efficacy*	*Side Effects*	*Ref.*
Bromocriptine (BRC)	Ergot derivative	Controls prolactin in 80-90% of microprolactinomas, 70% of macroprolactinomas	Nausea, vomiting, dizziness, postural hypotension	[112], [113], [114]
Cabergoline (CAB)	Ergot derivative	Normalizes prolactin in 86% of patients; reduces tumor size in 67%; improves visual field abnormalities in 70% of patients	Possible cardiac valve disease	[115], [116], [117]
Quinagolide (QG)	Non-ergot derivative	Controls prolactin in 81% of patients, reduces tumor size	More frequent side effects than cabergoline	[118], [119], [120]

Bromocriptine (BRC), the oldest treatment for prolactinomas, is an ergot alkaloid with D2 receptor agonist and D1 antagonist properties [121]. It remains a widely used alternative to cabergoline [122]. Bromocriptine is typically administered orally 2-3 times daily, with doses ranging from 2.5 to 15.0 mg per day, and up to 30 mg per day for resistant

cases [123], [124], [125], [126], [127], [128], [129]. It controls hyperprolactinemia in 80-90% of patients with microprolactinomas and 70% of those with macroprolactinomas, which improves gonadal function and reduces tumor size [113], [114]. However, rapid intestinal absorption of bromocriptine results in several adverse effects, including headache, dizziness, nausea, vomiting, and postural hypotension [112].

Cabergoline (CAB), a selective D2 receptor agonist, is highly effective in treating hyperprolactinemia [130]. It is generally administered orally at a starting dose of 0.5 mg per week for idiopathic hyperprolactinemia or microprolactinomas [118], with lower doses (0.25 mg per week) for macroprolactinomas to prevent excessively fast tumor shrinkage [120], [131]. A large study involving 455 patients found that cabergoline treatment normalized prolactin levels in 86% of all patients, including 92% of 244 patients with microprolactinomas or idiopathic hyperprolactinemia and 77% of 181 patients with macroadenomas. Additionally, tumor shrinkage was observed in 67% of patients, and 70% experienced improvements in visual field abnormalities [115]. However, some research has indicated that cabergoline might negatively impact the cardiac valves [116], [117].

Quinagolide (QG), a non-ergot dopamine agonist with selective D2 receptor activity, is administered orally once daily and effectively controls prolactin levels and tumor growth in patients with hyperprolactinemia [132]. Studies have shown that quinagolide normalizes prolactin levels and reduces tumor size in patients with macroprolactinomas [119]. After 24 weeks of treatment, quinagolide achieved biochemical control of prolactin excess in 81% of patients, compared to 70% for bromocriptine [120]. Nevertheless, quinagolide is less effective and has more frequent side effects compared to cabergoline [118].

Surgery is considered when medication is ineffective or the patient cannot tolerate the side effects of the drugs [105]. The most common surgical approach is transsphenoidal surgery, where the tumor is removed through the nasal passages [105]. This procedure is typically used when tumors are large or causing significant symptoms.

Radiation therapy is a rare third option used when both medication and surgery fail to control prolactin levels [133], [134]. This treatment involves targeting and destroying tumor cells with radiation [135], [136]. Due to its potential side effects and the availability of other treatments, it is not commonly used [137], [138].

Receptors often can recognize multiple ligands, while individual ligands can bind to various receptors [139]. In many cases, each ligand triggers a specific biological response by binding to a different site on the receptor's surface, which activates a specific signaling pathway. Occasionally, a receptor can interact with different ligands using the same amino acid sequence but still produce different biological effects [140]. The prolactin receptor is a well-known example of this flexibility in ligand binding, as it binds to prolactin (PRL) [26], [141] and growth hormone (GH) [142], [143] (Figure 5), also known as somatotropin, utilizing the same group of amino acids in its EC. Despite this, each hormone triggers different responses in the body. This dual binding ability makes it challenging to develop treatments that can selectively influence receptor activity [140]. To address the challenge of the prolactin receptor's dual binding ability, Rizk et al. [140] have explored the use of fragment antigen-binding region (Fab), a portion of synthetic antibodies (sABs), to control the activity of the human prolactin receptor, indicating the potential for future medical treatments (Figure 6).

Rizk et al. introduced a novel Fab that could distinguish between the different conformational states of the receptor when bound to prolactin or growth hormone. The Fabs were developed using phage display, a technique that created a large library of antibodies on bacteriophages. By incubating the EC of the PRLR with a phage display, researchers identified four unique Fab clones: A4, A8, A9, and A10. Clones A8, A9, and A10 showed strong binding affinities to the human prolactin receptor extracellular domain, whereas A4 showed minimal detectable binding and was therefore used as a negative control in further experiments [140].

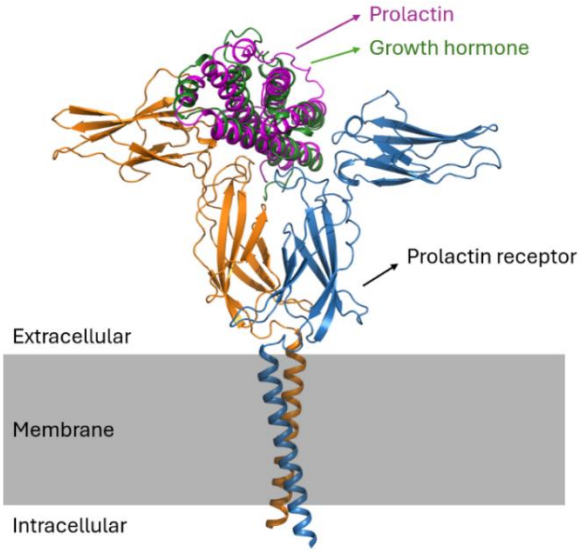

Figure 5: Superimposition of the structures of prolactin (PRL) and growth hormone (GH) bound to the full-length prolactin receptor (PRLR). The prolactin receptor (PRLR) is shown in blue for the monomer, orange for the homodimer, magenta for prolactin (PRL), and green for growth hormone (GH). The receptor is presented in its active, ligand-bound state. Adapted from AlphaFold3 [144]. Alignments were done using prolactin receptor L26-F268 (RMSD = 0. 973 Å) to show the difference between prolactin and growth hormone in a receptor-bound state. Illustrated in PyMOL v2.3.4. [66]

The structural studies revealed that these Fabs bound to an epitope on the PRLR that was distinct from the hormone-binding site. For instance, the crystal structure of the Fab A8 in complex with PRLR (PDB: 4I18) [140] showed that the Fab bound to a region away from the hormone-binding site, inducing a conformational change that favored GH binding over PRL [142]. This allosteric modulation suggested that Fabs could selectively induce receptor activity by stabilizing specific receptor conformations.

The ability of these Fabs to modulate receptor activity had

significant therapeutic potential. For example, in breast cancer cells, Fabs that inhibited PRLR function could potentially reduce tumor growth driven by prolactin signaling. The research showed that Fabs A8, A9, and A10 effectively blocked prolactin-induced receptor internalization and downstream signaling pathways, such as the STAT5, ERK, and AKT pathways, which were crucial for cell proliferation and survival. This inhibition was more effective for prolactin than for growth hormone, highlighting the specificity of these Fab fragments in regulating receptor activity [140].

In therapeutic applications, Fabs could be used to selectively inhibit prolactin signaling in diseases where prolactin played a pathogenic role, such as in certain types of breast cancer or prolactinomas. By preventing prolactin from activating its receptor, these fragments could reduce the growth and proliferation of cancer cells that relied on prolactin signaling for survival. This approach could lead to new treatments, providing a targeted way to combat diseases associated with abnormal prolactin signaling [140].

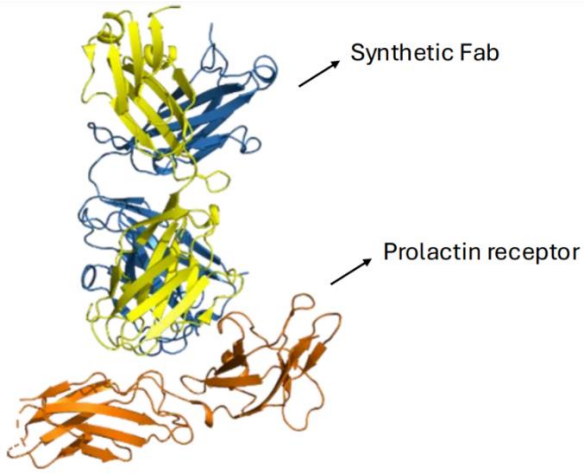

Figure 6: Fab binding to the prolactin receptor. The Fab, a portion of a synthetic antibody, binds to an epitope on the extracellular region of the prolactin receptor (orange), separate from the hormone-binding site (PDB: 4I18)[140]. The light chain and heavy chain of the Fab are colored in yellow and blue, respectively. This interaction induces a conformational change in the prolactin receptor, leading to allosteric inhibition of prolactin receptor signaling. This binding ability selectively alters receptor activity, offering the potential for targeted therapies in diseases driven by prolactin signaling, such as certain types of breast cancer. Illustrated in PyMOL v2.3.4. [66]

The development of Fabs that selectively modulated the PRLR offered a possible approach to therapeutic intervention. These Fab fragments, developed by using phage display, could selectively distinguish between various receptor conformations due to the hormone binding. This facilitated the targeted inhibition of specific signaling pathways. This approach could enable the development of new treatments for conditions associated with abnormal prolactin signaling, providing a new strategy for managing diseases like breast cancer [140].

Here, we discussed the potential diagnostic pitfalls in hyperprolactinemia, focusing on the high-dose effect and macroprolactinemia. The high-dose hook effect, also known as the prozone phenomenon, occurs in immunoassays when assay antibodies fail to form a complete sandwich structure in the presence of very high prolactin concentrations. The excessive levels of prolactin saturate antibodies, hindering proper antigen-antibody complex formation. This saturation can yield artificially low prolactin readings, potentially causing false-negative diagnosis [95]. Conversely, macroprolactinemia involves the presence of macroprolactin in blood, which is a large molecular weight form of prolactin. Macroprolactin is often less biologically active but can still be detected by standard immunoassays [12]. This can cause false-positive results, suggesting hyperprolactinemia even in cases where clinical symptoms may not correlate with prolactin activity [45].

8. Conclusion

Prolactin is a key hormone with significant roles in lactation and reproductive health. Diagnostic challenges, such as the high-dose hook effect and macroprolactinemia, complicate the accurate measurement of prolactin levels. While current treatments like dopamine agonists are effective, emerging therapies hold promise for more targeted interventions. Future research should focus on improving diagnostic accuracy and developing novel therapies to address prolactin-related disorders.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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